Available online at www.sciencedirect.com
SCIENCE DIRECT®
Coordination Chemistry Reviews 249 (2005) 2437–2457
COORDINATION CHEMISTRY REVIEWS
www.elsevier.com/locate/ccr

Review

Metal compounds and small molecules activation – case studies

Wojciech Macyk [a], Alicja Franke [a,b], Grażyna Stochel [a,*]

[a] *Faculty of Chemistry, Jagiellonian University, ul. Ingardena 3, 30-060 Kraków, Poland*
[b] *Institute of Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstr. 1, D-91058 Erlangen, Germany*

Received 15 December 2004; accepted 9 June 2005
Available online 26 August 2005

Contents

Abbreviations: Φ_Δ, quantum yield of $^1O_2$ formation; Cbl, cobalamin; C*c*O, cytochrome *c* oxidase; cyclam, 1,4,7,9-tetraazacyclotetradecane; DNICs, dinitrosyl iron complexes; DTC, dithiocarbamate; dtpa^{5-}, diethylenetriaminepentaacetate; edampda^{2-}, *N*,*N*′-bis(2-pyridylmethyl)ethylenediamine-*N*,*N*′-diacetate; EDRF, endothelial-derived relaxing factor; edta^{4-}, ethylenediaminetetraacetate; FTTP, tetra(*m*-trifluoromethylphenyl)porphyrin; hedtra^{4-}, hydroxyethylenediaminetriacetate; IFET, interfacial electron transfer; IRP, iron regulatory protein; mida^{2-}, methyliminodiacetate; metMb, metmyoglobin; NADH, reduced nicotinamide adenine dinucleotide; NHase, nitrile hydratase; nta^{3-}, nitrilotriacetate; OEP, octaethylporphyrin; P450$_{cam}$, cytochrome P450 from *Pseudomonas putida*; pida^{2-}, 2-pyridylmethyliminodiacetate; Por, porphyrin; RBS, Roussin's Black Salt; RNOS, reactive NO species; ROS, reactive oxygen species; RRS, Roussin's Red Salt; salen, *N*,*N*′-bis(salicylidene)ethyelenediamine dianion; SNP, sodium nitroprusside; Sol, solvent; tim, 2,3,9,10-tetramethyl-1,4,8,11-tetraazacyclodeca-1,3,8,10-tetraene; TMPS, tetra(sulfonatomesityl)porphine; TmTP, tetra-*m*-tolylporphine; tpa, tris-2-pyridylmethylamine; tpen, *N*,*N*,*N*′,*N*″-tetra-(2-pyridylmethyl)ethylenediamine; TPP, tetraphenylporphyrin; TPPS, tetra(4-sulfonato-phenyl)porphine; ttha^{6-}, triethylenetetraaminehexaacetate

* Corresponding author. Tel.: +48 12 663 2243; fax: +48 12 634 0515.
E-mail address: stochel@chemia.uj.edu.pl (G. Stochel).

doi:10.1016/j.ccr.2005.06.018

Abstract

The activation of small molecules has a significant impact in biology, medicine, industrial catalysis and environmental protection. Two molecules, especially, oxygen and nitric oxide have attracted considerable interest for many years. Reactive oxygen species (ROS) and reactive nitric oxide species (RNOS) may be generated thermally or photochemically in systems consisting of metal compounds. In homogeneous systems metal species serve as a coordination and/or electron (eventually energy) transfer centre. In heterogeneous systems small molecules can undergo adsorption followed by electron or energy transfer processes with semiconductor participation. This review presents recent trends in studies on the metal assisted processes in which activated forms of NO or O_2 are formed and play a key biological and medical role.

Keywords: ROS; RNOS; Photosensitiser; Semiconductor; Activation; Mechanism; Bioregulation; Phototherapy; Photodetoxification; Photocatalysis

1. Introduction

Small molecule activation (NO, O_2, CO, CO_2, N_2, etc.) has attracted scientific attention for several years. It has a significant impact in biology, medicine, industrial catalysis and environmental protection. Each activation process has its own requirements depending primarily on the desired effect such as change of the reaction pathway, selectivity, effectiveness, yields, new processes or reactions which are not allowed thermodynamically and/or kinetically from the substrate ground state, lower energy input, etc. These goals could be achieved in a thermal or photochemical manner in homogeneous or heterogeneous systems as direct or indirect processes. Metal ions and compounds play a crucial role in thermal and photochemical activation of small molecules. They not only can mediate the actual active form of the small molecule, but also control its spatial concentration dynamics.

In homogeneous system an activated molecule is usually coordinated to a metal centre while in heterogeneous systems adsorption processes play a key role. The bound molecule shows different physical and chemical properties (geometry, electron density, reactivity, etc.) as compared to those of the free one. The coordinated or adsorbed molecule also has a different mobility: the bound moiety can be transported to other places where, under certain conditions, it can be released. Finally, access to a coordinated or adsorbed molecule may be limited to a certain type of substrate due to possible steric hindrance and limited size of the cavity in which the molecule is embedded. Data on the electronic and structural character of the active form as well as on the thermodynamics and kinetics of the particular activation process is necessary not only for understanding its mechanism, but also for enabling a systematic tuning of the studied systems to achieve the best results.

Considerations in this paper are focused on two biologically important molecules: nitric oxide, NO, and dioxygen, O_2. The solubility and transport properties of NO and O_2 are similar but these two molecules in their ground states display quite different reactivity. Nitric oxide, a stable-free radical with an unpaired electron in the π^* orbital, achieves a wide variety of effects through its interaction with active centres via redox and addition chemistry and therefore can be considered as one of the 'reactive nitric oxide species' (RNOS). NO can reversibly bind to a metal ion and plays the role of an electron donor or an electron acceptor. However, it is neither a strong one-electron oxidant nor a strong one-electron reductant. The properties of O_2 molecule are different. Its ground triplet state shows much lower activity and therefore must be excluded from the 'reactive oxygen species' (ROS) family. Moreover, for NO and O_2 there are different priorities for activation modes. In the case of nitric oxide electron transfer processes are the most important source of different RNOS whereas in the case of ROS, energy transfer processes are as important as electron transfer processes. ROS in systems containing metal compounds can be achieved either through thermal or photochemical paths. As a result of photochemical activation electron or energy transfer occurs. In the case of the electron transfer, radicals are mainly generated while energy transfer is responsible rather for singlet oxygen generation. This differentiation, however, is not very strict since several consecutive thermal reactions influence the formation of various ROS. An additional advantage of the application of light is the high selectivity of supported energy which cannot be realised by thermal methods. The selection of an appropriate light energy helps to achieve the desired excited state and therefore the desired activity. For instance, oxygen activation leads to formation of one of two forms of singlet oxygen, $^1\Sigma_g$ or $^1\Delta_g$, having different energy.

Coordination compounds or solid surface participating in the activation process support an appropriate reaction site. The geometry of this site may play a key role, for instance in enzymatic reactions. The metal centre controls redox properties and therefore the nature of coordinated (or chemisorbed) small molecule. In the case of photoactivated systems the presence of metal ion assures appropriate spectroscopic properties of the sensitiser. A metal ion may influence the lifetimes of a triplet excited state of a sensitiser enhancing the efficiency of small molecule photoactivation.

The aim of this paper is not to review the state of art in NO and O_2 activation research. Several excellent reviews covering these topics appeared both for NO [1–6] and O_2 [6–12] activation. Here we want to emphasise selected aspects of metal assisted RNOS and ROS generation in very different homo- and heterogeneous systems (Fig. 1). In the case of NO we focus on exemplifying the role of biological metal centres and possible applications of exogenous metal compounds in bioregulatory functions of nitric oxide and its activation by redox or substitution reactions. On the other hand, for dioxy-

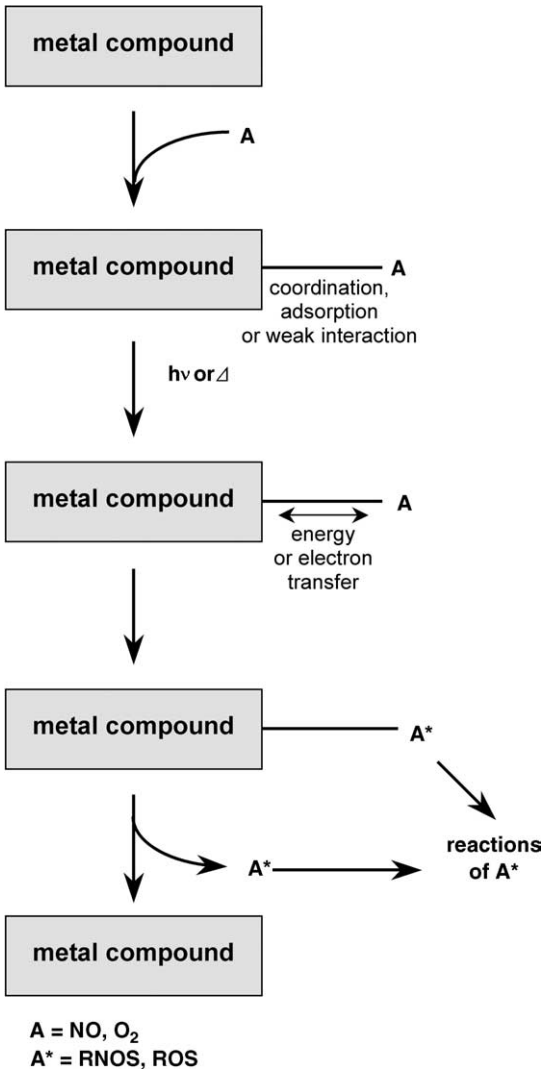


Fig. 1. Metal compounds in NO and O_2 activation processes.

gen we mostly focus on the role of metal compounds in the photochemical/photophysical activation of O_2 and selected examples of the biological and environmental applications.

2. Nitric oxide activation

2.1. Biological and medical aspects of nitric oxide

The discoveries that nitric oxide is responsible for an astonishing range of physiological and pathological processes in humans have attracted considerable scrutiny over the past decade and led to renewed interest in the chemistry of NO and its derivatives such as metal nitrosyl complexes or other reactive nitric oxide species. It is now well established that nitric oxide plays a key role in vascular system regulation [13,14], signalling between nerves in both the peripheral and central nerve system [15,16] and in immune response to pathogens [17,18]. At the tissue level, a local and temporary change in nitric oxide concentration is translated into a cellular signal. The kinetics and mechanisms that determine NO concentration dynamics in tissues are still poorly understood. Generally, NO mediates its effects by stimulating or inhibiting transition metal-containing proteins and by post-translational modification of proteins (e.g. formation of nitrosothiol adducts) [17,19–21]. Interactions of nitric oxide with iron centres are undoubtedly the most important biological reactions in which NO participates. Both guanylate cyclase regarded as the major target for NO, and NO-synthase, the enzyme which catalyses biosynthesis of nitric oxide, are iron heme proteins [22–24]. The border line between the physiological and pathological activities of NO is a matter of controversy, but tissue redox environment, supramolecular organisation and compartmentalisation of NO targets are important figures in determining NO actions. Reactive oxygen species and metal ions can drastically modify NO activity. For example, oxidative and nitrosative stress or neurodegenerative diseases (e.g. Alzheimer's disease, Huntington's disease, Parkinson's disease) may have the origin in the dynamic imbalance between these three types of species (ROS, RNOS, M^{n+}) [25,26]. Concentrations of NO produced in vivo for bioregulatory purposes are very low, usually less than micromolar, whereas concentrations of this species synthesised during defence against microorganisms and tumours are much higher.

Since nitric oxide serves so many important roles in mammalian bioregulatory and immunology it is not surprising that breakdown in the regulation of its biosynthesis and metabolism results in a number of diseases states such as hypertension, diabetes, arthritis, epilepsy or septic shock [27–30]. This has stimulated extensive research activity into search for new therapeutics and NO delivery methods to regulate the formation, metabolism and function of nitric oxide under physiological conditions. A number of such pharmaceutical agents capable of regulating the NO level in vivo have been used successfully, including inhibitors of nitric oxide synthase and prodrugs of the nitrovasodilators class such as nitroglycerin, organic and inorganic nitrites and nitrates, sodium nitroprusside, nitrosamines, hydrazines, etc. [31–33]. Since much of the biochemistry of NO involves metal nitrosyl complexes, a novel approach is based on the application of transition metal complexes in the regulation of the NO level in biological systems. Some of them, such as those of Fe and Ru, have been proved to be capable to release or binding of NO in vivo in the situation of its under- or overproduction, respectively. Also of great interest is the use of photochemistry of metal nitrosyl complexes as a mode for delivering NO to specific targets on demand in biological systems.

2.2. Reactive nitric oxide species (RNOS)

The reactive nitric oxide species (RNOS) which are generated, transferred and consumed within biological and medical processes may be viewed as $NO^{\bullet}$, NO^{-} and NO^{+} species. They show a large variety of behaviours, from oxi-

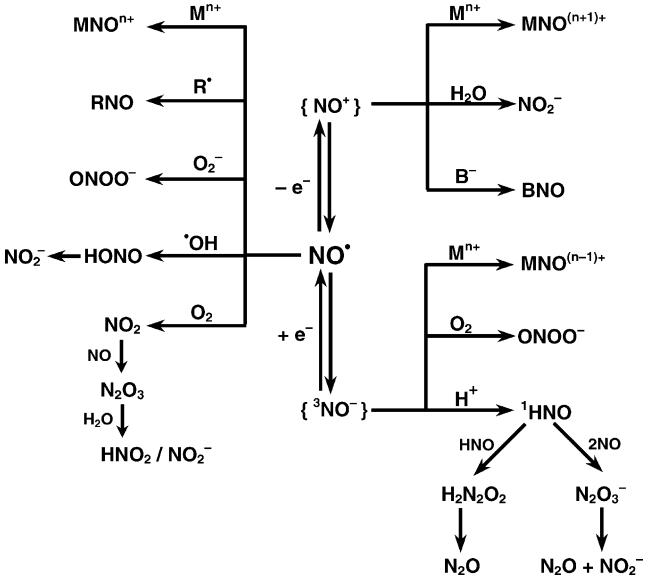


Fig. 2. Reactive nitric oxide species (RNOS) and some of their reactions (M^{n+}: metal ion, $R^{\bullet}$: radical, B^-: base).

dising to reducing, from nucleophilic to electrophilic, from σ donors to large contribution of π acceptance. Some main paths of their reactivity are presented in Fig. 2. In biological micro-environments (pH, redox milieu, hydrophobicity, hydrophilicity, compartmentalisation, etc.) from the three interrelated redox forms only $NO^{\bullet}$ is stable enough to directly play its role. The two other active redox forms (NO^- and NO^+) must be stabilised via interactions with other molecules, especially O_2 and metal complexes.

$NO^{\bullet}$ as a stable-free radical participates very readily in one-electron events such as coupling to other free radicals (e.g. $OH^{\bullet}$, $O_2^{\bullet-}$, lipid peroxyl radicals, thiol radicals, etc.) Its reactions with $OH^{\bullet}$ (to give nitrite) or with $O_2^{\bullet-}$ (to give peroxynitrite) proceed at rates near the diffusion limit. NO is also very reactive with redox active and substitution labile metal centres. Generally, substitution reactions of NO on metal centres as well as reactions with radicals display kinetic rate laws with a first order dependence on NO concentration. In the contrary reactions where substrate undergoes two electron changes, 2 equiv. of NO are required and kinetic rate laws are third-order with second-order dependence on NO concentration. For example reaction of NO with O_2 in aqueous media (to give nitrite, Eq. (1)) is slow under physiological conditions (low-submicromolar NO concentrations) (Eq. (2), $4k_{aq} \approx 9 \times 10^6\ M^{-2}\ s^{-1}$) [34–37]:

$$4NO + O_2 + 2H_2O \rightarrow 4H^+ + 4NO_2^- \quad (1)$$

$$-d[NO]/dt = 4k_{aq}[NO]^2[O_2] \quad (2)$$

In hydrophobic media the product of NO autooxidation is not NO_2^-, but the much more reactive (especially as nitrosating agent) nitrogen dioxide NO_2. The aqueous redox chemistry of NO is highly sensitive to pH as both nitrite reduction to NO and NO reduction to N_2O (via HNO) are proton coupled reactions (Eqs. (3) and (4), and Fig. 2) [38–40]:

$$NO_2^- + 2H^+ + e^- \rightarrow NO + H_2O \quad (3)$$

$$NO + H^+ + e^- \rightarrow {}^1HNO \quad (4)$$

As a consequence NO is a relatively strong reducing agent at high pH and nitrous acid a strong oxidant at low pH. During immune response NO is produced in much higher concentrations than is typical for bioregulatory functions and under these conditions reactive nitrogen species such as peroxynitrite ($ONOO^-$) and N_2O_3 may have biological importance [3,19,41]. Peroxynitrite has received considerable attention as a possible toxic/mutagenic agent, but from the other side reaction between NO and $O_2^{\bullet-}$ (Eq. (5)) could be considered as cytoprotective due to a less damaging biological effect of $ONOO^-$ than $O_2^{\bullet-}$:

$$NO + O_2^{\bullet-} \rightarrow ONOO^- \quad (5)$$

NO^- behaves as a reducing agent and base. NO^- is isoelectronic with O_2 and like dioxygen has a triplet ground state. The recent study shows that $^3NO^-$ is more stable than $^1NO^-$ and ^1HNO is more stable than ^3HNO or $^3NO^-$ [39,40]. The effective pK_a for the transformation $^1HNO = {}^3NO^- + H^+$ is relatively high (11.3), so in biological media ^1HNO will be dominant. This is important because $^3NO^-$ reacts with $^3O_2$ at a near diffusion-limited rate to form peroxynitrite ion $ONOO^-$ whereas reaction of $^3O_2$ with the conjugate acid ^1HNO is slow. In the longer time scale, nitrous oxide (N_2O) may be formed from ^1HNO either via dimerisation of ^1HNO and dehydration or via intermediate the $N_3O_3^-$ species which decomposes to N_2O and NO_2^-.

NO^+ is a strong oxidant. In water it undergoes rapid hydrolysis to nitrite NO_2^-. The NO^+ species behaves mainly as an acid generating the nitroso compounds B–NO with bases (B). The nitrosation is a consequence of a nucleophilic attack of S-, N-, O- and C-donors at the nitrogen atom of the nitrosonium ion. From biological standpoint especially important are *S*-nucleophiles like thiols (R-SH) which serve among others for NO^+ reservoir [42].

Although the diverse redox NO forms demonstrate various reactivities, each of them is able to generate $[MNO]^n$ complexes (Fig. 2). This apparently strange behaviour results from the uncommon properties of the NO-ligand which can be treated not only as 2-electron, but also as 3-electron donor, which forms both σ and π bonds and shows a wide variety of coordination geometries. Formation and breaking of M–NO bonds and reactions of coordinated nitrosyl ligand are the key factors for reactive nitric oxide species (RNOS) formation and decay as well as their biological functions.

The NO/NO^+ and NO/NO^- self exchange rates are quite slow, therefore the kinetics of nitric oxide electron transfer reactions are strongly effected by transition metal complexes, especially redox active and substitution labile. The reactivity of NO with O_2 is dramatically effected upon coordination of

the one of diatomic molecule (NO or O_2) to a metal centre. These reactions are very important for the bioregulatory role of NO. For example trapping of NO by HbO_2 or MbO_2 is a very fast process (second-order kinetics) in comparison to the third order autooxidation of NO. The results of HbO_2 (or MbO_2) reactions with NO are simultaneous oxidation of Fe^{II} to Fe^{III} and NO to NO_3^-. On the other hand reactions of Fe^{IV}=O species with NO to give Fe^{III}(ONO) lead to reduction of metal along with oxidation of NO. The reaction with HbO_2 is generally believed to be an important sink for NO in the cardiovascular system, while trapping of ferryl intermediates (or other strong oxidants) by NO may play a role in reducing oxidative stress [3,43–45].

Coordination to a metal center very often activates NO toward either nucleophilic or electrophilic attack depending on the nature of the metal complex and its oxidation state. The metal centre may also promote NO reactivity toward disproportionation or substrate oxidation [1,46].

2.3. NO as a ligand in comparison to O_2 and CO

Biochemistry of NO as well as O_2 and CO molecules is largely mediated by transient binding to the metal centres of the diverse biomolecules. The low-valent transition metal ions are able to bind these diatomic molecules because the unoccupied XO π^* orbitals are well matched, spatially and energetically, to occupied metal d_π orbitals. The binding of XO to metal ion results from a synergic flow of electrons from metal to ligand in the π system (backbonding) and from ligand to metal in the σ system. These interactions have been adapted by nature to utilise NO, O_2 and CO, either as reactant or as biochemical signals. The size, charge (neutral) and hydrophobicity of these three small molecules are similar suggesting similar access to the cellular metal pools.

Nitric oxide has uncommon properties as a ligand. In a complex with a metal centre nitrosyl ligand can adopt a variety of metal binding configurations. Due to a very small energy difference between transition metal d and NO π^* orbitals, determination of the metal oxidation state in a nitrosyl complex is complicated. A generalised description of the metal–NO interaction was offered some years ago by Enemark and Feltham [47], who proposed the notation $\{MNO\}^x$, where x represents the sum of the electrons in the metal d and NO π^* orbitals. In most mononuclear complexes, a more simple approach can be used to explain the nature of the M–NO linkage and to determine the formal metal oxidation state [48]. According to this, in a nitrosyl complex the character of NO can range from that of a nitrosyl cation (NO^+) which binds to the metal in the linear mode (donation of an electron by NO), to that of a nitroxyl anion (NO^-), for which a bond angle M–NO of ∼120° can be considered (acceptance of an electron by NO). In the NO^+ (linear) type of nitrosyl coordination, π-acceptance (donation of electron density from occupied metal d orbitals into the π^* antibonding orbitals of the NO ligand) is considered to play an important role. The nitrosonium cation, NO^+, is isoelectronic with carbon monoxide [49,50]. It binds to transition metals with a similar coordination mode as CO. However, the NO^+ ligand due to its higher electronegativity can be considered as a weaker σ-donor and better π-acceptor than CO. Moreover, in the M–NO link the M–N bond is usually strong and N–O bond relatively weak, contrasting with M–CO link, where M–C bond is relatively weak and C–O bond is strong [49]. In the NO^- type of coordination π-back bonding is not very important. A very large *trans*-labilising effect induced by this ligand [48–50] is a result of the restricted π-overlap in NO^- bonding and also of the strong σ-donation.

Interaction of the nitric oxide with iron(II) heme results in the coupling of the electron on a metal atom and nitrosyl complex formation described formally as Fe^{III}–NO^- (bond angle Fe–NO close to 120°) [51,52]. A similar coordination geometry is observed for the oxygen Fe^{II} heme complexes [21,51]. The affinity of O_2 for ferrous heme is very high and similar to that of NO (of the order 10^7 to $10^8\ M^{-1}\ s^{-1}$) [4,53,54]. CO in the reaction with ferrous heme atom prefers a linear geometry but due to steric hindrance is frequently forced to bind with a non-preferred bond angle. These steric factors as well as the intrinsic weakness of the Fe^{II}–CO bond compared to that of $\{FeNO\}^7$ contribute to the increased affinity of hemoproteins for NO, rather than CO. The greater affinity to NO over CO for ferrous heme is reflected in both a higher NO formation rate constant and a lower NO dissociation rate constant [53].

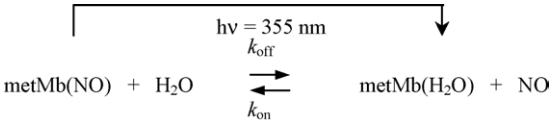
In strong contrast to CO and O_2, NO in general, binds more tightly to ferrous iron in the absence of a *trans* ligand [55]. A very large *trans*-labilising effect induced by nitroxyl ligand results from the restricted π-overlap in NO bonding and from the strong σ-donation. Thus, NO binding to ferrous heme will frequently dissociate the *trans* ligand forming a five-coordinate complex, or alternatively, forcing a *trans* ligand to bind to a five-coordinate nitrosyl complex can weaken the Fe–NO bond and lead to the NO dissociation. For example, under physiological conditions nitric oxide binding to guanylate cyclase displaces the proximal histidine inducing protein conformational changes which increase enzyme activity [56].

Unlike CO and O_2, nitric oxide can also bind reversibly to ferric iron forming a nitrosyl complex with a linear geometry of the $\{FeNO\}^6$ bond. Due to the transfer of unpaired electron from NO to the iron atom, a spin-coupled ferrous-nitrosonium formulation Fe^{II}–NO^+ is more accurate in this case [4,52–54,57].

2.4. Mechanistic studies on selected NO–metallobiomolecule systems

Biomedical roles of nitric oxide have drawn considerable attention to the solution-phase reactions of NO under biological relevant conditions. Elucidation of the mechanisms by which nitric oxide undergoes coordination to, or

release from, the metal centres is relevant to the physiological roles of NO and will also substantially facilitate the search for new pharmaceutical agents capable of regulating NO level in vivo. In this context an overview of selected mechanistic investigations involving binding of nitric oxide to metallobiomolecules is presented here.



Scheme 1.

2.4.1. Interactions of nitric oxide with iron centres

The most significant property of nitric oxide in its biological chemistry is its interactions with iron proteins. Many heme and non-heme iron proteins have been suggested to have their activity modulated by NO in vivo.

2.4.1.1. Hemoproteins. The mammalian guanylate cyclase and bacterial nitric oxide reductase regarded as the important target enzymes of NO action in living organisms both utilise heme-cofactors. Heme centres are also involved in the in vivo synthesis of NO by oxidation of arginine catalysed by nitric oxide synthase [23,24,58–60]. Moreover, due to the similarity of nitric oxide and dioxygen, NO is able to inhibit many enzymes that under physiological conditions bind and/or activate O_2, including cytochrome oxidase, hemoglobin, myoglobin and the cytochrome P450 superfamily [61–64]. Nitric oxide binds both to the iron(II) and iron(III) hemoproteins with association rate constants ranging from 10^3 to $10^8\,M^{-1}\,s^{-1}$, significantly larger for the ferrous complexes [52–54]. The high NO association rate constants observed for the ferroheme proteins can be ascribed to the vacant coordination site at the high spin heme iron(II) atom. Inversely, the rate constants for the dissociation of nitric oxide from these complexes appear to be much lower than for the ferric complexes. The high values of NO binding rate constants accompanied by very small values of NO dissociation rate constants result in very large association constants for the ferroheme proteins. The question to address and to answer is how proteins with high spin iron(II) heme centres can avoid being inhibited by NO under physiological conditions. It is general thought that the problem of the strong ferrous nitrosyl bonds in heme proteins has been overcome by nature via prevention of $\{FeNO\}^7$ formation and/or increase of the $\{FeNO\}^7$ dissociation rate [53,65].

An important topic when considering the chemistry of the nitrosyl iron(II)/(III) hemoproteins is the relative effect of the proximal ligand (*trans*-coordinated to the nitrosyl group) of the heme iron atom on both NO binding mechanism and the nature of the Fe–NO bond formed. In this context, studies were directed towards mechanistic aspects of the reactions between nitric oxide and two important iron(III) hemoproteins, e.g. imidazole-ligated metmyoglobin [66] and thiolate-ligated cytochrome $P450_{cam}$ [67].

The iron(III) centre of metmyoglobin (metMb) is six coordinate, with imidazole and water molecules occupying the fifth and sixth coordination positions. Nitric oxide binding to metmyoglobin involves reversible substitution of the water molecule by NO as indicated in the following equation:

$$\mathrm{metMb(H_2O) + NO \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} metMb(NO) + H_2O} \quad (6)$$

From UV–vis spectroscopic changes and electrochemical measurements with the use of an NO-electrode the equilibrium constant for Eq. (6) was determined to be $2.4 \times 10^3\,M^{-1}$ at 20 °C and pH 7.4. The Fe–NO linkage in the reaction product, metMb(NO), formally has a linear character, which means that during the binding process partial charge transfer from NO ligand to iron atom occurs (Fe^{II}–NO^+).

The kinetics of the association and dissociation processes were followed by both laser flash photolysis and stopped-flow techniques at varying temperature and pressure. Laser flash photolysis of the metMb/NO solution displaces the system from equilibrium (Scheme 1) so that the back relaxation kinetics can be easily followed spectrophotometrically.

The large and positive values of activation entropy and activation volume found for the forward reaction of metMb and NO are consistent with the operation of a limiting dissociative ligand substitution mechanism in which dissociation of water molecule must precede formation of the Fe–NO bond according to the following equations:

$$\mathrm{metMb(H_2O) \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} metMb + H_2O} \quad (7)$$

$$\mathrm{metMb + NO \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} metMb(NO)} \quad (8)$$

The kinetics of the back reaction were determined via NO-trapping experiments (with the use of $[Ru^{III}(edta)H_2O]$ as a NO-trapping agent for the labilised NO) which gave the association parameters again consistent with a limiting dissociation mechanism. The reverse process is dominated by the dissociation of NO from the $\{FeNO\}^6$ species accompanied by a charge transfer from metal to nitrosyl to give five-coordinate metMb plus NO, and a concomitant spin change (Fig. 3a).

A similar mechanistic study with the use of the stopped-flow and laser flash photolysis techniques was undertaken in order to elucidate mechanism of the NO binding to cytochrome $P450_{cam}$ in the absence and presence of substrate molecule (1*R*-camphor) [67]. Cytochrome $P450_{cam}$ isolated from the bacterium *Pseudomonas putida* catalyses the regio- and stereospecific hydroxylation of camphor to the 5-exo alcohol and like other P450 cytochromes utilises the thiolate as the proximal ligand of the heme iron atom. It is considered to be the best model system for understanding the reaction

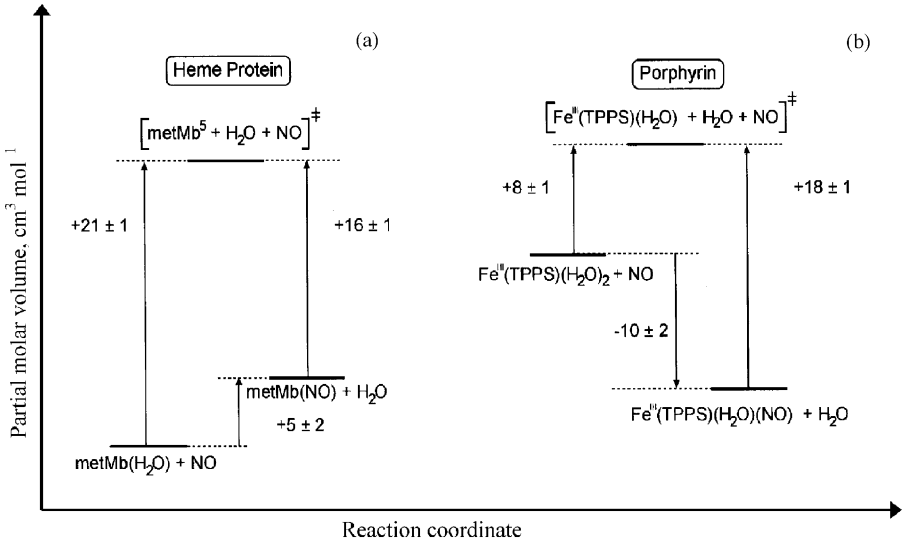


Fig. 3. Volume profiles for the reactions of: (a) metmyoglobin with nitric oxide (data from Ref. [66]); (b) $[Fe^{III}(TPPS)(H_2O)_2]$ with nitric oxide (data from Ref. [68]).

mechanism in which small molecules (NO, O_2) undergo coordination to the iron centres of many important biomolecules.

The substrate-free ferric $P450_{cam}$ is a low-spin ($S = 1/2$) six coordinate complex with a water cluster bound at the distal site *trans* to the cysteine axial ligand in the proximal position. Resonance Raman studies indicated that the addition of nitric oxide to this form of $P450_{cam}$ leads to a nitrosyl complex with a linear Fe–NO group. The resulting diamagnetic $\{FeNO\}^6$ complex has formally the Fe^{II}–NO^+ character. The binding of NO to substrate-free $P450_{cam}$ displays biphasic kinetics, which can be interpreted in terms of an equilibrium between conformational substrates in cytochrome $P450_{cam}$. The different substrates in substrate-free $P450_{cam}$ have already been confirmed by FTIR studies [69,70] and are caused by a different hydrogen bonding network between differently packed water molecules in the heme pocket. The values of activation parameters for both reaction steps were found to be large and positive, and as in the case of NO binding to metMb consistent with the operation of a limiting dissociative ligand substitution mechanism, where the lability of coordinated water dominates the reactivity of the iron(III) centre with NO. The activation parameters found for the release of NO from nitrosyl complex of $P450_{cam}$ clearly show that the reverse reaction also follows a rate-limiting dissociative mechanism, in agreement with the principle of microscopic reversibility. The overall reaction volume for the NO binding to substrate-free cytochrome $P450_{cam}$ is close to zero (Fig. 4a) and very similar to that found for the reaction between NO and imidazole-ligated metmyoglobin (Fig. 3a).

The binding of the 1*R*-camphor to low-spin, six-coordinate $P450_{cam}$ leads to a spin state change to the high-spin $P450_{cam}$ form. The resulting complex is five-coordinate with no water occupying the sixth position of the iron(III) centre. Second-order rate constants for the binding of nitric oxide to camphor-bound $P450_{cam}$ were found to be much larger than

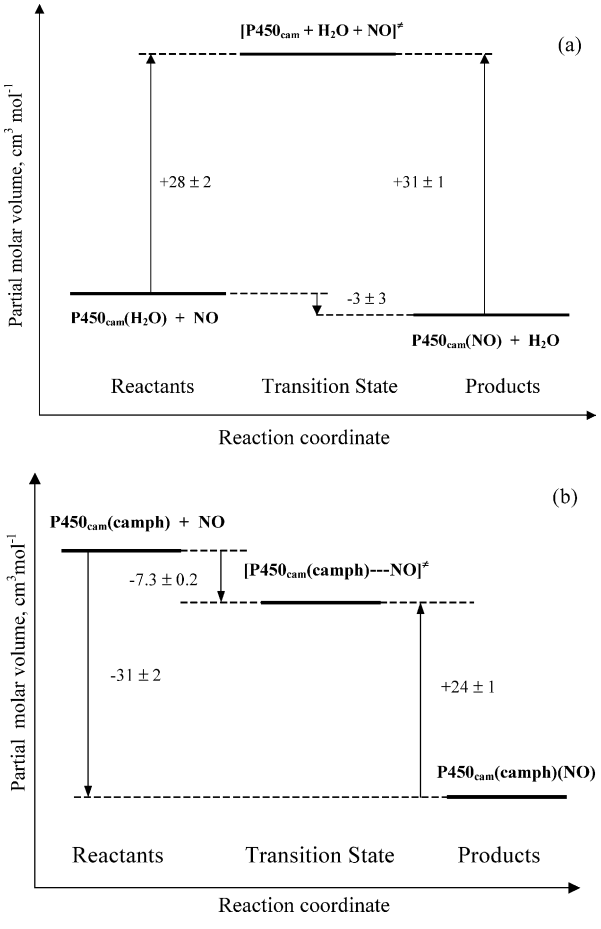


Fig. 4. Volume profiles for the reversible binding of NO to: (a) substrate-free cytochrome $P450_{cam}$; (b) camphor-bound cytochrome $P450_{cam}$ (adapted from Ref. [67]).

those determined for the substrate-free form. This observation can be ascribed to the vacant position in the coordination sphere of the substrate-bound Fe^{III}-heme. Negative values of activation entropy and activation volume determined for the formation of the nitrosyl complex of $P450_{cam}$ in the presence of camphor support a mechanism dominated by the bond formation process. In such a mechanism an encounter complex, {cyt $P450_{cam}$‖NO}, is formed prior to the ligand bond formation, according to the following equation:

$$\text{cyt P450}_{\text{cam}} + \text{NO} \underset{k_{-D}}{\overset{k_D}{\rightleftarrows}} \{\text{cyt P450}_{\text{cam}}\|\text{NO}\} \xrightarrow{k_a} \text{cyt P450}_{\text{cam}}(\text{NO}) \quad (9)$$

where k_D is the rate constant for the diffusion-limited formation of the encounter complex, k_{-D} is the rate constant for the diffusion apart of the encounter complex, and k_a is that for the activation step in which the Fe–NO bond is formed. The activation parameters for the release of NO from the nitrosyl complex of camphor-bound $P450_{cam}$ are consistent with a reaction mechanism in which the Fe–NO bond cleavage is the rate-limiting step. The volume profile for the reaction between nitric oxide and camphor-bound form $P450_{cam}$ differs totally from that obtained for the NO binding to the substrate-free form of $P450_{cam}$ (Fig. 4) or metmyoglobin (Fig. 3a). In this case a drastic volume decrease on going from the reactant to the product states is observed, which can be partly ascribed to the high-spin to low-spin transition of the iron(III) during the NO binding process.

Comparison of the kinetics and mechanism of NO binding to metmyoglobin and to cytochrome $P450_{cam}$ (in the substrate-free and camphor-bound form) leads to conclusions that factors like the lability of the leaving group (or its absence), protein structure and accessibility of the heme active site play an important role in the dynamics of the NO reactions with metal centres of biological relevance. It seems also very likely that the reactions of NO release from nitrosyl complexes of iron hemoproteins are largely affected by the nature of NO binding and dissociation mechanism, as well as by factors stabilising NO binding in the protein active site. Interestingly, the reaction dynamics of NO binding to metalloproteins appears to be more dominated by the nature of the metal centres than by the free radical character of nitric oxide, which behaves in such systems like other typical Lewis base donors.

2.4.1.2. Synthetic iron(II)/(III) porphyrins. The interactions of synthetic metalloporphyrins with small molecules like O_2, NO and CO have long been of interest supporting the studies of the oxygenation reactions of some hemoproteins [11,12,51]. The synthetic metalloporphyrins can be used as model systems for proteins responsible for O_2 transport and storage (like hemoglobin and myoglobin) [71–73]. Biological roles of nitric oxide have turned considerable attention to the reactions of the metalloporphyrin nitrosylation and resulted in extensive kinetic studies during the past decades [4,74,75] on NO substitution processes involving [M(Por)] systems (where M = $Fe^{II/III}$, Co^{II}, Mn^{II}, and Por = TPPS, TMPS, TPP, OEP). Since NO photodissociation from [M(Por)(NO)] complexes is commonly reversible with the quantum yields Φ_{dis} close to unity, pulsed laser techniques have been well suited to investigate the kinetics of the nitrosylation reactions of such complexes [4]. Water soluble iron(II)/(III) porphyrins readily bind nitric oxide with the second-order rate constants much larger than those measured for the analogous reactions with hemoproteins. The fast rates for the reactions of NO binding to model porphyrin complexes can be accounted for in terms of the lability of the iron(II)/(III) porphyrins resulting from the absence of the protein pocket limiting access to iron in the heme centre. Since NO dissociation rate constants determined for the model complexes are also significantly larger than those for the hemoproteins, it seems that the presence of the surrounding polypeptide chain efficiently hinders the release of nitric oxide from nitrosyl hemoproteins. Comparison of the NO association and NO dissociation rate constants obtained for the iron(II)/(III) hemoproteins with those determined for their model porphyrin complexes can lead to the conclusion that the most likely the role of the protein pocket is to regulate rates of NO binding and release processes to the physiological requirements. Indeed, activation parameters found for the nitrosylation reactions of iron hemoproteins and water-soluble iron model complexes clearly reflect the influence of the protein structure in the heme binding pocket on the NO binding kinetics. For example, the value of activation volume determined for the nitric oxide binding to metmyoglobin [66] is significantly larger than that reported for the analogous reaction with the ferric porphyrin model complex, $[Fe^{III}(TPPS)(H_2O)_2]$ (Fig. 3b) [68]. The larger value of $\Delta V^{\#}$ for metMb/NO system suggests that the protein pocket may undergo some structural rearrangements during the formation of the five-coordinate metMb. The EXAFS structural study on this reaction confirmed this observation indicating steric strain in the linear coordination of nitric oxide to iron(III) centre ($\{FeNO\}^6$), which would result from an increase in the protein pocket size prior to the NO binding.

2.4.1.3. Non-heme iron proteins. In general, non-heme iron proteins bind ligands at the iron site less tightly than hemoproteins. Due to the lack of clear optical spectroscopic signals the reactions of non-heme enzymes with nitric oxide are less clear as compared to the nitrosylation reactions of heme systems. However, EPR spectroscopy indicated that these enzymes can form well characterised nitrosyl complexes in which high spin ferric iron is antiferromagnetically coupled to NO^- [76].

Non-heme enzymes (e.g. soybean lipoxygenase, estradiol dioxygenases, putidamonooxin, etc.) reacting with oxygen under physiological conditions are also able to bind nitric oxide [77–79]. However, inhibition of these enzymes by nitric oxide requires much higher NO concentrations than those found under physiological or even pathophysiological

conditions. Not only iron enzymes but iron storage proteins (ferritin) can also easily react with nitric oxide. Furthermore, these interactions may be responsible for the NO induced release of iron from ferritin stores [80]. Similarly, EPR study revealed that nitric oxide can modulate the biological activity of two non-heme diiron proteins, e.g. hemerythrin and ribonucleotide reductase [81,82]. Hemerythrin, the binuclear oxygen protein can form reversible adducts with NO at the (Fe^{II}–Fe^{II}) deoxy and (Fe^{II}–Fe^{III}) semi-met oxidation levels. The reaction of the NO molecule with ribonucleotide reductase, the enzyme that converts ribonucleotides to the deoxyribonucleotides necessary for DNA synthesis involves radical coupling reaction between a tyrosinal radical and NO ($Tyrosine^{\bullet} + NO \rightarrow$ Tyrosine–NO) rather than direct binding to the iron centre [83,84].

It has been revealed that nitrile hydratase (NHase), a bacterial metalloenzyme catalysing the hydration of nitriles to corresponding amides, is a photoreactive enzyme that is inactivated by nitrosylation of the non-heme iron centre and activated by photodissociation of nitric oxide [85]. The iron-containing NHase is the first enzyme with a mononuclear low-spin non-heme iron(III) which is thought to be involved in the catalysis.

Another group of non-heme iron proteins representing an important biological target for nitric oxide contain iron–sulfur (Fe–S) centres [86]. Reactions of nitric oxide with the iron–sulfur clusters result in alteration of the functional properties of the proteins, e.g. a loss, a decline or an increase in their activity. For example, the NO binding to the Fe–S cluster of nitrogenase and formation of Fe–S–NO complexes lead to complete inhibition of nitrogen fixation [71]. In contrast to the reactions with the heme iron, the interactions of nitric oxide with Fe–S clusters as a rule lead to the destruction of the clusters [87,88]. This may happen by virtue of the cytotoxic action of NO, as several other key enzymes, such as cytoplasmic and mitochondrial aconitase, NADH:ubiquinone oxidoreductase and NADH-succinal reductase, also contain iron–sulfur clusters [19]. The effect of nitric oxide on cytoplasmic and mitochondrial aconitase, the enzymes from a class of metalloregulatory proteins called *iron regulatory proteins* (IRP) is especially interesting and intriguing [88]. Some groups suggest that peroxynitrite is the reactive species [89,90] whereas others have suggested that NO itself is sufficient [91]. This confusion in the literature clearly shows that inactivation of the (Fe–S) cluster proteins in biological systems should be very carefully examined in the context of relative reactivities of NO, $ONOO^-$ and $O_2^{\bullet -}$ species with other available substrates.

Due to the extremely high reactivity of nitric oxide, paracrine actions of NO require intracellular transfer of this small molecule from the donor cells to the target cells. It has been assumed that biologically produced NO might be stabilised and stored in vivo as a dinitrosyl–iron(II) complex (DNIC) with protein thiols, and can be released from cells in the form of a low molecular weight dinitrosyl–iron(II)–dithiolate by intra- and extracellular thiols. Thus, the dinitrosyl–iron(II) complex with low molecular weight thiols acts as a diffusible NO carrier to facilitate paracrine actions of NO [92]. DNICs are readily detectable by EPR spectroscopy (with the *g* region of 2.03–2.04) and have therefore been much studied [93]. They have been detected in animal tissues and microorganisms in a wide variety of conditions from cancerous tissue to the substantia nigra of Parkinson's disease brain tissue. Moreover, there is evidence that dinitrosyl–iron(II)–L-cysteine complex possesses pharmacological properties similar to those described to EDRF (endothelial-derived relaxing factor) [94,95].

2.4.2. *Interactions of nitric oxide with cobalt centres*

Cobalt, present in human organisms in the form of cobalamin cofactors (Vitamin B_{12} derivatives) plays a vital role in many enzymatic biotransformations involving enzyme-catalysed molecular rearrangements and methyl group transfer reactions [96,97]. The common structural feature of these biomolecules is the presence of the equatorial corrin chelate coordinated to the cobalt atom. Vitamin B_{12} derivatives containing cobalt in the higher oxidation state (+3) are six-coordinate with various ligands (such as water molecule in aquacobalamin) occupying the sixth coordination site whereas the reduced form of this biomolecule is a five-coordinate complex with a low-spin cobalt(II) centre. One of the recent interests in the biological functions of Vitamin B_{12} concerns the involvement of aquacobalamin ($Cbl(III)H_2O$) and its reduced form (Cbl(II)) in modification of physiological actions of nitric oxide. According to the observations reported in the literature [98], $Cbl(III)H_2O$ may react with NO in vivo to produce the Cbl(III)NO complex. However, recent detailed spectroscopic and kinetic studies have shown that no direct interaction between NO and aquacobalamin occurs, which is why it cannot account for the observed physiological effects. The UV–vis spectroscopic changes observed on introducing NO into aqueous solution of $Cbl(III)H_2O$, result from the reaction of cobalt(III) with nitrite present as an impurity in aqueous NO solution [99].

The reduced form of Vitamin B_{12}, also present under physiological conditions, efficiently binds nitric oxide to yield the stable nitrosyl adduct, Cbl(II)NO [100,101]. Thus, this interaction may account for the observed biological effects. The nitrosylation reaction of Cbl(II) may influence the physiological activity of cobalamin-dependent enzymes. For example, the NO binding to Cbl(II) leads to inactivation of cobalamin-dependent methionine synthase.

The Cbl(II)NO complex belongs to the class of the $\{M–NO\}^8$ species and can be formally described as $Cbl(III)NO^-$. The kinetics of NO binding to Cbl(II) were recently studied with the use of laser flash photolysis [101]. The rate constants for the forward reaction of nitric oxide with the reduced form aquacobalamin were found to be very high (of the order of $\sim 10^8\ M^{-1}\ s^{-1}$) whereas NO dissociation rate constants appeared to be too small for laser flash measurements. It was, however, possible to measure values of back

rate constants directly using the NO-trapping method. NO binding to the vacant coordination site of the Cbl(II) should be very fast and exhibit a significantly negative activation volume as a result of bond formation and a partial oxidation of the Co^{II} centre in the rate-determining step. However, although the NO binding reaction is very fast, it is almost two orders of magnitude slower than the limiting rate constants for a diffusion-controlled reaction in water. Moreover, it exhibits a small but significantly positive value of volume activation, suggesting the operation of a dissociative interchange (I_d) ligand substitution mechanism for the forward reaction. A detailed investigation of this reaction allowed explanation of the apparent discrepancy and showed the important role of solvent molecules in determining the mechanism of NO binding to metal centres. The intermediate produced in laser flash experiments of the Cbl(II)/NO system differs from that in the thermal reaction leading to the different intimate reaction mechanisms for the photoinduced and thermal nitrosylation of reduced cobalamin. Kinetic data for the reaction of NO release from Cbl(II)NO showed that the dissociation of nitric oxide is slow and a high energy barrier must be overcome to break the Co^{III}–NO^- bond. A positive activation volume observed for this process can be accounted for in terms of a dissociative interchange mechanism.

2.4.3. Interactions of nitric oxide with copper centres

Nitric oxide can act as a ligand for copper centres as well and may be involved in redox chemistry with the metal once bound [102]. This dual character of NO interactions with copper complexes in biological systems is sometimes difficult to distinguish, especially as a subtle, temperature-dependent, equilibrium between these two extremes has been detected, recently. Under physiological conditions (e.g. at low NO concentration and presence of oxygen) redox reactions between NO and copper proteins can be very fast. Hence, these interactions may be of physiological importance. Furthermore, since unpaired electron located on the π^* orbital of NO can couple with the unpaired electron on Cu^{2+}, nitric oxide has also been used extensively as a spectroscopic probe to elucidate the nature of the active sites of copper-containing enzymes and proteins such as hemocyanin, tyrosinase, ceruloplasmin, ascorbate oxidase, laccase, azurin, halocyanin and the iron/copper binuclear centre of cytochrome *c* oxidase [103–106]. The copper centres in copper proteins are generally divided into three main types according to their EPR and optical features (T1, T2 and T3). It is assumed that these different types of Cu centres react differently with nitric oxide [82,107]. Of particular interest seems to be the use of NO to study dioxygen reactive centres constituted either by copper pairs or by mixed Cu/Fe pairs, as in the binuclear centre of the cytochrome *c* oxidase (C*c*O) [102,108]. Cytochrome *c* oxidase, the terminal electron acceptor of the respiratory chain in the mitochondrion and in some bacteria, contains two heme groups (Fe_a and Fe_{a3}) and two copper centres (Cu_A and Cu_B). Both Fe_{a3} and Cu_B, when reduced, can bind NO. NO binding to C*c*O is responsible for the reversible inhibition of mitochondrial respiration, and this reaction may play a role in modulating the rate of O_2 consumption under physiological conditions. The reaction of C*c*O with nitric oxide is very fast and much more complex than those with other copper-containing proteins. In general, it involves formation of the Cu_B^+–NO^+ species which is subsequently hydroxylated to nitrous acid and the reduced form of Cu_B. The electron residing on the Cu_B can then be transferred to other redox centres of C*c*O.

Many nitrosyl copper complexes were studied in the context of their susceptibility to photodissociation [107]. Nitric oxide complexes with T3 copper sites in ceruloplasmin and hemocyanin are inert to light, while T1 types of copper sites form photolabile complexes with nitric oxide. The photolability of nitrosyl complexes at the T1 copper site has been exploited to investigate the temperature dependence of the ligand binding equilibrium and the kinetics of the association reaction after photodissociation in azurin and halocyanin [109,110].

2.5. Metal complexes as NO-donors and acceptors

The pathological events resulting from the dysfunction in nitric oxide metabolism are fatal in more than 50% of cases. This is why the search for new compounds able to selectively enhance or inhibit the synthesis of NO, to modify its effects or eliminate it from physiological media, is a major challenge for medicinal chemists, and is also a potentially profitable area for the pharmaceutical industry. The traditional approach to the search for new therapeutic agents is via organic chemistry testing of a wide class of compounds potentially capable of switching the nitric oxide synthase [111]. However, many inorganic chemists have recently pursued a different approach to the problem by exploiting knowledge of the role of metal compounds in activation and deactivation nitric oxide processes. Hence, NO overproduction or deficiency under pathophysiological conditions can be regulated by delivery within a biological environment of respective metal complexes which are able to bind (NO-scavengers) or release nitric oxide (NO-donors).

2.5.1. Nitrosyl metal complexes – exogenous source of nitric oxide

Nitrovasodilators are NO-donating compounds that mimic the action of an endothelium-derived relaxing factor (EDRF) imitating the physiological blood vessel dilatation process. Long before NO was identified as the EDRF a number of compounds containing NO, NO_2^- or NO_3^- groups were recognised as vasodilators and use in the treatment of angina pectoris without fully understanding the mechanism of their action [31,32]. NO-donors differ in their need for specific cofactors to release nitric oxide as well as in susceptibility to changes of factors such as pH, oxygen, light and temperature. Depending on the chemical nature of both nitrovasodilators and cofactors, the pathways of bioactivation are clearly

different. For example, some compounds require enzymatic catalysis, whereas others produce NO in a non-enzymatic way. Moreover, among NO-donors some are spontaneous NO generators and some need to interact with thiols or be reduced or oxidised as a prerequisite for a release of NO. The amount of NO as well as the rate and period/profile of its release from NO-donating compounds determine the biological effectiveness of NO.

Sodium nitroprusside (SNP, $Na_2[Fe(CN)_5NO]$) is clinically used as a nitrovasodilator [112]. It is used as an infusion for the control of blood pressure and as a ready source of NO in physiological experiments. Its nitro analogue, $K_3[Fe(CN)_5NO_2]$ is also assumed to have potential application as a hypotensive agent, but its clinical use has not been tested yet [113,114]. The thermal reactivity of SNP has been widely investigated for its spontaneous release of NO in biological fluids [112–116]. In fact, the formation constant of $[Fe(CN)_5NO]^{2-}$ is very high and so its spontaneous decomposition is unlikely. Two different pathways for the SNP metabolism have been suggested [113]. One of them involves a direct nucleophile attack on SNP and the subsequent formation of an unstable complex $[Fe^{II}(CN)_5NONucl]^{3-}$. The decomposition of this intermediate complex produces either Fe^{I} compounds or oxidised nucleophile or/and Fe^{II} complex and NONucl species. The latter is thought to be a source of NO or NO^+. The other pathway of the SNP metabolism includes its reduction in cells as the first step, followed by release of one of the CN^- ligands and formation of $[Fe(CN)_4NO]^{2-}$. Because of the NO^+ character of the nitrosyl ligand in the latter complex, a nucleophilic attack on the NO^+ ligand is expected to be the most probable [113]. Although SNP and its nitro analogue are very valuable NO-donors, their use is problematic due to reduction in their activity provoked by photolysis [117,118] and to their oxidative breakdown provoked by an activated immune system. The release of cyanide from SNP in blood can also be caused by the action of UV or visible light from room light. This means that great caution must be exercised when working with solutions of $Na_2[Fe(CN)_5NO]$ during medical therapies. Intensive studies of the photodecomposition of SNP have the objective to provide more stable pharmaceutical preparations achieved with use of various procedures, examples being the addition of small amounts of Vitamin B_{12} to 5% dextrose solution of SNP or administration of a substance consuming photochemically generated radicals [119,120].

Recently, a family of simple analogues of SNP, characterised by the ability to maintain the ligand set while altering metal ions, has been investigated [113,121]. From a series of $[M(CN)_xNO_y]^{n-}$ complexes, where M = V, Mn, Cr, Co, Fe^{I}, Fe^{II}, Fe^{III}, only iron complexes appeared to be pharmacologically active. The Cr and Mn complexes did not exhibit any hypotensive action. Although these SNP analogues contain the toxic cyanide ligand and are therefore clinically unacceptable, the investigation of this family of compounds helped to interpret the mechanism of nitric oxide donation from the $[M(CN)_xNO_y]^{n-}$ complexes [113].

The interaction of light with medicinally important metal compounds provides the possibility to trigger the desired action at the desired point, which makes light the key factor in developing photochemical strategies for targeted NO delivery systems. The NO photolabilisation method is especially suitable at sites in cells and preparations where diffusion is restricted and the uptake by conventional perfusion techniques distorts both the time course and steady-state concentration of the physiological ligand. In view of the high instability of NO and its permeability across cell membranes from extracellular to intracellular domains, the photolabilisation method generating NO rapidly at known concentrations within biological preparations at specific locations is of great value. The NO photolabilisation strategy is based on the application of precursors of low thermal activity, which are photochemically active to give NO when subjected to electronic excitation [64]. The photosensitive nature of the precursor compounds adds an important new dimension to their therapeutic potential, that is, it helps to improve the perfusion of selected vascular beds and to deliver potentially cytotoxic quantities of NO to a specific target site. For instance, the photochemistry of metal nitrosyl complexes has been used as a vehicle for delivering NO to hypoxic cell cultures in order to sensitise gamma-radiation damage [122].

A class of metal complexes which has received considerable attention is an iron–sulfur–nitrosyl cluster anion of the type $[Fe_xS_y(NO)_z]^{n-}$. The Roussin's Red Salt, $[Fe_2S_2(NO)_4]^{4-}$ and Roussin's Black Salt, $[Fe_4S_3(NO)_7]^-$ contain in their ions a large number of NO ligands and are known as highly effective nitrovasodilators with unusual pharmacological profiles. Although ionic, they enter endothelial cells remarkably quickly and accumulate therein, releasing NO slowly. Nitric oxide can be released from these compounds thermally or photochemically. Exposure to light greatly enhances the vasodilator actions of iron–sulfur–nitrosyl clusters. This is due to an oxygen-dependent photochemical reaction, which accelerates the release of ligated nitrosyl groups as free NO [123]. Roussin's Black Salt anion has many qualities desirable for the biological delivery of NO to specific targets, e.g. water solubility, absorption across the visible light region and stability to biological conditions, and therefore is a good candidate for a photochemical precursor that can deliver nitric oxide to biological targets on demand [124]. In fact, RBS at low concentration (10 nM) showed photocytotoxicity towards melanoma cells and two neoplastic cell lines: human (SK-MEL188) and mouse (S91). Cellular death was caused by short-lived Roussin's Black Salt photoproducts, mainly NO, but not by other RBS photoproducts [125].

An interesting group of potential photochemical precursors includes the nitrosyl derivatives of ruthenium. It was shown that doubly charged NO compounds, $K_2[RuCl_3NO]$ and $K_2[RuCl_5NO]$, are thermally stable but photolabile, releasing nitric oxide on exposure to near-UV light [126,127]. They are water soluble and so unlikely to cross biological membranes. Irradiation of these compounds in the range

300–350 nm leads to rapid liberation of NO with a rate greater than $10^5\ s^{-1}$ and product quantum yields of 0.012 and 0.06 for $[RuCl_3NO]$ and $K_2[RuCl_5NO]$, respectively. The efficacy of ruthenium nitrosyl complexes in biological systems was revealed by the relaxation of contracted aortic rings brought about by photoreleased NO. The effect is identical with that of EDRF, where the action of NO is well established [128].

Another class of light-sensitive nitrosyl complexes are metalloporphyrins [129–131]. The nitrosyl complexes of metalloporphyrins release nitric oxide after absorption of photons by the porphyrin macrocyclic π-system with quantum yields varying from virtually zero to unity. Due to empty d-orbitals of transition metal complexes which can couple to the porphyrin π-orbitals forming states of intermediate energy, the electronic excited states in these systems are inherently short-lived. Both the rapid deactivation of the excited porphyrin states (π, π^*) and the spectroscopic inaccessibility of the internal metal states result in difficulties in determination of the factors controlling the denitrosylation processes. However, it has been suggested that the identity of the bound metal is one of the most important features in determining the photoinduced deligation yields [129]. Hoffman and Gibson examined ligand photorelease for a wide variety of hemoproteins and their metal-substituted analogues containing three different metals, e.g. Fe, Co and Mn. They found that metalloporphyrin–ligand systems with a total occupancy of the metal d orbitals and ligand π^* orbitals of $x = 6$ are highly photolabile, whereas those of $x \geq 7$ are not photolabile, examples being $\Phi \approx 1$ for $MbFe^{III}$–NO and $\Phi \leq 10^{-4}$ for $MbCo^{II}$–NO complex. It was also suggested that the linear, d^6 metal–ligand fragments are relatively photolabile, while the systems with bent fragments and higher electron occupancy are relatively photoinert [132]. Morlino and Rodgers have investigated photoinduced deligation in two protein-free nitrosylmetalloporphyrins, $TPPFe^{II}NO$ and $TPPCo^{II}NO$. The difference in the denitrosylation yields of these nitrosyl adducts was accounted for in terms of energy partitioning in the upper excited state of the porphyrin. Only those excited states that relax via a CT state result in the loss of nitric oxide [133,134].

The photochemical studies on the nitrosyl porphyrin complexes of the first-row transition metals revealed that application of these compounds in the photochemical delivery to specific targets on demand is hampered due to their lability and oxygen sensitivity [4]. In this context, interest was focused on the nitrosyl ruthenium porphyrin complexes of the type Ru^{II}(Por)(NO)(ONO) (where Por = TPP, OEP, TmTP and FTTP), anticipated to be more stable. Ford et al. showed that the flash photolysis of the nitrosyl nitro complexes of ruthenium indicate the operation of at least two pathways leading to the formation of a photoreaction intermediate, Ru(Por)(ONO) and Ru(Por)(NO) formed by photolabilisation of nitric oxide and nitrito ligand, respectively [46,135].

The salen-type complexes, [Ru(R-salen)(X)(NO)], where R-salen is a derivative of the *N*,*N*′-bis(salicylidene)ethylenediamine dianion, are regarded as exceptionally promising systems as precursors for photochemical NO delivery to various targets [136]. Near-UV irradiation of a representative member of this family, e.g. [Ru(salen)(Cl)(NO)], leads to the NO photolabilisation and formation of the solvento species, [Ru(salen)(Cl)(Sol)].

Another group of compounds studied in context of their potential application as NO-donors are macrocyclic systems with an extremely stable, sequestered metal core. Complexes of the type *trans*-$[Ru^{II}(X)(NO)(cyclam)]^{2+}$ (where X = halo or hydroxo ligand and cyclam = 1,4,8,11-tetraazacyclotetradecane) release nitric oxide very slowly and therefore can be considered as promising controlled-release NO prodrugs, acting as long-lasting, although softer vasodilators [137,138].

The problem of thermal instability and oxygen sensitivity of potential NO-donors compounds in the aerobic aqueous environments under physiological conditions has been overcome by exploration of a new strategy of NO generation from an air-stable, water soluble [*trans*-Cr^{III}(cyclam)$(ONO)_2]^+$ complex via the photolytic cleavage of coordinated nitrite [139]. The photolysis of this complex in aqueous solution results in the formation of an intermediate complex, believed to be [*trans*-Cr^{IV}(cyclam)(O)(ONO)]$^+$ with the concurrent production of nitric oxide.

2.5.2. *Metal complexes as nitric oxide scavengers in biological systems*

A number of transition metal complexes have been tested for NO-trapping and for antisepsis potential. The following chemical properties of these complexes make them suitable for the application: (i) transition metals have variable oxidation states, (ii) their ligands are coordinated in a precise spatial arrangement and (iii) more importantly, by changing the ligands the complexes can be adjusted to control pharmacological interactions in biological systems.

The complexes that possess most of these characteristics are polyaminocarboxylate complexes of Ru and Fe. The parent compound K[Ru^{III}(Hedta)Cl] has emerged as a nitric oxide scavenger [140,141]. It is water soluble, which is why it cannot cross lipophilic cell membranes. Recently, pre-clinical studies have proved pharmacological activity of $[Ru^{III}(edta)H_2O]^-$ in biological systems ranging from cultured cells to sophisticated disease models [27]. Ru^{III}–edta complex possesses low toxicity and many desired pharmacokinetic properties. Therefore, it appeared to be a promising candidate as a nitric oxide scavenger. In aqueous solution, at physiological pH 7.4 $[Ru^{III}(Hedta)Cl]^-$ hydrolyses rapidly to $[Ru^{III}(edta)H_2O]^-$. Recent kinetic study revealed that nitric oxide binds rapidly to $[Ru^{III}(edta)H_2O]^-$ to form a very stable nitrosyl complex, characterised by a high complex-formation constant (estimated as about $10^8\ M^{-1}\ s^{-1}$) and very low dissociation rate constant [142]. The NO binding is extremely fast and occurs within the dead time of the stopped-flow instrument. Attempts to determine the complex-formation rate constant using laser flash photolysis technique

were complicated due to the occurrence of unwanted side reactions.

Among inorganic compounds tested for antisepsis potential, iron-based complexes have been brought back to light. Aminocarboxylate complexes of iron(II) are known for their ability to bind NO rapidly, and have been used in industry to enhance the solubility of NO in aqueous solutions to facilitate the removal of NO from exhaust gases [143–146]. The potential medical application of these complexes as clinically useful agents for the control of blood NO levels during septic shock has recently been considered. Shepherd et al. synthesised and characterised $[Fe^{II}L(NO)]$ complexes of the aminocarboxylate and pyridyl-based ligand systems with L = edta^{4-} (ethylenediaminetetraacetate), nta^{3-} (nitrilotriacetate), pida^{2-} (2-pyridylmethyliminodiacetate), tpa (tris-2-pyridylmethylamine) and tpen (*N*,*N*,*N*′,*N*″-tetra-(2-pyridylmethyl)ethylenediamine) [147]. Although Fe^{II}-aminocarboxylate or pyridyl-based complexes bind to nitric oxide rapidly and tightly, forming relatively stable NO-adducts, the parent $Fe^{II}L$ complexes are inherently reactive with oxygen. On the other hand, independent studies revealed that iron(III) complexes, such as Fe^{III}–dtpa (dtpa^{5-} = diethylenetriaminepentaacetate), bind readily to NO upon reduction to Fe^{II} at physiologically relevant potentials, and are able to protect mice against death caused by septic shock [148].

Recently, the binding mode and oxidation state of NO in the nitrosyl iron(II) aminocarboxylate complexes $Fe^{II}(L)(NO)$, where L = edta^{4-}, hedtra^{4-} (hydroxyethylenediaminetriacetate), nta^{3-}, mida^{2-} (methyliminodiacetate), dtpa^{5-} and ttha^{6-} (triethylenetetraaminehexaacetate) have been studied with the use of UV–vis and ATR-IR spectroscopy [149]. The equilibrium constants K_{NO} for the nitrosylation processes were determined by combined spectrophotometric and potentiometric techniques. In general, a good correlation between the oxygen sensitivity of $[Fe^{II}(L)_x]$ complexes and the stability constants of their corresponding nitrosyl complexes was found, in which the higher values of K_{NO} for the nitrosyl complex is accompanied by the shorter "qualitative oxidation time". The tendency of $[Fe^{II}(L)_x]$ to reversibly bind NO correlated directly with the oxygen sensitivity of the Fe^{II} complexes, suggesting that [Fe(L)NO] is stabilised in the form of $[Fe^{III}(L)(NO^-)]$ similar to that found for the binding of dioxygen, viz. $[Fe^{III}(L)(O_2^{\bullet-})]$. Kinetic studies on the formation and dissociation reactions for the Fe^{II}(L)/NO systems were performed using a combination of laser flash photolysis and stopped-flow (NO-trapping method) techniques [150–152]. For a series of ligands, the formation rate constants vary between 1.0×10^6 and $2.4 \times 10^8\ M^{-1}\ s^{-1}$, whereas the dissociation rate constants vary between 0.11 and $3.2 \times 10^3\ s^{-1}$. The activation parameters determined from the temperature and pressure dependence of the forward and back rate constants suggest the operation of a dissociative interchange (I_d) mechanism, which is also in agreement with the results obtained from the water exchange experiments. Although NO is a radical it behaves as a normal nucleophile during such ligand displacement reactions. Its radical nature, however, does require a formal charge transfer process to occur in order to stabilise the nitrosyl reaction product. The results illustrate the important influence of aminocarboxylate chelates on the values of the rate constants and overall equilibrium constant, as well as on the nature of the mechanism for the forward and back reactions. There seems to be no simple correlation between these constants and the selected groups or charge on the iron(II) complexes, and electronic/structural features must control the coordination number of the complexes and as a result their mechanistic behaviour.

Considering the properties of the iron(II) aminocarboxylate complexes studied in terms of their therapeutic application as NO-scavengers in the human body, the following conclusions can be drawn: (1) The highest value of the rate constant for the NO binding was found in the case of $[Fe^{II}(edta)H_2O]^{2-}$, which is ca. 4 times higher than that for the NO binding to $[Fe^{II}(hedtra)H_2O]^{2-}$. However, taking into account the 22 times faster NO release from $[Fe^{II}(edta)H_2O]^{2-}$, the nitrosyl complex of the hedtra complex is around 5 times more stable than the nitrosyl complex of the edta. The nitrosyl complexes of Fe^{II}(ttha) and Fe_2(ttha) appear to be comparably as stable as $[Fe^{II}(hedtra)H_2O]^{2-}$. (2) The oxygen sensitivity of the complexes studied appears to be a limitation of their utility as NO-scavengers in the bloodstream. On the other hand, this limitation seems to be only apparent problem keeping in mind the fact that at physiological redox potentials in the cell, these complexes could occur in their reduced Fe^{II} form. Presently, from the series of metal aminocarboxylate complexes, the $[Ru^{III}(edta)H_2O]^-$ appears to be the promising candidate for practical application as an efficient NO scavenger in the human body [142].

Two other iron-based complexes possessing several properties of a desirable antisepsis agent have recently been described in the literature. One of them is an unsaturated macrocyclic water-soluble Fe^{II} complex, $[Fe^{II}(tim)(CH_3CN)_2](PF_6)_2$ (tim = 2,3,9,10-tetramethyl-1,4,8,11-tetraazacyclodeca-1,3,8,10-tetraene). It reversibly binds nitric oxide forming a six-coordinated nitrosyl complex similar in its EPR behaviour to iron(II) porphyrin nitrosyls. Although the macrocyclic complex of Fe^{II} satisfies some of the criteria as a possible NO scavenger [153] its utility is strongly limited by the small value of the formation constant, which is ca. 10 orders of magnitude smaller than that found for the NO binding to Fe^{II}(heme) [2].

The other iron(II) complex is $[Fe^{II}(edampda)]$ (edampda^{2-} = *N*,*N*′-bis(2-pyridylmethyl)ethylenediamine-*N*,*N*′-diacetate) [154]. $[Fe^{II}(edampda)]$ binds NO reversibly with a formation rate constant of about $1 \times 10^2\ M^{-1}\ s^{-1}$ and the dissociation rate constant of $7.7 \times 10^{-3}\ s^{-1}$, at 25 °C. The resulting stability constant is equal to $1.3 \times 10^4\ M^{-1}$. Although $[Fe^{II}(edampda)]$ is less stable than other nitrosyl iron(II) complexes, its greater resistance to oxidation is an advantageous property when compared to other oxygen sensitive iron(II) complexes. Additionally, neutral

[Fe^{II}(edampda)] complex more easily diffuses through membranes, which is a beneficial property for its use in NO removal in the state of sepsis. Investigation of the synthesis and structural characterisation of the Ru^{II} and Ru^{III} analogues of [Fe^{II}(edampda)] are in progress.

Another class of iron complexes under consideration are iron–dithiocarbamate complexes, Fe(DTC). The nitrosyl complexes of Fe(DTC) were studied extensively until the 1970s because of their unique magnetic and electronic properties. Now their high reactivity towards NO and high stability of the resultant nitrosyl complexes, has attracted attention as a possibility for detection and analysis of biologically produced nitric oxide [155,156].

Studies on the vasodilation properties of complexes of the type *trans*-[Ru^{II}(X)(NO)(cyclam)]$^{2+}$ (see previous section) led to consideration of the chemistry of these compounds from the other point of view. Because of the slow dissociation of NO, *trans*-[Ru^{II}(X)(NO)(cyclam)]$^{2+}$ and related complexes might be exploited in the removal of nitric oxide in blood [137,138].

3. Oxygen activation

Oxygen activation has been reviewed in several papers [6–9]. Usually the authors concentrate only on oxygen activation by metal complexes in homogeneous phase or on properties of O_2 adsorbed at the solid (heterogeneous catalysis). In the latter systems photocatalytic activity at a semiconductor surface can be considered. Here we attempt to present only selected examples of using these two different types of systems, homo- and heterogeneous, for reactive oxygen species generation.

Reactive oxygen species involve several molecules, ions and radicals such as $^1O_2$, $OH^\bullet$, $OOH^\bullet$, HO_2^-, $O_2^{\bullet-}$ and H_2O_2. These mostly unstable species can lead to oxidation of several organic molecules, in particular biomolecules. ROS are generated also in environmental systems and photosystems in the presence of metal ions like Fe^{III}, Fe^{II}, Cu^{II}, Cr^{III} [157]. Contrary to the behaviour of the NO molecule, which is very reactive due to its electronic configuration in the ground state, the dioxygen molecule is relatively stable. Therefore, in general, oxygen activation requires an energy input which can be derived by a thermal (e.g. Fenton-like reactions) or photochemical way (e.g. photoassisted Fenton reaction).

A particular role of photogenerated reactive oxygen species can be found in medicine. One of the most promising anticancer therapies is photodynamic therapy (PDT). A photochemically active substance (photosensitiser) is delivered and accumulated in the ill tissue. Excitation of the photosensitiser causes formation of its triplet excited state (T_1, Fig. 5) which can lead to initiation of one of two types of photodynamic reactions. In the photoprocess of type I electron (eventually hydrogen) transfer occurs. As a result radical intermediates are formed. The photoprocess of type II involves an energy transfer from the photosensitiser to $^3O_2$ molecule leading to $^1O_2$ generation. The reactions of type II are usually dominant in PDT. The singlet oxygen and other ROS (especially radicals) so generated in consecutive steps are responsible for the tumour necrosis.

At present porphyrins, chlorins, bacteriochlorins, phthalocyanines and naphthalocyanines are the most commonly studied photosensitisers. There are also some attempts to use TiO_2-based heterogeneous photosensitisers.

3.1. Oxygen as an energy or electron acceptor in homogeneous systems

Many dioxygen complexes have been characterised. Since an electron transfer between O_2 ligand and metal centre may occur the formal state of oxygen in such complexes may be O_2, $O_2^{\bullet-}$ or O_2^{2-}. In addition, the metal-dioxygen centre can adopt several different geometries [7].

The O_2 coordination to metal centres enables oxygen transport and storage in living organisms [8]. Iron- and copper-containing metalloproteins are mainly recognised to be oxygen carriers or reservoirs. These biomolecules may be divided into three classes: (i) heme-containing proteins

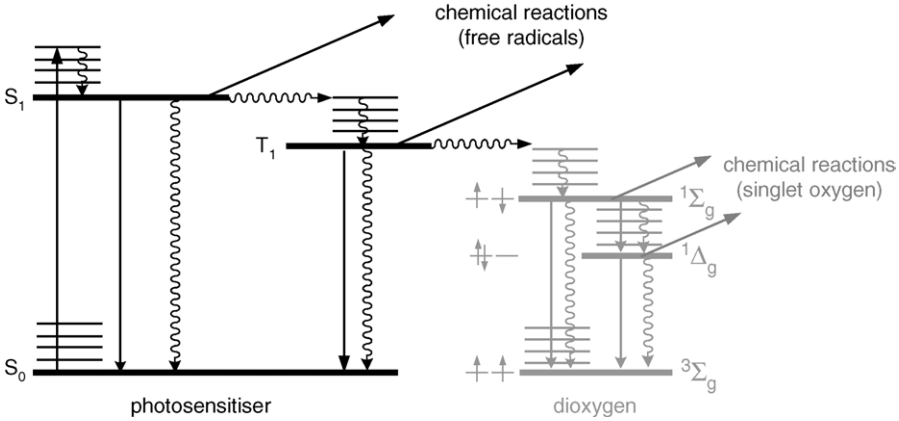


Fig. 5. Photophysical and photochemical principles of the photodynamic action.

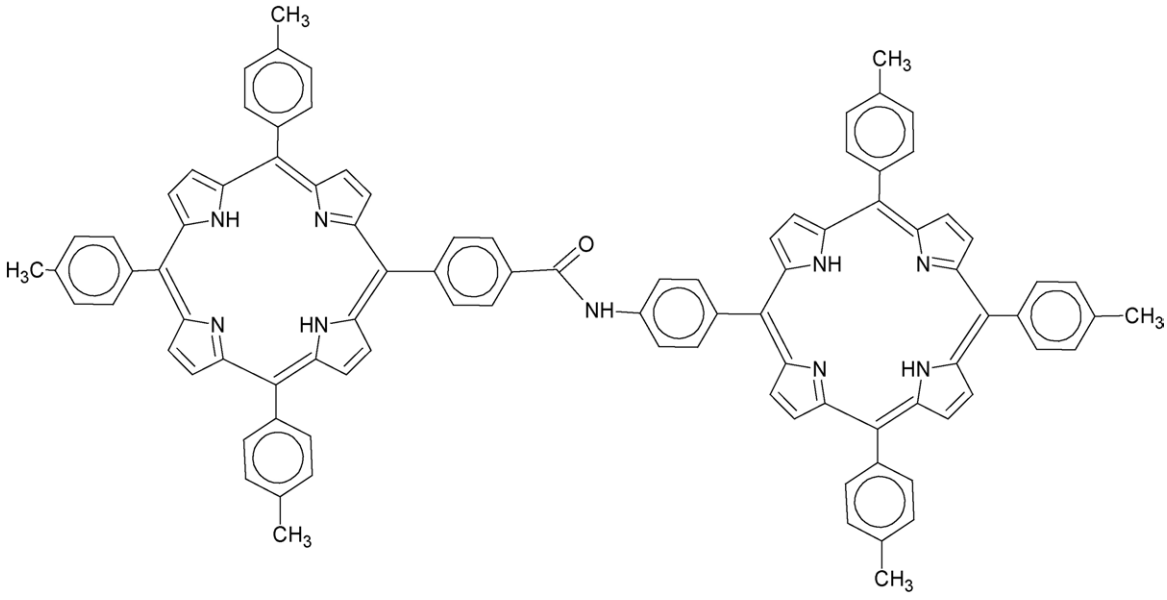


Fig. 6. Structure of T-D: 10,15,20-tritolylporphyrin-5-(4-amidophenyl)-[5-(4-phenyl)-10,15,20-tritolylporphyrin].

(hemoglobin, myoglobin), (ii) non-heme-containing proteins with a di-iron site (hemerythrin, myohemerythrin) and (iii) non-heme-containing proteins with a di-copper site (hemocyanin).

Several organic dyes (e.g. methylene blue, bengal rose, etc.) are very effective in photosensitised singlet oxygen generation. Suitable T_1 state energies and their lifetimes assure good quantum yields of $^1O_2$ photoproduction (Φ_Δ) which can reach values of ca. 0.75 in the case of bengal rose [158]. In the case of metalloporphyrins the Φ_Δ values are even higher, for instance 0.88 for PdTPP in benzene [10]. The additional advantage of porphyrins and their derivatives is their low cytotoxicity in the dark and absorption in a broad range of UV and visible light. The red light absorption is another feature of an ideal photosensitiser since in this spectroscopic region tissues show the deepest light penetration. Phthalocyanines are characterised by Q-band maxima at 670–700 nm [10].

Recently the synthesis, photochemical and photophysical properties and preliminary studies on biological effects of a new, photostable tritolylporphyrin dimer (T-D; Fig. 6) were described [159]. T-D in its excited state activates dioxygen to singlet oxygen with a quantum yield of 0.8, i.e. a significantly higher efficiency as compared to a clinically used hematoporphyrin derivative – Photofrin (Φ_Δ = 0.25–0.28) [160]. The photodynamic properties of the dimer were examined by following the growth of SK-MEL188 (human melanoma) cells irradiated with red light. Using a 30 times lower T-D concentration and 2 times lower light dose as compared to experimental conditions applied in tests with hematoporphyrin derivatives or Photofrin, the effect of photocatalytic killing was comparable for all three photosensitisers.

Since the applicability of a photosensitiser in PDT strongly depends on properties of the first triplet state (T_1), long lifetimes of the T_1 states of photosensitisers are expected. For instance, the metallosubstituted chlorophylls show T_1 lifetimes of a few hundred nanoseconds in the presence of air and 10–100 μs in the absence of oxygen [161]. However, in the case of a platinum(II) complex [162] the triplet state lifetime remained very short, in the range of 2–4 μs. The heavier central ions, due to magnetic interactions with the electrons of ligand, exert the so-called heavy atom effect, which (via spin-orbit coupling) affects the parameters of the intersystem crossing in the complex.

In addition to organic and metalloorganic molecules, inorganic complexes can also photosensitise singlet oxygen formation. Among others complexes of Ru^{II}, Os^{II}, Pt^{II}, Pd^{II} with bipyridine or phenanthroline derivatives as ligands have been tested [10]. In some cases the Φ_Δ values are close to 1.

Simple transition metal complexes are able to generate other reactive oxygen species. A good example is the chemistry and photochemistry of iron(II)/iron(III) systems. In presence of hydrogen peroxide, hydroxyl radical generation takes place. Depending on the oxidation state of iron ions and irradiation the following systems can be considered: H_2O_2/Fe^{II} (Fenton), H_2O_2/Fe^{III} (Fenton-like) and $H_2O_2/Fe^{II}(Fe^{III})$/UV (the photo-assisted Fenton system). Irradiation with UV light in the latter case results in very efficient $OH^\bullet$ radical formation [163]:

$$Fe(OH)^{2+} \xrightarrow{h\nu} Fe^{2+} + OH^\bullet \quad (10)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet \quad (11)$$

In acidic media $HO_2^\bullet$ radicals can also be formed [164]:

$$Fe^{3+} + H_2O_2 \rightleftarrows H^+ + FeOOH^{2+} \quad (12)$$

$$FeOOH^{2+} \rightarrow Fe^{2+} + HO_2^\bullet \quad (13)$$

Due to ROS generation the oxidation of various organic compounds can be realised by photo-assisted Fenton and related processes.

3.2. Oxygen as an energy or electron acceptor in heterogeneous systems

In heterogeneous systems in which oxygen is adsorbed at a semiconductor (usually TiO_2) surface ROS can also be formed. It is a result of *interfacial electron transfer* (IFET), a consecutive step of light absorption and generation of the electron in the conduction band and the hole in the valence band. The ability of TiO_2 in ROS photogeneration opens a variety of possible environmental, biological and medical applications. For instance, water and air purification, smart self-cleaning surfaces are only a few examples. The mechanism of heterogeneous photocatalysis has been described in detail in textbooks and review papers [165–170]. Here only selected aspects of ROS photogeneration in such systems will be discussed.

3.2.1. Reactive oxygen species (ROS) formation

The main process in which hydroxyl radicals are formed is water or hydroxyl group oxidation by valence band holes (h^+):

$$h^+ + H_2O \rightarrow OH^\bullet + H^+ \quad (14)$$

or

$$h^+ + OH^- \rightarrow OH^\bullet \quad (15)$$

This process at titania surface is possible due to ca. 0.2–0.3 V higher redox potential of h^+ as compared to the redox potential of $OH^\bullet/OH^-$ pair.

A variety of ROS may be formed via the so-called reductive pathway. An electron from the conduction band of titania reduces adsorbed dioxygen molecule to superoxide anion [171–174]. A series of consecutive reactions leads to formation of further ROS, such as hydrogen peroxide, $HO_2^\bullet$ and $OH^\bullet$ radicals:

$$e^- + O_2 \rightarrow O_2^{\bullet-} \quad (16)$$

$$O_2^{\bullet-} + H^+ \rightarrow HO_2^\bullet \quad (17)$$

$$O_2^{\bullet-} + HO_2^\bullet \rightarrow O_2 + HO_2^- \quad (18)$$

$$HO_2^\bullet + HO_2^\bullet \rightarrow H_2O_2 + O_2 \quad (19)$$

$$HO_2^- + H^+ \rightarrow H_2O_2 \quad (20)$$

$$H_2O_2 \xrightarrow{h\nu} 2OH^\bullet \quad (21)$$

$$H_2O_2 + O_2^{\bullet-} \rightarrow OH^\bullet + OH^- + O_2 \quad (22)$$

$$H_2O_2 + e^- \rightarrow OH^\bullet + OH^- \quad (23)$$

This series of equations demonstrates the possibility of hydroxyl radical formation in the reductive pathway. The formation of one $OH^\bullet$ radical on this path requires three electrons from the conduction band. The presence of ROS close to the semiconductor surface plays an indispensable role in the consecutive steps of organic species degradation.

Singlet oxygen can also be formed upon TiO_2 irradiation. The mechanisms of $^1O_2$ formation usually discussed are

(i) energy transfer [175]:

$$TiO_2 \xrightarrow{h\nu} TiO_2^* \xrightarrow{O_2} TiO_2 + {}^1O_2 \quad (24)$$

(ii) $HO_2^\bullet$ radical recombination [176]:

$$TiO_2 + O_2 \xrightarrow{h\nu} O_2^{\bullet-} \xrightarrow{H^+} HOO^\bullet \quad (25)$$

$$2HOO^\bullet \rightarrow H_2O_2 + {}^1O_2 \quad (26)$$

(iii) ion-annihilation [177,178]:

$$TiO_2 + O_2 \xrightarrow{h\nu} O_2^{\bullet-} \xrightarrow{h^+} {}^1O_2 \quad (27)$$

The singlet oxygen is most likely formed according to the first mechanism, i.e. in photosensitation process [175].

The main disadvantage of TiO_2, however, is its broad band gap limiting the absorption to UV light. This problem can be at least partially solved by sensitisation of titania to visible light. As an example a series of TiO_2-based photocatalysts modified with platinum(IV) chloride complexes (Pt^{IV}/TiO_2) can be considered. The materials efficiently catalyse photodegradation of 4-chlorophenol (4-CP) with visible light [179–182]. Upon visible light irradiation an oxidising weakly bound chlorine atom ($E = 1.3$–2.4 V) and a reducing Pt^{III} species (ca. -0.7 V) are formed (Fig. 7). The transient Pt^{III}

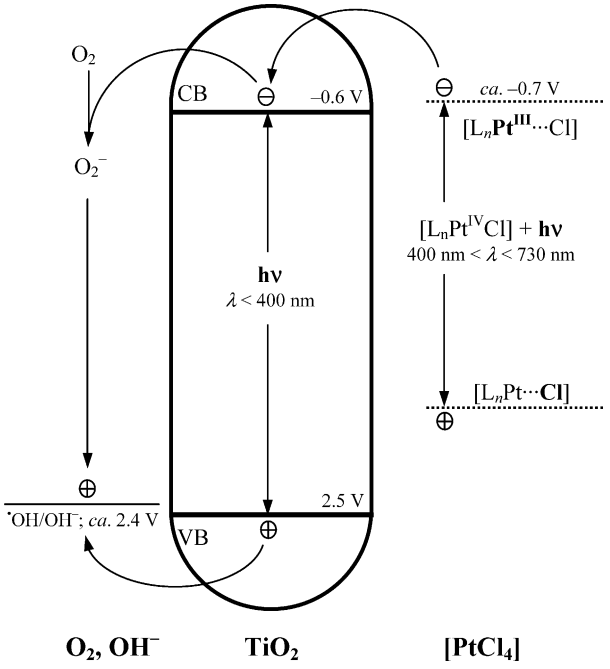


Fig. 7. Proposed mechanism of TiO_2 sensitisation by $[Pt^{IV}Cl_x]^{4-x}$ complexes.

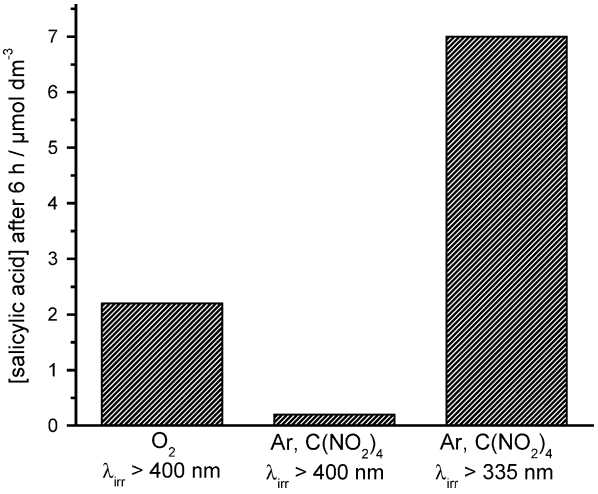


Fig. 8. Formation of salicylic acid upon irradiation of 1% [$PtCl_4$]/P25 suspended in benzoic acid solution: aerated suspension ($\lambda_{irr} > 400$ nm), in Ar-saturated suspension in the presence of $C(NO_2)_4$ ($\lambda_{irr} > 400$ nm and $\lambda_{irr} > 335$ nm).

is capable of an electron injection into the conduction band of TiO_2.

Irradiation with UV-light leads to photogeneration of electrons in conduction band and holes in the valence band. However, since the latter is not produced upon visible light irradiation the formation of hydroxyl radicals on the oxidative pathway is not possible. In a series of experiments of the formation salicylic acid from benzoic acid and $OH^\bullet$ radicals $OH^\bullet$ radicals are formed mainly on the reductive pathway in this system, i.e. the reduction of O_2 and further consecutive steps as described above. In this experiment the suspension of the Pt^{IV}/TiO_2 in the solution of benzoic acid (10^{-2} M) was irradiated with visible light ($\lambda_{irr} > 400$ nm, Fig. 8). Substantial amounts of salicylic acid were detected in the presence of oxygen. When tetranitromethane (10^{-2} M) was used as an electron scavenger (instead of oxygen) only trace amounts of salicylic acid were detectable and the relatively stable yellow $C(NO_2)_3^-$ anion was identified through its typical absorbance at 350 nm [183–185]. Contrary to this, a fast formation of salicylic acid occurred when the latter experiment was conducted with UV light ($\lambda_{irr} > 335$ nm). In this case light absorption by the titania matrix generates valence band holes which oxidise water or surface-hydroxyl groups to the $OH^\bullet$ radicals.

3.2.2. *Photocatalytic mineralisation of organic pollutants*

The oxidation of organic pollutants can occur under relatively mild conditions: at room temperature, in the presence of a photocatalyst most commonly based on TiO_2 and upon irradiation with UV light (wavelengths shorter than 400 nm). When TiO_2 material is sensitised, UV light can be substituted with visible light. Pollutants containing carbon, hydrogen, nitrogen, sulfur, halogen atoms and others are converted to CO_2, H_2O, NO_3^-, SO_4^{2-}, halide anions, etc. The process leading to these end-products is called mineralisation. There are reports on photocatalysed degradation of hundreds of organic compounds [166,186]. Several pilot plants for photocatalytic water cleaning already operate in sunny places.

One of the model pollutants used in studies on photodegradation is 4-chlorophenol (4-CP) [187]. The route of 4-CP degradation at a titania surface has been extensively studied [166,188–193]. The oxidation is initiated by hydroxyl radical or directly by the hole. This step leads to hydrogen atom abstraction or introduction of an additional –OH group to the aromatic ring. In consecutive steps the C–Cl bond cleavage, the introduction of the next hydroxyl groups and ring opening take place.

Photomineralisation does not always mean photooxidation. Quite often the oxidation process must be preceded by reduction steps. A good example is the transformation of CCl_4 to CO_2 and Cl^-. The end-products can be formed only after the reduction of carbon(IV) followed by its re-oxidation [166]. Therefore the redox properties of excited semiconductor together with possibility of ROS generation play a crucial role.

An interesting, very stable pollutant attracting particular attention is cyanuric acid. It is the final product of atrazine degradation in most photocatalytic systems based on TiO_2. Its degradation was observed on the previously described Pt^{IV}/TiO_2 photocatalyst [180] or at titania with the surface modified with fluoride anions [166,194]. The extraordinary properties of the latter photocatalyst were attributed to hydroxyl radicals formed in the homogeneous phase which were stronger oxidants as compared to hydroxyl radicals adsorbed at the titania surface [166,194].

3.2.3. *Semiconductors as photosensitisers in PDT*

Similar to homogeneous systems capable of ROS photogeneration, heterogeneous photocatalysts can also be considered as potential photosensitisers in photodynamic therapy. Such applications (mainly of TiO_2-based materials) have been recently tested [195].

The role of ROS photogenerated in heterogeneous systems is similar to ROS produced in solution. The first target of reactive oxygen species is the cellular membrane which can be damaged and its permeability can be changed [196–198]. Further damage inside the cell include the ROS attack on nucleic acids [199–202] and enzymes [203]. Oxidation of proteins and aminoacids photocatalysed by TiO_2 has also been observed by Muszkat et al. [204]. This process responsible for the cell damage can also be applied for purification of biocontaminated waters.

Photoexcited TiO_2 particles induced primary DNA damage and structural chromosome aberrations in cultured mouse lymphoma L5178Y cells [201]. Since no induction of gene mutations was observed upon irradiation in microbial or mammalian cells systems treated with TiO_2, it was suggested that DNA lesion causes a chromosomal aberration rather than gene mutations.

The cytotoxicity of TiO_2 upon UV irradiation, attributed to photogenerated $OH^{\bullet}$ radicals, was also observed against Chinese hamster ovary cells [205,206]. Photoinduced killing of T-24 human bladder cancer cells in the presence of titania was reported by Fujishima and co-workers [196,207,208]. The mechanism of ROS photogeneration, particularly singlet oxygen and superoxide anion formation was investigated by Inoue and co-workers [175]. The energy transfer from radiationless recombination of photogenerated electron/hole pair to an adsorbed oxygen molecule was proposed as the most likely process of singlet oxygen formation.

3.2.4. Photocatalytic microorganisms killing

Reactive oxygen species photogenerated at the surface of excited TiO_2 may also be responsible for killing microorganisms. There are reports on the killing (or at least growth inhibition) of bacteria [186,189,200–204] and algae [209] at the surface of irradiated TiO_2. These properties of TiO_2 layers are already applied in the production of sterile tiles and other surfaces. Photogenerated ROS attack cell membrane (the process called lipids peroxidation), nucleic acids, proteins (enzymes deactivation). Similar mechanisms are involved in PDT.

Photogeneration of ROS outside the microorganism cell may cause a severe damage of the cell membrane. The process involves breaking of three main membrane layers: outer membrane, peptidoglycan and cytoplasmic membrane [210]. Slow damage of the outer membrane changes the permeability to ROS. Then the cytoplasmic membrane is attacked by radicals leading to peroxidation of membrane lipids. This mechanism, however, assumes the radical attack not only at the semiconductor surface but also at a certain distance from particles. In fact, the bactericidal effect of illuminated TiO_2 film on *E. coli* was observed even at a distance of 50 μm from the film [211]. Once the cellular membrane is damaged (open) small photocatalyst particles can reach the inner part of the cell causing severe, efficient oxidation of the cell content [200,212].

The oxidation power of ROS is high enough to oxidise aminoacids, peptides [204] and enzymes [203]. Destruction of nucleic acids was also observed [199–202]. It can be monitored as pyrimidine dimer formation [213], DNA structural changes [202], decrease of DNA molecular weight [200] or hydroxylation of guanine bases [199].

Bactericidal properties of some metals (e.g. Ag, Cu) merged with photocatalytic properties of titania can be combined in hybrid materials with the metal islands deposited at the TiO_2 surface. Expected increased efficiency of *E. coli* killing was observed for Ag/TiO_2 system with 1% (w/w) silver content [189]. Peroxidation of lipids in this system was significantly faster when compared to the system with pure titania. Besides the bactericidal activity of the silver, the metal was also a trap for photogenerated charges.

A similar effect can be achieved using copper instead of silver [195,214]. Fujishima et al. described ceramic tiles covered with TiO_2 layers which were sprayed with metal salt solutions followed by photoreduction of ions to form metal islands [195]. The activity of such surfaces can be divided into two main actions: (i) bacteria killing by metal ions (in dark) and/or by photogenerated ROS; (ii) photodecomposition of organic material, in particular endotoxins, lipids, etc. Removal of organic matter from the surface prevents the growth of new microorganisms because of the lack of available nutrients.

4. Conclusions

Although activation processes of small molecules like NO and O_2 have been studied for many years, a lot of questions still remain to be answered. The most effective methods to use metal ions and metal compounds as electron and/or energy transfer mediators in activation processes are still of great interest. Improvement of our knowledge on biological processes involving interplay of reactive oxygen and nitric oxide species with metal ions is essential for design and tuning of artificial systems for medical, industrial and environmental applications.

Acknowledgements

The authors thank the Foundation for Polish Science, “Fastkin” No. 8/97 (GS) and fellowship (WM), State Committee for Scientific Research (KBN), DAAD (AF) and DFG (WM).

References

[1] J.A. Olabe, L.D. Slep, Comprehens. Coord. Chem. II 1 (2003) 603.
[2] Y. Chen, M.A. Sweetland, R.A. Shepherd, Inorg. Chim. Acta 260 (1997) 163.
[3] P.C. Ford, L.E. Laverman, I.M. Lorkovic, Adv. Inorg. Chem. 54 (2004) 203.
[4] M. Hoshino, L.E. Laverman, P.C. Ford, Coord. Chem. Rev. 187 (1999) 75, and references cited herein.
[5] G. Stochel, A. Wanat, E. Kulis, Z. Stasicka, Coord. Chem. Rev. 171 (1998) 203.
[6] R. van Eldik, Coord. Chem. Rev. 182 (1999) 373.
[7] R.E. Berry, Comprehens. Coord. Chem. II 1 (2003) 625, and references cited therein.
[8] D.M. Kurtz, Comprehens. Coord. Chem. II 8 (2003) 229, and references cited therein.
[9] D.T. Sawyer, J.S. Valentine, Acc. Chem. Res. 14 (1981) 393.
[10] M.C. DeRosa, R.J. Crutchley, Coord. Chem. Rev. 233/234 (2002) 351.
[11] D. Mansuy, P. Battioni, in: K.M. Kadish, K.M. Smith, R. Guilard (Eds.), The Porphyrin Handbook, vol. 4, Academic Press, Amsterdam, 2000, p. 1.
[12] J.P. Collman, R. Boulatov, C.J. Sunderland, in: K.M. Kadish, K.M. Smith, R. Guilard (Eds.), The Porphyrin Handbook, vol. 11, Academic Press, Amsterdam, 2003, p. 1.
[13] S. Moncada, R.M.J. Palmer, A. Higgs, Pharmacol. Rev. 43 (1991) 109.
[14] R.F. Furchgott, J.V. Zawadzki, Nature 288 (1980) 373.

[15] K. Shibuki, Neurosci. Res. 9 (1990) 69.
[16] H.H.W. Schmidt, U. Walter, Cell 78 (1994) 919.
[17] A.R. Butler, D. Lyn, H. Williams, Chem. Soc. Rev. 22 (1993) 233.
[18] S.R. Maraj, S. Khan, X.-Y. Cui, R. Cammack, C.L. Joannou, M.N. Hughes, The Analyst 120 (1995) 699.
[19] S.S. Stamler, D.J. Singel, J. Loscalzo, Science 258 (1992) 1898.
[20] R.J.P. Williams, Chem. Soc. Rev. 25 (1996) 77.
[21] G. Stochel, E. Ilkowska, M. Pawelec, A. Wanat, M. Wolak, ACH-Models Chem. 135 (1998) 847.
[22] P.L. Feldman, O.W. Griffith, D.J. Stuehr, Chem. Eng. News 71 (1993) 26.
[23] M.A. Marleta, J. Biol. Chem. (1993) 12231.
[24] E. Wasielewska, M. Witko, G. Stochel, Z. Stasicka, Chem. Eur. J. 3 (1997) 609.
[25] E.J. Nelson, J. Connolly, P. McArthur, Biol. Cell 95 (2003) 3.
[26] A. Ledo, J. Frade, R.M. Barbosa, J. Laranjinha, Mol. Aspects Med. 25 (2004) 75.
[27] S.P. Fricker, Platinum Met. Rev. 39 (1995) 150.
[28] C. Nathan, FASEB J. 6 (1993) 3051.
[29] R.J. Gryglewski, R.M. Botting, J.R. Vane, Hypertension 12 (1988) 530.
[30] R.J. Gryglewski, Thromb. Haemostas 19 (1993) 158.
[31] T.L. Brun, Lancet 2 (1867) 97.
[32] M. Feelisch, E.A. Noack, Eur. J. Pharmacol. 139 (1987) 19.
[33] M. Feelisch, E.A. Noack, H. Schroeder, Eur. Heart J. 9 (Suppl. A) (1988) 57.
[34] D.A. Wink, J.F. Darbyshire, R.W. Nims, J.E. Saveedra, P.C. Ford, Chem. Res. Toxicol. 6 (1993) 23.
[35] H.H. Awad, D.M. Stanbury, Int. J. Chem. Kinet. 25 (1993) 375.
[36] P.C. Ford, D.A. Wink, D.M. Stanbury, FEBS Lett. 326 (1993) 1.
[37] D.D. Thomas, X. Liu, S.P. Kantrow, J.R.J. Lancaster, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 355.
[38] K.Y. Lee, D.J. Kuchynka, J.K. Kochi, Inorg. Chem. 29 (1990) 4196.
[39] V. Shafirovich, S.V. Lymar, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 7340.
[40] M.D. Bartberger, W. Liu, E. Ford, K.M. Miranda, C. Switzer, J.M. Fukuto, P.J. Farmer, D.A. Wink, K.N. Houtz, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 10958.
[41] A.A. Nedospasov, J. Biochem. Mol. Toxicol. 16 (2002) 109.
[42] H. Lipton, Y.B. Choi, N.J. Sucher, H.S. Chen, Biofactors 8 (1998) 33.
[43] S. Herold, FEBS Lett. 439 (1998) 85.
[44] M.A. DeLeo, P.C. Ford, J. Am. Chem. Soc. 121 (1999) 1980.
[45] S. Herold, F.-J.K. Rehmann, J. Biol. Inorg. Chem. 6 (2001) 543.
[46] I.M. Lorkovic, P.C. Ford, Inorg. Chem. 38 (1999) 1467.
[47] J.H. Enemark, R.D. Feltham, Coord. Chem. Rev. 13 (1974) 339.
[48] B.F. Johnson, B.L. Haymore, J.R. Dilworth, in: G. Wilkinson (Ed.), Comprehensive Coordination Chemistry, vol. 2, Pergamon Press, Oxford, 1987, p. 99.
[49] G.B. Richter-Addo, P. Legzdings, Metal Nitrosyls, Oxford University Press, Oxford, 1992.
[50] E.I. Ochiai, J. Chem. Educ. 73 (1996) 130.
[51] L. Cheng, G.B. Richter-Addo, in: K.M. Kadish, K.M. Smith, R. Guilard (Eds.), The Porphyrin Handbook, vol. 4, Academic Press, Amsterdam, 2000, p. 219.
[52] M. Wolak, R. van Eldik, Coord. Chem. Rev. 230 (2002) 263.
[53] C.E. Cooper, Biochim. Biophys. Acta 1411 (1999) 290, and references cited therein.
[54] A. Wanat, M. Wolak, Ł. Orzeł, M. Brindell, R. van Eldik, G. Stochel, Coord. Chem. Rev. 229 (2002) 37, and references cited herein.
[55] T.G. Traylor, V.S. Sharma, Biochemistry 31 (1992) 2847.
[56] J.R. Stone, M.A. Marletta, Biochemistry 33 (1994) 5636.
[57] A.W. Addison, J.J. Stephanos, Biochemistry 25 (1986) 4104.
[58] P.L. Feldman, O.W. Griffith, D.J. Stuehr, Chem. Eng. News 71 (1996) 26.
[59] R.G. Knowles, S. Moncada, Biochem. J. 298 (1994) 249.
[60] N. Newton, Biochim. Biophys. Acta 185 (1969) 316.
[61] R. Cassoly, Q.H. Gibson, J. Mol. Biol. 91 (1975) 301.
[62] E.G. Moore, Q.H. Gibson, J. Biol. Chem. 251 (1976) 2788.
[63] P. Ascenzi, M. Coletta, R. Santucci, F. Polizio, A. Desideri, J. Inorg. Biochem. 53 (1994) 273.
[64] M. Hoshino, K. Ozawa, H. Seki, P.C. Ford, J. Am. Chem. Soc. 115 (1993) 9568.
[65] B.A. Averill, Chem. Rev. 96 (1996) 2951.
[66] L.E. Laverman, A. Wanat, J. Oszajca, G. Stochel, P.C. Ford, R. van Eldik, J. Am. Chem. Soc. 123 (2001) 285.
[67] A. Franke, G. Stochel, C. Jung, R. van Eldik, J. Am. Chem. Soc. 126 (2004) 4181.
[68] L.E. Laverman, M. Hoshino, P.C. Ford, J. Am. Chem. Soc. 119 (1997) 12663.
[69] C. Jung, H. Schulze, E. Deprez, Biochemistry 35 (1996) 15088.
[70] C. Jung, O. Ristau, H. Schulze, S.G. Sligar, Eur. J. Biochem. 235 (1996) 660.
[71] Y. Henry, C. Ducrocq, J.-C. Drapier, D. Servent, C. Pellat, A. Guissani, Eur. Biophys. J. 20 (1991) 1.
[72] V.S. Sharma, H.M. Ranney, J. Biol. Chem. 253 (1978) 6467.
[73] M.P. Doyle, J.W. Hoekstra, J. Inorg. Biochem. 14 (1981) 351.
[74] L.E. Laverman, P.C. Ford, J. Am. Chem. Soc. 123 (2001) 11614.
[75] P.C. Ford, I.M. Lorkovic, Chem. Rev. 102 (2002) 993, and references cited therein.
[76] R. Cammack, C.E. Cooper, Meth. Enzymol. 227 (1992) 353.
[77] M.J. Nelson, J. Biol. Chem. 262 (1987) 12137.
[78] B. Kalyanaraman, Meth. Enzymol. 268 (1996) 168.
[79] H. Twilfer, F.H. Bernhardt, K. Gersonde, Eur. J. Biochem. 147 (1985) 171.
[80] N.E. LeBrun, S.C. Andrews, G.R. Moore, A.J. Thomson, Biochem. J. 326 (1997) 173.
[81] C.J. Haskin, N. Ravi, J.B. Lynch, E. Münck, J.R. Que, Biochemistry 34 (1995) 11090.
[82] A.C.F. Gorren, E.D. Boer, R. Wever, Biochim. Biophys. Acta 916 (1987) 38.
[83] M. Lepoivre, F. Fieschi, J. Coves, L. Thelander, M. Fontecave, Biochem. Biophys. Res. Commun. 179 (1991) 442.
[84] B. Roy, M. Lepoivre, Y. Henry, M. Fontecave, Biochemistry 34 (1995) 5411.
[85] M. Tsujimura, N. Dohmae, M. Odaka, Chijimatsu, K. Takio, M. Yohda, M. Hoshino, S. Nagashimo, I. Endo, J. Biol. Chem. 272 (1997) 29454.
[86] V.S. Sharma, M.R. Schmidt, H.M. Ranney, J. Biol. Chem. 251 (1976) 4267.
[87] P. Kostka, Anal. Chem. 67 (1995) 411.
[88] A.R. Butler, F.W. Flitney, D.L.H. Williams, TiPS 16 (1995) 18.
[89] A. Hausladen, I. Fridovich, J. Biol. Chem. 47 (1994) 29405.
[90] L. Castro, M. Rodriguez, R. Radi, J. Biol. Chem. 269 (1994) 29409.
[91] J. Stadler, T.R. Billiar, R.D. Curran, D.J. Stuehr, J.B. Ochoa, R.L. Simmons, Am. J. Physiol. 260 (1991) 910.
[92] A.F. Vanin, Biochemistry (Moscow) 63 (1998) 782.
[93] K. Szaciłowski, J. Oszajca, A. Barbieri, A. Karocki, Z. Sojka, S. Sostero, R. Boaretto, Z. Stasicka, J. Photochem. Photobiol. A: Chem. 143 (2001) 99.
[94] H.W. Foster, J.A. Cowan, J. Am. Chem. Soc. 121 (1999) 4093.
[95] T. Ueno, T. Yoshimura, Jpn. J. Pharmacol. 82 (2000) 95.
[96] S.S. Greenberg, J. Xie, J.M. Zatarain, D.R. Kapusta, M.J.S. Miller, J. Pharm. Exp. Ther. 273 (1995) 257.
[97] K.M. Jenkinson, J.J. Reid, M.J. Rand, Eur. J. Pharmacol. 275 (1995) 145.
[98] M. Brouwer, W. Chamulitrat, G. Ferruzzi, D.L. Sauls, J.B. Weinberg, Blood 88 (1996) 1857.
[99] M. Wolak, G. Stochel, M. Hamza, R. van Eldik, Inorg. Chem. 39 (2000) 2018.
[100] D. Zheng, R. Birke, J. Am. Chem. Soc. 123 (2001) 4637, and references cited therein.

[101] M. Wolak, A. Zahl, T. Schneppensieper, G. Stochel, R. van Eldik, J. Am. Chem. Soc. 123 (2001) 9780.
[102] J. Torres, M.T. Wilson, Biochim. Biophys. Acta 1411 (1999) 310, and references cited therein.
[103] R. Wever, R. Boelens, E.D. Boer, B.F. van Gelder, A.C.F. Gorren, H. Rademaker, J. Inorg. Biochem. 23 (1985) 227.
[104] R. Boelens, H. Rademaker, R. Wever, B.F. van Gelder, Biochim. Biophys. Acta 765 (1984) 196.
[105] G. Musci, S.D. Marco, M.C. Bonaccorsi di Patti, L. Calabrese, Biochemistry 30 (1991) 9866.
[106] C.T. Martin, R.H. Morse, R.M. Kanne, H.B. Gray, B.G. Malstrom, S.I. Chan, Biochemistry 20 (1981) 5147.
[107] F.X.R. van Leeuwen, B.F. van Gelder, Eur. J. Biochem. 87 (1978) 305.
[108] G.W. Brudwig, T.H. Stevens, S.I. Chan, Biochemistry 19 (1980) 5275.
[109] D. Ehrenstein, G.U. Niehaus, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 9681.
[110] D. Ehrenstein, M. Filiaci, B. Scharf, M. Engelhard, P.J. Steinbach, G.U. Niehaus, Biochemistry 34 (1995) 12170.
[111] A. Petros, D. Bennet, P. Vallence, Lancet 338 (1991) 1557.
[112] A.R. Butler, C. Glidewell, Chem. Soc. Rev. 16 (1987) 361.
[113] J. Oszajca, G. Stochel, E. Wasielewska, Z. Stasicka, R.J. Gryglewski, A. Jakubowski, K. Cieslik, Inorg. Biochem. 69 (1998) 121.
[114] A. Wanat, R. van Eldik, G. Stochel, J. Chem. Soc., Dalton Trans. (1998) 2497.
[115] K. Szaciłowski, G. Stochel, Z. Stasicka, H. Kisch, New J. Chem. 21 (1997) 893.
[116] M. Feelisch, Eur. Heart J. 14 (1993) 123.
[117] I.H. Tuzel, J. Clin. Pharmacol. 14 (1974) 494.
[118] J.M. Campbell, F. McCrae, J. Reglinski, R. Wilson, W.E. Smith, R.D. Sturrock, Biochim. Biophys. Acta 1156 (1993) 327.
[119] T. Martin, J.A. Patel, Am. J. Hosp. Pharm. 26 (1969) 51.
[120] G.E. Schumacher, Am. J. Hosp. Pharm. 23 (1966) 532.
[121] J. Reglinski, A.R. Butler, C. Glidewell, Appl. Organometall. Chem. 8 (1994) 25.
[122] J. Bourassa, W. DeGraff, S. Kudo, D.A. Wink, J.B. Mitchell, P.C. Ford, J. Am. Chem. Soc. 117 (1997) 2853.
[123] F.W. Flitney, I.L. Megson, J.L.M. Thomson, G.D. Kennovin, A.R. Butler, Br. J. Pharm. 117 (1996) 1549.
[124] J. Bourassa, B. Lee, S. Berbard, J. Schoonover, P.C. Ford, Inorg. Chem. 38 (1999) 2947.
[125] A. Janczyk, A. Wolnicka-Glubisz, A. Chmura, M. Elas, Z. Matuszak, G. Stochel, K. Urbanska, Nitric Oxide 10 (2004) 42.
[126] A.B. Cox, R.M. Wallace, Inorg. Nucl. Chem. Lett. 7 (1971) 1191.
[127] P.N. Komozin, V.M. Kazakova, I.V. Miroshnichenko, N.M. Snitsyn, Russ. J. Inorg. Chem. 28 (1983) 1806.
[128] N. Bettache, T. Carter, J.E.T. Corrie, D. Ogden, D. Trentham, Meth. Enzymol. 268 (1996) 266.
[129] M. Hoshino, M. Kogure, J. Phys. Chem. 93 (1989) 5478.
[130] Q.H. Gibson, S. Ainsworth, Nature 180 (1957) 1416.
[131] M. Hoshino, S. Arai, M. Yamaji, Y. Hama, J. Phys. Chem. Soc. 90 (1986) 2109.
[132] E.J. Rose, B.M. Hoffman, J. Am. Chem. Soc. 105 (1983) 2866.
[133] E.A. Morlino, M.A.J. Rodgers, J. Am. Chem. Soc. 118 (1996) 11798.
[134] E.A. Morlino, M.A.J. Rodgers, Prog. React. Kinet. 23 (1998) 91.
[135] K.M. Miranda, X. Bu, I.M. Lorkovic, P.C. Ford, Inorg. Chem. 36 (1997) 4838.
[136] C.F. Works, P.C. Ford, J. Am. Chem. Soc. 122 (2000) 7592.
[137] D.R. Lang, J.A. Davis, L.G.F. Lopes, A.A. Ferro, L.C.G. Vasconcellos, D.W. Franco, E. Tfouni, A. Wieraszko, M.J. Clarke, Inorg. Chem. 39 (2000) 2294.
[138] M.H. Krieger, A. Souza-Torsoni, F.G. Marcondes, D.W. Franco, J.C. Toledo, E. Tfouni, A.A. Ferro, M. Haun, M. Sumitami, Hypertension 33 (1999) 159.
[139] M.D. Leo, P.C. Ford, J. Am. Chem. Soc. 121 (1999) 1980.
[140] N.A. Davies, M.T. Wilson, E. Slade, S.P. Fricker, B.A. Murrer, N.A. Powell, G.R. Henderson, Chem. Commun. (1997) 47.
[141] S.P. Fricker, E. Slade, N.A. Powell, O.J. Vaughan, G.R. Henderson, B.A. Murrer, I.L. Megson, S.K. Bisland, F.W. Flitney, J. Pharmacol. 122 (1997) 1441.
[142] A. Wanat, T. Schneppensieper, A. Karocki, G. Stochel, R. van Eldik, J. Chem. Soc., Dalton Trans. (2002) 941.
[143] J.F. Demmik, I.C.F. van Gils, A.A.C.M. Beenackers, Ind. Eng. Chem. Res. 36 (1997) 4914.
[144] V. Zang, R. van Eldik, Inorg. Chem. 29 (1990) 4462.
[145] E. Sada, H. Kumazawa, Y. Takada, Ind. Eng. Chem. Fundam. 23 (1984) 60.
[146] E. Sada, H. Kumazawa, H. Machida, Ind. Eng. Chem. Res. 26 (1987) 1468.
[147] R.E. Shepherd, M.A. Sweetland, D.E. Junker, J. Inorg. Biochem. 65 (1997) 1.
[148] W.M. Kazimierski, G. Wolberg, J.G. Wilson, S.R. Smith, D.S. Williams, H.H. Horp, L. Molina, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 9138.
[149] T. Schneppensieper, S. Finkler, A. Czap, R. van Eldik, M. Heus, P. Nieuwenhuizen, C. Wreesmann, W. Abma, Eur. J. Inorg. Chem. 9 (2001) 491.
[150] T. Schneppensieper, A. Wanat, G. Stochel, S. Goldstein, D. Mayerstein, R. van Eldik, Eur. J. Inorg. Chem. 9 (2001) 2317.
[151] A. Wanat, T. Schneppensieper, G. Stochel, R. van Eldik, B. Eckhard, K. Wieghardt, Inorg. Chem. 41 (2002) 4.
[152] T. Schneppensieper, A. Wanat, G. Stochel, R. van Eldik, Inorg. Chem. 41 (2002) 2565.
[153] D.A. Baldwin, R.M. Pfeiffer, Reichgott, N.J. Rose, J. Am. Chem. Soc. 95 (1973) 5152.
[154] M.S. Ward, R.E. Shepherd, Inorg. Chim. Acta 286 (1997) 197.
[155] S. Fuji, T. Yoshimura, Coord. Chem. Rev. 198 (2000) 89.
[156] L.A. Reinke, D.R. Moore, Y. Kotake, Anal. Biochem. 243 (1996) 8.
[157] P. Cieśla, P. Kocot, P. Mytych, Z. Stasicka, J. Mol. Catal. A: Chem. 224 (2004) 17.
[158] R.W. Redmond, J.N. Gamlin, Photochem. Photobiol. 70 (1999) 391.
[159] A. Drzewiecka, K. Urbańska, Z. Matuszak, M. Pineiro, L.G. Arnaut, J. Habdas, A. Ratuszna, G. Stochel, Acta Biochim. Polon. 48 (2001) 277.
[160] S. Kimel, B.J. Tromberg, W.G. Roberts, M.W. Berns, Photochem. Photobiol. 50 (1989) 175.
[161] A. Drzewiecka-Matuszek, A. Skalna, G. Stochel, L. Fiedor, J. Biol. Inorg. Chem., in press, published online: 26 May 2005.
[162] L. Fiedor, G. Stochel, K. Urbanska, Patent, Poland, submitted for publication.
[163] J. Kiwi, C. Pulgarin, P. Peringer, M. Graetzel, Appl. Catal. B: Environ. 3 (1993) 85.
[164] J.J. Pignatello, Environ. Sci. Technol. 26 (1992) 944.
[165] A. Mills, S. Le Hunte, J. Photochem. Photobiol. A: Chem. 108 (1997) 1.
[166] M.R. Hoffmann, S.T. Martin, W. Choi, D.W. Bahnemann, Chem. Rev. 95 (1995) 69.
[167] M.A. Fox, M.T. Dulay, Chem. Rev. 93 (1993) 341.
[168] A.L. Linsebigler, G. Lu, J.T. Yates Jr., Chem. Rev. 95 (1995) 735.
[169] P.V. Kamat, Chem. Rev. 93 (1993) 267.
[170] N. Serpone, E.E. Pelizzetti, Photocatalysis. Fundamentals and Applications, Wiley, New York, 1989.
[171] R.B. Cundall, R. Rudham, M.S. Salim, J. Chem. Soc., Faraday Trans. 1 72 (1976) 1642.
[172] P.R. Harvey, R. Rudham, S. Ward, J. Chem. Soc., Faraday Trans. 1 79 (1983) 1381.
[173] M. Fujihira, Y. Satoh, T. Osa, Bull. Chem. Soc. Jpn. 55 (1982) 666.

[174] K. Okamoto, Y. Yamamoto, H. Tanaka, M. Tanaka, A. Itaya, Bull. Chem. Soc. Jpn. 58 (1985) 2015.
[175] R. Konaka, E. Kasahara, W.C. Dunlap, Y. Yamamoto, K.C. Chien, M. Inoue, Free Radic. Biol. Med. 27 (1999) 294.
[176] H. Sugimoto, D.T. Sawyer, J.R. Kanofsky, J. Am. Chem. Soc. 110 (1988) 8707.
[177] E.A. Mayeda, A.J. Bard, J. Am. Chem. Soc. 95 (1973) 6223.
[178] S.P. Pappas, R.M. Fischer, J. Paint Technol. 46 (1974) 65.
[179] W. Macyk, H. Kisch, Chem. Eur. J. 7 (2001) 1862.
[180] W. Macyk, G. Burgeth, H. Kisch, Photochem. Photobiol. Sci. 2 (2003) 322.
[181] G. Burgeth, H. Kisch, Coord. Chem. Rev. 230 (2002) 40.
[182] H. Kisch, G. Burgeth, W. Macyk, Adv. Inorg. Chem. 56 (2004) 241.
[183] P. Boarini, V. Carassiti, A. Maldotti, R. Amadelli, Langmuir 14 (1998) 2080.
[184] K.D. Asmus, H. Henglein, Ber. Bunsen-Ges. Phys. Chem. 68 (1968) 348.
[185] H. Henglein, Ber. Bunsen-Ges. Phys. Chem. 86 (1982) 241.
[186] D.F. Ollis, H. Al-Ekabi (Eds.), Photocatalytic Purification and Treatment of Water and Air, Elsevier, Amsterdam, 1993.
[187] A. Mills, R.H. Davies, D. Worsley, Chem. Soc. Rev. (1993) 417.
[188] J. Theurich, M. Lindner, D.W. Bahnemann, Langmuir 12 (1996) 6368.
[189] M. Barbeni, E. Pramauro, E. Pelizzetti, E. Borgarello, M. Grätzel, N. Serpone, Nouv. J. Chim. 8 (1984) 547.
[190] H. Al-Ekabi, N. Serpone, E. Pelizzetti, C. Minero, M.A. Fox, R.B. Draper, Langmuir 5 (1989) 250.
[191] A. Mills, S. Morris, R. Davies, J. Photochem. Photobiol. A: Chem. 70 (1993) 183.
[192] U. Stafford, K.A. Gray, P.V. Kamat, J. Phys. Chem. 98 (1994) 6343.
[193] J. Cunningham, G. Al-Sayyed, J. Chem. Soc., Faraday Trans. 86 (1990) 3935.
[194] Y.-C. Oh, W.S. Jenks, J. Photochem. Photobiol. A: Chem. 162 (2003) 323.
[195] A. Fujishima, K. Hashimoto, T. Watanabe, TiO_2 Photocatalysis. Fundamentals and Applications, BKC Inc., Tokyo, 1999.
[196] H. Sakai, E. Ito, R.X. Cai, T. Yoshioka, Y. Kubota, K. Hashimoto, A. Fujishima, Biochim. Biophys. Acta 1201 (1994) 259.
[197] M. Xu, N. Huang, Z. Xiao, Z. Lu, Supramol. Sci. 5 (1998) 449.
[198] M. Sökmen, F. Candan, Z. Sümer, J. Photochem. Photobiol. A: Chem. 143 (2001) 241.
[199] W.G. Wamer, J.-J. Yin, R.R. Wei, Free Radic. Biol. Med. 23 (1997) 851.
[200] N. Huang, M. Xu, C. Yuan, R. Yu, J. Photochem. Photobiol. A: Chem. 108 (1997) 229.
[201] Y. Nakagawa, S. Wakuri, K. Sakamoto, N. Tanaka, Mutat. Res. 394 (1997) 125.
[202] T. Ashikaga, M. Wada, H. Kobayashi, M. Mori, Y. Katsumura, H. Fukui, S. Kato, M. Yamaguchi, T. Takamatsu, Mutat. Res. 466 (2000) 1.
[203] T. Hancock-Chen, J.C. Scaiano, J. Photochem. Photobiol. B: Biol. 57 (2000) 193.
[204] L. Muszkat, L. Feigelson, L. Bir, K.A. Muszkat, J. Photochem. Photobiol. B: Biol. 60 (2001) 32.
[205] T. Uchino, H. Tokunaga, M. Ando, H. Utsumi, Toxicol. In Vitro 16 (2002) 629.
[206] P.-J. Lu, I.-C. Ho, T.-C. Lee, Mutat. Res. 414 (1998) 15.
[207] H. Sakai, R. Baba, K. Hashimoto, Y. Kubota, A. Fujishima, Chem. Lett. (1995) 185.
[208] Y. Kubota, T. Shuin, C. Kawasaki, M. Hosaka, H. Kitamura, R. Cai, H. Sakai, K. Hashimoto, A. Fujishima, Br. J. Cancer 70 (1994) 1107.
[209] C.A. Linkous, G.J. Carter, D.B. Locuson, A.J. Ouellette, D.K. Slattery, L.A. Smitha, Environ. Sci. Technol. 34 (2000) 4754.
[210] K. Sunada, T. Watanabe, K. Hashimoto, J. Photochem. Photobiol. A: Chem. 156 (2003) 227.
[211] Y. Kikuchi, K. Sunada, T. Iyoda, K. Hashimoto, A. Fujishima, J. Photochem. Photobiol. A: Chem. 106 (1997) 51.
[212] Z. Huang, P.-C. Maness, D.M. Blake, E.J. Wolfrum, S.L. Smolinski, W.A. Jacoby, J. Photochem. Photobiol. A: Chem. 130 (2000) 163.
[213] Y. Kubota, C. Niwa, T. Ohnuma, Y. Ohko, T. Tatsuma, T. Mori, A. Fujishima, J. Photochem. Photobiol. A: Chem. 141 (2001) 225.
[214] P. Amezaga-Madrid, G.V. Nevarez-Moorillon, E. Orrantia-Borunda, M. Miki-Yoshida, FEMS Microbiol. Lett. 211 (2002) 183.