ELSEVIER

Coordination Chemistry Reviews 229 (2002) 37–49

COORDINATION CHEMISTRY REVIEWS

www.elsevier.com/locate/ccr

Laser flash photolysis as tool in the elucidation of the nitric oxide binding mechanism to metallobiomolecules

A. Wanat [a], M. Wolak [a], Ł. Orzeł [a], M. Brindell [a], R. van Eldik [b], G. Stochel [a,*]

[a] *Faculty of Chemistry, Jagiellonian University, Ingardena 3, Cracow 30060, Poland*
[b] *Institute for Inorganic Chemistry, University of Erlangen-Nürnberg, Egerlandstr. 1, 91058 Erlangen, Germany*

Received 6 September 2001; accepted 15 March 2002

Contents

Abstract

The article presents a sampling of mechanistic studies on nitric oxide binding to metallobiomolecules. The main emphasis falls on the application of ambient and high pressure laser flash photolysis techniques in the elucidation of the mechanism of the reaction of NO with metals in active centres of biomolecules and complexes of potential medicinal application.

Keywords: Laser flash photolysis; Metallobiomolecules; Nitric oxide, kinetics and mechanism

1. Introduction

Bioinorganic chemistry is a rapidly developing field with a high potential for application in medicine. It offers the possibilities to design novel therapeutic and diagnostic agents for the treatment and understanding of diseases which are currently intractable. Progress in inorganic medicinal chemistry depends strongly on understanding both thermodynamics (structure and equilibria) and kinetics (mechanism) of reactions of metal complexes, especially under biologically relevant conditions. The bioinorganic chemistry of nitric oxide belongs to the current areas with exciting clinical potential. Despite numerous investigations of NO interactions with transition metal complexes, many questions

* Corresponding author. Tel.: +48-12-633-6377x2243; fax: +48-12-633-53-92.
E-mail address: stochel@chemia.uj.edu.pl (G. Stochel).

0010-8545/02/$ - see front matter
PII: S0010-8545(02)00091-7

concerning the fundamental chemical kinetics and mechanism by which nitric oxide undergoes co-ordination to the metal centres of biological relevance still remain unanswered. Elucidation of these mechanisms will substantially facilitate the search for new drugs to treat nitric oxide-mediated diseases.

2. Biological and medical aspects of nitric oxide

More than two decades ago, nitric oxide was just another toxic molecule, one of many environmental pollutants found in the exhaust fumes of petrol-driven vehicles, photochemical smog and cigarette smoke. A major conceptual change about nitric oxide took place upon the discovery that it is synthesised and secreted by a number of mammalian cells. The endogenous formation of nitric oxide plays a key role in many bioregulatory systems including the control of cardiovascular function, signalling between nerves in both the peripheral and central nerve system, and defence against micro-organisms and tumours. This expression of a wide variety of effects is achieved by nitric oxide mainly through its interaction with transition metals or thiols strategically located at either allosteric or active sites of NO responsive signalling proteins, ion channels, receptors, enzymes and transcription factors.

A breakdown in the regulation of the metabolism of NO leads to a number of diseases including hypertension, epilepsy, diabetes, arthritis and septic shock. Some of the methods to regulate nitric oxide formation, metabolism and function, have been in clinical use for more than a century, examples being organic nitrates and nitroglycerin applied in the treatment of angina pectoris. A novel approach is based on the application of transition metal complexes. Some of them, such as those of Fe and Ru, are capable of regulating the level of NO in biological systems without affecting NO-synthase action, by the release or binding of nitric oxide in the situation of its under- or over-production, respectively.

3. Ambient and high pressure laser photolysis

The kinetic approach to elucidate the mechanism of a studied reaction involves the measurement of the reaction rates and rate constants as a function of chemical and physical variables. The time coverage for the various kinetic techniques used in such measurements is shown in Fig. 1a. Since photolabilisation of nitric oxide from nitrosyl complexes of biological relevance is often a reversible process, nanosecond pulsed laser techniques are well suited for investigating the kinetics of nitrosylation reactions. In such studies, flash photolysis of an equilibrium mixture of M(L) and M(L)NO

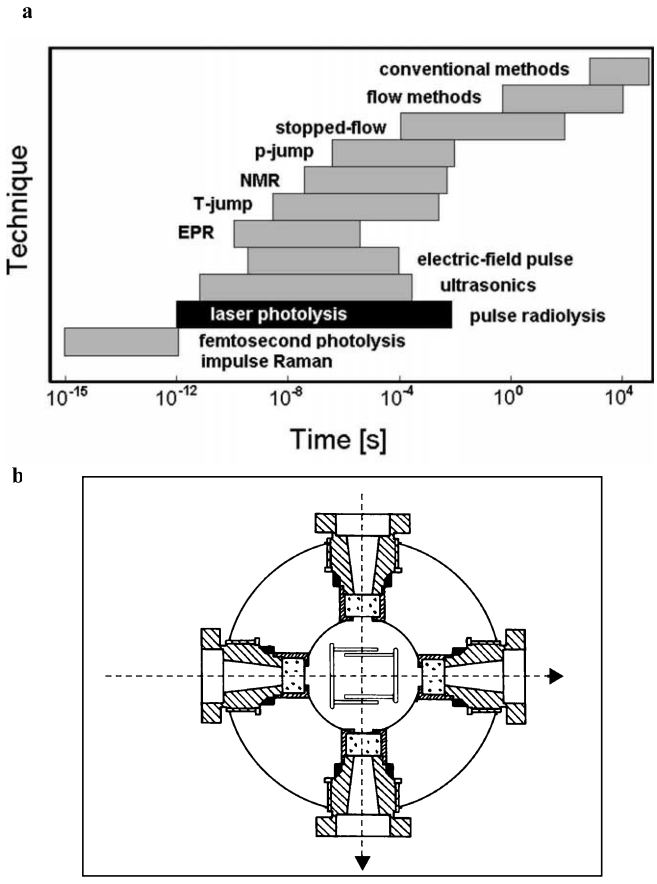
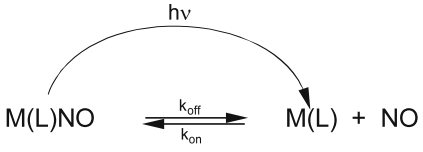


Fig. 1. (a) The time scale of the various kinetic techniques. (b) Photolysis high pressure vessel.



Scheme 1.

leads to the labilisation of NO from the latter complex, and if no permanent photoproduct is formed, relaxation of the non-steady state system back to the equilibrium position can be followed by spectrophotometric methods Scheme 1.

In order to obtain further mechanistic information from the analysis of nitrosylation processes in terms of volume changes along the reaction coordinate, the combination of laser flash photolysis with high pressure techniques has been employed (Fig. 1b). The additional physical parameter of pressure adds not only a decisive dimension to the mechanistic study of NO reactions, but also enables the construction of reaction volume profiles.

4. Mechanistic studies on NO binding to metallobiomolecules

4.1. Iron

In terms of biological activity, the most significant property of NO is its interaction with the metal centre in heme- and non-heme iron proteins. Clearly, an understanding of the nitric oxide interactions with iron centres is essential to shed new light on the nature of biological activity of nitric oxide in the human body, and to facilitate the search for new medicines to treat nitric oxide-mediated diseases.

4.1.1. Iron(II)/(III) hemoproteins and their model complexes

Both guanylate cyclase, regarded as the main receptor for NO, and NO-synthase, the enzyme catalysing the biosynthesis of NO, are hemoproteins [1,2]. Heme centres are also involved in NO inhibition of many metalloenzymes such as cytochrome P_{450} [3], cytochrome oxidase [4], catalase [5], nitrile hydratase [6] as well as in the control of the NO concentration in blood by conversion to NO_3^- [7]. NO-hemoprotein interactions also play an important role in vasodilating effects of a ferriheme salivary protein (nitrophorins) of blood sucking insects [8]. In addition, paramagnetic complexes of nitrosylhemoglobin have received considerable attention for their potential use for in vivo monitoring of NO production [9].

The application of laser flash photolysis techniques enable one to measure the rate constant for the binding of NO (k_{on}), and in some cases also the rate constant for the dissociation of NO (k_{off}) for a number of ferri- and ferro-hemoproteins. As can be seen from Table 1, the second order rate constants, k_{on}, for all studied proteins, with the exception of cytochrom $c^{II/III}$, are very high and range from 10^3 to 10^8 M^{-1} s^{-1} [10–19], being significantly larger for the ferrous complexes. As already mentioned in the literature, this range of rates can be ascribed to the fact that facile reactions of NO with heme proteins require either a very labile co-ordination site (such as observed for the model porphyrin complexes—see further discussion) or a vacant co-ordination site such as in high spin Fe(II) heme proteins [20]. The low reactivity of cytochrome *c* toward nitric oxide can be explained in terms of the absence of labile co-ordination sites at the metal heme centre of this protein since the fifth and sixth co-ordination sites are occupied by histidine and methionine residues in both oxidation states of cytochrome *c* [11]. The rate constants for the dissociation of nitric oxide from nitrosyl ferriheme proteins are significantly larger than for the ferrous complexes, the latter being too small to be determined by laser flash photolysis technique and, therefore, have to be measured by other means. The small values of the dissociation rate constants for the ferroheme proteins result in very large association constants, *K*. For example, nitric oxide binds to myoglobin (Mb(II)) with a *K* value eight orders of magnitude higher than for metmyglobin (Mb(III)).

Various flash photolysis studies of the nitrosyl hemoproteins have demonstrated that the quantum yields (Φ_{dis}) for the net photolabilisation of nitric oxide from the nitrosyl metalloproteins are very small [20]. From a mechanistic perspective, the inconveniently small values of Φ_{dis} often complicate kinetic studies on the nitrosylation of hemoproteins. The photochemistry of the nitrosyl metalloporphyrins was the subject of many investigations [20,21]. Despite many ambiguities existing in the literature concerning the explanation for the low quantum yields of nitric oxide photodissociation, there appears to be a general agreement that mechanical effects resulting from the presence of the

Table 1
Binding and dissociation rate constants for the reaction of nitric oxide with representative hemoproteins and model iron(II)/(III) porphyrins

Fe(II)/(III) porphyrin	Conditions	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	*K* (M^{-1})	References
Mb(II)	Phosphate buffer, pH 7.0	1.7×10^7	1.2×10^{-4}	1.4×10^{11}	[10]
Hb(II)	Phosphate buffer, pH 7.0	2.5×10^7	4.6×10^{-5}	5.3×10^{11}	[10]
Cyt c^{II}	H_2O	8.3	2.9×10^{-5}	2.9×10^5	[11]
Cytochrome oxidase	PH 7.0	1×10^8	1.2×10^{-2}	8.3×10^9	[12]
Guanylate cyclase	PH 7.0	7×10^8	7×10^{-4}	1.0×10^{12}	[13,14]
Mb(III)	H_2O, pH 6.5	1.9×10^5	13.6	1.4×10^4	[11]
	Tris buffer, pH 7.4	2.71×10^4	16.0	1.7×10^3	[15]
Hb(III)		4×10^3	1	4×10^3	[16]
Cyt c^{III}	H_2O, pH 6.5	7.2×10^2	4.4×10^{-2}	1.6×10^4	[11]
Cat(III)	H_2O, pH 6.5	3×10^7	1.7×10^2	1.8×10^5	[11]
Microperoxidase		1.1×10^6	3.4	3.2×10^5	[17]
Fe(II) (TPPS)	H_2O, pH 6.5	1.8×10^9	~ 0	$> 10^9$	[11]
Fe(II) (TMPS)	PH 7.0	1×10^9	–	–	[18]
Fe(III) (TPPS)	H_2O, pH 6.5	7.2×10^5	6.8×10^2	1.1×10^3	[11]
Fe(III) (TMPS)	H_2O pH 6.0	3×10^6	7.3×10^2	4.1×10^3	[19]

protein pocket may play an important role in such denitrosylation processes [20]. Valuable information on the role of the protein pocket in the binding of nitric oxide to hemoproteins can be obtained from kinetic studies on the nitrosylation reactions of heme models, i.e. protein-free iron porphyrin complexes. Water soluble iron(II)/(III) porphyrins readily bind nitric oxide (see Table 1) and the k_{on} values for these reactions can be conveniently measured using nanosecond flash photolysis techniques, the more so because the quantum yields Φ_{dis} observed for such processes are close to unity, i.e. are much higher than for the hemoproteins [18,19]. The fast rates for NO binding to model complexes reflect the lability of the iron(II)/(III) porphyrins. Clearly, in the absence of the protein pocket where access to the metal heme centre is not limited, more facile reactions can occur. The dissociation rate constants determined for the model complexes are in general also significantly larger than for the hemoproteins, indicating that dissociation of nitric oxide from the latter can be hindered by the presence of the protein surrounding [20]. If so, the question is how the presence of the protein pocket can affect the mechanistic behaviour of nitric oxide binding to hemoproteins.

Recently, we have performed detailed kinetic and mechanistic studies on the reversible binding of nitric oxide to metmyglobin (Mb(III)) using laser flash photolysis and stopped-flow techniques at ambient and high pressure [15]. The Fe(III) centre of metMb is six coordinate, with a water molecule occupying the site to which NO will coordinate. From a mechanistic perspective, the nitrosyl complex of metmyoglobin is of particular interest given that both association and dissociation of NO are conveniently observable under physiological temperatures and pH [11]. Nitric oxide binds reversibly to metmyoglobin according to (Eq. (1)).

$$\mathrm{metMb(H_2O) + NO \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} metMb(NO) + H_2O} \quad (1)$$

From UV–vis spectral changes and the use of an NO-electrode, the equilibrium constant for (Eq. (1)) was determined to be 2.4×10^3 M^{-1} at 20 °C and pH 7.4 (0.1 M Tris buffer) [15]. This value is consistent with the ratio of the 'on' and 'off' rate constants ($K = k_{on}/k_{off}$) determined under similar conditions (see below). When one of the reactants (in the present case NO) is used in large excess, the reaction depicted in (Eq. (1)) is expected to follow pseudo first order kinetics. The observed rate constant for such a process can be expressed as in (2).

$$k_{obs} = k_{on}[NO] + k_{off} \quad (2)$$

Accordingly, plots of k_{obs} versus [NO] were linear with slopes equal to k_{on} and non zero intercepts equal to k_{off}. Laser flash photolysis of equilibrated mixtures of metMb(NO)/metMb at pH 7.4 (Tris buffer) gave transient difference spectra consistent with the spectral differences between metMb and metMb(NO), i.e. with labilisation of coordinated NO to give non-equilibrium concentrations of these species. The transient spectra decayed exponentially to the original spectrum of metMb(NO), and no permanent photoproducts were observed [15].

The equilibrium constants K are sufficiently small that extrapolation to $[NO] = 0$ gives a measurable intercept, i.e. k_{off}. However, such intercepts are susceptible to extrapolation errors. Consequently, the reliability of the k_{off} values obtained from the [NO] dependence of k_{obs} was confirmed more directly by employing an NO-trapping method, in which an excess $[Ru(edta)H_2O]^-$ is used as a scavenger for the NO released during the dissociation of metMb(NO).

The activation parameters for the 'on' and 'off' reactions obtained from systematic measurements of k_{on} and k_{off} as a function of temperature and pressure, using the two different techniques, are summarised in Table 2. The respective values of the activation parameters indicate reasonable agreement between the two various techniques used [15]. The $\Delta H^{\neq}_{on}$, $\Delta S^{\neq}_{on}$ and $\Delta V^{\neq}_{on}$ values are large and positive, features also seen for the 'on' reactions of the water soluble model complexes Fe(III)(TPPS) and Fe(III)(TMPS) with NO [19]. The pattern of large and positive $\Delta S^{\neq}_{on}$ and, more diagnostically, large and positive $\Delta V^{\neq}_{on}$ values provides direct evidence for a dissociative ligand substitution mechanism, analogous to that observed for model iron(III) porphyrine complexes [19]. In agreement with this proposal is the reported rapid exchange between coordinated and bulk water molecules on Fe(III)(TPPS)$(H_2O)_2$ ($k_{ex} = 1.4 \times 10^7$ s^{-1} at 25 °C) [22], and the dissociative nature of this process is underlined by the large and positive activation entropy [22] and activation volume found for this reaction [23].

A similar mechanism accounts for the activation parameters for the nitrosylation of metMb, i.e.:

$$\mathrm{metMb(H_2O) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MetMb^5 + H_2O}$$

$$\mathrm{metMb^5 + NO \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} metMb(NO)} \quad (3)$$

where $metMb^5$ is the five-coordinate intermediate formed by dissociation of coordinated water. FTIR and Resonance Raman data indicate that the reaction product metMb(NO) formally has a linear Fe(II)–NO^+ character, which means that partial charge transfer from NO to Fe(III) occurs during the bonding process [24]. Recent multiple scattering XAFS analysis of metMb(NO) confirm this structural assignment [25].

Notably, the k_{-1} pathway occurs between two high spin Fe(III) species, so the volume change involves primarily formation of the Fe(III)–OH_2 bond. In contrast, the k_2 pathway not only involves Fe–NO

Table 2
Rate constants and activation parameters for the binding of NO to metMb (k_{on}) and the dissociation of NO from metMb(NO) (k_{off}) as determined by laser flash photolysis and stopped-flow techniques [a]

Methods	k_{on} (M^{-1} s^{-1})	K_{off} (s^{-1})	$\Delta H^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$(J mol^{-1} K^{-1})	$\Delta V^{\#}$(cm^3 mol^{-1})
Laser flash photolysis	4.7×10^4		63±2	55±8	20±6 [b]
		37	68±4	14±13	18±3 [b]
Stopped-flow	4.8×10^{-4}		71±2	82±7	21±1 [c]
		28	83±2	62±8	16±1 [c]
NO-trapping		29	78±2	46±7	20±1 [d]

[a] Data from [15].
[b] Determined at 20 °C.
[c] Determined at 5 °C.
[d] Determined at 10 °C.

bond formation but considerable charge transfer from NO to give (formally) a Fe(II)–NO^+ species and the change from a quintet spin state to a low-spin diamagnetic complex. For model porphyrin complexes, the high spin to low spin Fe(III) transformation is accompanied by a negative contribution to ΔV, [26,27] as is also for the co-ordination-induced high spin to low spin transformation of Fe(II) [28]. Differences in the activation parameters for the k_{-1} and k_2 steps will largely cancel out given the likely scenario of early transition states in going from $metMb^5$ to either of the six coordinate species. It may, therefore, be concluded that the reported values for $\Delta V^{\neq}_{on}$ mainly represent the volume changes associated with the k_1 pathway. These are significantly larger than reported for the model porphyrin systems [19]. This can further be seen from a comparison of the volume profiles for the metMb(H_2O)+NO and Fe(TPPS)(H_2O)$_2$+NO reactions shown in Fig. 2. Dissociation of a coordinated water molecule from an octahedral metal centre is expected to be accompanied by a maximum volume increase of 13 cm^3 mol^{-1} [26]. The larger value of $\Delta V^{\neq}_{on}$ reported here for metMb suggests that the protein may also undergo some structural rearrangement during the formation of the five-coordinate $metMb^5$. In fact, the XAFS structural findings referred to above [25] indicate steric strain in the linear coordination of NO to the Fe(III) centre, which could involve an increase in the size of the protein pocket prior to the binding of NO, and, therefore, a larger $\Delta V^{\neq}_{on}$ than that observed for the model porphyrin complexes. Microscopic reversibility argues that the reverse process will be dominated by the k_{-2} step, the dissociation of NO from the Fe(II)(NO^+) species accompanied by a charge transfer from metal to nitrosyl to give $metMb^5$ plus NO, and a concomitant spin change. As a result, the activation parameters must reflect the intrinsic entropy and volume changes associated with bond breakage and the solvational changes associated with solvent reorganisation concurrent with charge redistribution and the spin change. Such factors are consistent with the large and positive values of $\Delta S^{\neq}_{off}$ and $\Delta V^{\neq}_{off}$ demonstrated here for metMb and previously for the water soluble ferri-heme models [19]. In both the heme protein and model porphyrin systems, Fe(III)–NO has a Fe(II)–NO^+ character and bond cleavage is accompanied by a formal oxidation of Fe(II) to Fe(III), and thus should result in a similar volume increase in both cases. The more negative ΔV for the overall reaction in the case of the model compounds may reflect

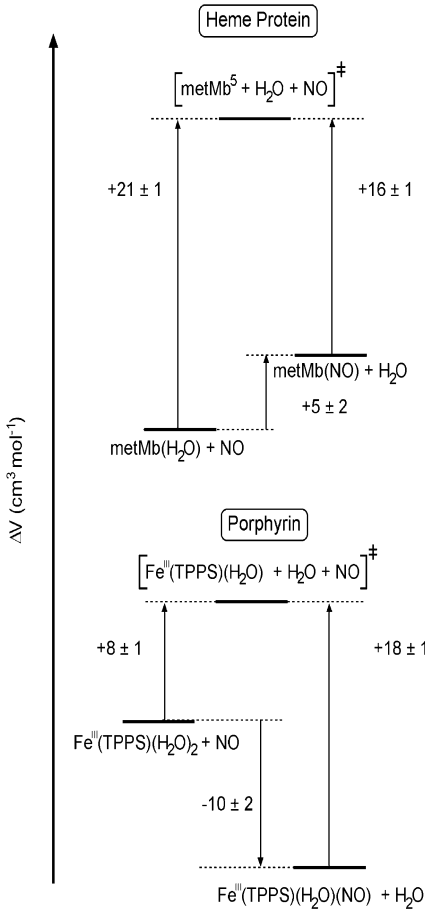


Fig. 2. Volume profiles for the reactions: Upper, metMb(H_2O)+NO⇄metMb(NO)+H_2O (data from ref. [15]); Lower, Fe(III)(TPPS)(H_2O)$_2$+NO⇄Fe(III)(TPPS)(H_2O)(NO)+H_2O (data from ref. [19]).

specific solvation of the NO^+ moiety. Similar solvation by water is less likely in the protein pocket of metMb, which excludes the bulk solvent.

4.1.2. Non-heme iron proteins

Metalloproteins containing non-heme iron centres represent an important biological target for nitric oxide [29]. Nitric oxide is able to bind to iron–sulphur (Fe–S) clusters, as well as to non-heme mononuclear iron proteins (soybean lipoxygenase, estradiol dioxygenase, isopenicillin N synthase, etc) [30]. Reactions of nitric oxide with the iron–sulphur clusters result in alteration of functional properties of proteins: a loss, a decline or an increase in their activity. For instance, EPR studies demonstrated that association of nitric oxide to the non-heme diiron centre of ribonucleotide reductase brings about the enzyme inhibition preventing DNA synthesis and cell proliferation [31]. The effect of NO binding to cytoplasmic aconitase, a metalloregulatory enzyme known as an 'iron regulatory protein' (IRP) is of special interest. It has been shown that by reaction with the Fe–S centre of the enzyme, nitric oxide can switch IRP into its mRNA binding mode, mimicking the response of iron starvation [32].

In contrast to the reactions with the heme iron centres, the reactions of NO with Fe–S clusters usually result in destruction of the clusters [29]. Recent studies indicate that NO, unlike O_2^- and $ONOO^-$, does not affect mitochondrial aconitase activity. This means that metal–NO species detected in cells may be markers rather than causes of cellular responses, and the inactivation of $[FeS]_n$ proteins in biological systems should be examined in the context of relative reactivities of NO, O_2^- and $ONOO^-$ with other available substrates [33].

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 nitric oxide might be stabilised in organisms by binding to iron(II) dithiolate complexes. Dinitrosyl iron complexes (DNICs) have been found in animal tissues and microorganisms due to their characteristic EPR signal. The biologically produced nitric oxide can be stabilised and stored as a dinitrosyl–iron(II) complex 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.

Kurepteva et al. have demonstrated that ferrous–ascorbate nitrosyl complexes (Fe–AA–NO) which can be detected under physiological conditions due to their characteristic EPR signal with a g-factor close to 2.02, can also act as carriers of NO and possibly of O_2 in the blood plasma. It is also suggested that these complexes can be a NO containing species involved in the tile blood vessel relaxation [34].

It has been revealed that nitrile hydratase (Nhase), a bacterial metalloenzyme catalysing the hydration of nitriles to the corresponding amides, is a photoreactive enzyme which is inactivated by nitrosylation of the non-heme iron centre and activated by photodissociation of nitric oxide. 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 [35].

4.2. Copper complexes

The reactions of nitric oxide with copper proteins as possible NO targets in vivo are not as well characterised as those with iron proteins. Perhaps the most characteristic feature of the interaction between NO and copper in biological systems is its dual nature in that NO can act as a ligand and/or as a redox partner. Recent studies indicate that redox reactions can be very fast even at low NO concentrations and in the presence of oxygen, and may, therefore, be of physiological relevance [36]. NO has also been used extensively as a spectroscopic probe to elucidate the nature of the copper sites in a number of copper proteins such as haemocyanin, tyrosinase, ceruloplasmin (Cp), ascorbate oxidase (AO), laccase, azurin, halocyanin and the iron/copper binuclear centre in cytochrome c oxidase [37]. In general it is assumed that different spectroscopic types of copper centres (T1, T2 and T3) react differently with NO.

Haemocyanin (Hc), an oxygen carrier protein found in invertebrates, possesses a pair of copper atoms, which are EPR silent (T3) in both the oxygenated and deoxygenated form. The addition of NO to deoxyHc gave rise to two types of EPR signals ([38a,38b]). Similar EPR signals were detected when NO was added to tyrosinase, a copper protein also possessing a pair of copper atoms. The reaction between deoxyHc and NO was interpreted in terms of two kinetically distinguishable processes: an initial reaction that results in metHc and N_2O formation, and a slower reaction leading to the nitrosyl derivative. However, the re-examination of this reaction led to the conclusion that the redox process observed is not due to NO but to NO_2, and the final derivative consists of a half-met centre with no NO bound to it ([38c]).

EPR studies on the reactions of nitric oxide with oxidised multicopper oxidases such as Cp or AO, established the stoichiometry of the active sites in these multicopper proteins. As a result it was found that the structures of both Cp and AO contain three T1 coppers and one trinuclear cluster comprising T2 and T3 copper sites. The longer incubation of multicopper centres with NO led to irreversible reduction of T1 copper [39].

The reaction of nitric oxide with laccase containing three types of copper atoms, resulted in the simultaneous reduction of T1 and T3 coppers and slower

reduction of the T2 copper centre. Experiments carried out with a derivative lacking the T2 copper, revealed that the T1 centre was reduced but not T3. The above and further studies on the NO binding to laccase confirmed that all these reactions occur via the T2 copper site ([37d]).

The reaction of NO with cytochrome *c* oxidase (C*c*O) containing two haem groups (Fe_a and Fe_{a3}) and two copper centres Cu_A and Cu_B, is much more complex than that with laccase. Both Fe_{a3} and Cu_B, when reduced, can bind nitric oxide [40a]. It was found that depending on the redox state of the enzyme under anaerobic conditions, C*c*O can catalyse reduction of NO to N_2O or oxidation to NO_2 [40b]. It was reported that 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 oxygen consumption under normal physiological conditions. The fast reaction between nitric oxide and C*c*O probably involves NO binding to Cu_B^{2+} with the formation of Cu_B^{1+}–NO^+ species. The latter is then hydroxylated giving nitrous acid (HNO_2) and the reduced form of Cu_B. The electron residing on Cu_B can be subsequently transferred to other redox centres of C*c*O, such as Fe_a, Cu_A or Fe_{a3} [36a,36c,12,41]. Based on these studies, it is suggested that NO may play a vital role in the regulation of important Type 2 copper containing enzymes.

The copper–NO complexes were also studied in the context of their susceptibility to photodissociation [39b]. The nitrosyl complexes with T3 copper sites in Cp and Hc occurred to be inert to light, whereas T1 copper sites were found to bind NO in photolabile complexes. This observation has been used to suggest that C*c*O Cu_B might resemble a T1 copper site in contrast to other views in which this centre is compared with a T2 type site. The photolability of nitrosyl complexes at the T1 copper site has also been used to investigate the temperature dependence of the ligand binding equilibrium and the kinetics of the association reaction after photodissociation in azurin and halocyanin over a range of temperatures [42]. These studies showed the presence of conformational substrates in the studied enzymes.

4.3. Cobalt complexes

Cobalt, present in human organisms in the form of cobalamin cofactors (vitamin B_{12} derivatives), participates in a variety of biological pathways involving enzyme–catalysed molecular rearrangements and methyl group transfer reactions. One of the recent interests in the biological functions of vitamin B_{12} concerns the modification of physiological actions of nitric oxide by vitamin B_{12} derivatives [43]. According to the observations reported in the literature, from the four most important cob(III)alamin derivatives (aquacobalamin, methylcobalamin, adenosylcobalamin and cyanocobalamin) only aquacobalamin (Cbl(III)(H_2O)) has been claimed to react with NO in vivo to produce nitrosylcob(III)alamin, Cbl(III)NO. Recent kinetic and spectroscopic studies have shown, however, that direct interaction between NO and aquacobalamin does not occur, and, therefore, cannot account for the observed physiological effects [42]. The UV–vis spectral changes observed on introducing NO into aqueous solutions of Cbl(III)(H_2O) have been shown to result from the reaction of Cbl(III) with nitrite (present as an impurity in aqueous NO solutions), rather than from the reaction of aquacobalamin with NO [43].

As indicated by UV–vis, NMR and resonance Raman spectroscopic studies, the reduced form of vitamin B_{12} reacts with NO to form a stable nitrosyl complex [42–44] according to the overall reaction given in (Eq. (4)).

$$\mathrm{Cbl(II)} + \mathrm{NO} \overset{k}{\rightleftarrows} \mathrm{Cbl(II)NO} \quad (4)$$

The large values of the formation constants for this complex ($K = 1.0 \times 10^8\ M^{-1}$ at pH 7.0 [43] and $K = 3.1 \times 10^7\ M^{-1}$ at 20 °C and pH 7.4 [44]) indicate that the binding of NO by cob(II)alamin is very efficient, and thus may account for the observed physiological effects. UV–vis, ^{1}H-, ^{31}P-and ^{15}N-NMR data further suggest that the reaction product under physiological conditions is a six-coordinate, 'base-on' form of the vitamin with a weakly bound α-dimethylbenzimidazole base and a bent nitrosyl ligand coordinated to cobalt at the β-site of the corrin ring. In analogy to porphyrin Co(II)–nitrosyl complexes, the cob(II)alamin nitrosyl adduct formed in reaction (4) can formally be described as $Cbl(III)NO^-$.

The kinetics of NO binding to cob(II)alamin was recently studied with the use of laser flash photolysis technique [44]. Irradiation of the $Cbl(III)NO^-$ complex in aqueous solution under pseudo-first order conditions with respect to NO led to the formation of Cbl(II) and free NO, after which the re-formation of $Cbl(III)NO^-$ could be observed. The photochemical reaction induced by the laser flash can, therefore, be expressed as in (5).

$$\mathrm{Cbl(III)NO^-} \xrightarrow{h\nu\ (532\mathrm{nm})} \mathrm{Cbl(II)} + \mathrm{NO} \overset{k_{on}}{\rightarrow} \mathrm{Cbl(III)NO^-} \quad (5)$$

The decay of the transient spectrum of the photo-induced Cbl(II) species in the presence of excess NO enables the measurement of the pseudo-first order rate constant for the formation of the Co–NO bond, k_{obs}, as a function of [NO].

$$k_{obs} = k_{on}[\mathrm{NO}] + k_{off} \quad (6)$$

Plots of the observed rate constant versus [NO] were linear under the studied conditions. No measurable intercepts, however, could be obtained from the plots indicating that the k_{off} values characterising the back reaction were too small to be observed in the laser flash experiment. It was, however, possible to measure these values directly using the previously mentioned NO

Table 3
Rate constants and activation parameters for the binding and release of NO from cob(II)alamin [a]

Kinetic parameter	'on' reaction	'off' reaction
k_{on} (M^{-1} s^{-1}) [b]	7.4×10^8 M^{-1} s^{-1}	
k_{off} (s^{-1}) [b]		5.6 s^{-1}
$\Delta H^{\neq}$ (kJ mol^{-1}) [c]	24.5±0.7	76±1
$\Delta S^{\neq}$ (J mol^{-1} K^{-1}) [c]	+7±2	+24±5
$\Delta V^{\neq}$ (cm^3 mol^{-1}) [c]	+5.4±0.2	+7.9±0.5

[a] Data from reference [44].
[b] Experimental conditions: 25 °C, pH 7.4.
[c] Determined at pH 7.4.

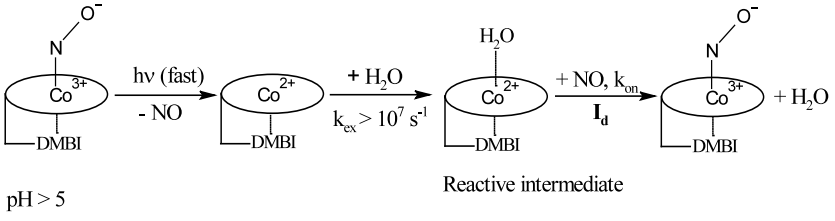
trapping method (see Section 4.1.1). The 'on' and 'off' rate constants and the corresponding activation parameters, $\Delta H^{\neq}$, $\Delta S^{\neq}$ and $\Delta V^{\neq}$ determined from the temperature and pressure dependence of k_{on} and k_{off}, are summarised in Table 3.

It is reasonable to expect that binding of NO to the vacant coordination site of the five-coordinate cobalt(II) centre in reduced vitamin B_{12} should be very fast and exhibit a significantly negative activation volume as a result of bond formation and a partial oxidation of the cobalt(II) centre in the rate-determining step. However, although the observed reaction is very fast, it surprisingly exhibits a small but significantly positive volume of activation, viz. +5.4 cm^3 mol^{-1}. This value cannot be correlated with a simple bond formation process at the vacant coordination site of cob(II)alamin, but provides evidence for the operation of a dissociative interchange (I_d) ligand substitution mechanism for the photo-induced 'on' reaction [26a,45]. It follows that the rate-determining step in the binding of NO apparently involves displacement of water at the Co(II) centre in the photo-induced reactive intermediate. This conclusion, although surprising at the first sight, could be explained by a detailed spectrophotometric and kinetic investigation of the studied system [44]. A thorough study of the transient spectra recorded immediately after the laser flash, revealed that the reactive Cbl(II) intermediate generated in aqueous medium differs from the intact Cbl(II) species, which binds NO in the thermal reaction. According to the most self-consistent reaction scheme based on all spectroscopic and kinetic measurements performed for the studied system [44], photodissociation of NO from nitrosylcob(II)alamin leads to the formation of a five-coordinate Cbl(II) species with a weakly coordinated DMBI ligand. This species rapidly binds a water molecule prior to the recombination with NO, forming a six-coordinate aqua intermediate (see Scheme 2) [44].

Binding of NO to this intermediate is controlled by displacement of the very labile water molecule according to an I_d mechanism, and accounts for the positive volume of activation found for the 'on' reaction. Such a water molecule is, however, not present in the intact cob(II)alamin complex at physiological pH [46], such that intimate reaction mechanisms are expected to differ for the photo-induced and thermal nitrosylation of reduced cobalamin. The latter process is presumably controlled by NO binding to the vacant coordination site on the intact Cbl(II) complex and is expected to be even faster than the corresponding photo-induced reaction, where the binding of NO involves substitution of coordinated water on the photo-generated Cbl(II) species.

Kinetic data for the 'off' reaction summarised in Table 3, clearly indicate that the dissociation of NO from nitrosylcob(II)alamin is slow and a high energy barrier (reflected in a large $\Delta H^{\neq}_{off}$ value) must be overcome to break the $Co(III)NO^-$ bond in the $\{Co–NO\}^8$ corrin nitrosyl. A positive activation volume observed for this process can be accounted for in terms of a dissociative interchange mechanism in which the breakage of the Co–NO bond is partially accompanied by the strengthening of the Co–N(DMBI) bond in the *trans* position to the leaving nitrosyl ligand.

Since the intermediate produced in the laser flash experiment differs from that in the thermal reaction, the data do not allow one to construct energy and volume profiles for the thermal reaction. It is, however, possible to construct a volume profile for the binding of NO to the photo-generated aqua complex at pH 7.4, as shown in Fig. 3. The volume profile once again illustrates the I_d character of the 'on' and 'off' reactions and the important role of solvent molecules in determining the mechanism of NO binding to the metal centre [44].



Scheme 2.

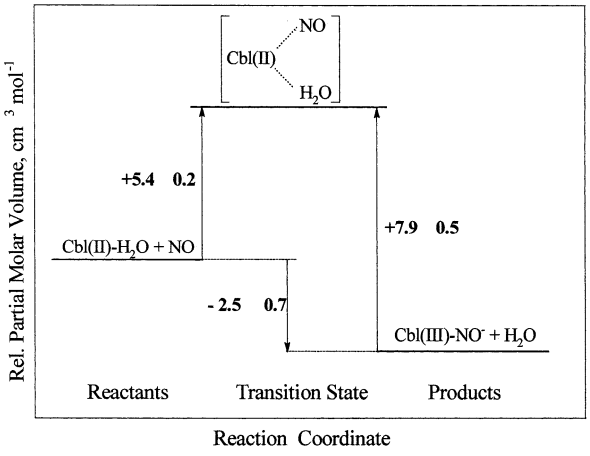


Fig. 3. Volume profile for the photo-induced reaction Cbl(II)–H_2O + NO⇄Cbl(III)–NO^- + H_2O (data from ref. [44]).

5. Nitric oxide donation and scavenging by transition metal complexes

Since nitric oxide is implicated in a number of diseases coupled to its over- or under-production, there is substantial interest in finding new pharmaceutical agents capable of regulating the NO-level in vivo. A class of compounds, which have recently received considerable interest for these purposes are transition metal complexes. The application of metal complexes as pharmaceutical agents creates a real challenge for the development of a new generation of drugs.

5.1. NO-donors

There is an intense interest in the efficient delivery of nitric oxide within a biological environment. In the commonly applied medical therapies, this is achieved by the use of stable NO compounds operating as vasodilators through the pro-drug philosophy. Also of great interest are photochemical strategies for targeted NO delivery to biological systems. The NO photolabilisation strategy is based on the application of precursors of low thermal reactivity, which are photochemically active to give NO when subjected to electronic excitation. 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 γ-radiation damage [47]. To study known and new potential photochemical precursors, continuous and flash photolysis techniques are commonly used.

A class of metal complexes which has received considerable attention in this respect, are iron–sulphur–nitrosyl cluster anions of the type $Fe_xS_y(NO)_z^{n-}$. The Roussin's Red Salt $[Fe_2S_2(NO)_4]^{2-}$ (RRS) and Roussin's Black Salt $[Fe_4S_3(NO)_7]^-$ (RBS) contain a large number of NO ligands, which can be released thermally or photochemically from these ionic compounds. Exposure to light greatly enhances the vasodilator actions of iron–sulphur–nitrosyl clusters. Quantitative photoreaction studies demonstrated that the anion of RRS undergoes photoconversion to the black anion with no other detectable Fe–S–NO products, whereas photolysis of the Black Salt in aerated solutions leads to the complete fragmentation of the RBS cluster with a relatively small quantum yield [48].

Another group of light-sensitive nitrosyl complexes is nitrosyl derivatives of ruthenium. Two-caged NO compounds, i.e. trichloronitrosylruthenium ($RuNOCl_3$) and dipotassium pentachloronitrosylruthenate ($K_2RuNOCl_5$), are thermally stable but photolabile, releasing nitric oxide on exposure to near-UV light [49]. Preliminary studies demonstrated that on irradiation in the range 300–350 nm, ruthenium nitrosyl compounds liberate NO with product quantum yields of 0.012 and 0.06 for $RuNOCl_3$ and $K_2RuNOCl_5$, respectively [50].

An interesting class of potential photochemical precursors, from which NO can be released by point irradiation are metalloporphyrins [51,52]. These complexes release nitric oxide with quantum yields varying from almost zero to unity, when photons are absorbed by the porphyrin macrocyclic π-system. The electronic excited states in these systems are inherently short-lived because empty d-orbitals of transition metals can couple to the porphyrin π-orbitals, forming states of intermediate energy. Both the rapid deactivation of the excited porphyrin states (π, π*) and the spectroscopic inaccessibility of the internal metal states make it extremely difficult to determine factors controlling the denitrosylation process [53,54].

In the course of probing photochemical precursors, it was found that potential utility of the nitrosyl porphyrin complexes of the first-row transition metals in the photochemical NO delivery to specific targets is severely hampered by their lability and oxygen sensitivity [20,54]. For that reason, attention was focused on nitrosyl ruthenium porphyrin complexes, anticipated to be more stable. This proposition has been the stimulus of investigations into the synthesis, structural characterisation and photochemical reactivities of several ruthenium porphyrin complexes of the type Ru(II)(P)(NO)(ONO), where P = TPP, OEP (octaethylporphyrin), TmTP (tetra(*m*-tolyl)porphyrin) and FTTP (tetra(*m*-trifluoromethylphenyl)porphyrin) [55–57]. Ford et al. showed that the flash photolysis of the nitrosyl nitrito complexes of ruthenium indicate the operation of at least two pathways leading to the formation of a photoreaction intermediate: Ru(P)(ONO) formed by NO photolabilisation and Ru(P)(NO) involving nitrito ligand photolabilisation [56].

Among nitrosyl ruthenium porphyrins and amines, i.e. compounds of potential use for photochemically activated labilisation of NO, a new synthetic platform for ruthenium nitrosyls has appeared, viz. the salen-type

complexes Ru(R-salen((X)(NO), where R-salen is a derivative of the *N*,*N*′-bis(salicylidene)ethylenediamine dianion. Preliminary studies on the photoreactivity of a representative member of this family, Ru(salen)(Cl)(NO), demonstrated that this complex undergoes NO labilisation upon near-UV irradiation to give the solvent species, Ru(salen)(Cl)(Sol). Thus, this system, especially when further modified, is exceptionally promising as a precursor for photochemical NO delivery to various targets [58].

Another group of potential NO-donors offered by coordination chemistry are compounds containing macrocyclic ligands. They provide an extremely stable, sequestered metal core, while also altering the metal ions reactivity. In this context, the synthesis, structural characterisation and chemical reactivity of complexes of the type *trans*-$[Ru(II)(X)(NO)(cyclam)]^{2+}$ (where X = halo or hydroxo ligand, and cyclam = 1,4,8,11-tetraazacyclotetradecane) have recently been studied. It was shown that such compounds can be promising controlled-release NO prodrugs, acting as long-lasting, although softer, vasodilators [59].

Studies on metallonitrosyl complexes as pharmaceutical agents indicated that thermal instability of certain systems in the aerobic aqueous environments under physiological conditions is a problem. As a consequence, Ford et al. have explored and described a new strategy of NO generation from an air-stable, water-soluble complex via the photolytic cleavage of coordinated nitrite. The complex studied was *trans*-$Cr(III)(cyclam)(ONO)_2^+$, which was indeed found to be thermally stable in aerated aqueous solution. The preliminary studies indicate the viability of NO delivery by photochemical extrusion of nitric oxide from the coordinated nitrite [60].

5.2. NO-scavengers

Overproduction of nitric oxide can be the basis for pathological events in the human body. Septic shock is the most dramatic manifestation of systematic acute inflammatory reactions. The design of new drugs that can reduce nitric oxide levels in this and other diseases is a current medical challenge. For that reason, a number of transition metal complexes have been tested for NO trapping and for antisepsis potential. Due to the rapid kinetics of nitrosylation processes, laser flash photolysis techniques have successfully been used in this area of investigations.

The ligands that possess most of the desired pharmacokinetic properties are polyaminocarboxylates [61]. The parent compound K[Ru(III)(Hedta)Cl] has emerged as a nitric oxide scavenger. Preliminary kinetic studies performed with use of stopped-flow techniques indicated that $[Ru(III)(edta)H_2O]^-$ binds nitric oxide very rapidly with a second rate constant higher than 10^8 $M^{-1}s^{-1}$ at body temperature, and very efficiently ($K > 10^8$ M^{-1}), forming a linear nitrosyl complex $Ru(II)–NO^+$ [62,63]. However, there still remain unanswered questions concerning the mechanism by which NO binds to the Ru(III)–edta complex. It is hoped that further mechanistic studies on this system will provide better insight into the underlying reaction mechanism and enable full elucidation of the nature of the nitrosylation process [64].

Among inorganic compounds tested for antisepsis potential, iron-based complexes have been investigated. 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 [65–67]. The potential medical application of these complexes as clinically useful agents for the control of NO levels in blood 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 [68,69]. Although these complexes bind nitric oxide rapidly and tightly, forming relatively stable NO-adducts, the parent Fe(II)L complexes are inherently sensitive to oxygen, which can limit the utility of these complexes as NO scavengers in the bloodstream since the corresponding Fe(III)L complexes do not bind NO at all.

Little mechanistic information is at present available on the nitrosylation reactions of Fe(II)(L) complexes, although some selected rate and stability constants have been reported in the literature [65–67]. In this context, we have performed systematic studies on the reversible binding of nitric oxide to several Fe(II)(L) complexes, where L = H_2O, edta (ethylenediaminetetraacetate), hedtra (hydroxyethylenediaminetriacetate), nta (nitrilotriacetate), mida (methyliminodiacetate), dtpa (diethylenetriaminepentaacetate), and ttha (triethylenetetraaminehexaacetate) [70–73]. The binding of NO to Fe(II)(L) complexes can be expressed by the overall reaction (7).

$$Fe(II)(L)H_2O + NO \underset{k_{off}}{\overset{k_{on}}{\rightleftarrows}} Fe(II)(L)NO + H_2O \qquad (7)$$

Laser flash photolysis and stopped-flow (NO-trapping method) techniques were applied in order to determine the k_{on} and k_{off} values, respectively. Laser flash photolysis of equilibrium Fe(II)(L)NO/Fe(II)(L) mixtures gave transient difference spectra (Fig. 4) consistent with the spectral differences between Fe(II)(L) and Fe(II)(L)NO, i.e. with labilisation of coordinated NO to give non-equilibrium concentrations of these species. The transient spectra decayed exponentially to the original spectra, and no permanent photoproducts were observed.

For a series of ligands the complex-formation rate constants varied between 1.6×10^6 and 2.4×10^8 M^{-1}

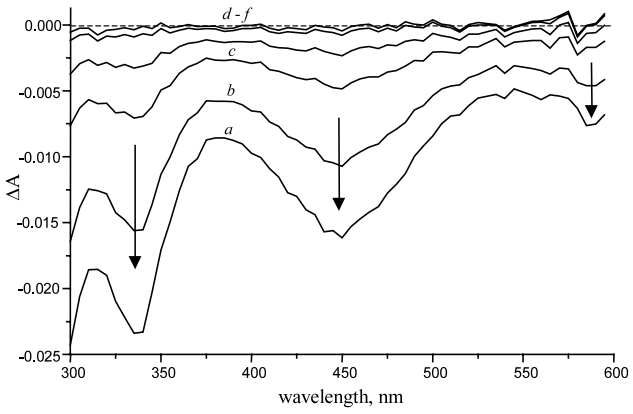


Fig. 4. Transient absorption difference spectra for $[Fe(H_2O)_5NO]^{2+}$ after laser flash photolysis at 532 nm. Experimental conditions: $[Fe(H_2O)_6^{2+}] = 0.012$ M., $[NO] = 3.6 \times 10^{-4}$ M, 0.2 M acetate buffer, pH 5.0, 22 °C. Curves: a, after 25 μs; b, after 50 μs; c, after 100 μs; d, after 150 μs; e, after 250 μs; f, after 450 μs (data from ref. [72]).

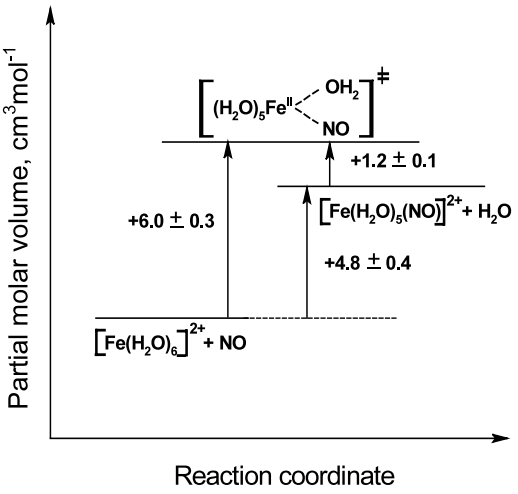


Fig. 5. Volume profile for the binding of NO to $[Fe(II)(H_2O)_6]^{2+}$ on the basis of the determined volumes of activation (data from ref. [72]).

s^{-1}, whereas the dissociation rate constants varied between 0.11 and 3.2×10^3 s^{-1} at 25 °C. In general, good agreement between the values of the equilibrium constants K calculated from the kinetic data (k_{on}/k_{off}) and those determined directly with the aid of a combined spectrophotometric and potentiometric (NO-electrode) technique was observed. The Fe(II)(edta) complex is the species with the highest complex-formation rate constant for NO and this result agrees well with literature data [65–67], whereas the lowest k_{on} values were found for the mida and aqua complexes.

The tendency of Fe(II)(L) complexes 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. $Fe(III)(L)(O_2^-)$. The mida complex shows relatively low oxygen sensitivity, exhibits a low binding affinity for NO, and releases NO almost completely on passing an inert gas through the solution. In contrast, the edta complex of Fe(II) is extremely oxygen sensitive, shows a very high binding affinity for NO, and releases NO only slowly when treated with an inert gas [70].

Systematic studies of k_{on} and k_{off} as a function of temperature and pressure enabled us to gain detailed insight into the mechanisms of the thermal reactions by which NO forms and breaks bonds with the iron centre in the Fe(II) model complexes studied [73]. Activation enthalpies and entropies obtained for the 'on' and 'off' reactions clearly indicate that the reversible binding of NO can best be described by an I_d mechanism, which is also in agreement with that found for water exchange reactions on these complexes [74]. The activation volumes are small positive and also in line with an interchange dissociative (I_d) ligand substitution mechanism (Fig. 5). The only exception in the selected series of complexes seems to be the Fe(II)(nta) system, and the reported data suggest the operation of an I_a mechanism.

6. Concluding remarks

Only a few subjects have grown as rapidly in their importance as the biological and medical role of nitric oxide. Despite the extensive studies on the metal–NO interactions, the fundamental chemistry of processes by which NO forms and breaks bonds with metal centres under biologically relevant conditions is still not well understood and requires more research. As shown in this paper, ambient and high pressure laser flash photolysis can be a very useful tool in such studies. Construction of volume profiles for the processes studied in addition to conventional and commonly used activation and reaction parameters, can bring a decisive dimension to mechanistic considerations and improve the design of metallodrugs for NO-mediated diseases.

Acknowledgements

The authors gratefully acknowledge financial support from the Polish Research Committee (KBN) under Grant Nos. PB 0371/T09/98/15 and 1605/T09/2000/19.

References

[1] S. Kim, G. Deinum, M.T. Gardner, M.A. Marletta, G.T. Babcock, J. Am. Chem. Soc. 118 (1996) 8769 (and references therein).
[2] P.-L. Feldman, O.W. Griffith, D. Stuehr, J. Chem. Eng. News 71 (1993) 26.

[3] Y. Minamiyama, S. Takemura, S. Imaoka, Y. Funae, Y. Tanimato, M. Inoue, J. Pharmacol. Exp. Therapeut. 283 (1997) 1479.
[4] M.W.J. Cleeter, J.M. Cooper, V.M. Darley-Usmar, S. Moncada, A.H.V. Scapira, FEBS Lett. 345 (1994) 50.
[5] G.C. Brown, Eur. J. Biochem. 232 (1995) 188.
[6] T. Noguchi, M. Hoshino, M. Tsujimura, M. Odaka, Y. Inoue, I. Endo, Biochemistry 35 (1996) 16777.
[7] L.J. Ignarro, J.M. Fukuto, J.M. Griscavage, N.E. Rogers, R.E. Byrns, Proc. Natl. Acad. Sci. USA 90 (1993) 8103.
[8] J.M.C. Ribiero, J.M.H. Hazzard, R.H. Nussenzveig, D.E. Champagne, F.A. Walker, Science 260 (1993) 539.
[9] Y.B. Henry, R. Banerjee, J. Mol. Biol. 73 (1973) 469.
[10] E.G. Moore, Q.H. Gibson, J. Biol. Chem. 251 (1976) 2788.
[11] M. Hoshino, K. Ozawa, H. Seki, P.C. Ford, J. Am. Chem. Soc. 115 (1993) 9568.
[12] (a) A. Giuffre, P. Sarti, E. D'Itri, G. Buse, T. Soulimane, M. Brunori, J. Biol. Chem. 271 (1996) 33404;
(b) P. Sarti, A. Giuffre, E. Forte, D. Mastronicola, M.C. Barone, M. Brunori, Biochem. Biophys. Res. Commun. 274 (2000) 183.
[13] V.G. Kharitonov, M. Russwurm, D. Magde, V.S. Sharma, D. Koesling, Biochem. Biophys. Res. Commun. 239 (1997) 284.
[14] J.R. Stone, M.A. Marletta, Biochemistry 35 (1996) 1093.
[15] L.E. Laverman, A. Wanat, J. Oszajca, G. Stochel, P.C. Ford, R. van Eldik, J. Am. Chem. Soc. 123 (2001) 285.
[16] V.S. Sharma, T.G. Traylor, R. Gardiner, H. Mizukami, Biochemistry 26 (1987) 3837.
[17] V.S. Sharma, R.A. Isaacson, M.E. John, M.R. Waterman, M. Chevion, Biochemistry 22 (1983) 3897.
[18] L.E. Laverman, P.C. Ford, Chem. Commun. (1999) 1843.
[19] L.E. Laverman, M. Hoshino, P.C. Ford, J. Am. Chem. Soc. 119 (1997) 12663.
[20] M. Hoshino, L.E. Laverman, P.C. Ford, Coord. Chem. Revs. 187 (1999) 75 (and references therein).
[21] A. Antonini, M. Brunori, Hemoglobin and Myoglobin in their Reaction with Ligands, North-Holland, Amsterdam, 1971.
[22] I.J. Ostrich, G. Liu, H.W. Dodgen, J.P. Hunt, Inorg. Chem. 19 (1980) 619.
[23] T. Schneppensieper, A. Zahl, R. van Eldik, Angew. Chem. Int. Ed. 40 (2001) 1678.
[24] (a) T.G. Traylor, V.S. Sharma, Biochemistry 31 (1992) 2847;
(b) Y. Wang, B.A. Averill, J. Am. Chem. Soc. 118 (1996) 3972;
(c) L.S. Miller, A.J. Pedraza, M.R. Chance, Biochemistry 36 (1997) 12199.
[25] A.M. Rich, R.S. Armstrong, P.J. Ellis, P.A. Lay, J. Am. Chem. Soc. 120 (1998) 10827.
[26] (a) A. Drljaca, C.D. Hubbard, R. van Edik, T. Asano, M.V. Basilewsky, W.J. le Noble, Chem. Rev. 98 (1998) 2167;
(b) G. Stochel, R. van Eldik, Coord. Chem. Rev. 187 (1999) 329.
[27] E.C. Constable, G. Baum, E. Bill, R. Dyson, R. van Eldik, D. Fenske, S. Kaderli, D. Morris, A. Neubrand, M. Neuburger, D.R. Smith, K. Wieghardt, M. Zehnder, A.D. Zuberbühler, Chem. Eur. J. 5 (1999) 498 (and references cited therein).
[28] T.G. Traylor, J. Luo, J.A. Simon, P.C. Ford, J. Am. Chem. Soc. 114 (1992) 4340.
[29] J.S. Stamler, D.J. Singel, J. Loscalzo, Science 258 (1992) 1898 (and references cited therein).
[30] V.J. Chen, A.M. Orville, M.R. Harpel, C.A. Frolik, K. Surerus, E. Muenck, J.D. Lipscomb, J. Biol. Chem. 264 (1989) 21677.
[31] C.J. Haskin, N. Ravi, J.B. Lynch, Biochemistry 27 (1988) 1014.
[32] A.R. Butler, F.W. Flitney, D.L.H. Williams, TiPS 16 (1995) 18.
[33] J.S. Stamler, Cell 78 (1994) 931.
[34] Z.V. Kuropteva, M.E. Kudryavtsev, Gen. Phys. Biophys. 16 (1997) 91.
[35] M. Tsujimura, N. Dohmae, M. Odaka, K. Chijimatsu, Takio, M. Yohda, M. Hoshino, S. Nagashimo, I. Endo, J. Biol. Chem. 272 (1997) 29454.
[36] (a) J. Torres, M.T. Wilson, Biochim. Biophys. Acta 1411 (1999) 310;
(b) J. Torres, C.E. Cooper, M.T. Wilson, Biochem. Soc. Trans. 450S (1996) 24;
(c) C.E. Cooper, J. Torres, M. Sharpe, M.T. Wilson, FEBS Lett. 414 (1997) 281;
(d) J. Torres, C.E. Cooper, M.T. Wilson, J. Biol. Chem. 273 (1998) 8756.
[37] (a) R. Wever, R. Boelens, E. De Boer, B.F. Van Gelder, A.C.F. Gorren, H. Rademaker, J. Inorg. Biochem. 23 (1985) 227;
(b) R. Boelens, H. Rademaker, R. Wever, B.F. Van Gelder, Biochim. Biophys. Acta 765 (1984) 196;
(c) G. Musci, S. Di Marco, M.C. Bonaccorsi di Patii, L. Calabrese, Biochemistry 30 (1991) 9866;
(d) C.T. Martin, R.H. Morse, R.M. Kanne, H.B. Gray, B.G. Malstrom, S.I. Chan, Biochemistry 20 (1981) 5147.
[38] (a) A.J.M. Schoot Uiterkamp, FEBS Lett. 20 (1972) 93;
(b) J. Verplaetse, P. Van Tornout, G. Defreyn, R. Witters, R. Lontie, Eur. J. Biochem. 95 (1979) 327;
(c) B. Solvato, G.M. Giacometti, M. Beltramini, F. Zilio, G. Giacometti, R.S. Magliozzo, J. Peisach, Biochemistry 28 (1989) 680.
[39] (a) F.X.R. Van Leeuwen, R. Wever, B.F. Van Gelder, Biochim. Biophys. Acta 315 (1973) 200;
(b) F.X.R. Van Leeuwen, B.F. Van Gelder, Eur. J. Biochem. 87 (1978) 305.
[40] (a) G.W. Brudvig, T.H. Stevens, S.I. Chan, Biochemistry 19 (1980) 5275;
(b) T.H. Stevens, G.W. Brudwig, D.F. Bocian, S.I. Chan, Proc. Nat Acad. Sci. USA 76 (1979) 3320.
[41] A. Giuffre, G. Staubauers, M. Brunori, P. Sarti, J. Torres, M.T. Wilson, J. Biol. Chem. 273 (1998) 32475.
[42] (a) D. Ehrenstein, G.U. Niehaus, Proc. Natl. Acad. Sci. USA 89 (1992) 9681;
(b) D. Ehrenstein, M. Filiaci, B. Scharf, M. Engelhard, P.J. Steinbach, G.U. Niehaus, Biochemistry 34 (1995) 12170.
[43] (a) D. Zheng, R. Birke, J. Am. Chem. Soc. 123 (2001) 4637 (and references therein);
(b) G. Wolak, Stochel, M. Hamza, R. vanEldik, Inorg. Chem. 39 (2000) 2018 (and references therein);
(c) K.M. Jenkinson, J.J. Reid, M.J. Rand, Eur. J. Pharmacol. 275 (1995) 145.
[44] M. Wolak, A. Zahl, T. Schneppensieper, G. Stochel, R. van Eldik, J. Am. Chem. Soc. 123 (2001) 9780.
[45] R. van Eldik, H. Cohen, D. Meyerstein, Inorg. Chem. 33 (1994) 1566.
[46] (a) G.N. Schrauzer, L. Lee, J. Am. Chem. Soc. 90 (1964) 6541;
(b) I. Sagi, M.D. Wirt, S. Frisbie, M.R. Chance, J. Am. Chem. Soc. 112 (1990) 8641;
(c) M. Giorgetti, I. Ascone, M. Berrettoni, P. Conti, S. Zamponni, R. Marassi, J. Biol. Inorg. Chem. 5 (2000) 156.
[47] J. Bourassa, W. DeGraff, S. Kudo, D.A. Wink, J.B. Mitchell, P.C. Ford, J. Am. Chem. Soc. 119 (1997) 2853.
[48] J. Bourassa, B. Lee, S. Bernard, J. Schoonover, P.C. Ford, Inorg. Chem. 38 (1999) 2947.
[49] A.B. Cox, R.M. Wallace, Inorg. Nucl. Chem. Lett. 7 (1971) 1191.
[50] N. Bettache, T. Carter, J.E.T. Corrie, D. Ogden, D. Trentham, Meth. Enzymol. 268 (1996) 266.
[51] T.G. Traylor, D. Magde, J. Marsters, K. Jongeward, G.Z. Wu, K. Walda, J. Am. Chem. Soc. 115 (1993) 4808.
[52] M. Yamaji, Y. Hama, Y. Miyazaki, M. Hoshino, Inorg. Chem. 31 (1992) 932.
[53] A. Antipas, M. Gouterman, J. Am. Chem. Soc. 105 (1983) 4896.
[54] P.C. Ford, J. Bourassa, K. Miranda, B. Lee, I. Lorkovic, S. Boggs, S. Kudo, L.E. Laverman, Coord. Chem. Rev. 171 (1998) 185.

[55] K.M. Miranda, X. Bu, I.M. Lorkovic, P.C. Ford, Inorg. Chem. 36 (1997) 4838.
[56] I.M. Lorkovic, K.M. Miranda, B. Lee, S. Bernhard, J.R. Schoonover, P.C. Ford, J. Am. Chem. Soc. 120 (1998) 11674.
[57] I.M. Lorkovic, P.C. Ford, Inorg. Chem. 38 (1999) 1467.
[58] C.F. Works, P.C. Ford, J. Am. Chem. Soc. 122 (2000) 7592.
[59] D.R. Lang, J.A. Davis, L.G. Lopes, A.A. Ferro, L.C.G. Vasconcellos, D.W. Franco, E. Tfouni, A. Wieraszko, M.J. Clarke, Inorg. Chem. 39 (2000) 2294.
[60] M. De Leo, P.C. Ford, J. Am. Chem. Soc. 121 (1999) 1980.
[61] M.J. Abrams, S.P. Fricker, B.A. Murrer, O.J. Vaughan, World Patent Appl. (1995) 95/5814A.
[62] N.A. Davies, M.T. Wilson, E. Slade, S.P. Fricker, B.A. Murrer, N.A. Powell, G.R. Henderson, Chem. Commun. (1997) 47.
[63] 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, Br. J. Pharmacol. 122 (1997) 1441.
[64] A. Wanat, T. Schneppensieper, A. Karocki, G. Stochel, R. van Eldik, J. Chem. Soc. Dalton Trans., 6 (2002) 941.
[65] V. Zang, R. van Eldik, Inorg. Chem. 29 (1990) 4462.
[66] V. Zang, M. Kotowski, R. van Eldik, Inorg. Chem. 27 (1988) 3279.
[67] E. Sada, H. Kumazawa, H. Machida, Ind. Eng. Chem. Res. 26 (1987) 1468.
[68] R.E. Shepherd, M.A. Sweetland, D.E. Junker, J. Inorg. Biochem. 65 (1997) 1.
[69] Y. Chen, M.A. Sweetland, R.A. Shepherd, Inorg. Chem. Acta 260 (1997) 163.
[70] T. Schneppensieper, S. Finkler, A. Czap, R. van Eldik, M. Heus, P. Nieuwenhuizen, C. Wreesmann, W. Abma, Eur. J. Inorg. Chem. (2001) 491.
[71] Schneppensieper, A. Wanat, G. Stochel, S. Goldstein, D. Meyerstein, R. van Eldik, Eur. J. Inorg. Chem. (2001) 2317 and references cited therein.
[72] A. Wanat, T. Schneppensieper, G. Stochel, R. van Eldik, B. Eckhard, K. Wieghardt, Inorg. Chem. 41 (2002) 4.
[73] T. Schneppensieper, A. Wanat, G. Stochel, R. van Eldik, Inorg. Chem. (2002) in press.
[74] L. Helm, A.E. Merbach, Coord. Chem. Rev. (1999) 187.