Degradation of apotransferrin by the myeloperoxidase system

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Human apotransferrin was examined for its sensitivity to myeloperoxidase (MPO), a haem enzyme and an oxidant in neutrophils. Exposure of apotransferrin to a system composed of MPO, hydrogen peroxide and chloride at moderately acidic pH, but not at neutral to alkaline pH, altered its ultraviolet absorption spectrum. This reaction was not observed when MPO, hydrogen peroxide or chloride was omitted, when MPO was replaced by heated MPO, or when an inhibitor of haem enzymes, cyanide or azide, was added to the reaction system. These findings suggest that the degradation of apotransferrin by the MPO-hydrogen peroxide-chloride system was due to oxidation and damage of the protein moiety of apotransferrin.


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Introduction

Myeloperoxidase (MPO), a haem enzyme and an oxidant present in lysosomes (azurophilic granules) of neutrophils, is considered to work in the body’s self-defence system in animals and to be a unique enzyme in having two optimum pHs11. MPO catalyzes both the peroxidation of phenolic hydrogen donors such as epinephrine12 in neutral or alkaline solution and the peroxidation of chloride at acidic to moderately acidic pH (4.8 to 5.0)13. The latter reaction results in the irreversible destruction of chemical and biological compounds such as fuchsir basic and acid red No. 1066, prostaglandin E15, DNA, RNA and their constituents6; inactivation of enzymes such as trypsin, papain, chymopapain, pepsin, collagenase, alkaline phosphatase, and lysozyme7; detoxication of antimycins A1, A2 and A37, which have a strong toxicity for the respiratory electron transport of mitochondria; chlorination of prostaglandin E1 and RNA9; oxygenation of the carcinogen aflatoxin B18; haemolysis of horse, pig and human erythrocytes8; and the cytolysis of cells such as B-16 melanoma
tumor cells\textsuperscript{10}. Thus MPO may act in two ways in the xenobiotic metabolism and disposition pathway mediated by phagocytes, neutrophils, in the body’s self-defence system\textsuperscript{14}. One is in a halide-dependent reaction system that scavenges xenobiotics in moderately acidic conditions in phagolysosomes acidified on stimulation by xenogenous substances. The other is in the peroxidation system in neutral or alkaline solution, a non-halide-dependent reaction system. Odajima\textsuperscript{13} has reported that the halide which MPO demands physiologically is probably chloride, on the basis of its dissociation constants in the reactions with chloride, bromide and iodide. Transferrin is a glycoprotein widely distributed in a variety of vertebrate body fluids such as plasma, and it functions to deliver iron to cells via a receptor-mediated endocytic process, to remove toxic free iron from the blood, and to provide an antibacterial, low-iron environment. Klausner et al.\textsuperscript{12} have reported that the transferrin receptor in K562 cells mediates endocytosis of transferrin. Free iron in the aerobic environment of the blood generates free radicals as a product of the conversion of ferrous iron to ferric iron, and the resultant ferric iron is of low solubility\textsuperscript{10}. It is well established that bacterial pathogenesis is dependent on the ability to acquire iron from transferrin or other host proteins. Rodriguez and Jungery\textsuperscript{16} have reported that the intraerythrocytic stage of Plasmodium falciparum appears to obtain iron from iron-transferrin complexes by synthesizing transferrin receptors that become localized in the cell surface of the infected erythrocyte. Dautry-Varsat et al.\textsuperscript{5} have reported that the pH of the endocytic vesicle in a hepatoma cell Hep G2 is lowered to 5.5 or below when the transferrin binds to cell-surface transferrin receptors of the hepatoma cell, and this transferrin-receptor complex is endocytosed by the hepatoma cell; this causes dissociation of iron from the transferrin-receptor complex, but apotransferrin (an iron-released form of transferrin) remains bound to its receptor. The iron remains within the cell, and the apotransferrin-receptor complex is recycled to the cell surface. Upon encountering the neutral pH of the medium, apotransferrin is dissociated from the receptor. Clark and Pearson\textsuperscript{10} have reported that at neutral pH the oxidation system composed of MPO, hydrogen peroxide, chloride and iodide inactivates transferrin’s iron-binding capacity. There is, however, no report on the degradation of apotransferrin by this system. The aim of this study was to investigate whether apotransferrin is degraded by exposure to the MPO-hydrogen peroxide-chloride system in the same pH range as that where chloride is peroxidized by the system, and to propose the mechanism of the MPO system-mediated reactions.

Materials and Methods

MPO (donor: H$_2$O$_2$ oxidoreductase, EC 1.11.1.7) was prepared from neutrophils of normal pig peripheral blood\textsuperscript{17,18}. The MPO concentration was calculated on the basis of an extinction coefficient of 95 mM$^{-1}$ cm$^{-1}$ at 430 nm. The MPO preparation typically exhibited an R.Z. value (A$_{430}$ nm/A$_{280}$ nm) of above 0.85 as an index of the purity and was previously confirmed to give a single band on polyacrylamide gel electrophoresis. Human apotransferrin (iron-free form of transferrin, MW, 75,000) was purchased from Sigma (St. Louis, MO) and used without further purification. The spectrophotometric and kinetic experiments were carried out with Hitachi spectrophotometers, types 200-20 and 557 in a quartz cuvette (10x10x45 mm) at 25°C. The buffers used were acetate (acetic acid/sodium acetate) for pH 4.0-5.8, phosphate (potassium phosphate, monobasic/sodium phosphate, dibasic) for pH 6.0-7.8 and borate (boric acid/sodium carbonate) for pH 8.0-9.0.

Results

Exposure of apotransferrin to a system composed of MPO, hydrogen peroxide and chloride at moderately acidic pH, but not at neutral to alkaline pH, changed the ultraviolet absorption spectrum of apotransferrin (Fig.1), which showed an increase at 256 nm and a decrease at 280 nm. The decrease in absorbance at 280 nm, which is attributable to aromatic amino acids, indicates that the degradation of apotransferrin by the MPO-hydrogen peroxide-chloride system was due to oxidation and damage of the protein moiety of apotransferrin. The pH optimum was observed at 5.0 (Fig.2A). The initial rate of increase in the absorbance of apotransferrin at 256 nm was dependent upon the concentrations of MPO, hydrogen peroxide and chloride (Figs.2B,C and D). The spectral change was abrogated by omitting MPO, hydrogen peroxide or chloride, by replacing MPO with heated MPO, or by adding an inhibitor of haem enzymes, cyanide or azide, to the reaction system. The results are summarized in Table 1. These findings indicate that apotransferrin is degraded by the MPO-hydrogen peroxide-chloride system at moderately acidic pH, probably due to oxidation and damage of the protein moiety of apotransferrin by the MPO system. The ultraviolet absorption spectrum of transferrin changed in the same way as that of apotransferrin during the reaction with the MPO-hydrogen peroxide-chloride system, and the pH optimum of this reaction was also observed at 5.0 (data not shown).
Discussion

This study provides an insight into the function, mechanism and biological significance of the MPO system in neutrophils as an important self-defence system in animals. The present study and our earlier findings show that MPO destructs target substances in the presence of hydrogen peroxide and chloride only at moderately acidic pH, the same pH span as that where chloride is peroxidized by MPO. This consistency indicates that MPO first catalyzes peroxidation of chloride and that consequent oxidized chloride species destruct apotransferrin, and that the peroxidation of chloride is the rate-limiting step in these processes. It is also worth noting that the acidic pH optima observed in the present study (pH 5.0) and in our previous studies (pH 5.0 to 5.5) lie within the limits of the pH changes, from slightly acidic to moderately acidic (pH 4.7 to 5.2), which occur in cytoplasm of leukocytes during phagocytosis, as reported by Pavlov and Solo'ev. This parallelism suggests that MPO localized in phagocytic leukocytes, neutrophils, naturally acts at acidic pH in the presence of a halide ion, probably chloride. From the present study, we concluded that the degradation of apotransferrin by the system composed of MPO, hydrogen peroxide and chloride was due to oxidation and damage of protein moiety of apotransferrin molecule by the MPO system. In nature, the cytoplasm of resting neutrophils is at neutral to moderately alkaline.

Table 1 Oxidation of apotransferrin by the myeloperoxidase systems

<table>
<thead>
<tr>
<th>Reaction systems</th>
<th>Components</th>
<th>Reaction</th>
</tr>
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<tbody>
<tr>
<td>Complete</td>
<td>MPO + H₂O₂ + Cl⁻</td>
<td>Detected</td>
</tr>
<tr>
<td>MPO omitted</td>
<td>H₂O₂ + Cl⁻</td>
<td>Not detected</td>
</tr>
<tr>
<td>H₂O₂ omitted</td>
<td>MPO + Cl⁻</td>
<td>Not detected</td>
</tr>
<tr>
<td>Cl⁻ omitted</td>
<td>MPO + H₂O₂</td>
<td>Not detected</td>
</tr>
<tr>
<td>Heated MPO</td>
<td>Heated MPO + H₂O₂ + Cl⁻</td>
<td>Not detected</td>
</tr>
<tr>
<td>N₂O₃ added</td>
<td>MPO + H₂O₂ + Cl⁻ + N₂O₃⁻</td>
<td>Not detected</td>
</tr>
<tr>
<td>CN⁻ added</td>
<td>MPO + H₂O₂ + Cl⁻ + CN⁻</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

Reaction mixture (2.5 ml) contained 170 μg/ml apotransferrin and enzyme system (20 mM NaCl, 0.1 mM H₂O₂ and 17.7 mM MPO) added successively. Sodium azide (N₂O₃) or potassium cyanide (CN⁻) used were 0.1 mM and 0.5 mM respectively. Heated MPO was prepared by heating MPO for 1 min in water bath at 85°C. Assays were carried out in 0.1 M buffer (pH 5.0) at 25°C.
pH. As shown in Figure 3, which is drawn from the results of Pavlov and Solov’ev, when neutrophils are stimulated by a xenogenous substance, the pH of the cytoplasm falls to the acidic to moderately acidic level, then returns to the neutral range during phagocytosis. Apotransferrin might be degraded by the MPO system under such acidic conditions in neutrophils.

The proposed mechanism of degradation of apotransferrin by the system composed of MPO, hydrogen peroxide and chloride is summarized in Figure 4. It involves a combination and revision of previously proposed reaction cycles between MPO compounds I and II and the hydrogen donor and the MPO-catalyzed oxidation of chloride. MPO compound I is a hydrogen peroxide-peroxidase. This intermediate is the first enzyme-substrate complex, and its oxidation state (represented as Fe(III)) is two equivalents higher than that of ferric enzyme (represented as Fe(III)). MPO compound II is a hydrogen peroxide-MPO, the second enzyme-substrate complex, and its oxidation state (represented as Fe(IV)) is one equivalent higher than that of the ferric enzyme. It is conceivable that chloride is oxidized by one-electron or by two-electron loss. The reactions between the peroxidase intermediate compounds I and II and the chloride, which are shown by dotted lines, generate two free radicals of chlorine. The reaction between compound I and the chloride generates one chlorine cation, which is also shown by dotted lines. Two free radicals of chlorine dimerize to form diatomic molecular chlorine. One chlorine cation reacts with a chlorine anion to form diatomic molecular chlorine. The diatomic molecular chlorine readily reacts with water and generates hypo-halite nonenzymatically. On the other hand, the reactions between compounds I and II and the hydrogen donor take place at alkaline pH without chloride and generate two free radicals of hydrogen donor. Then, in the presence of chloride at acidic pH, the products of chloride peroxidized by MPO and/or their by-products, which probably include chlorine radicals, chlorine cat-
ions, and diatomic molecular chlorine, may non-enzymatically attack the target apotransferrin, resulting in degradation of its protein moiety at acidic to moderately acidic pH.

References