Minireview

# Transition metal ion-catalyzed oxygen activation during pathogenic processes

Susanne Hippeli, Erich F. Elstner*

*Lehrstuhl für Phytopathologie, Labor für Angewandte Biochemie, Technische Universität München, Am Hochanger 2, 85350 Freising-Weihenstephan, Germany*

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**Abstract Most pathological processes include the production of activated oxygen species augmented or attenuated by transition metal ions catalyzing one electron transitions. Inhalation of airborne particles, infections, ingestion of toxins or liberation from endogenous stores represent biological pathways for the induction of pathogenic processes by these metal ions. In this short review basic reactions involving transition metal ions operating during oxidative stress in certain diseases will be discussed.**



*Key words:* Transition metal ion; Reactive oxygen species; Radical chain reaction; Disease

## 1. Introduction

Atmospheric oxygen has to be activated in order to react with organic molecules. This is due to the fact that molecular oxygen is an unreactive molecule in the triplet ground state ($^3O_2$) and has to be activated in order to react with atoms or molecules in the ('normal') singlet ground state thus circumventing spin-forbidden reactions. These processes of activation include very reactive intermediates. Therefore, aerobic cells must cope with, and to some extent also adapt to, oxidative stress provoked for example by infections or intoxications, where reactive intermediates of oxygen accumulate. Most visible or measurable disease symptoms are connected with oxygen activation where principally a transition from heterolytic (two electron transitions) to increased homolytic (one electron transitions) reactions is observed. Homolytic reactions produce free radicals, which are generally counteracted by a parallel increase of intrinsic radical scavenging processes or by compounds administered with food thus warranting metabolic control within certain limits. Most transition metals belonging to the fourth period due to their electron motility in the third shell which is filled up in the series from scandium (8 electrons) to zinc (18 electrons) can easily undergo one electron redox reactions. These may include electron donations to oxygen forming superoxide or to hydrogen peroxide, forming the extremely reactive OH radical thus inducing or strongly increasing oxygen stress in cells, tissues, organs and finally organisms visible or measurable as disease symptoms.

In a recent review Saran et al. [1] stated that: "there is probably not a single radical chain process in vivo that proceeds without the participation of some metal in loose or bound form, whether as a side effect or even playing a dominant role" thus emphasizing a biochemical dogma based on a wealth of publications of the last 20 years.

## 2. Oxygen activation and reactive oxygen species (ROS)

Deficiencies in transition metal ions (TMI) can cause severe disease symptoms, with iron deficiency causing anemia as the best known example. Overload by TMI, on the other hand, due to intoxication or an inherited disease also results in pathological symptoms often ameliorated by sequestration of the corresponding TMI by chelators. Many of the well-known diseases – independent of an overload or genetic deficiency – such as diabetes, arthritis, ischemia-reperfusion damage, cancer, arteriosclerosis, sunburn and many others include, or are governed by, both ROS and TMI. The interaction of ROS and TMI is complex and based on several initial and secondary processes [2]. The most important reactions concerning this subject will be briefly addressed; the whole field of oxygen biochemistry has been frequently reviewed and several books on this topic have appeared [3–5]. This short review will not go too deeply into details of the chemistry of oxygen-TMI interactions, since this field has been covered exhaustively by Martell and Sawyer [2].

### *2.1. Homolytic and heterolytic reactions: how are free radicals created?*

During heterolytic reactions electron pairs are transferred, either utilizing or creating ions. Homolytic reactions create radicals through the transfer of single electrons, represented as a dot. A radical is a compound containing an unpaired electron. There are stable and unstable radicals. Most free radicals are highly reactive creating new radicals thus initiating chain reactions. This holds especially for lipids in membranes.

## 3. Mechanisms of oxygen activation

### *3.1. Photodynamic reactions*

Oxygen activation may occur via photodynamic reactions through activated pigment states (P*), where exciton transfer from the pigment (mostly aromatic or heterocyclic compounds) may form singlet oxygen (type II reaction). Singlet

*Corresponding author. Fax: (49) (8161) 714538.
E-mail: Elstner@lrz.tum.de

*Abbreviations:* DF, desferrioxamine, Desferal; EDTA, ethylene diamine tetraacetate; Hb, hemoglobin; IRP, iron regulatory protein; PMNL, polymorphonuclear leukocytes; RC, redox-cycling compound; ROS, reactive oxygen species; SOD, superoxide dismutase; TC, tungsten carbide; TMI, transition metal ions


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oxygen in contrast to atmospheric oxygen is not subject to the spin rule and reacts rapidly with most organic molecules (RH), especially at double bonds of unsaturated fatty acids, producing hydroperoxides which can be activated by $Fe^{2+}$ reductively released from endogenous stores [6].

Photodynamic reactions not involving physical transfer of excitation energy via P* and $^1O_2$ but undergoing charge separation within the excited pigment are called photodynamic reactions type I.

### 3.2. Reductive oxygen activation: formation of primary oxygen radicals

In the presence of reductants of sufficient affinity for oxygen and an appropriately negative redox potential ($E_o'$ of the redox pair $O_2/O_2^{\bullet-} = -330$ mV), superoxide may be formed from atmospheric oxygen. Superoxide dismutates at neutral pH in aqueous media with a rate constant $k = 2\times10^5$ (L $M^{-1}$ $s^{-1}$), yielding hydrogen peroxide. Hydrogen peroxide can be monovalently reduced by certain electron donors yielding the extremely reactive hydroxyl radical, $OH^{\bullet}$. Electron donors may be semiquinones or reduced transition metal ions such as $Fe^{2+}$ or $Cu^{+}$. The most prominent candidate is $Fe^{2+}$, stemming from $Fe^{3+}$ reduced by superoxide, thiols or ascorbate (Haber-Weiss-Fenton chemistry; see below). The OH radical has a very positive redox potential (close to +2 V) and a life time in biological media of approximately 1 μs thus reacting closely to the site of its generation producing 'site-specific' oxidative damage (see Section 5). The oxygen species $OH^{\bullet}$ is the major target of the antioxidative power of phenolics since, due to its thermodynamic and kinetic properties it is not under the control of specific enzymes (see Section 6.2).

### 3.3. Peroxynitrite (ONOOH): a strong oxidant similar to $OH^{\bullet}$

The simple molecule $NO^{\bullet}$ plays a very important role in the regulation of vascular tone. It activates the enzyme guanylate cyclase which produces the vasorelaxing cGMP. NO is synthesized from the amino acid arginine catalyzed by the enzyme NO synthase (NOS; EC 1.14.13.39). NO synthesis seems to proceed in a two step reaction: the first reaction converts arginine into $N^{G}$-hydroxy-L-arginine and is catalyzed by NOS. The second step is apparently independent of NOS and seems to be driven by superoxide generated by NADPH oxidases or by XOD.

Peroxynitrite $ONOO^{-}$ is formed from nitrogen monoxide and superoxide in an extremely fast reaction ($k = 6.7\times10^9$ L $M^{-1}$ $s^{-1}$) indicating that ONOOH formation is only diffusion limited. ONOOH, which is mainly produced under pathophysiological conditions (rapid superoxide formation by XOD or activated neutrophils together with NOS activation) inhibits NOS where bovine serum albumin, glutathione or tyrosine act as protectants. ONOOH initiates lipid peroxidation, inhibits mitochondrial electron transport, inactivates glyceraldehyde 3-phosphate dehydrogenase as well as Na/K-ATPases and membrane sodium channels.

Experiments with model reactions and inhibitors could demonstrate that only in the presence of both radicals, $NO^{\bullet}$ and $O_2^{\bullet-}$ and through their interaction, damage of endothelial cells occurred. Several authors used different NO generators such as *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), sodium nitroprusside or 3-morpholino-sydnonimine (SIN-1), where SIN-1 produces simultaneously both $NO^{\bullet}$ and $O_2^{\bullet-}$ [7].

Table 1
The most important reactive oxygen species

| | |
|---|---|
| Free radicals | Atmospheric oxygen, $^{\bullet}O_2^{\bullet}$ (diradical) |
| | Superoxide radical anion, $O_2^{\bullet-}$ |
| | Hydroperoxyl radical, $HO_2^{\bullet}$ |
| | OH radical, $OH^{\bullet}$ |
| | Alkyl and alkoxyl radicals, $R^{\bullet}$, $RO^{\bullet}$ |
| | Peroxyl radicals, $ROO^{\bullet}$ |
| | Nitrogen monoxide or dioxide, $NO^{\bullet}$ and $NO_2^{\bullet}$ |
| Non-radical compounds | Hydrogen peroxide, $H_2O_2$ |
| | Organic peroxides, ROOH |
| | Hypohalous acids or their salts, such as HOCl, $OCl^{-}$ and organic chloramines such as taurine chloramine |
| | Peroxynitrite, ONOOH |
| | Singlet oxygen, $^1O_2$ |

### 3.4. Hypohalous acids

Catalyzed by myeloperoxidases (EC 1.11.1.7), halides such as $Cl^{-}$ or $Br^{-}$ are converted into hypohalous acids (HOCl; HOBr) which are strongly oxidizing or halogenating agents, produced by activated leukocytes and secreted into the phagosome or tissues adjacent to these activated blood cells thus contributing to the degradation of ingested particles or to inflammations and tissue damage.

All the above mentioned ROS may interact thus producing an almost undifferentiable set of superimposed oxidants. Methods suitable for the differentiation of various ROS such as ONOOH, HOCl and Fenton oxidants have been recently reported [8,9].

The most important ROS are summarized in Table 1.

## 4. Transition metal-catalyzed oxidations and hydroxylations

At the end of the last century Fenton [10] described the ability of hydrogen peroxide to oxidize aromatic compounds in the presence of iron salts (Fenton's reagent). Later on Haber and Weiss [11] concluded that the reactivity of Fenton's reagent was due to the formation of OH radicals according to the following reaction sequence:

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

(II) $OH^{\bullet} + H_2O_2 \rightarrow H_2O + H^{+} + O_2^{\bullet-}$

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

(IV) $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + 2H^{+} + O_2^{\bullet-}$

(V) $Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$

Details of the thermodynamics of the Fenton-driven Haber-Weiss and related reactions have been described in [12].

The Udenfriend group reported in 1954 (see reference in [13]) that at neutral pH and room temperature, aromatic compounds are hydroxylated by molecular oxygen in the presence of $Fe^{2+}$, ascorbic acid and EDTA. This mixture, designated the 'Udenfriend system', was later described as also hydroxylating saturated hydrocarbons yielding alcohols and forming epoxides from olefins [13]. The oxidizing species of this reaction mixture is supposed to be a complex of oxygen, $Fe^{2+}$ and ascorbic acid transferring triplet oxygen to the substrate (S).

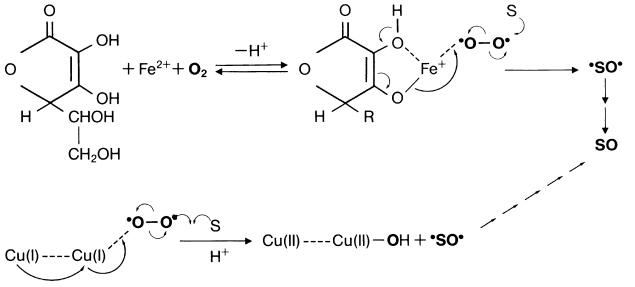


Fig. 1. Possible reaction mechanisms of oxidations by the ascorbate-iron (Udenfriend) system and of substrate oxidations by Cu(I).

The general reaction mechanism may be formulated as shown in Fig. 1 (upper part).

In analogy, ascorbate can be replaced by tetrahydropteridines or by catechol (references in [13]) which hydroxylates aromatics in the presence of $Fe^{3+}$ ('Hamilton system').

It has been shown with several model systems that substrate oxidations and hydroxylations can be observed in the absence of a reducing agent if the reduced TMI is present at high enough concentrations. In this respect iron can be replaced by Cu(I), V(II), Ti(III) or Sn(II). In this type of reaction two metal ions are electronically connected by a bridging group allowing overlapping of molecular orbitals of both TMIs (references in [13]) (Fig. 1, lower part).

## 5. OH radicals, ferryl-perferryl (cupryl-percupryl) complexes, 'active site' complexes or crypto-OH? The long-lasting dispute on the nature of the strongly oxidizing TMI-ROS species

The interactions between TMI and oxygen or ROS are extremely complex and not yet understood in all details: oxygen transport and oxygen activation producing ROS as well as ROS detoxification [2] and complex, biologically important metal-metal interactions [14] have to be distinguished. In this context only the production of extremely strong oxidants of the OH radical type will be addressed. There is no doubt that the free OH radical exists in the atmosphere [15] and is produced in radiation chemistry [16]. In biological systems, however, the ongoing dispute in the past was and is the question if, in the presence of TMI, superoxide and hydrogen peroxide, free $OH^{\bullet}$ is the species which brings about the damage or whether transient reaction products between TMI and superoxide or hydrogen peroxide might be reactive enough to rapidly ($k > 10^8$) interact with molecules in the near neighborhood ('active site chemistry'). Such transient products might be designated 'cage molecules' as postulated for peroxynitrite [17], as electron donor-hydrogen peroxide complex [18] or 'crypto-OH' [19]. Whatever might be the most appropriate name for such a species derived from superoxide or hydrogen peroxide in the presence of TMI, the 'active site chemistry' might be drawn as outlined below [20].

$$\text{(VI) } \mathrm{Cu(II)-Biol} + \mathrm{O_2^{\bullet -}} \rightarrow \mathrm{Cu(I)-Biol} + \mathrm{O_2}$$

$$\text{(VII) } \mathrm{Cu(I)-Biol} + \mathrm{H_2O_2} \rightarrow \mathrm{OH^-} + \{\mathrm{Cu(II)-Biol{-}OH^{\bullet}}\}$$

where {Cu(II)-Biol–$OH^{\bullet}$} stands for 'active site' copper ion creating an OH-type radical near the target molecule. This species might also be designated 'crypto-$OH^{\bullet}$' formed in the solvent cage as stated above.

The corresponding activities of different TMI-ROS complexes are not identical, however. Kocha et al. [21] compared the effects of copper ions with the effects of iron ions in the presence of hydrogen peroxide and EDTA as to oxidative albumin degradation. This was taken as the basis for the assumption that copper binds site-specifically according to the Czapski scheme ([20]; reactions VI and VII) while Fe-EDTA generates an OH-type oxidant in solution.

## 6. TMI mobilization from storage proteins and regulation of homeostasis

### 6.1. TMI mobilization

Under reductive conditions (ascorbate; superoxide; quinoid redox cyclers; cf. [22]) iron is released from storage or transport proteins (such as transferrins and ferritin) or, in the case of copper, by peroxynitrite from ceruloplasmin [23]. Most of the transferrin-bound iron is used for hemoglobin (Hb) synthesis and released again from Hb after senescence of erythrocytes and internalization by macrophages. Released iron is then rebound by transferrin thus completing the iron cycle. During this cycle iron concentrations exceeding the requirement for hemoglobin synthesis enter a "poorly characterized labile intercellular pool" [24].

Iron release from Hb is observed after allyl alcohol intoxication where desferrioxamine (Desferal, DF) inhibits hemolysis and lipid peroxidation [25]. Reperfusion after open heart surgery results in hemolysis yielding free heme and iron accompanied by formation of OH radicals presumably via the xanthine oxidase reaction [26].

Malaria parasites may release iron from Hb via acidification of their food vacuoles initiating Fe-redox cycling which is enhanced by vitamin C [27]. In a rabbit lung reperfusion model damaging Fe ions seem to stem from cytochrome $P_{450}$ [28].

Iron release from ferritin driven by superoxide causes lipid peroxidation [29] where the rate constant of the reaction between superoxide and iron-ferritin has been determined to be ca. $2\times10^6$ L $M^{-1}$ $s^{-1}$ [30].

### 6.2. ROS detoxification and regulation of TMI homeostasis

As already mentioned, detoxification by enzymic processes is only possible if the reactivity of the oxygen species in question is reasonably low under physiological conditions so that the enzymic reaction allows at least one $k$ order of magnitude between the reaction under enzyme catalysis and the non-catalyzed spontaneous reaction between the oxygen species and any reaction partner in its 'molecular' neighborhood. Therefore, the reactions of $OH^{\bullet}$, $^1O_2$, $RO^{\bullet}$, $ROO^{\bullet}$ and $HOO^{\bullet}$ are not under enzymic control since their reaction constants with potential partners in their typical 'environments' are too fast (generally $k \gg 10^8$) for enzyme catalysis. Thus, the reactions of biomolecules with these oxygen species have to be 'amended' after their reaction, i.e. damage. In order not to 'flood' these repair process antioxidative small molecules serve as scavengers and quenchers of activated states [1,3,5,31].

Enzyme-catalyzed detoxifications mainly concern superoxide, peroxides and epoxides (produced by cytochrome $P_{450}$ activities) as more or less 'stable' reduced oxygen species.

In most aerobic cells catalase, superoxide dismutases

(SOD), ascorbate peroxidase, mono- or dehydro-ascorbate reductases, glutathione peroxidase, glutathione reductase, and different peroxidases either individually or cooperatively remove stable ROS [3,5].

Homeostasis of TMI in aerobic cells is modulated by regulatory proteins controlling uptake, transport, storage and release. Metallothioneins in animals [32] and phytochelatins in plants [33] are sulfhydryl-rich proteins involved in metal binding and thus detoxification by sequestering free heavy metal ions and also TMI. Iron metabolism in animals is regulated by iron regulatory proteins (IRPs). These proteins bind to iron-responsive elements of mRNA for ferritin and to the transferrin receptor. As regulators of IRP activities, available cellular iron [34], cellular redox status [35], ROS [36,37] and NOS activity [38] have been described. IRP-1, which contains a [4Fe-4S] cluster [39] and acts as a cytoplasmic aconitase, is rapidly inactivated by iron-releasing redox cyclers such as the doxorubicin metabolite. This reaction might partially explain the cardiotoxicity of these anticancer compounds [40]. Belinkova and coworkers recently reported that inactivated aconitase is identical with the active oxaloacetate keto-enol tautomerase [41].

TMI in the bad sense may be converted into good: DF converts 'toxic' Fe into the redox-inactive Fe-DF, Mn into a SOD mimetic; similarly penicillamine converts free Cu into SOD-active Cu-penicillamine [42].

## 7. Pathological processes

TMI can be either inhaled as fibers, dust, soot or aerosols and thus exert toxic reactions or mobilized by microorganisms after infection or ingested as toxins produced by them. For all three cases a few examples will be given.

### *7.1. Intoxications and infections*

*7.1.1. Inhaled particles.* Airborne particulate matter such as soot particles from diesel engines, coal or oil burners, or fibers such as asbestos may cause inflammatory responses in the respiration tract via activation of alveolar macrophages. Besides geometrically determined interactions these particles contain substantial amounts of TMI and thus act catalytically as 'immobilized' converters interacting with different ROS [43,44]. These particles in many cases are emitted together with sulfur dioxide which exerts a cooperative effect on the destructive potential together with a change in the basic reaction mechanism. This holds for both crocidolite asbestos fibers and diesel soot particles [45,46]. Stimulation of biochemical model reactions (NADH/diaphorase system: the enzyme generates superoxide at the expense of NADH, so this model simulates the respiratory burst of activated leukocytes), indicative of oxidative destructions, their stimulation by EDTA and their inhibition by DF and SOD, is taken as proof for the involvement of both iron and superoxide in these reactions (Fig. 2) [46]. According to a recent report Fe seems to cooperate with NO in asbestos-catalyzed mutagenicity and carcinogenicity [47].

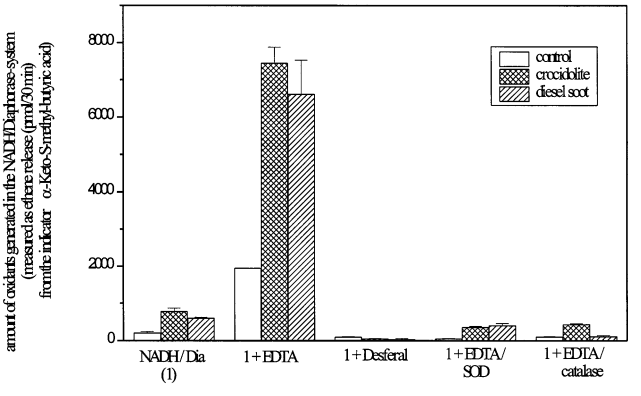


Fig. 2. The oxidative capacity of crocidolite and diesel soot in the NADH/diaphorase system.

Hard metal lung diseases are caused by exposure to toxic metal mixtures consisting of Co metal and tungsten carbides (TC). Toxicity is only exerted in the presence of both components, Co and TC [48]. The mechanism by which toxicity is mediated is rather complex, involving metal-metal surface interactions in the particle-biological medium interface [49]. In a first reaction metallic Co is ionized accompanied by TC-catalyzed oxygen reduction:

$$\text{(VIII)}\ \mathrm{Co-TC \rightarrow Co^{2+} - TC + 2e^-}$$

$$\text{(IX)}\ \mathrm{2O_2 + 2e^- - (TC - O_2^{\bullet -}) \rightarrow 2O_2^{\bullet -}}$$

*7.1.2. Intoxications.* Ingestions of a toxic load of TMI with food or drinks and thus development of disease symptoms are not a matter of discussion in this short review. There is yet another possibility of evoking TMI toxicity, namely the uptake of poisonous redox catalysts (RC) either willingly (chemotherapy; antimalarials) or unwillingly (paraquat; orellanine). In these cases TMI may be mobilized by the above mentioned redox mechanisms. In this context the herbicide paraquat and the fungal poison orellanine have to be mentioned.

**Paraquat**, like other low potential bipyridyls, reduces oxygen monovalently after its reduction by diverse oxidoreductases (diaphorases). As shown for paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride) [19] and very recently also for lucigenin (bis-*N*-methylacridinium) [50], formation of OH-type oxidants under aerobic conditions is strongly enhanced by the addition of Fe salts and lipid peroxidation by bipyridyls is directly dependent on their potential to reduce $Fe^{3+}$ [51].

Iron strongly enhances paraquat toxicity in living individuals such as *Escherichia coli* [52], animals such as flies (*Drosophila melanogaster* [53]) or mice, where toxicity was abolished by DF [54]. In vitamin E-deficient rats, however, DF, in contrast to the lipid-soluble iron chelator CP51 (a hydroxypyridin-4-one derivative), had no significant effect on mortality [55]. One mechanism of paraquat toxicity in copper-tolerant cell lines (hepatocytes) may be copper-dependent glutathione depletion [56], a reaction which has been shown to proceed via a peroxide- or a superoxide-dependent pathway starting from a $Cu^{2+}(GS^-)_2$ complex [57].

**Orellanine** ([2,2′-bipyridine]-3,3′,4,4′-tetrol-1,1′-dioxide) is a toxin produced by the fungus *Cortinarius orellanus* causing lethal nephrotoxicity. In vitro it forms a stable ferric complex which is easily reducible. At neutral pH it undergoes one electron transition producing the *o*-semiquinone anion radical. In the presence of $Fe^{2+}$ orellanine causes rapid oxygen consumption by facilitating $Fe^{2+}$ autoxidation [58].

**Bleomycin** (Bl), like **doxorubicin** and numerous other anti-

cancer drugs, exerts both desired and undesired health effects on the basis of iron binding (Bl-Fe) and thus 'site-specific' oxygen activation. After intercalation with DNA preferentially in tumors, Bl-Fe causes strand breaks and degradation. In contrast to other oxidative destructions this process is apparently stimulated by glutathione [59], glutathionyl-hydroquinone (a toxic benzene metabolite [60]) and antioxidants such as ferulic acid, epicatechin and ± catechins [61].

Inhibitors of Bl-Fe- or doxorubicin-catalyzed damage include compounds such as baicalein (5,6,7-trihydroxy-2-phenyl-4*H*-1-benzopyran-4-one [62]) and the bispiperazinedione, dextrazoxane (ICRF-187), which form inert complexes with iron in several model systems and are thus used as organ-protective drugs during chemotherapy [63].

Several phenolics and flavonoids known as potent antioxidants exhibit equivocal responses, however: mono- and trihydroxyethylrutosides inhibited negative inotropic effects of doxorubicin treatment [64] while BL-Fe-induced DNA damage was augmented by catechins and ferulic acid [61].

*7.1.3. Infection.* The so-called hospitalism bug *Pseudomonas aeruginosa* causes severe lung disease by releasing both the proteinaceous iron chelator pyochelin (PYCH) and the redox-cycling phenazine derivative, pyocyanin (PYO). Iron sequestration and cell toxicity are apparently based on a common mechanism: superoxide and hydrogen peroxide formation by PYO, iron release from ferritin by $O_2^{\bullet-}$, oxidation of $Fe^{2+}$ by ceruloplasmin, complexation of $Fe^{3+}$ by PYCH and its reduction by $O_2^{\bullet-}$ and finally reduction of $H_2O_2$ with formation of $OH^{\bullet}$ driven by PYCH-$Fe^{2+}$. The toxicity of this system has been shown by following $^{51}Cr$ release from prelabelled lung epithelial cells [65].

### 7.2. General pathoprocesses involving TMI

In the following a few selected examples of the (possible) involvement of TIM and ROS in certain well known diseases will be presented.

*7.2.1. Atherosclerosis (AS).* Low density lipoprotein (LDL) oxidation in vitro is strongly enhanced by iron or copper and modulated by pH [66], thiol compounds and antioxidants such as tocopherols, carotenoids and ubiquinol. Oxidized TMI such as $Cu^{2+}$ or $Fe^{3+}$ are readily reduced by macrophages thus facilitating monovalent lipid hydroperoxide (LOOH) decomposition and chain peroxidation [67]. The Bruneck study [68] provides strong epidemiological evidence for a role of iron in AS events since "ferritin and LDL-cholesterol showed a synergistic association with cardiovascular disease and death". It has also been shown that electrophoretic mobility of LDL (as a marker of its oxidation) in women correlates with increased ferritin [69]. These reports are in contrast, however, with a report that iron overload in rabbits decreases AS rather than increasing it [70]. Therefore a role of TMI in AS is indicated but the mechanisms are anything but clear.

Much attention has recently been given to elevated homocysteine and TMI levels [71].

*7.2.2. Skin inflammation and sunburn.* Inflammatory skin diseases have been discussed in context with ROS from infiltrating polymorphonuclear leukocyts (PMNL), TMI and UV radiation [72]. In the case of sunburn it has been shown that chronic UV exposure increases free iron in the skin [6] and that the iron chelator 2-furildioxime and sunscreen provide synergistic protection [73].

*7.2.3. Neurological disorders.* ROS are involved in the development of neurological disorders such as Parkinsonism, Alzheimer's disease (AD), amyotrophic lateral sclerosis, dementia and depressive situations. In addition to Fe and Cu, aluminum and manganese seem to play a crucial role in pathogenesis [74]. In AD, similar to atherosclerosis (AS, see above), Cu reduction seems to be achieved by factors involved in the development of the corresponding disease: in the case of AS macrophages [67] and in AD the amyloid precursor protein [75]. As indicated in Fig. 1 TMIs catalyze one electron transfer from ortho-diols to oxygen. It is thus quite obvious that catecholamines are readily oxidized by TMI producing ROS, *O*-methylation or addition of melatonin may protect against ROS production and CA oxidation [76].

Both deficiency and excess of Mn experimentally causes neurotoxicity via ROS generation [77] where subchronic oral exposure causes changes in monoaminergic systems and increases in uric acid levels [78].

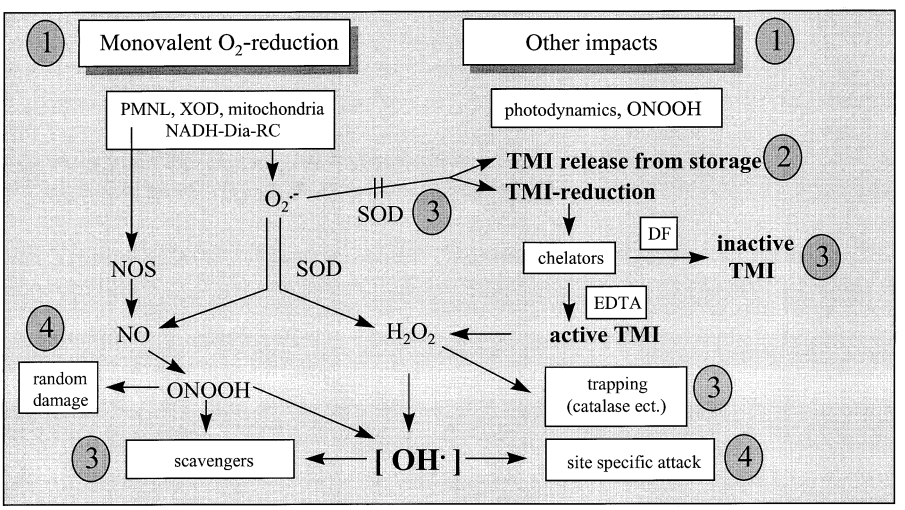


Fig. 3. Phases of TMI-catalyzed ROS toxicity. For more details see text.

*7.2.4. Joint diseases and rheumatism.* Rheumatism, osteoarthritis, gouty inflammations and finally arthrosis are inflammatory diseases involving activated PMNL and TMI during symptom development, counteracted by Zn (as macrophage immobilizer), Se (furnishing the active center of glutathione peroxidase) and the chain-breaking antioxidant, vitamin E [79].

Serum copper is increased in chronic juvenile arthritis [80] and the copper chelator penicillamine (see Section 5) similar to Wilson's disease (see below) is helpful in its treatment [81].

Fe- and XOD- (or PMNL-NADPH oxidase-) catalyzed oxidative depolymerization of the joint lubricant hyaluronic acid [82] during arthritis is seen as a predisposition for arthrosis.

During gouty inflammation, incomplete and thus redox-active complexation of Fe by urate crystals and enhancement of ROS production have been described as possible causes for symptom expression [83].

*7.2.5. Ischemia-reperfusion.* schemia-reperfusion damage of lung, intestine, heart and other organs is connected with ROS formation (especially by the xanthine oxidase reaction and activated PMNL), iron mobilization and thus Fenton/Haber-Weiss chemistry [84]. Reperfusion injury can be ameliorated by iron chelators such as DF, lipophilic siderophores of *Mycobacterium tuberculosis* ('exochelins' [85]; in analogy to the hydrophilic pyochelins of *P. aeruginosa*, see Section 7.1.3).

Other natural products representing both antioxidant and TMI chelator activity ameliorating reperfusion injury are procyanidines from grape (*Vitis vinifera*) seeds [86].

*7.2.6. Other diseases.* Elevated copper levels have been observed in context with ocular inflammation (uveitis) [87] and Wilson's disease as an inborn error in copper metabolism resulting in enhanced copper levels in the liver probably due to the deficiency in a Cu-transporting ATPase. An animal model for this disease has recently been presented [88].

A general simplified scheme characterizing the above mechanisms is shown in Fig. 3.

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