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Bromination and chlorination reactions of myeloperoxidase at physiological concentrations of bromide and chloride

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Abstract

Myeloperoxidase and eosinophil peroxidase use hydrogen peroxide to oxidize halides and thiocyanate to their respective hypohalous acids. Myeloperoxidase produces mainly hypochlorous acid and hypothiocyanite. Hypobromous acid and hypothiocyanite are the major products of eosinophil peroxidase. We have investigated the ability of myeloperoxidase to produce hypobromous acid in the presence of physiological concentrations of chloride and bromide. In accord with previous studies, between pH 5 and 7, myeloperoxidase converted about 90% of available hydrogen peroxide to hypochlorous acid and the remainder to hypobromous acid. Above pH 7, there was an abrupt rise in the yield of hypobromous acid. At pH 7.8, it accounted for 40% of the hydrogen peroxide. Bromide, at physiological concentrations, promoted a dramatic increase in bromination of human serum albumin catalyzed by myeloperoxidase. The level of 3-bromotyrosine increased to 16-fold greater than that for 3-chlorotyrosine. Chlorination of tyrosyl residues was not affected by bromide. With reagent hypohalous acids, bromination of tyrosyl residues was considerably more facile than chlorination. Hypochlorous acid promoted bromination to only a limited extent, which ruled out transhalogenation as a substantive route to 3-bromotyrosine. Chloramines and bromamines were also formed on albumin. Bromamines decayed much faster than chloramines and rapidly gave rise to protein carbonyls. We conclude that at physiological concentrations of chloride and bromide, hypobromous acid can be a major oxidant produced by myeloperoxidase. Its production in vivo will depend on pH and the concentration of bromide. Once produced, hypobromous acid will react with proteins to form bromamines, carbonyls, and brominated tyrosine residues. Consequently, 3-bromotyrosine should be considered as an oxidative product of myeloperoxidase and cannot be used as a specific biomarker for eosinophil peroxidase.

Keywords: Myeloperoxidase; Hypobromous acid; Hypochlorous acid; Chlorotyrosine; Bromotyrosine; Neutrophil; Eosinophil

Reactive oxidants generated by phagocytic white blood cells, such as neutrophils and eosinophils, have a major role in the killing of micro-organisms [1]. They also damage tissues at sites of inflammation. When stimulated, neutrophils and eosinophils consume oxygen in a respiratory burst that produces superoxide and hydrogen peroxide. Neutrophils also discharge myeloperoxidase while eosinophils release the related heme enzyme eosinophil peroxidase. Both enzymes use hydrogen peroxide to oxidize thiocyanate and bromide to their respective hypohalous acids but only myeloperoxidase uses chloride [2,3]. At physiological concentrations of these substrates, myeloperoxidase produces mainly hypochlorous acid and hypothiocyanite [2,4]. It was observed that 6% of the hydrogen peroxide it uses is converted to hypobromous acid [5]. The preferences for chloride and bromide as substrates are supported by their relative specificity constants [2], as well as their rates of reaction with compound I of myeloperoxidase [4]. Eosinophil peroxidase produces mainly hypothiocyanite and hypobromous acid [3,6,7].

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3-Chlorotyrosine and 3-bromotyrosine are formed when proteins are oxidized by hypochlorous acid and hypobromous acid, respectively [8]. Originally it was assumed that these halogenated amino acids could be used as specific biomarkers of myeloperoxidase [9,10] and eosinophil peroxidase [11,12], respectively. However, in an inflammatory model of sepsis, it was found that levels of 3-bromotyrosine were about one-third of those for 3-chlorotyrosine, and they were diminished in myeloperoxidase-knockout mice [13]. These results suggest that myeloperoxidase produces hypobromous acid during infection and inflammation. But they do not allow an estimate of the relative production of hypochlorous acid and hypobromous acid. This is because the rate constant for bromination of the tyrosine phenolic ring is 5000-fold greater than that for chlorination [14]. Hence, limited production of hypobromous acid may give high yields of brominated tyrosines compared to their chlorinated analogues.

Although there have been numerous investigations into the reactivity of hypochlorous acid with biological molecules, little attention has been focused on hypobromous acid. The relative efficiency of chlorination and bromination of protein tyrosine residues by either reagent hypohalous acids or myeloperoxidase has yet to be determined. Also, hypochlorous acid chlorinates tyrosyl residues via intermediate formation of chloramines [9,15]. This may also be the case for bromination. Hence, relative rates of halogenation of tyrosine residues may be substantially different for the hypohalous acids compared to protein chloramines and bromamines. For example, chloramines break down to form protein carbonyls in competition with their halogenation reactions [16,17]. Breakdown of bromamines to carbonyls has not been reported.

To gain a better appreciation of the oxidants myeloperoxidase is likely to produce in vivo, we have investigated its ability to produce hypohalous acids in the presence of physiological concentrations of halides. We have also characterized protein modifications when hypohalous acids react with human serum albumin. We show that at physiological pH, hypobromous acid is a major product of myeloperoxidase that gives rise to substantial bromination of proteins and formation of bromamines plus protein carbonyls.

Materials and methods

Materials

Human serum albumin (HSA)[1] was purchased from CSL, Parkville, Australia. Human leukocyte myeloperoxidase was purchased from Planta Natural Products (Austria). Its purity index (A_{430}/A_{280}) was 0.82 and its concentration was determined using its absorbance at 430 nm (ε_{430}, 89,000 $M^{-1}\,cm^{-1}$/heme) [18]. Its purity was also checked by electrophoresis on a native gel that was stained for peroxidase activity [2]. No other peroxidase bands besides myeloperoxidase were evident (data not shown). Hydrogen peroxide solutions were prepared daily and the concentration was calculated by measuring its absorbance at 240 nm (ε_{240}, 43.6 $M^{-1}\,cm^{-1}$) [19]. 5-Thio-2-nitrobenzoic acid (TNB) was prepared from 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as described previously [20]. Hypochlorous acid was purchased from Reckitt and Colman (NZ) Ltd. Its concentration was determined either by measuring its absorbance at 292 nm (pH 12, ε_{292} 350 $M^{-1}\,cm^{-1}$) [21] or by using 5-thio-2-nitrobenzoic acid as described previously [20]. Hypobromous acid was prepared daily by mixing equal volumes of 19 mM hypochlorous acid with 20 mM sodium bromide at pH 10.0 [21]. All other chemicals were purchased from the Sigma Chemical, St. Louis, USA.

Production of hypohalous acids by myeloperoxidase

Myeloperoxidase was incubated in phosphate buffer, pH 7.4, containing 5 mM taurine and 140 mM chloride plus varying concentrations of bromide, or just 1 mM bromide. All concentrations of bromide refer to added bromide and do not take into account bromide present in the buffer salts, which may contribute up to 5 μM [22]. Reactions were started by adding hydrogen peroxide. When reactions were complete, accumulated taurine chloramine and/or taurine bromamine were assayed using TMB. In this assay, 3,3′,5,5′-tetremethylbenzidine (TMB) is oxidized to a blue product that absorbs at 655 nm (ε_{655}, 30,000 $M^{-1}\,cm^{-1}$). Reactions were carried out in 400 mM acetate buffer, pH 5.4, containing 10% dimethylformamide and 2 mM TMB. Oxidation by taurine chloramine requires iodide as a catalyst but taurine bromamine reacts directly with TMB (unpublished result).

Treatment of human serum albumin (HSA) with hypohalous acids or myeloperoxidase

Hypohalous acids were added with vigorous vortexing to an equal volume of a 10 mg/ml solution of HSA in 10 mM phosphate buffer (pH 7.4), containing 140 mM sodium chloride (PBS) and varying concentrations of sodium bromide. The concentrations of hypohalous acids ranged from 0.4 to 2 mM which correspond to doses of 80–400 nmol/mg of protein. Haloamines were measured after 5 min. For the measurement of halogenated tyrosines and protein carbonyls, reactions were carried at room temperature (≈21 °C) and stopped by the addition of a 10-fold molar excess of methionine to scavenge haloamines.

[1] *Abbreviations used:* HSA, human serum albumin; TNB, 5-thio-2-nitrobenzoic acid; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; TMB, 3,3′,5,5′-tetremethylbenzidine; Tyr, tyrosine; Cl-Tyr, 3-chlorotyrsoine; Br-Tyr, 3-bromotyrosine.