Journal of Photochemistry & Photobiology, B: Biology 226 (2022) 112369

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Effect of ionizing radiation on human myeloperoxidase: Reaction with hydrated electrons

Daniel R. Ramos [a,*], M. Isabel Fernández [a], Paul G. Furtmüller [b], Christian Obinger [b], M. Victoria García [a], J. Arturo Santaballa [a], Moisés Canle [a,*]

[a] Universidade da Coruña, Chemical Reactivity & Photoreactivity Group (REACT!), Department of Chemistry, CICA & Faculty of Sciences, A Zapateira s/n, E-15071 A Coruña, Spain

[b] Department of Chemistry, Institute of Biochemistry, BOKU-University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria

ARTICLE INFO

Keywords:
Myeloperoxidase
Pulse radiolysis
Chlorinating activity
Oxidative stress
Inflammation

ABSTRACT

Myeloperoxidase (MPO) is a myeloid-lineage restricted enzyme largely expressed in the azurophilic granules of neutrophils. It catalyses the formation of reactive oxygen species, mainly hypochlorous acid, contributing to anti-pathogenic defense. Disorders in the production or regulation of MPO may lead to a variety of health conditions, mainly of inflammatory origin, including autoimmune inflammation.

We have studied the effect of ionizing radiation on the activity of MPO, as measured by the capacity retained by the enzyme to produce hypochlorous acid as reactive oxygen species after exposure to successive doses of solvated electrons, the strongest possible one-e$^-$ reducing agent in water. Chlorination activity was still present after a very high irradiation dose, indicating that radiation damage does not take place at the active site, hindered in the core of MPO structure. Decay kinetics show a dependence on the wavelength, supporting that the process must occur at peripheral functional groups situated on external and readily accessible locations of the enzyme.

These results are relevant to understand the mechanism of resistance of our innate anti-pathogenic defense system and also to get insight into potential strategies to regulate MPO levels as a therapeutic target in autoimmune diseases.

1. Introduction

Myeloperoxidase (MPO), a heme-containing dominant granule enzyme largely expressed in the azurophilic granules of neutrophils, plays a fundamental role in the production of reactive oxygen species, mainly hypochlorous acid (HClO), and drives cell-mediated antimicrobial activity in the human innate immune system. [1,2] Disorders in the production or regulation of MPO may lead to a variety of health conditions, mainly of inflammatory origin, including autoimmune inflammation. [3]

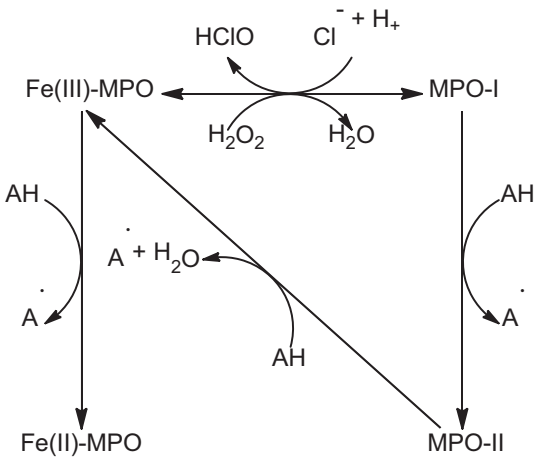
General halogenation and peroxidase activity of MPO is summarized in Scheme 1. Native ferric state of MPO (Fe(III)-MPO) reacts with hydrogen peroxide (H_2O_2) to form a ferryl π-cation radical complex called compound I (MPO-I). One-electron reduction of this redox intermediate leads to compound II (MPO-II), which may undergo another one-e$^-$ reduction to yield starting native Fe(III)-MPO. Furthermore, ferric compound may also undergo one- e$^-$ reduction to produce the ferrous species Fe(II)-MPO.

The main reaction mediated by MPO under physiological conditions is the oxidation of Cl^- by compound I to yield the highly-reactive chlorinating agent HClO [4] and/or the chlorinating complex MPO-I-Cl. [5] The high reduction potential of MPO-I allows the two- e$^-$ oxidation of Cl^-, [6] which confers MPO a unique role within the peroxidase-cyclooxygenase superfamily. [7–9] The major function of the initially formed chlorinating agents as well as derived chloramines, which also have oxidizing and chlorinating activity, [10] is probably to attack essential microbial cell elements, in a defense mechanism.

Most cellular components, including proteins, are susceptible of being damaged by harmful chemicals such as reactive oxygen species (ROS). [11] In fact, MPO promotes the generation of several ROS; furthermore, concurrent with MPO release from neutrophils, a burst of NADPH oxidase activity converting oxygen to superoxide anion ($O_2^{\bullet-}$) takes place. [12] Thus, MPO is particularly exposed to these highly reactive species that may modify the protein arrangement, then

* Corresponding authors.
E-mail addresses: daniel.rramos@udc.es (D.R. Ramos), moises.canle@udc.es (M. Canle).

https://doi.org/10.1016/j.jphotobiol.2021.112369
Received 9 August 2021; Received in revised form 31 October 2021; Accepted 24 November 2021
Available online 27 November 2021



Scheme 1. Normal chlorination and peroxidase cycle as applied to MPO, also showing formation of ferrous intermediate and HClO.

affecting its normal function. Because of its very nature and function, MPO structure should be rather resistant to oxidative environments, but this is possibly not the case under highly reductive conditions. Here, we examine the behaviour of MPO when exposed to hydrated electrons (e^-_{aq}), [13,14] and its sensibility towards reaction with this highly-reactive and non-selective species, which is the strongest possible reductant in water ($E^\circ = -2.9$ V *vs.* NHE). [15] Assessment of the enzymatic activity was performed based on the chlorination activity of the system after successive doses of ionizing radiation.

Even though abundant literature is available on the effect of ionizating radiation on biomolecules, [16,17] there is a lack of studies showing the effect of ionizing radiation (including high energy UV) on enzyme activity of peroxidases. Some researchers studied the photochemical modification of the chromophore of MPO, [18] while other studies focused on the reactions of superoxide anion with several peroxidases [19–21] including the reactions with the redox intermediates of MPO shown in Scheme 1, extensively investigated by Kettle, Winterbourn, and coworkers. [12,22,23] Reactions promoted by hydroxyl radicals ($HO^\bullet$) have also been reported. [21,24,25]

However, the processes involving e^-_{aq} have not been investigated so thoroughly [26,27] and their interaction with MPO has not been examined to date. We discuss the effect of e^-_{aq} on MPO activity in aqueous solution at pH = 5.0, at which *in vivo* MPO chlorination activity is at its maximum, [28–30] and at which phagocytosis takes place, [31] or at pH = 7.0, typical of peroxidation reactions [28,29].

Pulse radiolysis is a very powerful technique for studying short-lived free radicals and excited states using a beam of highly accelerated electrons, [32] as it allows the selective generation of a given radical using appropriate scavenging conditions to get rid of the undesired species, keeping only those of interest. MPO was reacted with e^-_{aq}, to test the potential effect of one- e^- reduction processes on Fe(III)-MPO, MPO-I, and MPO-II and the consequent effect on its chlorination activity.

2. Material and Methods

Highly purified MPO of a purity index (A_{430}/A_{280}) $\geq$ 0.84 was obtained from Planta Natural Products (http://www.planta.at), a stock solution of *ca.* 0.60 μM and pH = 7 (5 mM phosphate buffer) was used. Its concentration was determined spectrophotometrically using ε_{430} = 91,000 $M^{-1}{\cdot}cm^{-1}{\cdot}heme^{-1}$ for the Soret band of native ferric state. [33]

Stock solutions of glycine (Gly, Fischer Chemicals, Analytical Reagent Grade), potassium chloride (KCl, Sigma-Aldrich, ACS Reagent), and hydrogen peroxide (H_2O_2, Aldrich, 27.5 wt%) were prepared daily and kept in the dark at 4 °C. The concentration of H_2O_2 was determined using ε_{240} = 39.4 $M^{-1}{\cdot}cm^{-1}$. [34] Citric acid (Sigma, anhydrous) / phosphate (Na_2HPO_4, BDH, AnalaR grade) buffer, 0.5 M, was used at pH 5.0, while phosphate buffer (NaH_2PO_4, BDH, AnalaR grade), 0.1 M, was employed at pH 7.0. Potassium thiocyanate (KSCN, BDH, AnalaR grade) was used for the dosimetry, and *tert*-butanol (*t*-BuOH, Sigma, HPLC grade) as $HO^\bullet$ scavenger. All solutions were prepared with deionized water.

Pulse radiolysis experiments were carried out at the Free Radical Research Facility at the SRS Daresbury Laboratory (Cheshire, UK) using 2 μs, *ca.* 2 kRad (=20 Gy) pulses, from a 12 MeV linear electron accelerator (LINAC), such that *ca.* 12 μM of primary species (H^+, $HO^\bullet$ and e^-_{aq}) were produced. Transient absorption spectra were obtained using a Xe source and either a gated diode array or a photomultiplier for detection. Reaction kinetics were monitored by optical detection, and the dosimetry carried out with N_2O-saturated 10 mM KSCN solutions, taking $\varepsilon((SCN)_2^{\bullet-}$ at 500 nm) = 7100 $M^{-1}{\cdot}cm^{-1}$ and $G(HO^\bullet) = 6.0$.[1] [35] All solutions were deaerated with ultrapure N_2. The temperature was in all cases kept to within ±0.1 K using a thermostatted cell (*ca.* 298 K).

Ionizing radiation on aqueous solution promotes the generation of primary species H^+, $HO^\bullet$ and e^-_{aq} according to (1):

$$H_2O \rightsquigarrow H^+ + HO^\bullet + e^-_{aq} \quad (1)$$

Subsequent to water irradiation, H^+ is conveniently neutralized with a buffer solution, while $HO^\bullet$ is effectively scavenged with *t*-BuOH as in (2):

$$HO^\bullet + (CH_3)_3COH \rightarrow H_2O + {}^\bullet CH_2C(CH_3)_2OH \quad (2)$$

In this way, e^-_{aq} remain available for reaction with MPO (reaction 3):

$$e^-_{aq} + MPO \rightarrow MPO^{\bullet-} \rightarrow \ldots \rightarrow \ldots \quad (3)$$

Detailed description of pulse radiolysis experiments has been described elsewhere. [16,36]

2.1. Reduction of MPO-I

According to preliminary experiments, [37] formation of compound I was achieved by reaction of native enzyme with the adequate amount of H_2O_2. 1 mL samples of MPO-I containing 100 nM of the enzyme, a 10-fold excess of H_2O_2, 1 mM of phosphate buffer (pH = 7.0), and 1 vol% *t*-BuOH were readily prepared in a 2.5 cm-pathlength Suprasil quartz cuvette. They were bubbled with and kept under N_2. This cuvette was directly placed in the axis of the LINAC source so that it was aligned with the analysing beam. Vanishing of MPO-I (430 nm) and formation of MPO-II (456 nm) were monitored spectroscopically.

2.2. Reduction of MPO-II

Higher ratio of H_2O_2 leads to formation of compound II. [38] Thus, samples of MPO-II were prepared in the same way as above but containing a 1000-fold excess of H_2O_2. Upon irradiation the resulting process was followed at the wavelengths that correspond to the two Soret band maxima.

2.3. Reduction of Fe(III)-MPO

Finally, this reaction was studied by preparing the corresponding aqueous solutions of MPO at pH 7, with *t*-BuOH but without H_2O_2. The fading of the ferric species was followed at 430 nm.

[1] *G-value* is the basic unit of radiation chemical yield. $G = 1$ means that one entity (*e.g.*, one radical or one e^-_{aq}) is formed or destroyed for each 100 eV that are absorbed by the medium.

2.4. Overall Reaction of MPO with e^-_{aq}

Time-resolved spectra of the reaction with e^-_{aq} were obtained in the UV/Vis range (350–550 nm) after an initial number of pulses, once the response became fairly stable. This quantity was variable, at least 10 pulses (thus, total initial dose ≥200 Gy) and it was determined empirically every time for each wavelength: hardly any signal was observed during the first shots, which afterwards increased and rapidly stabilized; and then the reaction was measured. The measurement of transients with low absorption coefficients produces a poor signal-to-noise ratio, for which more concentrated samples containing 500 nM of MPO and 500 μM of H_2O_2 were used, all other parameters were kept constant. Decay kinetic traces were adequately fitted by a first-order kinetic model, and the corresponding rate constants were obtained for every single wavelength.

2.5. Reduction of Chlorinating Activity of the MPO/H_2O_2/Cl^- Enzymatic System

The damage produced by irradiation on MPO was quantified by monitoring its chlorinating activity loss. This was done by measuring the change in the rate of chlorination of an adequate substrate after different doses of irradiation. For this, the simplest α-amino acid, Gly, was chosen as target for the MPO/H_2O_2/Cl^- enzymatic system.

In vivo MPO chlorination activity takes place at a pH near 5, thus this value was probed, using 100 mM citric/phosphate buffer. [Cl^-] in plasma is as high as 100–140 mM, [39] hence 100 mM of Cl^- was used in all cases. [MPO] was taken as 20 nM, while [Gly] = 100 mM and [H_2O_2] = 1.0 mM were chosen as this combination yielded appropriate pseudo-first order kinetic traces.

Radiolysis pulses were applied on N_2-saturated aqueous solutions of MPO *ca.* 40 nM containing 1% (v:v) *t*-BuOH in a 1.0 cm-pathlength quartz cuvette. The residual chlorination activity of MPO was determined by measuring its ability to react with Gly to yield (*N*—Cl)-Gly in a very fast and selective reaction. [40,41] Thus, activity experiments were performed immediately after applying the total irradiation dose. The cuvette was placed into a diode-array UV/visible spectrophotometer and the rest of reactants were added to the required concentration. Addition of H_2O_2 triggered chlorination and spectra were measured between 200 and 500 nm; formation of (*N*—Cl)-Gly was followed at λ = 260 nm.

t-BuOH was shown not to interfere with the very reactive active site of MPO, and the chlorination process showed the same reaction rate in the absence and presence of the scavenger.

3. Results and Discussion

The dose applied to the samples with each 2 μs radiolysis pulse corresponds to ~2 kRad (=20 Gy), while the total dose applied in the study of the irradiation effect on enzymatic activity was over 600 Gy. One- e^- reduction of compounds I and II, and ferric MPO yields compound II, native ferric enzyme, and ferrous species, respectively, according to reactions (4)–(6):

$$\mathrm{MPO-I} + \mathrm{e^-_{aq}} + \mathrm{H^+} \rightarrow \mathrm{MPO-II} \quad (4)$$

$$\mathrm{MPO-II} + \mathrm{e^-_{aq}} + \mathrm{H^+} \rightarrow \mathrm{Fe(III)-MPO} + \mathrm{H_2O} \quad (5)$$

$$\mathrm{Fe(III)-MPO} + \mathrm{e^-_{aq}} + \mathrm{H^+} \rightarrow \mathrm{Fe(II)-MPO} \quad (6)$$

Ferric MPO and MPO-I show Soret bands with a maximum at 430 nm, although with rather different extinction coefficients. Soret peaks at 456 nm and 476 nm are distinctive of MPO-II and Fe(II)-MPO, respectively. Therefore, samples of MPO-I and MPO-II were irradiated and subsequent processes were followed by UV/visible absorption spectroscopy in the region of those two wavelengths, while pulse radiolysis of Fe(III)-MPO was followed at λ = 430 nm.

Formation of MPO-II according to (4) involves an important absorption increase at λ = 456 nm, whereas a decrease is observed at λ = 430 nm, as in reduction of Fe(III)-MPO (6). On the other hand, production of Fe(III)-MPO following (5) features the opposite behaviour. However, absorption traces obtained upon reaction of e^-_{aq} with all oxidation intermediates show hardly any change (Fig. 1).

These results are not consistent with reactions (4)–(6); despite the

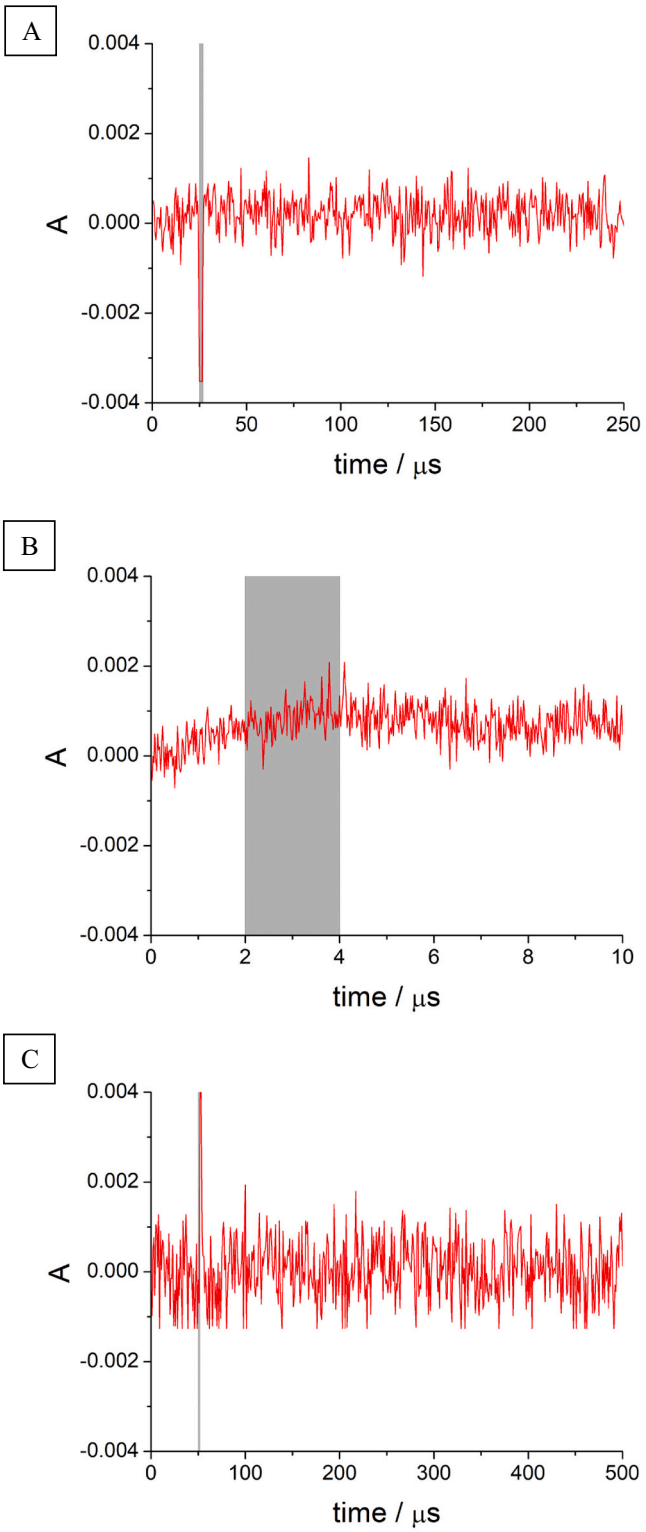


Fig. 1. Absorption traces measured upon irradiation of (A) MPO-I at λ = 430 nm, (B) MPO-II at λ = 450 nm, and (C) Fe(III)-MPO at λ = 430 nm.

enzyme concentration is very low, the heme group is an important chromophore so that all enzymatic compounds present high extinction coefficients, *i.e.*: if a one- e^- reduction processes takes place, a significant response is expected.

Surprisingly, the signal increased with accumulated pulses; samples that had been previously irradiated with a great dose showed a much higher response to additional pulses, some traces are shown in Fig. 2. These experiments were replicated repeatedly and, in all cases, the absorption signal only developed after several pulses.

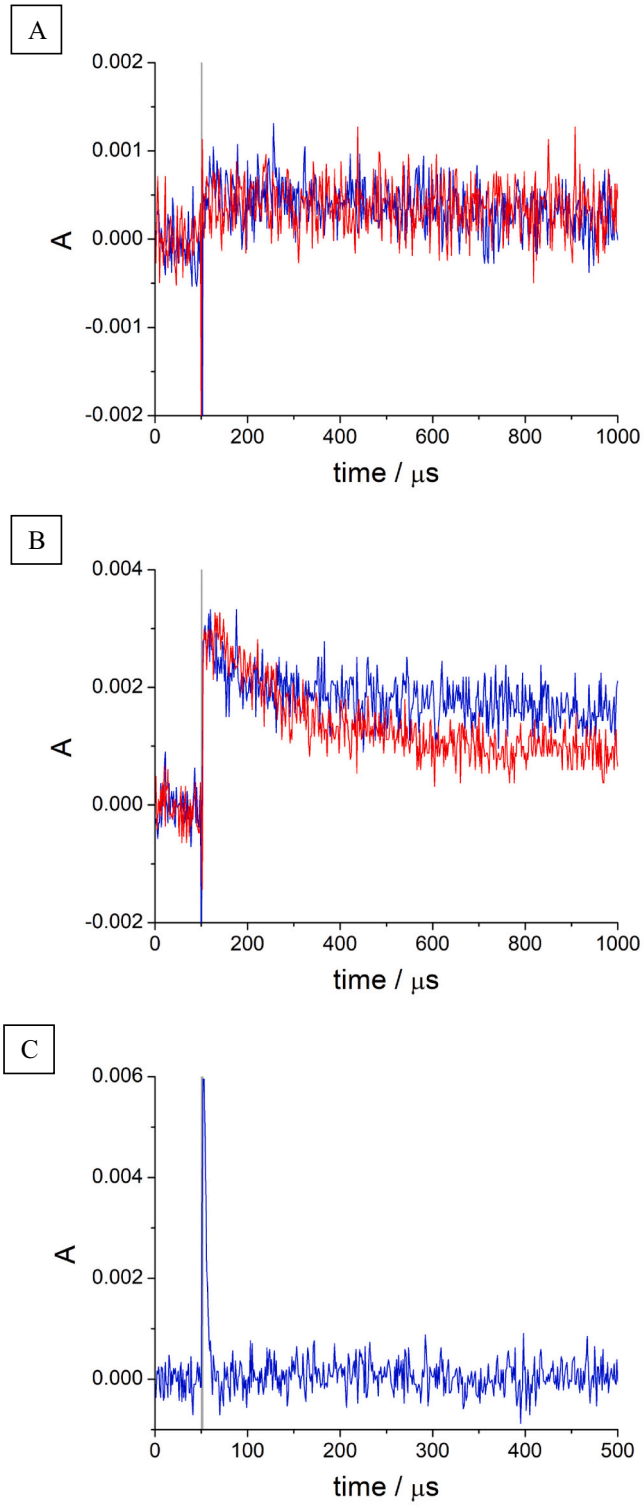


Fig. 2. Kinetic traces obtained for the first process upon reaction of e^-_{aq} with (A) MPO-I, (B) MPO-II, and (C) Fe(III)-MPO. Data acquired at λ = 430 nm (—) or 450 nm (—).

These traces still cannot account for reactions (4)–(6). The absorbance jump measured for irradiation of MPO-I (Fig. 2A) is very low, and the obtained product seems to be reasonably stable. However, data collected for MPO-II (Fig. 2B) correspond to typical relaxation kinetics, stable compounds are not formed and the absorbance change, although higher, is still too low to explain the reduction to Fe(III)-MPO and it is positive at both wavelengths.

Considering that the process observed is different for both compounds and that reactions (4) and (5) do not take place, a plausible explanation could be a reaction of e^-_{aq} with H_2O_2, present in a much higher concentration in the MPO-II sample. This process has been already studied [42]:

$$e^-_{aq} + H_2O_2 \rightarrow HO^- + HO^\bullet \quad (7)$$

and it shows a rate constant ($k = 1.1 \cdot 10^{10}$ $M^{-1} \cdot s^{-1}$) close or in the diffusion-control limit in water. Thus, it may have some relevance, but the products are rapidly removed by the buffer and the scavenger according to (2). The possibility of $HO^\bullet$ produced in (1) and (7) reacting with H_2O_2 could be also considered, as in (8):

$$HO^\bullet + H_2O_2 \rightarrow H_2O + HO_2^\bullet \quad (8)$$

One may argue that this process ($k = 2.7 \cdot 10^7$ $M^{-1} \cdot s^{-1}$) may compete with the reaction with the *t*-BuOH scavenger ($k = 6 \cdot 10^8$ $M^{-1} \cdot s^{-1}$), [42] but the rate constant of the reaction of $HO^\bullet$ with *t*-BuOH is still one magnitude higher than with H_2O_2, and the concentration of *t*-BuOH is much higher, so it will effectively remove all $HO^\bullet$ and (8) can be neglected.

Moreover, if the process observed in Fig. 2B corresponds to any reaction involving H_2O_2, it should be detectable from the first pulse, which was not the case (Fig. 1B). Furthermore, a significant signal was also observed with highly preirradiated samples of Fe(III)-MPO (Fig. 2C). The observed absorbance increase is even higher than with MPO-II and the subsequent decay shows much faster kinetics. This sample does not contain H_2O_2, so this fast-decay kinetics could be also ascribed to the disappearance of e^-_{aq}. However, the process observed in the first pulse (Fig. 1C) was much faster, and it should also be detected with MPO-I and MPO-II, either in the first pulse or after pre-irradiation (Figs. 1 and 2). Thus, it seems that this decay may correspond to another process that just involves the enzyme.

Some similar effects have been previously detected with other proteins upon pre-irradiation. Thus, e^-_{aq}-pre-irradiation of ribonuclease [43] and serum albumin, especially in the presence of ethanol and formate, [44] leads to faster rate constants in subsequent radiation pulses; although, in contrast to this study, the process can be observed from the first pulses. On the other hand, a similar behaviour was obtained in the reaction of ribonuclease with the formate radical anion ($HCO_2^\bullet$). [45] In this case, it has been observed that a signal centred at λ = 340 nm increases with pre-irradiation. This radical damages the protein structure, leading to some conformational changes that uncover the disulphide linkages, thus becoming more accessible for the reaction with further pulses, and the protonated form of the reduced $SS^{\bullet-}$ radical has an absorption band at 340 nm.

To get further information about the reactions taking place upon irradiation, UV/visible spectra of a MPO-II sample were obtained after a number of pulses once the response of further shots was stabilized, as previously explained (Fig. 3). The spectrum is very broad, extended over the entire monitored range, showing unresolved weak maxima at λ = 350 and 460 nm, both fading almost completely in a lapse of *ca.* 150 μs to leave a broad structureless absorption.

This observation can be interpreted either in terms of a highly delocalized spin through the molecule, or in the sense that the undergoing reactions do take place at different functional groups on the

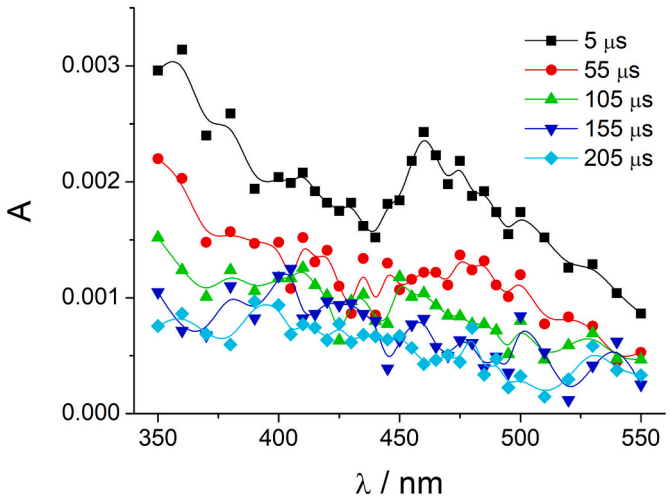


Fig. 3. Time-resolved spectra following unspecific reduction of MPO-II by e^-_{aq}.

surface of MPO. This would be in agreement with the rich fine vibronic structures that can be intuited for the observed electronic transitions, showing a variety of transitions from different vibrational modes.

Kinetic traces resemble typical relaxation processes. The kinetic analysis of the decay traces yielded rate constants that are wavelength dependent (Fig. 4). This supports that e^-_{aq} do not one- e^- reduce a single type of functional group but several different functionalities. Therefore, if multiple reactions take place with e^-_{aq} in a very complex molecule as MPO, and the protein structure gets progressively damaged by repetitive accumulative irradiation, the process is unlikely to be reproducible, but random effects should be then obtained. Thus, although this experiment was not entirely repeated, some replications were performed at several wavelengths and they were slightly different albeit consistent. This could also explain the diverse results obtained for the three samples (Figs. 1 and 2), although a similar behaviour was observed at different replications, the conclusions inferred can be maintained.

The fact that the initial pulses did not show any response is not yet explained. The band observed at 350 nm resembles that produced by the reaction of formate radical anion with disulphide bridges. [45] However, e^-_{aq} is more reducing and much less selective than $CO_2^{\bullet-}$. Also, the absorption band in that study was only detected under acidic pH conditions, which is not the case here, where the experiments are performed with 7.0 phosphate buffer. Besides, the spectrum presents a second maximum at 460 nm, whereas with ribonuclease the band shifted to 410 nm at neutral or slightly basic pH. Thus, an analogous behaviour between these two systems appears not very likely, but this possibility cannot be fully discarded. The precise origin of the signal becoming evident only after preirradiation remains elusive, but it may be due to either any undetectable reaction of e^-_{aq} with other species present (phosphate buffer, H_2O_2, remaining dissolved O_2, or trace amounts of impurities) during the first pulses, or even with MPO. It can be inferred from previous findings that the enzyme has the capacity to scavenge e^-_{aq} through very unspecific reactions: there is not a very noticeable light absorption in the studied region, either the developed radicals present extremely low absorption coefficients, or e^- are largely delocalized in the protein moiety. But the so-formed radical anions may intramolecularly react within the peptide and after some pulses the protein structure does not accept more electrons and starts getting damaged. Then, other functional groups are affected and the observed signal rises. With the purpose of getting an insight into this damage, we analysed the effect of high irradiation doses on the enzymatic activity.

Samples of MPO were irradiated with different doses, and the residual chlorination activity of the enzyme was determined by measuring its ability to oxidize the α-amino acid Gly to the corresponding (*N*—Cl)-Gly. MPO reacts with H_2O_2, Cl^- and Gly according to reactions (9)–(10):

$$MPO + H_2O_2 + Cl^- + H^+ \rightarrow MPO + HClO + H_2O \quad (9)$$

$$HClO + Gly \rightarrow (N\text{—}Cl) - Gly + H_2O \quad (10)$$

The mechanism taking place through reactions (9)–(10) involves the formation of free HClO, while in an alternative mechanism through reactions (11)–(12), an enzymatic intermediate (MPO-I-Cl) with chlorinating ability reacts directly with the amino acid: [30].

$$Fe(III) - MPO + H_2O_2 + Cl^- \rightarrow MPO - I - Cl + H_2O \quad (11)$$

$$MPO - I - Cl + Gly + H^+ \rightarrow Fe(III) - MPO + (N\text{—}Cl)\ Gly + H_2O \quad (12)$$

The overall reaction is the same in both mechanisms, and they do not affect the outcomes inferred from the data obtained in this study.

The initial rate of chlorination is represented *versus* the total absorbed dose in Fig. 5. Actually, the kinetics of MPO-mediated chlorination show a complex profile with an extremely fast initial rate that rapidly decreases as a steady-state is achieved. This initial burst is not observed here, but the initial part of the rate under equilibrium conditions (see Ref. [30] for a more detailed discussion). The observed rate of chlorination decreases with increasing radiation doses but some activity still remains after very intense irradiation, which supports the hypothesis

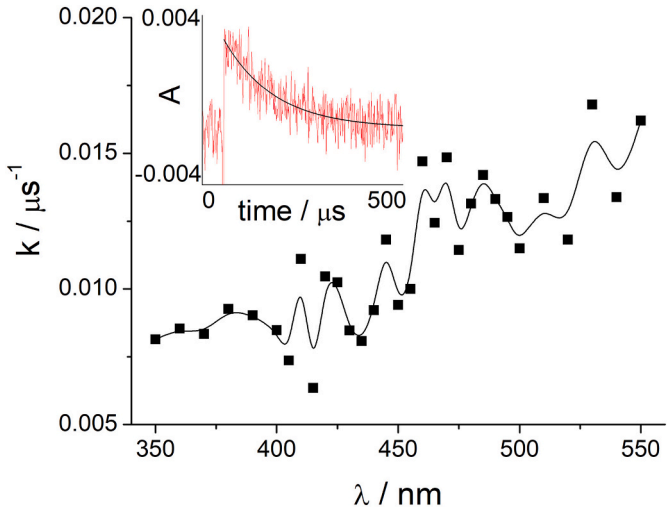


Fig. 4. Obtained rate constant *vs.* wavelength for the decay process shown in Fig. 3. Inset shows a typical first-order exponential fit.

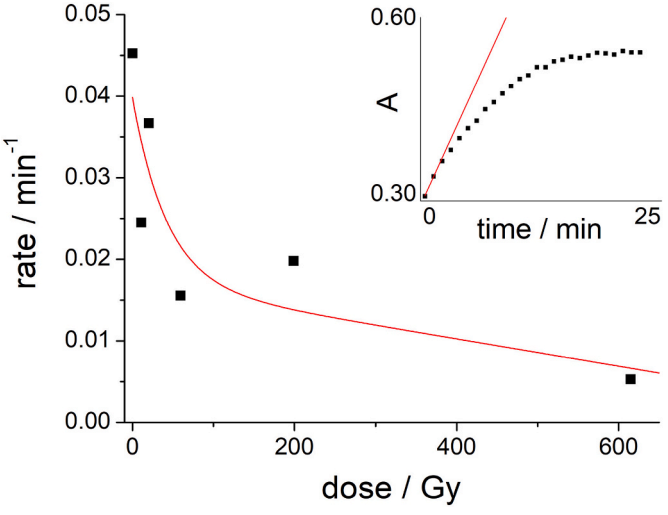


Fig. 5. Initial chlorination rate of Gly *vs* irradiation dose. Inset shows a typical fit of the initial rate.

that e^-_{aq} does not react at the active site but at surrounding peptide chains on the surface of MPO. Previous results already revealed that e^-_{aq} did not reduce heme, but these data also suggest that e^-_{aq} do not modify the protein structure at the active site as, in this case, a dramatic effect on the enzymatic activity would be expected.

The substrate binding site of MPO is hidden inside the enzyme, situated in the inner part of the molecule, [46–49] with a narrow channel connecting it to the outer medium and sterically hindered for bulky substrates. [5] Typically, e^-_{aq} show a cavity or excluded volume ~ 2.5 Å in radius in the structure of liquid water, with the e^- surrounded by a cluster of water molecules that may impede the access through this funnel-shaped passage.

Furthermore, e^-_{aq} are the strongest reducing agent in water and are extremely reactive, so it is reasonable to assume that if entering the narrow substrate channel, they may react with any available group situated in the walls that is susceptible of reduction, at a short distance away, before reaching the distal cavity and the active site therein. Therefore, e^-_{aq} do not enter and react with or in the close proximity of the active site as it remains functional. All these results indicate that e^-_{aq} scavenging takes place with different functional groups on the surface of MPO, including reaction sites close to the substrate channel entry. These areas develop damage affecting the protein tertiary structure, hence affecting the oxidation potential of the enzyme, or hindering the access of substrates to the heme pocket, accounting for the slower chlorination rate. This shows that one of main functions of the protein packing around the heme is to shield it from reactive species, as observed also in myoglobin, [50] allowing MPO to keep its anti-pathogenic function even after receiving large doses of ionizing radiation.

4. Conclusions

Pulse radiolysis experiments were performed to study the reactions of e^-_{aq} with MPO. One- e^- reduction of different oxidation intermediates did not produce any significant signal, indicating that the reaction does not take place at the strong chromophoric heme group, which is hindered in the core of MPO superstructure, but rather on other reactive sites of the molecule.

The spectrum observed after irradiation was very broad indicating either that the spin is highly delocalized or that multiple functional groups undergo reaction with e^-_{aq}. Initial decay kinetics show wavelength-dependent rates, supporting that different processes are being followed and, therefore, e^-_{aq} must react with several functional groups placed at more accessible locations of the enzyme.

The effect of irradiation dose on the chlorinating activity of MPO was analysed to clarify whether the heme herein, and consequently the active site, keep unchanged. At low doses, the enzyme itself acts as a scavenger, accepting electrons while showing a modest decrease in its activity. After high irradiation doses, MPO was still able to induce chlorination of the amino acid Gly, indicating the absence of serious damage at the active site, hindered at the end of the substrate channel, and that the protein folding around the active site plays a protective role to keep the anti-pathogenic vital function of MPO.

Funding Sources

This work was supported by: the Spanish *Ministerio de Ciencia y Tecnología* (CTQ2004-00534/BQU), the European Commission through the Access to Large-Scale Scientific Facilities Program (ref 41365), and the regional government of the *Xunta de Galicia* (Project GPC ED431B 2020/52).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We are indebted to personnel at Synchrotron Radiation Source at Daresbury (Cheshire, UK) for their kind technical assistance. We also thank Lucas Canle de Andrés for his design of the graphical abstract.

References

[1] C.C. Winterbourn, M.C.M. Vissers, A.J. Kettle, Myeloperoxidase, Curr. Opin. Hematol. 7 (2000) 53–58, https://doi.org/10.1097/00062752-200001000-00010.
[2] S.J. Klebanoff, Myeloperoxidase: friend and foe, J. Leukoc. Biol. 77 (2005) 598–625, https://doi.org/10.1189/jlb.1204697.
[3] A. Strzepa, K.A. Pritchard, B.N. Dittel, Myeloperoxidase: a new player in autoimmunity, Cell. Immunol. 317 (2017) 1–8, https://doi.org/10.1016/j.cellimm.2017.05.002.
[4] J.E. Harrison, J. Schultz, Studies on the chlorinating activity of myeloperoxidase, J. Biol. Chem. 251 (1976) 1371–1374, https://doi.org/10.1016/S0021-9258(17)33749-3.
[5] D.R. Ramos, M.V. García, J.A. Santaballa, P.G. Furtmüller, C. Obinger, Myeloperoxidase-catalyzed chlorination: the quest for the active species, J. Inorg. Biochem. 102 (2008) 1300–1311, https://doi.org/10.1016/j.jinorgbio.2008.01.003.
[6] J. Arnhold, P.G. Furtmüller, C. Obinger, Redox properties of myeloperoxidase, Redox Rep. 8 (2003) 179–186, https://doi.org/10.1179/135100003225002664.
[7] A. Taurog, Molecular evolution of thyroid peroxidase, Biochimie 81 (1999) 557–562, https://doi.org/10.1016/s0300-9084(99)80110-2.
[8] M. Zamocky, C. Jakopitsch, P.G. Furtmüller, C. Dunand, C. Obinger, The peroxidase–cyclooxygenase superfamily: reconstructed evolution of critical enzymes of the innate immune system, Proteins 72 (2008) 589–605, https://doi.org/10.1002/prot.21950.
[9] M. Zámocký, S. Hofbauer, I. Schaffner, B. Gasselhuber, A. Nicolussi, M. Soudi, K. F. Pirker, P.G. Furtmüller, C. Obinger, Independent evolution of four heme peroxidase superfamilies, Arch. Biochem. Biophys. 574 (2015) 108–119, https://doi.org/10.1016/j.abb.2014.12.025.
[10] X.L. Armesto, M. Canle, M.V. García, J.A. Santaballa, Aqueous chemistry of *N*-halo-compounds, Chem. Soc. Rev. 27 (1998) 453–460, https://doi.org/10.1039/A827453Z.
[11] B.S. Berlett, E.R. Stadtman, Protein oxidation in aging, disease, and oxidative stress, J. Biol. Chem. 272 (1997) 20313–20316, https://doi.org/10.1074/jbc.272.33.20313.
[12] C.C. Winterbourn, A.J. Kettle, Reactions of superoxide with myeloperoxidase and its products, Jpn. J. Infect. Dis. 57 (2004) S31–S33.
[13] G.L. Hug, Optical Spectra of Nonmetallic Inorganic Transient Species in Aqueous Solution, U.S. Department of Commerce, National Bureau of Standards, Washington, 1981.
[14] J. Jortner, R.M. Noyes, Some thermodynamic properties of the hydrated electron, J. Phys. Chem. 70 (1966) 770–774, https://doi.org/10.1021/j100875a026.
[15] H.A. Schwarz, Free radicals generated by radiolysis of aqueous solutions, J. Chem. Educ. 58 (1981) 101–105, https://doi.org/10.1021/ed058p101.
[16] M. Canle-López, J.A. Santaballa, S. Steenken, Photo- and radiation-chemical generation and thermodynamic properties of the aminium and aminyl radicals derived from *N*-phenylglycine and (*N*-chloro,*N*-phenyl)glycine in aqueous solution: Evidence for a new photoionization mechanism for aromatic amines, Chem. Eur. J. 5 (1999) 1192–1201, https://doi.org/10.1002/(SICI)1521-3765(19990401)5:4<1192::AID-CHEM1192>3.0.CO;2-M.
[17] K. Bernhard, J. Geimer, M. Canle-López, J. Reynisson, D. Beckert, R. Gleiter, S. Steenken, Photo- and radiation-chemical formation and electrophilic and electron transfer reactivities of enolether radical cations in aqueous solution, Chem. Eur. J. 7 (2001) 4640–4650, https://doi.org/10.1002/1521-3765(20011105)7:21<4640::AID-CHEM4640>3.0.CO;2-E.
[18] H. Hori, M. Ikeda-Saito, Photochemically modified myeloperoxidase, with optical spectral properties analogous to those of lactoperoxidase, retains its original catalytic activity, Biochemistry 29 (1990) 7106–7112, https://doi.org/10.1021/bi00482a023.
[19] N. Shimizu, K. Kobayashi, K. Hayashi, Kinetics of the reaction of superoxide anion with ferric horseradish peroxidase, Biochim. Biophys. Acta 995 (1989) 133–137, https://doi.org/10.1016/0167-4838(89)90071-x.
[20] L. Gebicka, J.L. Gebicki, Reactions of radiolytically-generated superoxide anion with higher oxidation states of lactoperoxidase, Int. J. Radiat. Biol. 63 (1993) 565–568, https://doi.org/10.1080/09553009314450741.
[21] L. Gebicka, J.L. Gebicki, Scavenging of oxygen radicals by heme peroxidases, Acta Biochim. Pol. 43 (1996) 673–678.
[22] A.J. Kettle, R.F. Anderson, M.B. Hampton, C.C. Winterbourn, Reactions of superoxide with myeloperoxidase, Biochemistry 46 (2007) 4888–4897, https://doi.org/10.1021/bi602587k.
[23] A.J. Kettle, A. Maroz, G. Woodroffe, C.C. Winterbourn, R.F. Anderson, Spectral and kinetic evidence for reaction of superoxide with compound I of myeloperoxidase, Free Radic. Biol. Med. 51 (2011) 2190–2194, https://doi.org/10.1016/j.freeradbiomed.2011.09.019.
[24] L. Gebicka, J.L. Gebicki, Modification of horseradish peroxidase induced by hydroxyl radicals, The influence of oxygen, Biochimie 78 (1996) 62–65, https://doi.org/10.1016/0300-9084(96)81330-7.

[25] C.C. Winterbourn, Myeloperoxidase as an effective inhibitor of hydroxyl radical production. Implications for the oxidative reactions of neutrophils, J. Clin. Invest. 78 (1986) 545–550, https://doi.org/10.1172/JCI112607.
[26] L. Gebicka, J.L. Gebicki, Reactions of hydrated electrons with horseradish peroxidase: a pulse radiolysis study, Int. J. Radiat. Biol. 59 (1991) 617–623, https://doi.org/10.1080/09553009114550551.
[27] L. Gebicka, J.L. Gebicki, Redox transformations in peroxidases studied by pulse radiolysis technique, Radiat. Phys. Chem. 39 (1992) 113–116, https://doi.org/10.1016/1359-0197(92)90182-F.
[28] I.I. Vlasova, J. Arnhold, A.N. Osipov, O.M. Panasenko, pH-dependent regulation of myeloperoxidase activity, Biochem. Mosc. 71 (2006) 667–677, https://doi.org/10.1134/s0006297906060113.
[29] H. Spalteholz, O.M. Panasenko, J. Arnhold, Formation of reactive halide species by myeloperoxidase and eosinophil peroxidase, Arch. Biochem. Biophys. 445 (2006) 225–234, https://doi.org/10.1016/j.abb.2005.06.025.
[30] D.R. Ramos, M.V. García, L. Canle, J.A. Santaballa, P.G. Furtmüller, C. Obinger, Myeloperoxidase-catalyzed taurine chlorination: initial versus equilibrium rate, Arch. Biochem. Biophys. 466 (2007) 221–233, https://doi.org/10.1016/j.abb.2007.07.024.
[31] M.S. Jensen, D.F. Bainton, Temporal changes in pH within the phagocytic vacuole of the polymorphonuclear neutrophilic leukocyte, J. Cell Biol. 56 (1973) 379–388, https://doi.org/10.1083/jcb.56.2.379.
[32] M. Canle, M.I. Fernández, J.A. Santaballa, Differential features of short-lived intermediates: structure, properties and reactivity, Adv. Phys. Org. Chem. 54 (2020) 99–118, https://doi.org/10.1016/bs.apoc.2020.07.001.
[33] T. Odajima, I. Yamazaki, Myeloperoxidase of the leukocyte of normal blood. I. Reaction of myeloperoxidase with hydrogen peroxide, Biochim. Biophys. Acta 206 (1970) 71–77, https://doi.org/10.1016/0005-2744(70)90083-5.
[34] R.F. Beers, I.W. Sizer, A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase, J. Biol. Chem. 195 (1952) 133–140, https://doi.org/10.1016/S0021-9258(19)50881-X.
[35] B.H. Milosavljevic, J.A. LaVerne, Pulse radiolysis of aqueous thiocyanate solution, J. Phys. Chem. A 109 (2005) 165–168, https://doi.org/10.1021/jp046088a.
[36] M. Canle López, M.I. Fernández, S. Rodríguez, J.A. Santaballa, S. Steenken, E. Vulliet, Mechanisms of direct and TiO_2-photocatalysed UV degradation of phenylurea herbicides, ChemPhysChem 6 (2005) 2064–2074, https://doi.org/10.1002/cphc.200500004.
[37] P.G. Furtmüller, U. Burner, C. Obinger, Reaction of myeloperoxidase compound I with chloride, bromide, iodide, and thiocyanate, Biochemistry 37 (1998) 17923–17930, https://doi.org/10.1021/bi9818772.
[38] P.G. Furtmüller, C. Obinger, Y. Hsuanyu, H.B. Dunford, Mechanism of reaction of myeloperoxidase with hydrogen peroxide and chloride ion, Eur. J. Biochem. 267 (2000) 5858–5864, https://doi.org/10.1046/j.1432-1327.2000.01491.x.
[39] C.J.V. Dalen, M.W. Whitehouse, C.C. Winterbourn, A.J. Kettle, Thiocyanate and chloride as competing substrates for myeloperoxidase, Biochem. J. 327 (1997) 487–492, https://doi.org/10.1042/bj3270487.
[40] X.L. Armesto, L. Canle, J.A. Santaballa, α-Amino acids chlorination in aqueous media, Tetrahedron 49 (1993) 275–284, https://doi.org/10.1016/S0040-4020(01)80525-1.
[41] X.L. Armesto, L. Canle, M.V. García, M. Losada, J.A. Santaballa, *N* reactivity *vs*. *O* reactivity in aqueous chlorination, Int. J. Chem. Kinet. 26 (1994) 1135–1141, https://doi.org/10.1002/KIN.550261108.
[42] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (·OH/·O^-) in aqueous solution, J. Phys. Chem. Ref. Data 17 (1988) 513–886, https://doi.org/10.1063/1.555805.
[43] H. Schuessler, M. Ebert, J.V. Davies, The reaction rates of electrons with native and irradiated ribonuclease, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 32 (1977) 391–396, https://doi.org/10.1080/09553007714551141.
[44] H. Schuessler, J.V. Davies, Radiation-induced reduction reactions with bovine serum albumin, Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med. 43 (1983) 291–301, https://doi.org/10.1080/09553008314550331.
[45] R.H. Bisby, R.B. Cundall, J.L. Redpath, G.E. Adams, One-electron reduction reactions with enzymes in solution. A pulse radiolysis study, J. Chem. Soc. Faraday Trans. 72 (1976) 51–63, https://doi.org/10.1039/F19767200051.
[46] T.J. Fiedler, C.A. Davey, R.E. Fenna, X-ray crystal structure and characterization of halide-binding sites of human myeloperoxidase at 1.8 A resolution, J. Biol. Chem. 275 (2000) 11964–11971, https://doi.org/10.1074/jbc.275.16.11964.
[47] M. Blair-Johnson, T. Fiedler, R. Fenna, Human myeloperoxidase: structure of a cyanide complex and its interaction with bromide and thiocyanate substrates at 1.9 A resolution, Biochemistry 40 (2001) 13990–13997, https://doi.org/10.1021/bi0111808.
[48] X. Carpena, P. Vidossich, K. Schroettner, B.M. Calisto, S. Banerjee, J. Stampler, M. Soudi, P.G. Furtmüller, C. Rovira, I. Fita, C. Obinger, Essential role of proximal histidine-asparagine interaction in mammalian peroxidases, J. Biol. Chem. 284 (2009) 25929–25937, https://doi.org/10.1074/jbc.M109.002154.
[49] I. Grishkovskaya, M. Paumann-Page, R. Tscheliessnig, J. Stampler, S. Hofbauer, M. Soudi, B. Sevcnikar, C. Oostenbrink, P.G. Furtmüller, K. Djinović-Carugo, W. M. Nauseef, C. Obinger, Structure of human promyeloperoxidase (proMPO) and the role of the propeptide in processing and maturation, J. Biol. Chem. 292 (2017) 8244–8261, https://doi.org/10.1074/jbc.M117.775031.
[50] A. Arkhipov, R. Braun, Y. Yin, Case Study: Myoglobin. https://www.ks.uiuc.edu/Training/CaseStudies/pdfs/myoglobin.pdf>, 2008 (accessed 31 July 2021).