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Myeloperoxidase-catalyzed taurine chlorination: Initial *versus* equilibrium rate

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Abstract

Myeloperoxidase (MPO) catalyzes the two-electron oxidation of chloride, thereby producing hypochlorous acid (HOCl). Taurine (2-aminoethane-sulfonic acid, Tau) is thought to act as a trap of HOCl forming the long-lived oxidant monochlorotaurine [(N-Cl)–Tau], which participates in pathogen defense. Here, we amend and extend previous studies by following initial and equilibrium rate of formation of (N-Cl)–Tau mediated by MPO at pH 4.0–7.0, varying H_2O_2 concentration. Initial rate studies show no saturation of the active site under assay conditions (*i.e.* $[H_2O_2] \geqslant 2000 [MPO]$). Deceleration of Tau chlorination under equilibrium is quantitatively described by the redox equilibrium established by H_2O_2-mediated reduction of compound I to compound II. At equilibrium regime the maximum chlorination rate is obtained at $[H_2O_2]$ and pH values around 0.4 mM and pH 5. The proposed mechanism includes known acid–base and binding equilibria taking place at the working conditions. Kinetic data ruled out the currently accepted mechanism in which a proton participates in the molecular step (MPO-I + Cl$^-$) leading to the formation of the chlorinating agent. Results support the formation of a chlorinating compound I–Cl$^-$ complex (MPO-I–Cl) and/or of ClO$^-$, through the former or even independently of it. ClO$^-$ diffuses away and rapidly protonates to HOCl outside the heme pocket. Smaller substrates will be chlorinated inside the enzyme by MPO-I–Cl and outside by HOCl, whereas bulkier ones can only react with the latter.
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Myeloperoxidase (MPO)[1] is a heme-containing dominant granule enzyme present in circulating polymorphonuclear neutrophils, which represent the cornerstone of cell-mediated antimicrobial activity in the human innate immune system. Neutrophils endocytose pathogens and deposit their granule contents intracellularly into target phagosomes [1]. The principal reaction catalyzed by MPO under physiological conditions is thought to be the oxidation of Cl$^-$ by H_2O_2 to yield the highly-reactive oxidizing and chlorinating agent HOCl [1]. In this respect MPO is unique among the members of the animal peroxidase superfamily [2] because the high reduction potential of its redox intermediate compound I (MPO-I) allows the two-electron oxidation of Cl$^-$ [3]. HOCl can participate in subsequent nonenzymatic reactions such as oxidation and chlorination of target cell components, and can also react with substances in the immediate environment that

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[1] *Abbreviations used:* MPO, myeloperoxidase; MPO–H, protonated species of MPO; MPO–H–Cl, protonated myeloperoxidase–chloride complex; MPO-I, compound I; MPO-I–H, protonated species of compound I; MPO-I–Cl, compound I–chloride complex; MPO-I–Cl–H, protonated species of compound I–chloride complex; MPO-II, compound II; MPO-II–HO₂, compound II–hydroperoxide complex; Tau, taurine; (N-Cl)–Tau, monochlorotaurine.

modulate its biological effects. For example HOCl chlorinates ammonia and amines to yield chloramines, which also have oxidizing and chlorinating activity [4]. The most important role of HOCl and chloramines in leukocyte function is probably to attack essential microbial cell components.

Taurine (Tau, 2-aminoethane-sulfonic acid) is one of the most abundant free amino acids in mammalian tissues, and accounts for a large part of the amines that are available for reaction with chlorinating reagents. Intracellular concentrations between 22 and 26 mM were reported for human leukocytes [5,6]. Although Tau is found in the cytoplasm of neutrophils and not released into phagosomes, it has been proposed that it could play a role in scavenging of HOCl released from the phagosomes [4]. Thereby, the long-lived oxidant monochlorotaurine [(N-Cl)–Tau] is formed, which is a hydrophilic anionic membrane-impermeable oxidant with relatively low toxicity [7]. Monochlorotaurine was reported to be uptaken by red blood cells and, finally, reduced by intracellular components, such as glutathione or hemoglobin, to Tau [4], which accumulates inside these cells [8]. These results suggest that red blood cells contribute to elimination of (N-Cl)–Tau in vivo, preventing its accumulation in the extracellular phase and protecting other blood cells, plasma components, and tissues from oxidative attack [8].

At pH 7.0 the formation of (N-Cl)–Tau from Tau and HOCl (Reaction 3 in Table 1) takes place with a measured rate constant of $4.8 \times 10^5 \, M^{-1} \, s^{-1}$ [9], whereas at pH 4.7 the rate constant is $3.5 \times 10^3 \, M^{-1} \, s^{-1}$ [10]. Further chlorination to Tau–dichloramine, which is more toxic than (N-Cl)–Tau and has significant cytolytic activity [8], is relatively slow. Generally, dichlorination is much slower than monochlorination. This is best demonstrated by the fact

that formation of (N-Cl)–methylamine ($2 \times 10^8 \, M^{-1} \, s^{-1}$) is about 5 orders of magnitude faster than the consecutive reaction between HOCl and (N-Cl)–methylamine [11], or by the published rate of ammonia chlorination by HOCl ($6.1 \times 10^6 \, M^{-1} \, s^{-1}$), which is about 4 orders of magnitude faster than further chlorination of monochloroamine [12]. Since Tau is not an electron donor for either compound I (MPO-I) or compound II (MPO-II), it is an ideal molecule to investigate the mechanism of its chlorination by MPO [10,13].

The chlorination of Tau by MPO has been studied by Marquez and Dunford [10,13]. However, in order to avoid consideration of the complex scenario of reactions outside the chlorination cycle of MPO (Table 1), the authors conducted most of their measurements at low pH (4.7) and focused only on initial rate data of Tau chlorination to eliminate the effect of formation of Tau–dichloramine [10,13]. From their kinetic data they could conclude that the chlorination reaction mediated by MPO in vivo may involve an enzyme intermediate species rather than free HOCl. Nevertheless, the authors did not include MPO-typical reactions, which play an important role in catalysis in vitro and in vivo, and thus significantly affect chlorination reactions, especially at high pH. These reactions need to be considered when investigating chlorination of Tau over the whole period of the reaction. This is possible since, as mentioned above, formation of Tau–dichloramine is negligible. Additionally, the influence of pH should be studied in order to determine whether protons intervene in the reaction mechanism.

The reactions involved in the so-called chlorination cycle of MPO are the formation of MPO-I, which has been described to be slightly reversible (Reaction 1 in Table 1) [14,15] and its direct reaction back to the ferric enzyme (Reaction 2 in Table 1). Moreover, both Fe(III)–MPO and MPO-I could participate in protonation reactions (Reactions 4 and 5 in Table 1). In addition to a redox reaction with H_2O_2, protonated native MPO can also bind Cl^- forming a high-spin complex (MPO–H–Cl, Reaction 6 in Table 1); this binding constant has been accurately measured as a function of pH [16–18]. Very important is the role of H_2O_2 as one-electron donor reducing MPO-I to MPO-II and forming $HO_2^{\cdot}$ (hydroperoxyl) radical (Reaction 7 in Table 1). Compound II is outside the halogenation cycle. The rate constant for the reduction of MPO-I to MPO-II by H_2O_2 was reported to be $8.2 \times 10^4 \, M^{-1} \, s^{-1}$ [14,15] at pH 7.0, so this reaction is competitive with MPO-I reduction by Cl^- at neutral pH regions [19]. The only alternative of a compound I to compound II transition in the system MPO/H_2O_2/Cl^-/Tau (i.e. in the absence of an exogenous one-electron donor) is intramolecular electron transfer that quenches the porphyryl radical of compound I and oxidizes an amino acid of the protein matrix. This is known to occur with MPO but is extremely slow and thus of no significance in the presence of H_2O_2 [14,15].

The one-electron oxidation of H_2O_2 produces superoxide [14,15] which has been reported to mediate MPO-II

Table 1
Mechanisms of MPO-catalyzed Tau chlorination proposed to fit experimental kinetic data

Mechanism	Reactions	No.
I	$MPO + H_2O_2 \overset{K_1}{\rightleftharpoons} MPO\text{-}I + H_2O$	1
	$MPO\text{-}I + Cl^- + H^+ \overset{k_2}{\rightarrow} MPO + HOCl$	2
	$HOCl + Tau \overset{k_3}{\rightarrow} (N\text{-}Cl)\text{--}Tau + H_2O$	3
	$MPO + H^+ \overset{K_4}{\rightleftharpoons} MPO\text{--}H$	4
	$MPO\text{-}I + H^+ \overset{K_5}{\rightleftharpoons} MPO\text{-}I\text{--}H$	5
	$MPO\text{--}H + Cl^- \overset{K_6}{\rightleftharpoons} MPO\text{--}H\text{--}Cl$	6
	$MPO\text{-}I + H_2O_2 \overset{K_7}{\rightleftharpoons} MPO\text{-}II + HO_2^{\cdot} + H^+$	7
	$MPO\text{-}II + HO_2^{\cdot} + H^+ \overset{k_8}{\rightarrow} MPO + H_2O + O_2$	8
II	$MPO + H_2O_2 \overset{K_1}{\rightleftharpoons} MPO\text{-}I + H_2O$	1
	$MPO\text{-}I + Cl^- \overset{k_{2b}}{\rightarrow} MPO\text{-}I\text{--}Cl$	2b
	$MPO\text{-}I\text{--}Cl + Tau + H^+ \overset{k_{3b}}{\rightarrow} (N\text{-}Cl)\text{--}Tau + MPO + H_2O$	3b
	$MPO + H^+ \overset{K_4}{\rightleftharpoons} MPO\text{--}H$	4
	$MPO\text{-}I + H^+ \overset{K_5}{\rightleftharpoons} MPO\text{-}I\text{--}H$	5
	$MPO\text{--}H + Cl^- \overset{K_6}{\rightleftharpoons} MPO\text{--}H\text{--}Cl$	6
	$MPO\text{-}I + H_2O_2 \overset{K_7}{\rightleftharpoons} MPO\text{-}II + HO_2^{\cdot} + H^+$	7
	$MPO\text{-}II + HO_2^{\cdot} + H^+ \overset{k_8}{\rightarrow} MPO + H_2O + O_2$	8