Kinetics of Interconversion of Redox Intermediates of Lactoperoxidase, Eosinophil Peroxidase and Myeloperoxidase

Paul Georg Furtmüller, Walter Jantschko, Martina Zederbauer, Christa Jakopitsch,
Jürgen Arnhold[1] and Christian Obinger*

Metalloprotein Research Group, Division of Biochemistry, Department of Chemistry, BOKU-University of Natural Resources and Applied Life Sciences, [1]Institute of Medical Physics and Biophysics, School of Medicine, University of Leipzig, Leipzig, Germany

SUMMARY: Myeloperoxidase, eosinophil peroxidase and lactoperoxidase are heme-containing oxidoreductases, which undergo a series of redox reactions. Though sharing functional and structural homology, reflecting their phylogenetic origin, differences are observed regarding their spectral features, substrate specificities, redox properties and kinetics of interconversion of the relevant redox intermediates ferric and ferrous peroxidase, compound I, compound II and compound III. Depending on substrate availability, these heme enzymes path through the halogenation cycle and/or the peroxidase cycle and/or act as poor (pseudo-) catalases.

Today - based on sequence homologies, tertiary structure and the nature of the heme group - two heme peroxidase superfamilies are distinguished, namely the superfamily containing enzymes from archaea, bacteria, fungi and plants (1) and the superfamily of mammalian enzymes (2), which contains myeloperoxidase (MPO), eosinophil peroxidase (EPO), lactoperoxidase (LPO) and thyroid peroxidase (TPO). An important feature of mammalian peroxidases is the covalent link of the heme group to the protein that contributes to the differences in optical properties as well as redox properties and substrate specificities between the two peroxidase superfamilies and within the mammalian peroxidases. In LPO the heme is covalently attached to the protein via two ester linkages, namely, between the heme 1- and 5-methyl groups and a glutamate and aspartate, respectively. The cross-linking process is thought to occur autocatalytically, with the 5-hydroxymethyl bond formed before the 1-hydroxymethyl bond. Two ester linkages and a similar self-processing mechanism were also postulated for EPO and TPO. By contrast, in MPO - the only mammalian peroxidase for which a structure is available - three covalent links between the heme and the protein exist (3). In addition to the two ester links, a thioether sulfonium bond between the β-carbon of the 2-vinyl group and Met243 is present (3).

Structural differences govern differences in (pseudo-) halide oxidation by mammalian peroxidases. The ease of oxidation of halide ions is the following: $I^- > Br^- > Cl^-$. All peroxidases can oxidize iodide. At neutral pH, only MPO is capable to oxidize chloride at a reasonable rate (4), and it is assumed that chloride and thiocyanate are competing substrates in vivo. EPO can oxidize chloride only at acidic pH (5), and at normal plasma concentrations, bromide and thiocyanate function as substrates, whereas for LPO thiocyanate is the preferred substrate (6). Apparently, only MPO retains most of the oxidizing potential of hydrogen peroxide in the reaction in which compound I is formed (Fig. 1, Reaction 1). Recently, the standard reduction potential of the couple compound I/native peroxidase was shown to be 1.13 V for MPO and 1.10 V for EPO (7), the latter being similar to that of LPO (unpublished data). There is a correlation between redox properties and chloride oxidation (Table 1), but oxidation of bromide, iodide and thiocyanate seems to be governed mainly by structural features of these oxidoreductases (Table 1).

Compound I, a ferryl/porphyrin radical cation, of mammalian peroxidases takes part in the halogenation (Fig. 1, Reactions 1 & 2) and the peroxidatic cycle (Fig. 1, Reactions 1, 3a & 4a). With MPO an excess of H_2O_2 is necessary to build compound I (4), whereas in case of EPO (5) and LPO (6) its formation is already mediated with stochiometric amounts of H_2O_2. In the absence of an exogenous electron donor, mammalian peroxidase compound I decays to an

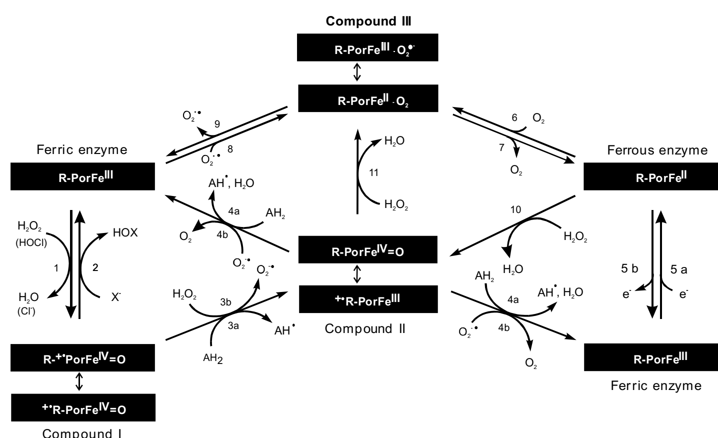

Fig. 1. General reaction scheme of mammalian peroxidases. In the first step H_2O_2 or HOCl (hypochlorous acid) is used for compound I formation (Reaction 1). Compound I is two oxidizing equivalents above the native enzyme with a porphyrin π-cation radical in combination with an iron(IV) center or an amino acid radical in combination with iron(IV). Compound I can react with halides or thiocyanate reducing the enzyme back to the ferric state (Reaction 2, halogenation activity). In the peroxidase reaction compound I is transformed in the first one-electron reduction to compound II, which contains an iron(IV) center (Reaction 3). Compound II is finally reduced back to ferric peroxidase in a second one-electron reduction (Reaction 4). Compound III (oxyperoxidase) is formed either from ferric peroxidase with superoxide (Reaction 8), from ferrous MPO with O_2 (Reaction 6) or from compound II with H_2O_2 (Reaction 11). It is a complex of ferrous-dioxygen in resonance with ferric-superoxide.

*Corresponding author: E-mail: christian.obinger@boku.ac.at

Table 1. Apparent second-order rate constant of the reactions between compound I of MPO, EPO and LPO with (pseudo-) halides (pH 7 and 15°C) and two-electron reduction potentials for the redox couple compound I/native enzyme and HOX/X^-, H2O at pH 7.0

	MPO (4)	EPO (5)	LPO (6)	
Compound I/ native Enzyme	1.16V (7)	1.10 V (7)	1.09 V (unpublished)	HOX/X^-, H_2O
	$\times 10^4$ ($M^{-1}s^{-1}$)	$\times 10^4$ ($M^{-1}s^{-1}$)	$\times 10^4$ ($M^{-1}s^{-1}$)	
Chloride	2.5	0.31	–	1.08 V
Bromide	110	1900	4.1	0.93 V
Iodide	720	9300	12000	0.57 V
Thiocyanate	960	10000	20000	–

intermediate with a compound II (ferryl-like) spectrum forming most likely an alternative compound I, a ferryl/protein radical species (see Fig. 1). MPO compound I is unique since it can also catalyze the one-electron reduction of compound I to compound II mediated by hydrogen peroxide, which is oxidized to superoxide (Fig. 1, Reaction 3b) that could function as electron donor of compound II (Fig. 1, Reaction 4b).

Compound I of mammalian peroxidases is a much better oxidant of typical aromatic peroxidase substrates than plant-type peroxidases (8). The standard reduction potential of the MPO redox couple compound I/compound II has been determined to be 1.35 V, whereas for the couple compound II/native enzyme it is 0.97 V at pH 7 and 25°C (9). As a consequence, big differences in rates of Reactions 3a & 4a are observed, suggesting that oxidation of substrates by MPO compound II but not compound I is strongly constrained by the nature of the substrate. By contrast, in LPO both standard reduction potentials are within the range 1.08-1.11 V (unpublished data).

Another peculiarity of MPO is the midpoint potential of the ferric/ferrous couple, which is +25 mV and thus similar to that of globins and much more positive than that of other heme peroxidases like LPO (−190 mV at pH 7 and 25°C), with the consequence that ferrous MPO could participate in enzyme turnover. It can react either with H_2O_2 (10) to compound II (Fig. 1, Reaction 10) or with dioxygen to compound III (Fig. 1, Reaction 6), which has a high dissociation rate constant reforming ferrous MPO rather than decaying to ferric MPO. Since the compound II to compound III transition is also mediated by H_2O_2 , a pseudo-catalase activity ($2\ H_2O_2 \rightarrow 2\ H_2O + O_2$) is obtained by Reactions 10, 11 and 7.

There are still many questions unanswered regarding the existence and role of compound I and compound II isomers (see Fig. 1) as well as observed differences in rates of Reactions 1-11 between MPO, EPO and LPO. Strongly needed is the 3D structure of EPO and LPO and further comprehensive mutational, kinetic and spectroscopic analyses.

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