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# Peroxynitritometal complexes

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## Contents



**Abstract**

Peroxynitrite as a ligand can be formed (1) from the reaction of a superoxometal complex with nitrogen monoxide, or (2) as an intermediate in the catalysis of the isomerization to nitrate or the reduction to nitrogen dioxide. With one exception, namely $[(CN)_5ONOOCo]^{3-}$, such complexes have not been isolated. The instances where peroxynitrito complexes have been described or invoked so far are limited to two proteins, (haemoglobin and myoglobin), three macrocyclic complexes, (a rhodium tetraazacyclotetradecane, and manganese and iron porphyrins), cobalt, chromium, and titanium. Upon formation of the complex, isomerization to nitrate can occur within the coordination sphere, or the O─O bond breaks to yield an oxometal complex and nitrogen dioxide. The latter two can recombine to form a nitratometal complex, which subsequently dissociates.


*Keywords:* Peroxynitrite; Isomerization; Catalyst; Peroxynitritometal complex; Haemoprotein

## 1. Introduction

This review describes a new development in the peroxynitrite field, namely the formation and properties of peroxynitritometal complexes, starting with haemoproteins, where such complexes were observed first, followed by metalloporphyrins and their analogs, and it ends with non-porphyrin peroxynitrito–metal complexes.

Peroxynitrite plays an important role in mechanisms of dioxygen toxicity. Research in this area has, over the years, centered on three important species, that are briefly reviewed

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in this introduction in connection with contributions by Professor Taube.

The first major development was the discovery of a function for erythrocuprein by McCord and Fridovich [1], namely catalysis of the dismutation of superoxide. Given the relative lack of reactivity of the superoxide anion, the nature of the oxidizing species was assumed to be the hydroxyl radical formed from the one-electron reduction of the dismutation product hydrogen peroxide by as yet unidentified iron complexes in a process known as the Fenton reaction. As to the mechanism of this reaction, Cahill and Taube favored oxoiron(2+) rather than the hydroxyl radical as a chain carrier based on experiments with labeled hydrogen peroxide [2]. In brief, the culprit is the hydroxyl radical or oxoiron(IV), and protection is afforded by superoxide dismutase and more importantly, catalase. Historical accounts of the reactions involved have been published [3,4].

The second phase started with the observation that superoxide was known to scavenge the endothelium-derived relaxing factor [5], which was subsequently identified as nitrogen monoxide [6,7], derived from arginine [8,9]. In 1990, Beckman et al. proposed that superoxide[1] and nitrogen monoxide, produced by activated macrophages, reacts rapidly to form peroxynitrite [12], which oxidizes and hydroxylates biomolecules. The case for this species was bolstered by observations that superoxide dismutase in many dioxygen-toxicity experiments was protective, without catalase, and that the Fenton reaction is relatively slow [13]. In contrast, the reaction of superoxide with nitrogen monoxide was shown to be diffusion-controlled [14]. Peroxynitrous acid is an unstable species that isomerizes to nitrate at a rate of $1.15\,s^{-1}$ at 25 °C; its p$K_a$ varies from 6.5 to 6.8, depending on ionic strength and temperature [15]. The mechanism of the isomerization is presently being debated, and homolysis to varying extents [16–22] and internal rearrangement [23–26] have been proposed. Anbar and Taube [27] showed 50 years ago that, when hydrogen peroxide was added slowly to excess nitrite, one labeled oxygen was found in nitrate. This observation shows that peroxynitrous acid is able to transfer an oxygen to nitrite, a result recently confirmed in a study of the reaction of [$^{15}$N]nitrite with peroxynitrous acid [28]. It is difficult, if not impossible, to explain this finding with a homolysis model. To the trio: superoxide, hydroxyl, and hydrogen peroxide now nitrogen monoxide, nitrogen dioxide and peroxynitrite had to be added. It should be emphasized that the production of peroxynitrite by activated macrophages and of hypochlorite by activated neutrophils within the context of the immunological response is beneficial to the organism.

The third stage started in 2000, when Wentworth et al. suggested that ozone may be produced by activated neutrophils and by antibodies, in both cases by way of singlet dioxygen [29,30]. Neutrophils may produce it via the well-known reaction of hypochlorite with hydrogen peroxide [31], and antibodies by energy transfer from UV-excited tryptophan to triplet dioxygen [29]. The formation of ozone involves the reaction of singlet dioxygen with water to form dihydrogen trioxide, which may subsequently be oxidized to ozone, or the reaction of hypochlorite with another singlet dioxygen, Reaction 1.

$$ClO^- + {}^1\Delta_g O_2 \rightarrow Cl^- + O_3, \quad -17.5\,kJ\,mol^{-1} \qquad (1)$$

Given that a rate for Reaction-1 has been determined by Yeatts and Taube as $k = 2 \times 10^{-3}\,M^{-1}\,s^{-1}$ at 0 °C [32], the rate for the forward direction is calculated to be ca. $2 \times 10^3\,M^{-1}\,s^{-1}$. Unfortunately, the reaction of ozone with hypochlorite is so fast that detection of the former has not been successful (Kissner and Koppenol, 2003, unpublished). In any case, it is clear that the mechanism of ozone formation has not yet been established. This is typical in the field of dioxygen toxicity: there have been, and there continue to be, lively discussions on the relative merits of various intermediates, i.e. the hydroxyl radical vs. oxoiron(IV), and peroxynitrous acid vs. nitrogen dioxide. All these species are short-lived and their presumed presence is based on complicated kinetic schemes and less than conclusive "footprints".

Metal complexes may stabilize peroxynitrite or accelerate its decay. Decay may involve, in principle, an isomerization to nitrate, a decomposition to nitrite and dioxygen, formation of a metal–nitrogen monoxide complex and superoxide, of a metaloxo complex and nitrogen dioxide, or of a metal–peroxide complex and the nitrosyl cation. We will show below that formation of a metal–nitrogen monoxide complex and the process of decomposition are not observed.

## 2. Haemoproteins

### *2.1. The O-bound peroxynitrite-complexes of metMb and metHb*

In analogy to the very fast reaction between nitrogen monoxide and superoxide, $(1.6 \pm 0.3) \times 10^{10}\,M^{-1}\,s^{-1}$ [14], the dioxygen iron complexes of horse heart oxymyoglobin (oxyMb) and of human oxyhaemoglobin (oxyHb) react rather rapidly with nitrogen monoxide, $(4–9) \times 10^7\,M^{-1}\,s^{-1}$ at pH 7.0 and 20 °C [33]. Because of the high reactivity of nitrogen monoxide towards the oxygenated haem center, diffusion of nitrogen monoxide into the distal pocket is considered to be the rate limiting step [34–36] for a reaction that yields nitrate and the iron(III) forms of myoglobin and haemoglobin. Since the dioxygen complex in oxyMb and oxyHb is best described as an intermediate form between an iron(II)–dioxygen and an iron(III)–superoxide complex [37], it has often been proposed that their reactions with nitrogen monoxide may proceed via iron(III)–peroxynitrite

[1] Systematic names (IUPAC) [10,11]: $O_2^{\bullet -}$, dioxide(•1−), superoxide is allowed; $HO^{\bullet}$, hydridooxygen(•), hydroxyl is allowed; $NO^{\bullet}$, nitrogen monoxide or oxonitrogen(•), nitric oxide is obsolete; $NO_2^{\bullet}$, nitrogen dioxide or dioxonitrogen(•); $ONOO^-$, oxoperoxonitrate(1−), trivial name peroxynitrite; $O_2NOO^-$, dioxoperoxonitrate(1−), trivial name peroxynitrate.

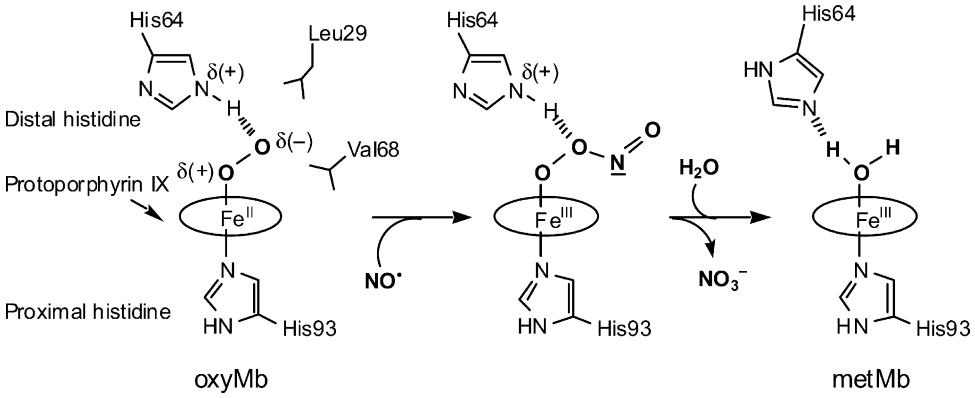
complexes [34,38–40]. In 1998, Herold presented evidence for these short-lived intermediates, which were characterized by rapid-scan UV–vis spectroscopy under alkaline conditions [41]. At neutral or low pH, the rapid decay of $MbFe^{III}OONO$ and $HbFe^{III}OONO$ to the corresponding aquo-complexes and nitrate does not allow for their spectroscopic detection [33,41]. The spectrum of $HbFe^{III}OONO$ (measured at pH 9.5 and 5 °C) displays three absorption maxima at 407 nm ($\varepsilon_{407}$ = 165 $mM^{-1}\,cm^{-1}$), 504 nm ($\varepsilon_{504}$ = 8.7 $mM^{-1}\,cm^{-1}$), and 636 nm ($\varepsilon_{636}$ = 5.4 $mM^{-1}\,cm^{-1}$) [41]. Almost identical absorption features were obtained for the myoglobin intermediate $MbFe^{III}OONO$ [410 nm ($\varepsilon_{410}$ = 138 $mM^{-1}\,cm^{-1}$), 504 nm ($\varepsilon_{504}$ = 8.0 $mM^{-1}\,cm^{-1}$), and 636 nm ($\varepsilon_{636}$ = 3.2 $mM^{-1}\,cm^{-1}$)]; its greater instability made it impossible to obtain an accurate spectrum [33]. These maxima are characteristic for high-spin metHb and metMb derivatives with anionic ligands [42–44].

Very recently, Olson and coworkers measured an EPR spectrum of the intermediate of the reaction between oxyHb and nitrogen monoxide by using rapid-freezing techniques [45]. The observed high-spin signal at $g$ = 6, obtained when oxyHb is mixed with one equivalent of nitrogen monoxide at pH 9.5, is consistent with the assignment of this intermediate as $HbFe^{III}OONO$. Moreover, the decay of the EPR signal correlates with that of the intermediate detected by optical spectroscopy.

Studies of the pH-dependence of the reactions of nitrogen monoxide with oxyMb and oxyHb showed that for both proteins the values of the second-order rate constants do not vary between pH 5.0 and 7.0, but significantly augment at alkaline pH. This increase may be due to a weakening of the hydrogen bond between the coordinated dioxygen and the distal histidine, which must always occur to allow for ligands to enter the distal pocket (Scheme 1) [34]. This weaker interaction may be the result of a general electrostatic effect, that is a consequence of an increase in negative charge on the protein. Alternatively, the acceleration at alkaline pH could be rationalized by assuming that a partial deprotonation of the proximal histidine takes place under basic conditions. This hypothesis is based on the observation that the acidity of the imino proton of imidazole increases on coordination to metal ions [46]. Deprotonation of the histidine bound *trans* to the oxygen ligand in the oxyproteins increases the stabilization of the iron(III)–superoxide form relative to the iron(II)–dioxygen form, weakens the bond between the iron and the oxygen, and thus may bring about the observed acceleration of the reaction at alkaline pH.

Interestingly, the peroxynitrite-complexes of the α- and the β-subunit of haemoglobin decay at different rates (36 and 7 $s^{-1}$ at pH 9.5 and 20 °C) [41]. Moreover, $MbFe^{III}OONO$ decays at an even larger rate (205 $s^{-1}$ at pH 9.5 and 20 °C) [44]. As mentioned above, the decay rates of these intermediates are highly pH-dependent and increase with decreasing pH. Taken together, these data indicate that the stability of the peroxynitrite complexes is regulated not only by the distal histidine, but also by the other amino acid residues present in the distal pocket, which are different in the two subunits of haemoglobin and in myoglobin. Indeed, Alayash and coworkers showed that the rates of decay of the intermediate Fe(III)-peroxynitrite complexes of the α- and the β-subunits of bovine haemoglobin are identical and approximately correspond to that of the slower component of human $HbFe^{III}OONO$ [47].

The decay rates of the metMb– and metHb–peroxynitrite complexes are all significantly larger than that of free peroxynitrite under the same conditions (for instance, 0.11 $s^{-1}$ at pH 9.5 and 25 °C) [48]. This difference can be rationalized by considering the influence of the distal histidine on the isomerization reaction. As depicted in Scheme 1, the distal histidine (His64 in myoglobin) may facilitate the cleavage of the O–O bond by interacting with one of the two oxygen atoms. The acceleration of the decay of the iron(III) peroxynitrite complex may therefore be a consequence of the presence of both the iron and this hydrogen bond which pull from two sides on the O–O bond. This mechanism may also explain the pH-dependence of the decay rates. Indeed, under basic conditions the distal histidine is deprotonated, and thus, cannot form the hydrogen bond responsible for the acceleration of the isomerization of the coordinated peroxynitrite.




Scheme 1. The role of the distal histidine (His64) for the isomerization of peroxynitrite bound to the iron(III) center of myoglobin.

Further mechanistic studies showed that, in contrast to previous reports [40], no free peroxynitrite is formed during the reactions of nitrogen monoxide with these oxyproteins [33]. Analysis of the proteins after cycling the oxidation by nitrogen monoxide and the reduction with ascorbic acid for 10 times indicates that less than 1% of the tyrosine residues are nitrated [33]. These results show that, when peroxynitrite is coordinated to the haem of myoglobin or haemoglobin, it rapidly isomerizes to nitrate, and thus cannot nitrate the tyrosine residues of the globin.

Interestingly, the iron(III)peroxynitrite complex is not detected when the aquoiron(III) forms of Mb and Hb (metMb and metHb) are allowed to react with peroxynitrite under neutral or alkaline conditions [49,50]. Indeed, the reactivity of metMb towards peroxynitrite is regulated by the presence of the distal histidine (His64 in myoglobin), which partly blocks the active site and stabilizes, via a strong hydrogen bond, the water ligand coordinated to the iron (see metMb structure in Scheme 1) [51]. In the presence of wild-type metMb the isomerization of peroxynitrite is only slightly accelerated ($k_{cat}$ = $(7.7 \pm 0.1) \times 10^4\,M^{-1}\,s^{-1}$ at pH 7.0 and 20 °C [50] and $k_{cat} = 1.03 \times 10^4\,M^{-1}\,s^{-1}$ at pH 7.6 [52]. It is conceivable that the first step of this reaction is represented by a ligand substitution reaction. The coordinated water is replaced by peroxynitrite and the peroxynitrito-complex $MbFe^{III}OONO$ is generated. As discussed above, since $MbFe^{III}OONO$ decays too rapidly to be detected under neutral conditions, no spectral changes would be expected in the course of this reaction and nitrate would be generated quantitatively. Indeed, it has been shown that in the presence of a large excess of metMb (750 μM), that is when the decay of peroxynitrite proceeds exclusively via the protein-catalyzed pathway, no nitrite is generated [50].

Groves and coworkers proposed an alternative mechanism for the metMb-catalyzed decay of peroxynitrite [52]. According to their hypothesis, the decay of $MbFe^{III}OONO$ proceeds in three steps: homolytic cleavage of the O–O bond of the peroxynitrito ligand with generation of nitrogen dioxide and the ferryl form of the protein, partial recombination to the nitrate complex, and finally dissociation of nitrate. Part of nitrogen dioxide eludes the reaction with ferrylMb and undergoes hydrolysis to nitrite and nitrate. This mechanism was supported by their observation that, in the presence of 100–200 μM metMb, the nitrate yields generated from the decay of 500 μM peroxynitrite leveled off around 11% [52]. Nevertheless, no experiments were carried out with higher metMb concentrations to confirm this hypothesis.

The metmyoglobin mutant in which His64 was replaced by alanine (H64A) is an efficient catalyst for the isomerization of peroxynitrite ($k_{cat}$ = $(1.3 \pm 0.1) \times 10^7\,M^{-1}\,s^{-1}$ at pH 7.0 and 20 °C) [53]. Ion chromatographic analysis of the nitrogen-containing products showed that in the presence of 0.01 equivalents of H64A peroxynitrite decays quantitatively to nitrate [51], in agreement with a concerted isomerization of the coordinated peroxynitrite without liberation of nitrogen dioxide. Moreover, HPLC-analysis revealed that 0.05 equivalents of H64A prevent nitration of free tyrosine by peroxynitrite almost completely. No changes are observed in the UV–vis spectra of H64A metMb treated with peroxynitrite. In particular, the intermediate peroxynitrite complex that must be formed in the first reaction step was never detected [51].

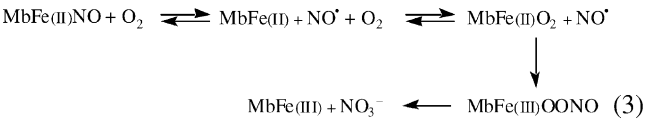
### 2.2. The N-bound peroxynitrite-complexes of metMb and metHb

The reactivity of dioxygen toward metal nitrosyl complexes has been thoroughly investigated, in particular because of the possible use of these complexes to activate dioxygen [54]. One of the conceivable pathways for this reaction may involve the formation of a N-bound peroxynitrite complex (Reaction 2).

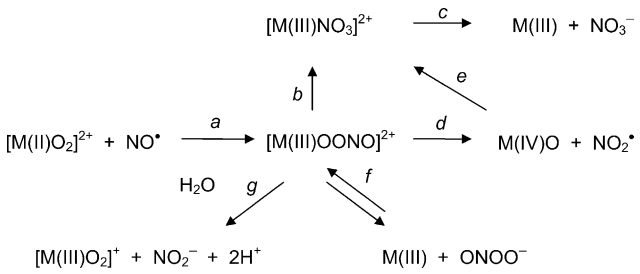
$$M^nNO + O_2 \rightarrow M^{(n+1)}N(O)OO \quad (2)$$

The oxidation of MbFe(II)NO, the pigment found in cured meat, has been studied since the 1950s, particularly to understand the processes responsible for its degradation during storage of meat [55,56]. In 1992, Skibsted and coworkers determined that the oxidation products of this reaction are metMb and nitrate [57]. Moreover, they found that the first-order rate constant of MbFe(II)NO oxidation is very similar to that of nitrate formation. No intermediates accumulated during the course of the reaction, but the slight increase of the oxidation rate with increasing partial pressure of oxygen led these authors to conclude that the reaction proceeds via initial binding of oxygen to MbFe(II)NO followed by an intramolecular rearrangement to nitrate and metMb [57]. However, the studies by Skibsted and coworkers were carried out in the presence of excess ascorbate. In 1996, Bohle and coworkers reinvestigated this reaction and showed that the oxidation of purified MbFe(II)NO by dioxygen is not a first-order transformation [58]. Detailed kinetic analysis of the results indicated that the reaction proceeds according to Reaction 2 via an intermediate, assigned as the corresponding N-bound peroxynitrite-iron(III) complex MbFe(III)N(O)OO [58]. The kinetics of this reaction were studied by measuring the absorbance changes between 390 and 680 nm. The spectrum of MbFe(III)N(O)OO, calculated by subjecting the measured spectra to factor analysis by singular value decomposition, is almost identical to that of the O-bound species discussed above [58]. The rate constants for the formation of MbFe(III)N(O)OO (from the reaction of MbFe(II)NO with an excess of dioxygen) and its isomerization to metMb and nitrate are both significantly lower than those for the corresponding O-bound complex, $1.34 \times 10^{-3}$ and $2.82 \times 10^{-3}\,s^{-1}$, at pH 7.0 and 37 °C [58]. However, the similarity of the rate of formation of MbFe(III)N(O)OO to that of nitrogen monoxide dissociation from MbFe(II)NO ($1.2 \times 10^{-4}\,s^{-1}$, at pH 7.0 and 22 °C [59]) may suggest that the reaction rather proceeds via an alternative pathway according to which dissociation of nitrogen monoxide is the rate-determining step. Dioxygen may then bind to iron(II) myoglobin, and the fast

reaction of oxyMb with nitrogen monoxide may generate the O-bound peroxynitrite complex and, finally, metMb and nitrate (Reaction 3) [54].




(3)




Scheme 3. Formation of peroxynitritometal complexes and products.

## 3. The O-bound peroxynitrite-complex of metalloporphyrins

### 3.1. Iron

Water-soluble iron porphyrin complexes such as 5,10,15,20-tetrakis-(*N*-methyl-4′-pyridyl)porphinatoiron(III), [Fe(III)(TMPyP)]$^{5+}$, 5,10,15,20-tetrakis-(2,4,5-trimetyl-3,5-sulfonatophenyl)porphinatoiron(III), [Fe(III)TMPS]$^{3-}$, and 5,10,15,20-tetrakis-(4′-sulfonatophenyl)porphinatoiron(III), [Fe(III)TPPS]$^{3-}$, have also been shown to efficiently catalyze the isomerization of peroxynitrite to nitrate [60–63]. The mechanism of this reaction is complex and involves two distinct pathways, one involving the iron(III) complex and the other the high-valent oxoiron(IV) complex (Scheme 2) [61]. When [Fe(III)(TMPyP)]$^{5+}$ was allowed to react with two equivalents of peroxynitrite (at pH 7.4 and 25 °C) the trace collected at 427 nm (the absorbance maximum of [oxoFe(IV)(TMPyP)]$^{4+}$) indicated the very rapid formation of an intermediate (estimated $k > 5 \times 10^7\,M^{-1}\,s^{-1}$), which was assigned as the peroxynitrite complex [Fe(III)(OONO)(TMPyP)]$^{4+}$ [61]. Unfortunately, no UV–vis spectrum of this species was reported to support this assignment. As shown in Scheme 2, this intermediate was proposed to generate [oxoFe(IV)(TMPyP)]$^{4+}$ and nitrogen dioxide by homolytic cleavage of the O–O bond (apparent decay rate 62.2 $s^{-1}$, at pH 7.4 and 25 °C).

### 3.2. Manganese

In 1995, Groves and Marla discovered that [Mn(III)(TMPyP)]$^{5+}$ reacts rapidly with peroxynitrite to generate [oxoMn(IV)(TMPyP)]$^{4+}$ [64]. In contrast to the analogous iron(III) complex, the manganese(III) compound is not a catalyst for peroxynitrite decay, because the reduction of [oxoMn(IV)(TMPyP)]$^{4+}$ to [Mn(III)(TMPyP)]$^{5+}$, required to close the catalytic cycle, proceeds very slowly (0.018 $s^{-1}$ at pH 7.4 and 28 °C) [65]. The formation of the high-valent intermediate is very likely to proceed via homolytic O–O bond cleavage of a Mn(III)–peroxynitrite complex. This reaction must be very fast, as the intermediate [Mn(III)(OONO)(TMPyP)]$^{4+}$ has never been observed.

In the presence of reducing agents such as ascorbate, glutathione, or Trolox, which rapidly reduce the oxoMn(IV)-intermediate, Mn(III)–porphyrin complexes are very efficient catalysts for peroxynitrite decay [66]. In the last decade, the potential use of Mn(III)–porphyrin complexes for pharmacological applications (peroxynitrite scavengers) has led to the design of numerous porphyrin ligands to optimize the reactivity of these catalysts under physiological conditions [67] (and references cited therein). In the same class of compounds fall manganese(II) texaphyrins, similar in structure to manganese porphyrins, which are oxidized by peroxynitrite, and, in the presence of a reductant, act catalytically to decompose peroxynitrite [68].

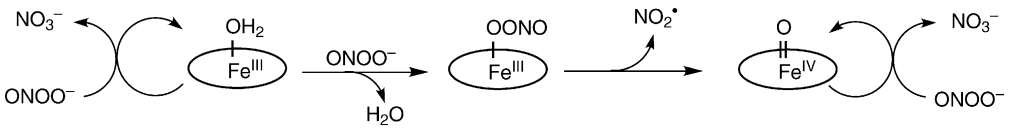
## 4. Non-porphyrin peroxynitritometal complexes

### 4.1. Cobalt

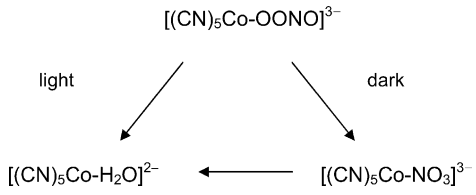
The observation of a fast reaction between nitrogen monoxide and oxy-haemoproteins with formation of a transient peroxynitrite complex [41] triggered the idea that such a reaction of nitrogen monoxide with pentacyanodioxygencobaltate(II) might yield a relatively stable pentacyanoperoxynitritocobaltate(III) complex, Reaction 4.

$$[(CH_3CH_2)_4N]_3[(CN)_5Co(II)O_2] + NO^{\bullet} \rightarrow [(CH_3CH_2)_4N]_3[(CN)_5Co(III)OONO] \quad (4)$$

The complex is indeed formed; it is soluble in water and absorbs in the UV with a shoulder at 280 nm [69]. In Scheme 3, this reaction is represented by process a and b. At pH 2, the coordinated peroxynitrite disappears with a rate constant of $9.6 \times 10^{-6}\,s^{-1}$. In the dark, this process is slower and results in the novel pentacyanonitratocobaltate complex.




Scheme 2. Simplified model proposed for the iron(III)porphyrin-catalyzed decomposition of peroxynitrite (adapted from references [61,62].




Scheme 4. Decay of the $[(CN)_5(OONO)Co]^{3-}$ complex.

At pH 6, the peroxynitrito complex appears to be stable, if kept in the dark (Scheme 4). Photolysis destroys the complex and the aquapentacyanocobaltate(III) complex is formed [70]. It is thought that the cyano ligands play an important role in diminishing the Lewis acid character of the cobalt ion, thereby stabilizing the peroxynitrito ligand. Were this not the case, the metal could simply catalyze the isomerization. Regrettably, no material suitable for X-ray analysis was obtained.

Attempts to isolate a peroxynitrito complex from the dioxygen complex of tris(pyrazolyl)boratecobalt(II) and nitrogen monoxide in tetrahydrofuran or toluene were not successful [71]. At temperatures below −61 °C an intermediate was observed, which may have been the desired peroxynitrito complex. Equimolar amounts of the nitrito and nitrato complex were obtained. The reaction of the corresponding dinitrogen−cobalt complex with nitrogen monoxide resulted in a novel nitrosyl compound, which, upon exposure to dioxygen, did not yield the peroxynitrito complex [71].

### 4.2. Rhodium

Upon mixing nitrogen monoxide with the dioxygen complex of β-*trans*-meso-$Me_6$-[14]-ane-$N_4$(aqua)rhodium(II), the absorption of the dioxygen complex at 271 nm disappears and an intermediate with a weaker absorption near 240 nm is formed within the mixing time of a stopped-flow spectrophotometer [72]. Because this intermediate does not appear to react with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{2-}$), iron(II) or *trans*-[14]-ane-$N_4$ nickel(II), it is thought to be nitrate bound to rhodium(III), and the rate constant of its formation, encompassing the reaction of nitrogen monoxide with the dioxygen rhodium complex and the isomerisation to nitrate, must be $10^6\,M^{-1}\,s^{-1}$ or higher (Reaction 5).

$$[14]\text{-ane-}N_4(H_2O)Rh(II)O_2 + NO^\bullet \rightarrow [14]\text{-ane-}N_4(H_2O)Rh(III)NO_3 \quad (5)$$

The "240 nm" intermediate decays slowly at acid pH, while, at higher pH, the rate constant approaches a limiting value of ca. $2 \times 10^{-3}\,s^{-1}$. The macrocyclic nickel complex has at low pH nitrate and water as axial ligands; loss of a hydron from the coordinated water appears to promote release of nitrate. Nitrate was found in solution; its increase with time matched the disappearance of the intermediate. Based on experiments where twice as much $ABTS^{2-}$ as the macrocyclic nickel complex (see 4.3.) was oxidized when the rhodium–dioxygen complex was mixed with nitrogen monoxide, it was concluded that the decay of the postulated initial peroxynitrite complex appears to involve, to an extent of ca. 40%, homolysis of the O–O bond of peroxynitrite to form oxorhodium(IV) and nitrogen dioxide. No kinetics could be established and the hypothesis is based on yields only. Thus, pathways a, b, c and a, d, e, c (Scheme 3) occur at the same time. Unexplained is a small amount of nitrate that is formed very rapidly [72]. It was possible to generate the intermediate from nitrogen monoxide that is formed slowly by the disproportionation of nitrous acid; as the other product of the disproportionation is nitrogen dioxide, also the peroxynitratorhodium(III) complex was produced [73].

### 4.3. Chromium

Photolysis at 266 nm of the nitrosylchromium(II) complex in the presence of dioxygen yields initially the (dioxido)chromium(II) complex that reacts rapidly – $k = 7 \times 10^8\,M^{-1}\,s^{-1}$ – with the just liberated nitrogen monoxide to form a transient peroxynitrito complex [74].

$$[CrNO]^{2+} + O_2 + h\nu \rightarrow [CrO_2]^{2+} + NO^\bullet \rightarrow [CrOONO]^{2+} \quad (6)$$

No spectrum of this intermediate could be established and the absorption at 293 nm decays rapidly to the background level. The products are nitratochromium(III), and chromium(III) and nitrate. The evidence for formation of peroxynitrite is based on the zero-order oxidation of *trans*-[14]-ane-$N_4$ nickel(II), presumably by nitrogen dioxide formed by homolysis of the bound peroxynitrite. Also the reaction in the presence of excess nitrogen monoxide is interpreted in terms of homolysis: three nitrogen monoxide molecules are consumed: one to form the peroxynitrito complex, one that reacts with nitrogen dioxide from the homolysis reaction and one that reacts with oxochromium(IV) (Scheme 3, reactions a and d). A direct reaction between the peroxynitritochromium(III) complex or the oxochromium(IV) and the nickel macrocycle cannot be excluded, but is deemed unlikely. The (dioxido)chromium(II) complex can be used as a catalyst for the co-oxidation of alcohols and nitrous acid [75]. Like the macrocyclic rhodium complex [72] discussed above, the (dioxido)chromium(II) complex also reacts with nitrogen dioxide, with a rate constant of $2.3 \times 10^8\,M^{-1}\,s^{-1}$ [74] and yields ultimately a nitratochromium complex [76].

### 4.4. Titanium

The reaction between excess oxotitanium(IV) and peroxynitrous acid in 0.9 M $H_2SO_4$ leads to an increase in absorption at 410 nm. The reaction proceeds with a rate constant of $k = 3.0 \times 10^2\,M^{-1}\,s^{-1}$ at 25 °C, as compared

to $4.9 \times 10^2\,M^{-1}\,s^{-1}$ for the reaction of oxotitanium(IV) with hydrogen peroxide [77]. At higher oxotitanium(IV) to peroxynitrous acid ratios saturation is observed. It seems likely that there is first the fast formation of a colorless end-on complex, hydroxido(peroxynitrito-$\kappa$O′)-titanium(2+), followed by rearrangement to a yellow side-on complex ($k \approx 20\,s^{-1}$), (peroxynitrito-$\kappa$O,$\kappa$O′)-titanium(3+). With peroxynitrous acid in excess, this rearrangement proceeds more slowly ($k \approx 0.1\,s^{-1}$), probably because multiple peroxynitrous acid molecules form end-on complexes with oxotitanium(IV) and hinder the rearrangement to the side-on complex. The absorption spectrum of the final product is that of yellow (dioxido)titanium(IV) (Scheme 3, reactions f and g). Presumably, during the rearrangement or later, the nitrosyl cation is lost, as has been observed to a minor extent for the peroxynitritorhodium complex [72]. These observations provide evidence for direct formation, previously unobserved, of a peroxynitrous acid-metal complex [78].

## 5. Concluding remarks

Formation of a peroxynitritometal complex, either directly or via the reaction of a dioxygenmetal ion with nitrogen monoxide, is in most cases inferred and not directly observed. This complex can isomerize to a nitratometal complex, or undergo homolysis to form the oxometal complex and nitrogen dioxide. More rarely, the peroxynitritometal complex loses a nitrosyl cation and a peroxide complex is formed. These possibilities are summarized in Scheme 3. Rapid catalysis of isomerization of peroxynitrite to nitrate by a metal ion would require fast formation of the peroxynitritometal complex, followed by isomerization within the coordination sphere of the metal and dissociation. In Scheme 3, this reaction sequence starts at the lower right, moves to the center and then up to the right, with nitrate and a catalysis-ready metal ion as products. As to homolysis, center line in Scheme 3, the results of the photolysis experiments with the peroxynitritocobalt complex suggest that it could be important to establish to what extent homolysis has been brought about by light.

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