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Review

# Reactive nitrogen species generated by heme proteins: Mechanism of formation and targets

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## Contents



**Abstract**

Nitration of tyrosine residues in proteins represents a pathological event that is associated with several human and animal diseases. Besides the classical pathways of formation of reactive nitrogen species (RNS) by NO oxidation, several studies show that heme peroxidases also play an important role in RNS generation. The mechanism of generation of these species has been studied in detail focusing on the nitration of several tyrosine and tryptophan derivatives. Also the $O_2$-storage and $O_2$-carrier heme proteins, myoglobin and hemoglobin, can induce RNS formation and promote self-nitration and oxidation. These reactions bear biological relevance and, therefore, the identification of the sites of endogenous modification of these proteins has been carried out by proteomic analysis.


*Keywords:* Peroxidase; Myoglobin; Hemoglobin; Reactive nitrogen species; Peroxynitrite; Nitrogen dioxide

## 1. Introduction

The small free radical species NO has been implicated in a variety of biological functions, including respiration, nerve transmission, apoptosis, modulation of vascular tone and homeostasis [1]. It can undergo oxidation or reduction reactions, leading to nitrosonium ($NO^+$) or nitroxyl ($NO^-$) ions, respectively [2,3]. $NO^+$ is responsible for a number of electrophilic reactions [4] but is too reactive to exist in aqueous solution where it immediately forms nitrous acid. On the other hand, $NO^-$ results from the ionization of HNO [5], that might occur in cells [6–8]. In addition, in analogy with protein phosphorylation, NO induces S-nitrosation of protein sulfhydryls as a widely used mode of transmitting cellular signals [9]. Evidence for the activity of NO as a modulator of protein functions has been amply reported in the literature for caspases (a family of proteolytic enzymes that execute programmed cellular death), transcription factors, matrix metalloproteases (implicated in the pathogenesis of stroke, neurodegenerative disease and cellular metastasis) and viral proteins [9]. On the other hand, overproduction of NO appears to contribute to tissue injury and inflammatory states, and collective evidence from in vitro and in vivo studies indicates that in these conditions several reactive species can be formed, either simultaneously or at different times, depending on cell type and the stimulus [10–12]. These species can be generated directly from NO or from nitrite, the major product of NO oxidative metabolism, which is more stable and thus longer lasting than NO [13].

*Abbreviations:* Hb, human hemoglobin; hhMb, horse heart myoglobin; HMb, human myoglobin; HRP, horseradish peroxidase; LPO, lactoperoxidase; RNS, reactive nitrogen species; ROS, reactive oxygen species

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Protein nitration is an easily detectable post-translational modification occurring in the pathogenesis of numerous diseases, including chronic inflammation, cardiovascular pathologies and neurodegenerative diseases [14–16]. This modification is a consequence of intense production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), when this overwhelms the compensatory antioxidant capacity of the cells. Nitrogen dioxide ($NO_2$) and peroxynitrite ($ONOO^-$) are powerful cytotoxic RNS that can be derived directly from NO [17,18]; they can trigger nitration or oxidation of aromatic or sulfur containing amino acid residues [19–21], DNA damage [22] and initiate lipid peroxidation [23]. An alternative pathway for the production of RNS which is receiving increasing attention is that dependent on peroxidases. This is typically associated with inflammatory processes, where activation of heme peroxidases represents the most important defense mechanism towards the infiltrated phagocytes [14], but recently the presence of peroxidase-like activity has been established also in the brain substantia nigra, where an enzyme apparently similar to lactoperoxidase seems to be involved [24,25]. Therefore, the general properties of peroxidases as well as their presence in a wide variety of cells and tissues [14,24] suggest a pivotal role for these enzymes in oxidative reactions and in the generation of RNS [17,26–29]. The key substrate for peroxidases in the generation of RNS is nitrite, the major product of NO metabolism, that has long been considered inert under physiological conditions [30,31]. The main targets of peroxidase dependent nitrations are tyrosine and tryptophan derivatives, including those contained in proteins [17,26,29]. Addition of a nitro group to tyrosine lowers the $pK_a$ of its phenolic group by 2–3 units and adds a bulky substituent; when this occurs in relevant tyrosines, nitration can alter protein function and conformation, impose steric restrictions and also inhibit tyrosine phosphorylation [27]. Even though nitrotyrosine formation is a well accepted footprint of the presence of NO-derived oxidants, a few proteins are currently known to acquire different functions after exposure to RNS. Among these, cytochrome *c* gains a strong peroxidase activity after nitration [32], nitrated fibrinogen accelerates clot formation [33] and protein kinase Cε becomes activated and translocates on nitration [34].

Nitration of tryptophan will likely have biological implications. In the first place, nitrotryptophan formation can inhibit generation of tryptophan-derived neurotransmitters (i.e. serotonin) or interfere with their action. Secondly, nitration of critical tryptophan residues in proteins may alter their function [35]. Loss of tryptophan residues in proteins incubated with RNS has also been shown to occur, by the disappearance of tryptophan fluorescence [36–38], amino acid analysis [39,40] and, more recently, by HPLC-MS measurements [41]. Yakamura et al. [40] reported that a partial inactivation of human recombinant Cu,Zn-superoxide dismutase in vitro is caused by modification of the single Trp32 residue by peroxynitrite in the presence of bicarbonate, although the reaction product was not identified. It has been suggested that nitrated free or protein-bound tryptophan may be able to propagate RNS damage, through a radical mechanism capable of inducing further oxidative stress [42].

Besides peroxidases, other metalloproteins are able to generate RNS and, in general, the same proteins producing RNS are potential targets for these species in the absence of scavenging substrates. Among others, Mb and Hb must be taken into account because of their abundance in tissues [43] and in the vasculature [44], respectively. Both proteins have considerable prooxidant activity, since they easily react with $H_2O_2$ [45,46] and $NO_2^-$ [46–49] promoting reactions that can be represented in a peroxidase-like cycle. For instance, following a period of ischemia, reperfusing cardiac tissue with oxygenated blood results in a damaging burst of oxidative stress [50]. The resulting myocardial injury is characterized by depletion of endogenous antioxidants and release of cardiac enzymes, proteins, including Mb, ROS and RNS into circulation [51]. Actually, recent studies have shown that both Mb and Hb play important roles in the pathology of certain disease states linked to the interaction of peroxides and reactive nitrogen species with heme proteins [46,52–54]. The discovery of heme to protein cross-linked forms of Mb and Hb in vivo are definitive markers of the participation of these proteins in oxidative reactions [46].

Here, we will give an overview of the mechanisms of the peroxidase catalyzed nitration of tyrosine and tryptophan derivatives focusing on our contribution to the field. We will also describe the covalent modifications of amino acid side chains and the heme prosthetic group of Mb and Hb induced by RNS under oxidative and nitrative stress conditions.

## 2. Mechanism of formation of RNS by peroxidases

Collectively, the in vitro and in vivo studies indicate that several nitrating species can be formed under nitrative stress conditions [10–12]. One of the mechanisms by which NO triggers its toxicity is its nearly diffusion-controlled reaction with superoxide anion to give peroxynitrite, $ONOO^-$ [55,56]. Alternatively, NO can be easily oxidized to nitrogen dioxide, e.g. by dioxygen, through $N_2O_3$. A third pathway includes nitrous acid formation from nitrite at acidic pH. However, recent studies have revealed that nitration pathways involving heme proteins are relevant in vivo [57–59]. In fact, both heme peroxidases [26,60,61], metMb [47,48] and metHb [62] have been shown to be able to oxidize nitrite in the presence of hydrogen peroxide to give RNS that, in turn, promote nitration of phenolic compounds and tyrosyl residues of proteins [26,47,48,61,62]. The most common mechanism of peroxidase dependent nitration occurs through the one-electron oxidation of nitrite by the classical enzyme intermediates known as compounds I and II to nitrogen dioxide (Fig. 1, pathway A). However, the results presented by Dunford and co-worker [60] and recently by us using a group of substrates derived from tyrosine [26,47,48,61] are incompatible with the classical peroxidase mechanism proceeding through nitrogen dioxide. Using the two molecules of *p*-cyanophenol and phenylacetic acid as mechanistic probes, we could show that two competing mechanisms are operative, depending on nitrite concentration. Phenylacetic acid is unreactive both towards $NO_2$ and the peroxidase/$H_2O_2$/$NO_2^-$ system when the concentration of nitrite is low; though, it is easily nitrated (and oxidized) by chemically generated peroxynitrite

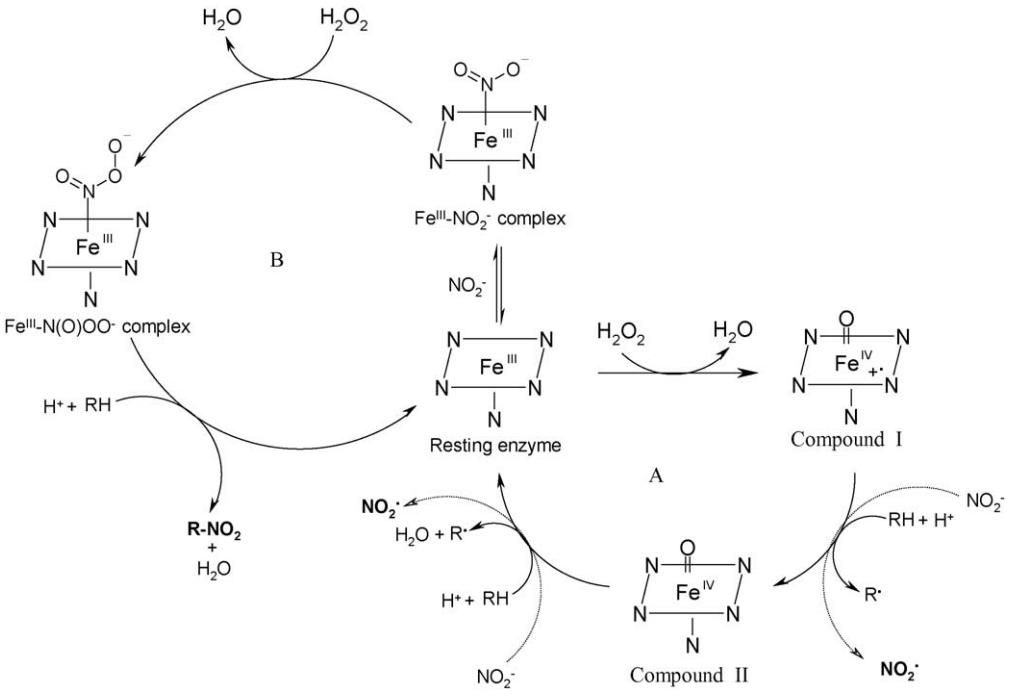


Fig. 1. Catalytic pathways in heme-protein induced nitration. (A) The classical peroxidase cycle including the ferric form and the compounds I and II intermediates. (B) Nitrite binds to the heme, and the resulting nitrite adduct reacts with $H_2O_2$ generating an unprecedented Fe(III)-N(O)OO$^-$ intermediate with unique spectroscopic and reactivity features.

and by the enzymatic system when the amount of nitrite is increased [61]. In contrast, *p*-cyanophenol is a poor substrate for chemically generated peroxynitrite whereas its nitration occurs during the enzymatic reaction promoted by peroxidases, even at low concentration of nitrite. Interestingly, the reaction is almost completely inhibited when the concentration of nitrite becomes very high (≈0.5 M). These observations suggest that nitration of *p*-cyanophenol can only occur by a radical coupling mechanism involving nitrogen dioxide [61]. Thus, the dominant mechanism for the enzymatic nitration of phenolic substrates is the classical peroxidase pathway, involving nitrogen dioxide, at low nitrite concentration (Fig. 1, pathway A), but it changes to another mechanism when the concentration of nitrite is higher. The latter mechanism involves an active species with reactivity similar to that of peroxynitrite (Fig. 1, pathway B). Interestingly, the range of nitrite concentrations where the change of mechanism occurs depends strongly on the nature of the enzyme: with the mammalian enzyme LPO, pathway B is dominating except at very low (e.g. below 1 mM) nitrite concentration, while with the plant enzyme HRP pathway A is dominating except at very high (e.g. above 0.1 M) nitrite concentration.

To gain information on the mechanism of enzymatic nitration, we tried to trap intermediates that are elusive at room temperature by studying the reaction of HRP in a mixed aqueous-organic solvent at −12 °C under turnover conditions [63]. At low nitrite concentration (<20 mM), both in the presence or absence of a phenolic substrate, upon addition of hydrogen peroxide, the spectrum of HRP corresponds to that of the compound II intermediate (Soret band at 418 nm, visible bands at 526 and 564 nm). At higher nitrite concentration (200 mM), HRP behaves differently if a phenol is present in solution or not. When only nitrite is present, the addition of hydrogen peroxide produces the typical compound II spectrum, but when also a phenol is present, the spectrum of an intermediate species that corresponds neither to compound I nor to compound II is produced, with Soret band at 419 nm (sharper and more intense with respect to compound II), visible bands at 536 and 568 nm [63]. This spectrum is very similar to that of the Fe(III)-$NO_2^-$ adduct of the enzyme (see Table 1) and, therefore, we attribute it to the intermediate species Fe(III)-N(O)OO$^-$, i.e. a *N*-bound peroxynitrite adduct, that presumably is a powerful nitrating agent.

## 3. Nitration of tyrosine and tryptophan derivatives

The main targets of RNS are lipids [23], DNA [22] and proteins [19–21]. Among the amino acids, RNS react promptly with tyrosine, cysteine, triptophan and methionine residues (see Fig. 2). We investigated in detail the kinetics of nitration of a series of tyrosine derivatives by HRP and LPO in the presence of nitrite and hydrogen peroxide [61]. These studies show that the reaction rates follow saturation behavior both with respect to phenol and nitrite, thus indicating that the reaction proceeds through a ternary complex between enzyme, nitrating species

Table 1
UV-Visible spectra of HRP derivatives in a solvent mixture of 10 mM phosphate buffer pH 7.5 and methanol (1:1) at −12 °C

| Derivative | Soret band (nm) | Visible bands (nm) |
|---|---|---|
| Compound II | 418 | 526, 564 |
| HRP Fe(III)-N(O)OO$^-$ | 419 | 536, 568 |
| HRP Fe(III)-$NO_2^-$ | 418 | 534, 568 |

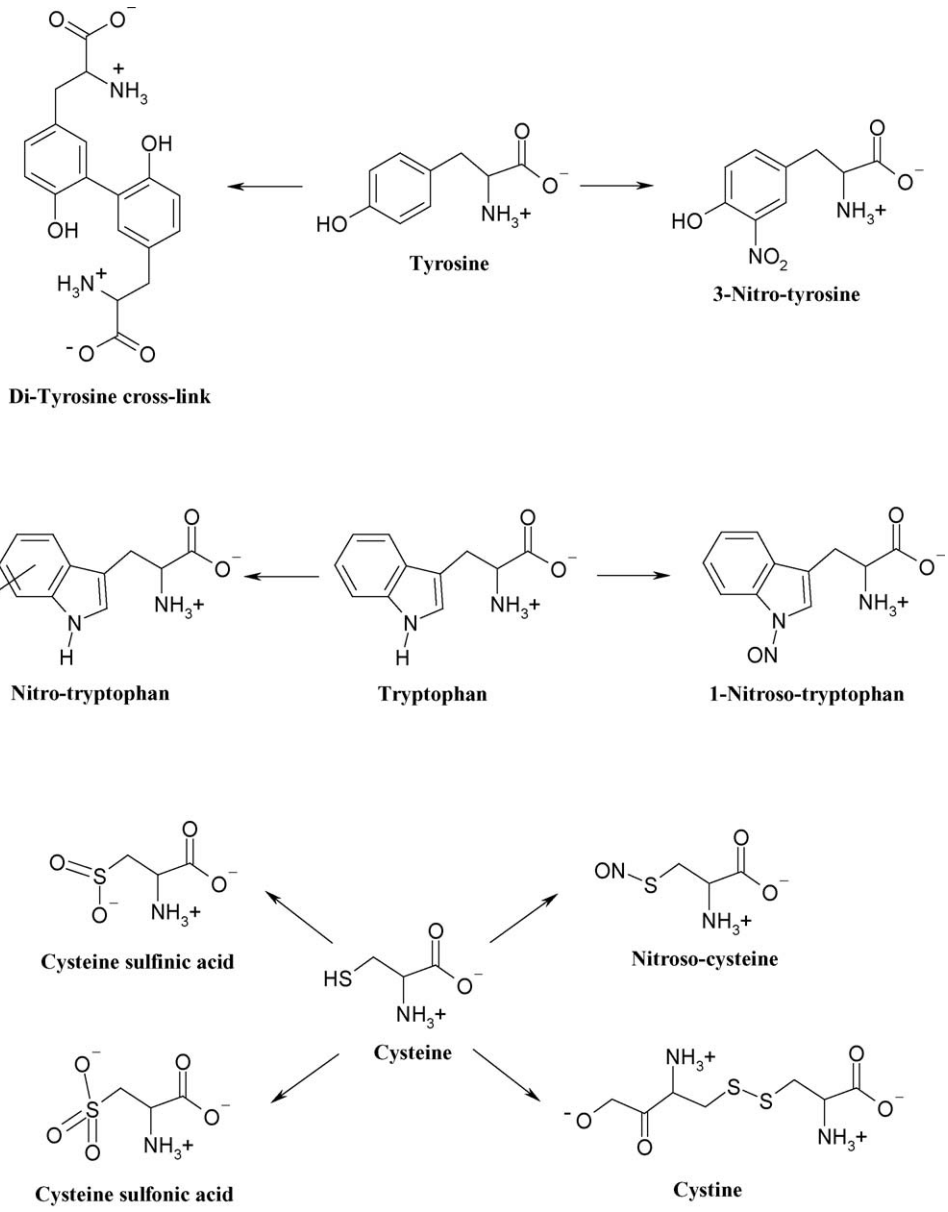


Fig. 2. Covalent modification of tyrosine, tryptophan and cysteine residues induced by RNS.

and the phenol. The nitration reaction is in competition with the classical peroxidase phenol coupling reaction, but the importance of the latter reaction decreases upon increasing nitrite concentration. The competition arises because the phenolic substrates undergo one-electron oxidation to phenoxy radicals by the peroxidase active species, according to the reaction scheme in Fig. 1, pathway A. The same enzyme intermediates oxidize nitrite to nitrogen dioxide and, therefore, phenoxy radicals can give coupling reaction to dimeric products or react with nitrogen dioxide to form nitrophenol, but $NO_2$ can also directly react with the phenol to extract a hydrogen atom and generate a phenoxy radical (Fig. 3). In the peroxynitrite pathway B, nitrophenol formation occurs by direct attack of the Fe(III)-N(O)OO$^-$ intermediate on the phenol, without generation of radical species. Interestingly, the $K_M$ values for the phenolic substrates in the nitration reactions are one to two orders of magnitude smaller

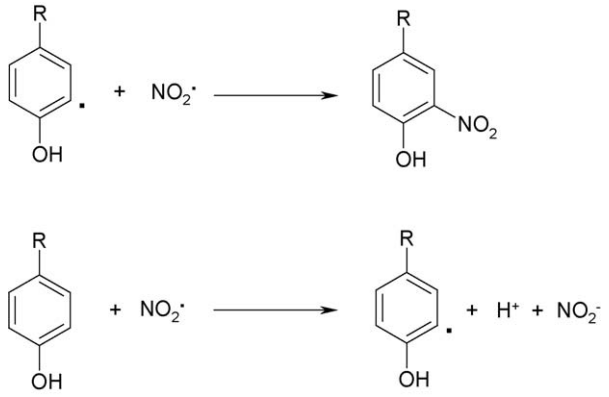


Fig. 3. Coupling between $NO_2$ and tyrsosyl radical leading to 3-nitrotyrosine; $NO_2$ can also directly extract a hydrogen atom from tyrosine and generate a phenoxy radical.

than the $K_M$ parameters of the same substrates in the classical peroxidase-catalyzed phenol coupling reactions [64,65]. This indicates that the enzymatic reactions occur with the substrate bound at different protein sites in the two cases, and in particular that the phenol must bind close to the heme in the normal peroxidase reaction, but not necessarily so in the nitration process, where enzyme-generated RNS can diffuse and react with the phenol bound near or at the protein surface.

Nitration of tyrosine derivatives by nitrite and hydrogen peroxide has been studied also using horse heart Mb [47], human Mb [48] and human Hb [62]. In general, the efficiency of the $O_2$ binding proteins in the catalytic reactions is much lower than that of peroxidases, and in this case the $K_M$ values are similar to those found for the corresponding catalytic oxidation of phenols to phenol dimers. This indicates that the same binding site for the phenol is maintained in the two types of processes. However, it is worth noting that the $k_{cat}$ values for phenol nitration are one order of magnitude larger than those for phenol oxidation [45].

The nitration of tryptophan derivatives is more complicated than that of tyrosine under many respects [66]. Due to its higher redox potential, tryptophan is less reactive than tyrosine towards RNS. Moreover, unlike tyrosine, the reaction of RNS with tryptophan produces nitrated isomers at various positions of the indole ring, and also nitrosation at the nitrogen atom and oxidative opening of the indole ring can occur. The enzymatic nitration of tryptophan derivatives performed with LPO or HRP in the presence of $NO_2^-$ and $H_2O_2$ showed that nitration occurs at positions 4-, 6-, 7- and $N^1$ of the indole ring, and that nitrosation occurs at the indole nitrogen atom (Fig. 4). Positions 2 and 5 of the indole nucleus appear to be unaffected. In these reactions, the product composition was found to change as a function of nitrite concentration. The nitrated isomer at position 6 was always the most abundant, but it is interesting that the amount of $N^1$-NO derivative was found to increase in the product mixture on increasing nitrite concentration. Two mechanisms could be hypothesized to account for the production of $N^1$-NO. The first one depends on the faster dimerization of $NO_2$ to $N_2O_4$ at high nitrite concentration, which is known to react with amines producing nitrosated and nitrated products [67]. The alternative pathway is the direct nucleophilic nitrosation of the N-H group by the enzyme generated peroxynitrite species [66]. A similar reaction has been observed for free peroxynitrite with secondary amines [67] and gluthatione [68]. In the enzymatic reactions, we found no evidence for other oxidation products, such as *N*-formylkynurenine, which has been often reported as one of the products of the tryptophan reaction with RNS [69].

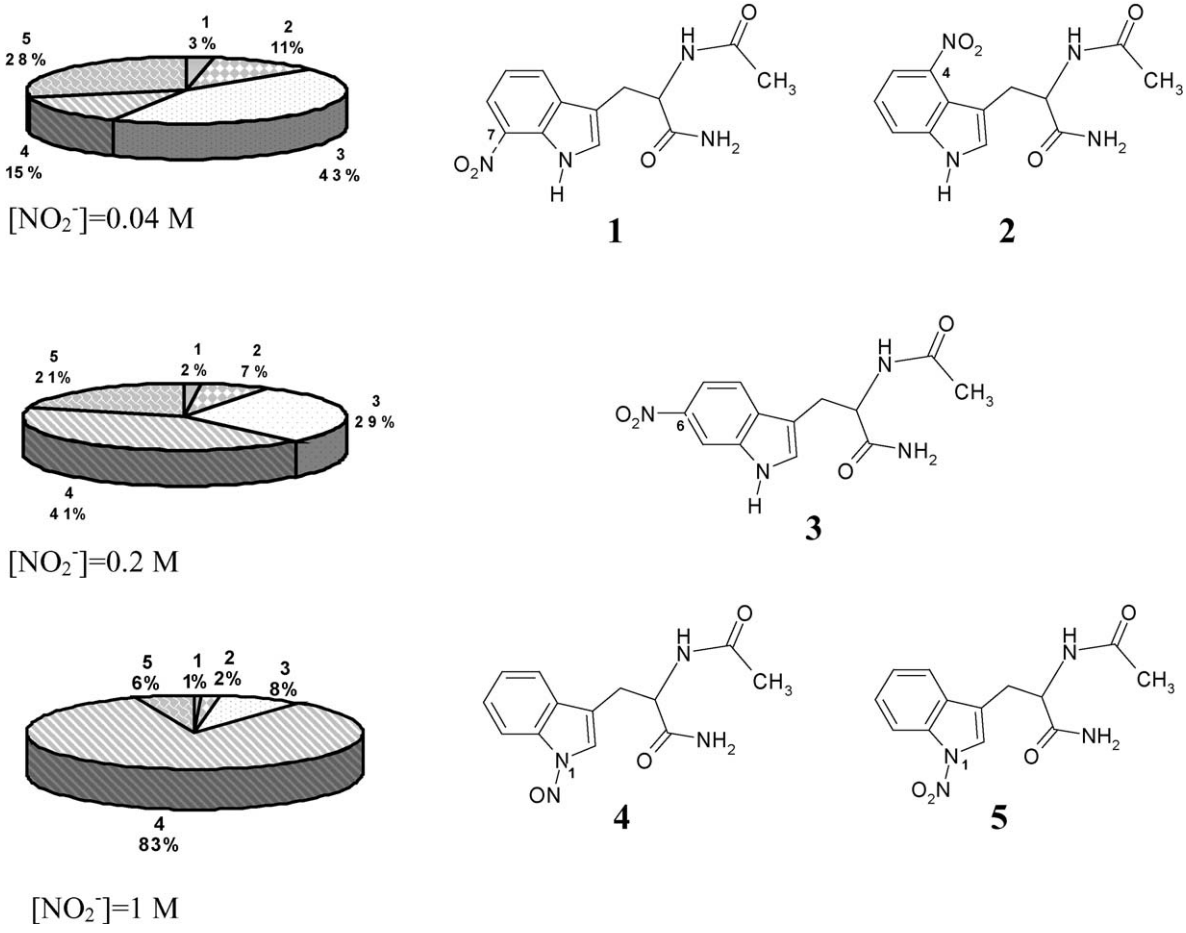


Fig. 4. Structure of the nitrotryptophan and nitrosotryptophan derivatives obtained in the peroxidase-catalyzed reaction. The percent yield of the products generated by HRP/$H_2O_2$/$NO_2^-$ at various nitrite concentrations is schematically indicated.

## 4. Nitration and oxidation of proteins by RNS

In the absence of an exogenous substrate, all the proteins generating RNS undergo self nitration and possibly oxidation at polar amino acid residues. Several factors influence the amount and the selectivity of the modification: (1) the proximity to the site of generation of the nitrating species; (2) the localization of the protein; (3) the abundance of tyrosine, cysteine and tryptophan residues in the amino acid sequence; (4) the presence of specific residues in the primary sequence that can assist formation of RNS [11]. We have investigated in detail the reactions undergone by the met forms of hhMb [47], HMb [48] and Hb [62] in the presence of nitrite and hydrogen peroxide. Myoglobins contain in their primary sequence at least four amino acid residues which can be target for RNS: Tyr103, Tyr146, Trp7 and Trp14. In addition, HMb contains a cysteine residue, Cys110, that could also undergo modification.

The experiments carried out in our group on hhMb showed that the tyrosine closest to the heme and more exposed to the surface, Tyr103, remains unaffected, while both the porphyrin and Tyr146, which is an inner residue farther from the heme, undergo nitration [47]. Tyr146 is located in the proximity of one of the four conserved cavities of Mb, the so called Xe1 cavity [70,71], where the nitrating species (i.e. $NO_2$ or $ONOO^-$), once produced by the heme, is able to diffuse, accumulate and suitably orient to perform the reaction leading to nitrotyrosine. Different results were obtained with HMb [48]. The human protein is much more prone to oxidation with respect to hhMb. Here the protein induced RNS promotes derivatization at the protein residues nearest to the heme, Tyr103 and Cys110, leading to nitrotyrosine and sulfinic acid formation, respectively (Fig. 5). Only when the nitrative conditions become more severe, both the heme prosthetic group and Tyr146 also undergo nitration. In analogy with hhMb, we have no evidence for tryptophan modification in the protein. In order to draw a comparison between hhMb and HMb (see Table 2), we have to take into account the presence of the unique Cys110 residue in the heme environment of HMb, that is able to capture the RNS and prevent its reaction with the porphyrin or diffusion to further sites of the protein [48]. Therefore, conversion of the heme cofactor to nitrated heme amounted to 50% of the starting protein for hhMb, while heme nitration occurs only to the extent of 8% in HMb under the same harsh conditions [47,48]. Nitrogen dioxide and peroxynitrite are so effective in the nitration and oxidation of tyrosine and cysteine in HMb that any possible intermolecular dityrosine or dicysteine cross-linking is prevented.

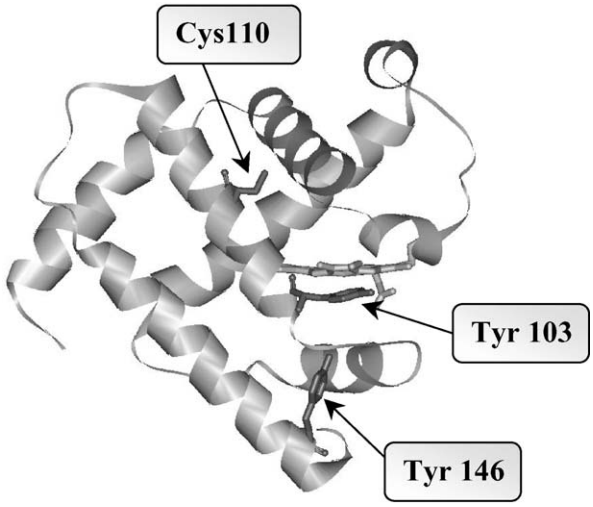


Fig. 5. Computer simulated three-dimensional structure of the K45R/C110A HMb mutant in which the alanine at position 1 1 0 has been replaced with a cysteine residue by the Swiss-PdbViewer Version 3.7 programme. The disposition of the side chains of Tyr103, Cys110 and Tyr146, representing the situation presumably present in native HMb, is shown.

The situation is completely different with Hb [62]. The reaction of the protein with nitrite and hydrogen peroxide gives rise to tyrosyl radicals which have sufficient lifetime to couple and generate dityrosine cross-linked dimers. The Tyr residues linked in these protein dimers have not been identified yet, but Edman analysis shows that the dimer is formed by coupling of the α and β subunits. No evidence for the presence of αα or ββ dimers has been obtained. In addition, RNS mediate nitration at Tyr residues of the αβ dimer, as confirmed by Western blot analysis, as well as at the other α and β subunits, and at the heme cofactors. According to MS data, nitration occurs at a single Tyr residue of the α-chain and at two Tyr residues of the β-chain. The modified residues have been identified as α-Tyr42, β-Tyr130 and β-Tyr145 (Fig. 6). Examination of the crystal structure of the protein reveals that α-Tyr42 is closest to the α-heme (∼4 Å), while both β-Tyr130 and β-Tyr145 are farther from β-heme, at about 10.5 and 8.6 Å distance, respectively. However, both tyrosines seem to lie in a suitable environment to undergo derivatization. In particular, among the residues in the neighbors of β-heme, β-Phe71 is part of an "hydrophobic tunnel" of Phe71-Trp15-Tyr130 which can possibly be involved in mediating RNS attack to β-Tyr145. Interestingly, none of the three tryptophan residues (α-Trp14, β-Trp15 and β-Trp37) has been nitrated, and no cysteine (α-Cys104, β-Cys93, β-Cys112)

Table 2
Endogenous protein targets for RNS in Mbs and Hb in the presence of nitrite and hydrogen peroxide

| Protein | $NO_2$-heme | $NO_2$-Tyr | Cys-$SO_2H$ |
|---|---|---|---|
| hhMb, harsh conditions (0.8 M $NO_2^-$, 1 mM $H_2O_2$) | (50) | Y146 (6) | [a] |
| HMb, mild conditions (0.1 mM $NO_2^-$, 1 mM $H_2O_2$) | (0) | Y103 (44) | C110 (76) |
| HMb, harsh conditions (0.8 M $NO_2^-$, 1 mM $H_2O_2$) | (8) | Y103 (54), Y146 (10–20) | C110 (95) |
| α-HHb | (∼50% total) | Y42 (30) | Not found |
| β-HHb | | Y130 (4), Y145 (63) | Not found |

Note that no nitrotryptophan has been detected under the conditions employed. The data in parenthesis refer to the percent modification relative to the total protein.
[a] hhMb does not contain cysteine residues.

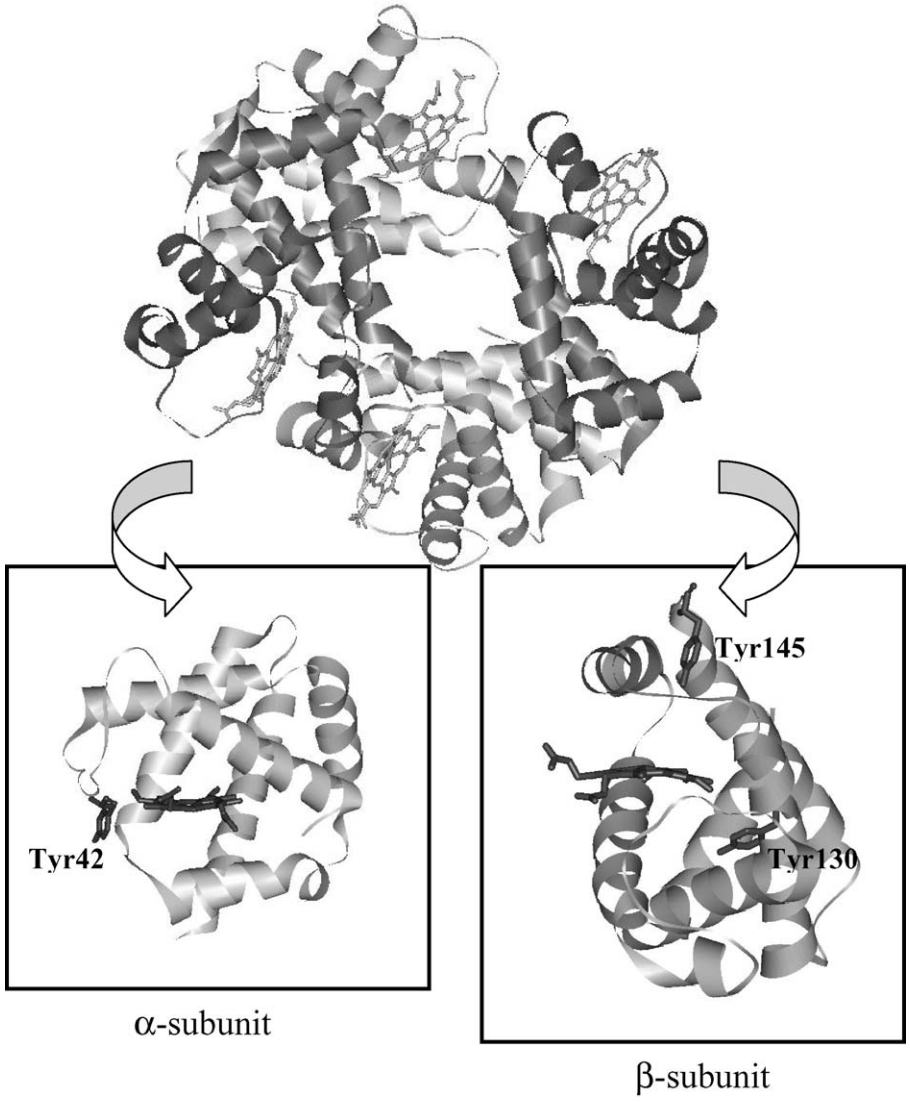


Fig. 6. Spatial arrangement of the tyrosine residues that undergo nitration in human Hb upon treatment with nitrite and hydrogen peroxide.

has been oxidized. But in Hb no cysteine occurs so close to the heme as it is in HMb.

It is worth noting that our data differ from those reported for the nitration reactions of Mb [72,73] and Hb [74] with chemically generated peroxynitrite. These differences may be ascribed to the fact that free peroxynitrite would primarily attack the outer, more exposed residues on the protein surface. In contrast, in our experiments the RNS ($NO_2$ or $ONOO^-$) are generated within the protein active site, in an inner pocket, and consequently the pattern of nitration is completely different.

## 5. Concluding remarks

In this review we summarized our contribution to the search of new reactivities by heme proteins in the presence of nitrite. Albeit with different efficiency, peroxidases, Mb and Hb promote nitrite oxidation to $NO_2$ and $ONOO^-$ in the presence of hydrogen peroxide. Exogenous tyrosine and tryptophan derivatives undergo nitration, and eventually nitrosation, with high yields. Moreover, heme proteins are, at the same time, potential sources and potential targets of the generated RNS. We have identified the sites of endogenous nitration or oxidation undergone by Mb and Hb by proteomic analysis. The different behavior of these proteins is determined by several factors, including: (i) the solvent exposure of tyrosine residues, (ii) the distance between the target residue and the heme center where the RNS is generated and (iii) the ability to convey oxidizing equivalents from the heme to nearby amino acid residues such as cysteine or tyrosines and from these residues to other targets, the latter processes involving long-range electron transfer or electron tunnelling.

## References

[1] L.J. Ignarro (Ed.), Nitric Oxide Biology and Pathobiology, Academic Press, San Diego, 2000.
[2] J.S. Stamler, M. Feelisch, in: M. Feelish, J.S. Stamler (Eds.), Methods in Nitric Oxide Research, Wiley, Chichester, UK, 1996, pp. 19–27.
[3] M.N. Hughes, Biochim. Biophys. Acta 1411 (1999) 263.

[4] D.R. Arnelle, J.S. Stamler, Arch. Biochem. Biophys. 318 (1995) 279.
[5] S.E. Bari, M.A. Martì, V.T. Amorebieta, D.A. Estrin, F. Doctorovich, J. Am. Chem. Soc. 125 (2003) 15272.
[6] J.M. Fukuto, A.J. Hobbs, L.J. Ignarro, Biochem. Biophys. Res. Commun. 196 (1993) 707.
[7] L. Chazotte-Aubert, S. Oikawa, I. Gilibert, F. Bianchini, S. Kawanishi, H. Ohshima, J. Biol. Chem. 274 (1999) 20909.
[8] W.K. Kim, Y.B. Choi, P.V. Rayudu, P. Das, W. Asaad, D.R. Arnelle, J.S. Stamler, S.A. Lipton, Neuron 24 (1999) 461.
[9] S. Miersch, B. Mutus, Clin. Biochem. 38 (2005) 777.
[10] J.S. Beckman, W.H. Koppenol, Am. J. Physiol. 271 (1996) C1424.
[11] H. Ischiropoulos, Biochem. Biophys. Res. Commun. 305 (2003) 776.
[12] S. Moncada, A. Higgs, N. Engl. J. Med. 329 (1993) 2002.
[13] L.J. Ignarro, J.M. Fukuto, J.M. Griscavage, N.E. Rogers, R.E. Byrns, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 8103.
[14] P.C. Dedon, S.R. Tannenbaum, Arch. Biochem. Biophys. 423 (2004) 12.
[15] I.V. Turko, F. Murad, Pharmacol. Rev. 54 (2002) 619.
[16] Y. Zhao, Z. Gao, H. Li, H. Xu, Biochim. Biophys. Acta 1675 (2004) 105.
[17] A. van der Vliet, J.P. Eiserich, B. Halliwell, C.E. Cross, J. Biol. Chem. 272 (1997) 7617.
[18] S. Pfeiffer, B. Mayer, B. Hemmens, Angew. Chem. Int. Ed. 38 (1999) 1714.
[19] A. Daiber, M. Mehl, V. Ullrich, Nitric Oxide 2 (1998) 259.
[20] R. Radi, J.S. Beckman, K.M. Bush, B.A. Freeman, J. Biol. Chem. 266 (1991) 4244.
[21] C. Quijano, B. Alvarez, R.M. Gatti, O. Augusto, R. Radi, Biochem. J. 322 (1997) 167.
[22] T. Douki, J. Cadet, B.N. Ames, Chem. Res. Toxicol. 9 (1996) 3.
[23] R. Radi, J.S. Beckman, K.M. Bush, B.A. Freeman, Arch. Biochem. Biophys. 288 (1991) 481.
[24] J. Everse, P.W. Coates, Free Radic. Biol. Med. 37 (2004) 839.
[25] L. Galzigna, A. De Iuliis, L. Zanatta, Clin. Chim. Acta 300 (2000) 131.
[26] L. Casella, E. Monzani, R. Roncone, S. Nicolis, A. Sala, A. De Riso, Environ. Health. Persp. 110 (2002) 709.
[27] R. Radi, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 4003.
[28] T.B. Brück, R.J. Fielding, M.C.R. Symons, P.J. Harvey, Eur. J. Biochem. 268 (2001) 3214.
[29] M. Brennan, W. Wu, X. Fu, Z. Shen, W. Song, H. Frost, C. Vadseth, L. Narine, E. Lenkiewicz, M.T. Borchers, A.J. Lusis, S.L. Hazen, J. Biol. Chem. 277 (2002) 17415.
[30] T. Lauer, M. Preik, T. Rassaf, B.E. Strauer, A. Deussen, M. Feelisch, M. Kelm, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 12814.
[31] D. Tsikas, J.C. Frölich, Clin. Sci. 103 (2002) 107.
[32] A. Cassina, R. Hodara, J. Souza, L. Thomson, L. Castro, H. Ischiropoulos, B.A. Freeman, R. Radi, J. Biol. Chem. 275 (2000) 21409.
[33] C. Vadseth, J.M. Souza, L. Thomson, A. Seagraves, C. Nagaswami, T. Scheiner, J. Torbet, G. Vilaire, J.S. Bennett, J.C. Marciano, V. Muzykantov, M.S. Penn, S.L. Hazen, J.W. Weisel, H. Ischiropoulos, J. Biol. Chem. 279 (2004) 8820.
[34] Z. Balafanova, R. Bolli, J. Zhang, Y. Zheng, J.M. Pass, A. Bhatnagar, X.L. Tang, O. Wang, E. Cardwell, P. Ping, J. Biol. Chem. 277 (2002) 15021.
[35] B. Alvarez, H. Rubbo, M. Kirk, S. Barnes, B.A. Freeman, R. Radi, Chem. Res. Toxicol. 9 (1996) 390.
[36] H. Ischiropoulos, A.B. Mehdi, FEBS Lett. 364 (1995) 279.
[37] J.P. Eiserich, J. Butler, A. van der Vliet, C.E. Cross, B. Halliwell, Biochem. J. 310 (1995) 745.
[38] Y. Kato, S. Kawakishi, T. Aoki, K. Itakura, T. Osawa, Biochem. Biophys. Res. Commun. 234 (1997) 82.
[39] K. Kikugawa, T. Kato, Y. Okamoto, Free Radic. Biol. Med. 16 (1994) 373.
[40] F. Yamakura, T. Matsumoto, T. Fujmura, H. Taka, K. Murayama, T. Imai, K. Uchida, Biochim. Biophys. Acta 1548 (2001) 38.
[41] T. Suzuki, H.F. Mower, M.D. Friesen, I. Gilibert, T. Sawa, H. Ohshima, Free Radic. Biol. Med. 37 (2004) 671.
[42] M.J. Davies, S. Fu, R.T. Dean, Biochem. J. 305 (1995) 643.
[43] M. Brunori, Trends Biochem. Sci. 26 (2001) 21.
[44] A. Denicola, J.M. Souza, R. Radi, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 3566.
[45] R. Roncone, E. Monzani, S. Nicolis, L. Casella, Eur. J. Inorg. Chem. (2004) 2203.
[46] J. Everse, N. Hsia, Free Radic. Biol. Med. 22 (1997) 1075.
[47] S. Nicolis, E. Monzani, R. Roncone, L. Gianelli, L. Casella, Chem. Eur. J. 10 (2004) 2281.
[48] S. Nicolis, A. Pennati, E. Perani, E. Monzani, A.M. Sanangelantoni, L. Casella, Chem. Eur. J. 12 (2005) 749–757.
[49] S. Herold, Free Radic. Biol. Med. 36 (2004) 565.
[50] R. Bolli, M.O. Jeroudi, B.S. Patel, O.I. Aroma, B. Halliwell, E.K. Lai, P.B. Mccay, Circ. Res. 65 (1989) 607.
[51] R. Bolli, Cardiovasc. Drugs Ther. 2 (Suppl.) (1991) 249.
[52] M.R. Gunther, V. Sampath, W.S. Caughey, Free Radic. Biol. Med. 26 (1999) 1388.
[53] A.I. Alayash, P.P. Rakesh, R.E. Cashon, Antiox. Redox Signal. 3 (2001) 313.
[54] B.J. Reeder, D.A. Svistunenko, C.E. Cooper, M.T. Wilson, Antiox. Redox Signal. 6 (2004) 954.
[55] N.V. Blough, O.C. Zafiriou, Inorg. Chem. 24 (1985) 3502.
[56] W.H. Koppenol, J.J. Moreno, W.A. Pryor, H. Ischiropoulos, J.S. Beckman, Chem. Res. Toxicol. 5 (1992) 834.
[57] J.B. Sampson, Y.Z. Ye, H. Rosen, J.S. Beckman, Arch. Biochem. Biophys. 356 (1998) 207.
[58] J.P. Eiserich, M. Hristova, C.E. Cross, A.D. Jones, B.A. Freeman, B. Halliwell, A. van der Vliet, J. Biol. Chem. 391 (1998) 393.
[59] U. Burner, P.G. Furtmüller, A.J. Kettle, W.H. Koppenol, C. Obinger, J. Biol. Chem. 275 (2000) 20597.
[60] R. Roman, H.B. Dunford, Eur. J. Biochem. 66 (1976) 607.
[61] E. Monzani, R. Roncone, M. Galliano, W.H. Koppenol, L. Casella, Eur. J. Biochem. 271 (2004) 895.
[62] M. Barbieri, R. Roncone, E. Monzani, L. Casella, in preparation.
[63] R. Roncone, E. Monzani, L. Casella, in preparation.
[64] L. Casella, S. Poli, M. Gullotti, C. Selvaggini, T. Beringhelli, A. Marchesini, Biochemistry 33 (1994) 6377.
[65] E. Monzani, A.L. Gatti, A. Profumo, L. Casella, M. Gullotti, Biochemistry 36 (1997) 1918.
[66] A. Sala, S. Nicolis, R. Roncone, L. Casella, E. Monzani, Eur. J. Biochem. 271 (2004) 2841.
[67] M. Masuda, H.F. Mower, B. Pignatelli, I. Celan, M.D. Friesen, H. Nishino, H. Hoshima, Chem. Res. Toxicol. 13 (2000) 301.
[68] A. van der Vliet, P.A. Hoen, P.S. Wong, A. Bast, C.E. Cross, J. Biol. Chem. 273 (1998) 30255.
[69] T.J. Timat, H. Steinhart, J. Agric. Food Chem. 46 (1998) 490.
[70] H. Frauenfelder, B.H. McMahon, P.W. Fenimore, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 8615.
[71] M. Brunori, Q.H. Gibson, EMBO Rep. 2 (2001) 674.
[72] J.L. Bourassa, E.P. Ives, A.L. Marqueling, R. Shimanovich, J.T. Groves, J. Am. Chem. Soc. 123 (2001) 5142.
[73] S. Herold, K. Shivashankar, M. Mehl, Biochemistry 41 (2002) 13460.
[74] M. Minetti, D. Pietraforte, V. Carbone, A.M. Salzano, G. Scorza, G. Marino, Biochemistry 39 (2000) 6689.