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The potentiation of myeloperoxidase activity by the glycosaminoglycan-dependent binding of myeloperoxidase to proteins of the extracellular matrix

Lukáš Kubala [a,b,c,*], Hana Kolářová [a,d], Jan Víteček [a,c], Silvie Kremserová [a,d], Anna Klinke [e], Denise Lau [e], Anna L.P. Chapman [f], Stephan Baldus [e], Jason P. Eiserich [b,g]

[a] Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, Brno, Czech Republic
[b] Department of Internal Medicine, University of California, Davis, CA, USA
[c] International Clinical Research Center, Center of Biomolecular and Cellular Engineering, St. Anne's University Hospital Brno, Brno, Czech Republic
[d] Department of Animal Physiology, Faculty of Science, Masaryk University, Czech Republic
[e] Department of Cardiology, University Heart Center Hamburg, University Hospital Eppendorf, Hamburg, Germany
[f] Centre for Free Radical Research, Department of Pathology, University of Otago, Christchurch, New Zealand
[g] Department of Physiology and Membrane Biology, University of California, Davis, CA, USA

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ABSTRACT

Background: Myeloperoxidase (MPO) is an abundant hemoprotein expressed by neutrophil granulocytes that is recognized to play an important role in the development of vascular diseases. Upon degranulation from circulating neutrophil granulocytes, MPO binds to the surface of endothelial cells in an electrostatic-dependent manner and undergoes transcytotic migration to the underlying extracellular matrix (ECM). However, the mechanisms governing the binding of MPO to subendothelial ECM proteins, and whether this binding modulates its enzymatic functions are not well understood.
Methods: We investigated MPO binding to ECM derived from aortic endothelial cells, aortic smooth muscle cells, and fibroblasts, and to purified ECM proteins, and the modulation of these associations by glycosaminoglycans. The oxidizing and chlorinating potential of MPO upon binding to ECM proteins was tested.
Results: MPO binds to the ECM proteins collagen IV and fibronectin, and this association is enhanced by the pre-incubation of these proteins with glycosaminoglycans. Correspondingly, an excess of glycosaminoglycans in solution during incubation inhibits the binding of MPO to collagen IV and fibronectin. These observations were confirmed with cell-derived ECM. The oxidizing and chlorinating potential of MPO was preserved upon binding to collagen IV and fibronectin; even the potentiation of MPO activity in the presence of collagen IV and fibronectin was observed.
Conclusions: Collectively, the data reveal that MPO binds to ECM proteins on the basis of electrostatic interactions, and MPO chlorinating and oxidizing activity is potentiated upon association with these proteins.
General significance: Our findings provide new insights into the molecular mechanisms underlying the interaction of MPO with ECM proteins.

1. Introduction

Neutrophil granulocytes, the largest group of polymorphonuclear leukocytes, are among the key cells active in host defense against invading pathogens. However, they also present a potential risk to the host by increasing oxidative burden. Myeloperoxidase (MPO), a highly abundant hemoprotein expressed mainly by neutrophil granulocytes, is thought to play a primary role in host defense [1–3]. MPO is also perceived as a critical mediator of inflammatory tissue injury. The significant pathological importance of MPO is recognized in chronic vascular diseases such as atherosclerosis and coronary artery disease. This is based on the observation that MPO mediates oxidative damage within the vessel wall initiating and promoting vessel remodeling, the

Abbreviations: ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; BAECs, bovine aortic endothelial cells; BSA, bovine serum albumin; CTAC, cetyltrimethylammonium chloride; DMF, N,N-dimethylformamide; DTNB, 5,5′-dithiobis(2-nitrobenzoic) acid; ECM, extracellular matrix; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GAGs, glycosaminoglycans; HRP, horseradish peroxidase; MCD, monochlorodimedon; MPO, myeloperoxidase; OD, optical density; PBS, phosphate buffered saline; RASMCs, rat aortic smooth muscle cells; RT, room temperature; SEM, standard error of the mean; TMB, 3,3′,5,5′-tetramethylbenzidine; TNB, 5-thio-2-nitrobenzoic acid

* Corresponding author at: Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Kralovopolska 135, CZ-612 65 Brno, Czech Republic. Tel.: +420 541 517 117; fax: +420 541 211 293.
E-mail address: kubalal@ibp.cz (L. Kubala).

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development of atherosclerotic lesions and endothelial dysfunction [1–4]. The most typically recognized mechanisms that are mediated by MPO and contribute to the development of these pathological processes are the catalysis of low-density lipoprotein oxidation, the oxidation of proteins altering their biological function, and the catabolism of nitric oxide contributing to endothelial dysfunction [2,3,5–7]. The particular importance of MPO in the impairment of vascular function is suggested for oxidative damage to vessel wall extracellular matrix [4,8–10]. Further, the pathological importance of MPO is supported by evidence that circulating levels of MPO are highly predictive of future vascular events associated with chest pain and acute coronary syndromes which correlate with overall patient outcome [1,3]. However, the overall picture of how MPO-driven pathological changes lead to the promotion of cardiovascular diseases is still incomplete.

During neutrophil granulocyte activation, MPO is released into the phagosome and the extracellular space where it can catalyze the production of a multitude of reactive species including hypochlorous acid, *N*-chloramines, reactive nitrogen species, and other oxidants [2–4,6,7]. Because MPO and markers of its enzymatic activity are generally increased at inflammatory foci, this pathway is thought to contribute to oxidant-dependent alterations in vascular and organ function during inflammation. For decades, an accumulation of MPO at inflammatory foci has been considered to be an index of inflammation, with increased tissue MPO activity thought to reflect neutrophil extravasation. However, the alternative process of MPO deposition within vascular tissue, independent of neutrophil diapedesis, was also shown [11–16]. Following the intraluminal release of highly cationic MPO, electrostatic interactions with the negatively charged endothelial plasma membrane are suggested to facilitate the binding of MPO with the vascular endothelium. Further, MPO can undergo apical to basolateral transcytosis across the vascular endothelium [8,11,14,15]. Immunohistochemical analysis of vascular MPO distribution revealed endothelial-associated MPO immunoreactivity on the endothelial surface, within endothelial cells, and in the extracellular matrix (ECM) of intima that was localized between the endothelial layer and the smooth muscle cell composed medial layer [11,17]. This ECM is synthesized by overlying endothelial cells forming a "basement membrane", a highly complex structure composed of various structural proteins, glycoproteins and proteoglycans including more abundantly present components such as collagen IV, laminins, nidogen/entactin, and perlecan, and less abundant components such as fibronectin [4,8,10,18]. The importance of MPO oxidative modification of the basement membrane structure was suggested by various authors particularly by authors Rees and Davies with their colleagues, who reported the MPO-dependent oxidation of extracellular matrix components particularly glycosaminoglycans (GAGs) [4,8–11,19–23].

The interaction of MPO with various types of plasma proteins including ceruloplasmin, apolipoproteins, and albumin has been demonstrated [14,24–28]. Interestingly, interactions of MPO with the ECM proteins perlecan and fibronectin were also reported [8,10]. The interaction of highly basic (cationic) MPO with other proteins has been suggested to be mostly dependent on electrostatic interactions, similar to the interaction of MPO with the negatively charged surfaces of endothelial cells or polymorphonuclear leukocytes [11–13,15,29]. In the case of MPO binding to the endothelium, the interaction was found to be dependent upon surface GAGs, since the exposure of MPO to an excess of external GAGs such as heparin, or a reduction in the surface presence of heparan profoundly reduced MPO binding to the endothelium and the consequent MPO transcytosis [11–13,15]. Interestingly, some proteins of subendothelial ECM or vessel wall ECM are, in general, proteoglycans or glycoproteins since a part of their molecules are sulfated anionic polysaccharides such as heparan sulfate [4,8,10,18].

It is well documented that MPO interaction with other biomolecules can modulate MPO activity. For example, the interaction of MPO with the plasma protein ceruloplasmin significantly decreases MPO activity [24,27,28]. Similarly, a decrease in MPO activity was reported after interaction with unfractioned and low molecular weight heparin [13]. In general, there is evidence that enzymatic activity of MPO sequestered in the subendothelial layer is preserved since products of MPO-dependent oxidation can be detected after transcytosis of MPO [8,11,13,17]. On the other side, studies evaluating the effects of MPO-derived oxidants on the proteoglycan perlecan and glycoprotein fibronectin reported significant modulation of these molecules by MPO [8,10]. However, the effects of MPO interaction with components of ECM on MPO activity are still not fully understood.

Overall, the fate of MPO sequestered from circulation by endothelial cells remains poorly understood in spite of clinical evidence of MPO localization within the subendothelial space and the suggested pathological consequences of oxidative damage mediated by MPO within the vessel wall. Further, since denudation of endothelial cells can occur as a consequence of acute inflammatory processes in the vessels, and the concomitant secretion of MPO along with an increase in plasma MPO levels can be expected, the exposed sub-endothelial matrix may be a likely direct target for MPO interaction. Thus, the aim of this study was to clarify the association of MPO with ECM proteins and to determine whether this association modulates MPO enzymatic activity.

2. Materials and methods

2.1. Chemicals and reagents

M199 and DMEM cell culture media were from Gibco (Invitrogen Corporation, CA, USA). Super Calf Serum and Fetal Bovine Serum were from Gemini Bio-Products (CA, USA). Purified MPO from human leukocytes was from Merck/Calbiochem (CA, USA) and from Planta Natural Products (Vienna, Austria). Chondroitin sulfate A from bovine trachea and polyclonal rabbit anti-human antibodies against MPO were from Merck/Calbiochem. Monoclonal mouse anti-human MPO antibodies were from Biomeda (CA, USA). Collagen type IV was from BD Biosciences (MA, USA). Trypsin/EDTA, penicillin/streptomycin, fibronectin from human plasma, heparin sodium salt from porcine intestine mucosa (207 IU/mg), alkaline phosphatase-conjugated anti-rabbit IgG antibodies, and mouse anti-fibronectin antibodies were from Sigma Chemical Co. (MO, USA). All other reagents were from Sigma Chemical Co. (MO, USA) or Fisher Scientific (CA, USA).

2.2. Cell cultures

Human aortic endothelial cells (HAECs) (Lonza, Germany) were maintained in complete Endothelial Cell Growth Medium-2 medium (Lonza). Bovine aortic endothelial cells (BAECs) from the European Collection of Cell Cultures (Health Protection Agency Culture Collections, UK) were maintained in medium 199 with 5% fetal bovine serum, 5% super calf serum, 25 U/ml penicillin, and 25 mg/ml streptomycin. Murine 3T3 fibroblasts and rat aortic smooth muscle cells A10 (RASMCs) were obtained from the American Type Culture Collection (USA) and were grown in DMEM containing 10% fetal bovine serum, 25 U/ml penicillin, and 25 mg/ml streptomycin. Cells were maintained at 37 °C in 5% CO_2 and the culture medium was changed every 2 days.

2.3. Isolation of cell-derived ECM proteins

Confluent cell cultures were maintained in microtiter plates for 6–12 days, with fresh medium being added every 2 days. Cells were washed with phosphate buffered saline (pH 7.4) (PBS) and gently lysed with PBS buffer containing Triton X-100 (0.5% v/v) and ethylenediaminetetraacetic acid (EDTA) (10 mM) at room temperature (RT) for 20 min. The extent of cell lysis was monitored periodically by microscopy, which revealed an immediate and complete loss of cell structure. Upon lysis of the cells, the remaining ECM was washed 5 times with PBS. Finally, the isolated matrix proteins were