Archives of Biochemistry and Biophysics 728 (2022) 109353

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Structure-biological activity relationships of myeloperoxidase to effect on platelet activation

I.V. Gorudko [a,*], D.V. Grigorieva [a], E.V. Shamova [a,b], N.P. Gorbunov [c,d], A.U. Kokhan [b], V.A. Kostevich [c,d], V.B. Vasilyev [c], O.M. Panasenko [d,e], N.V. Khinevich [f,g], H.V. Bandarenka [f,h], A.A. Burko [f,h], A.V. Sokolov [c,d]

[a] Department of Biophysics, Faculty of Physics, Belarusian State University, 4 Nezavisimosti Avenue, Minsk, 220030, Belarus
[b] Institute of Biophysics and Cell Engineering of National Academy of Sciences of Belarus, 27 Academicheskaya Str., Minsk, 220072, Belarus
[c] FSBRI "Institute of Experimental Medicine", 12 Acad. Pavlov Str., St. Petersburg, 197376, Russia
[d] Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, 1a Malaya Pirogovskaya Str., Moscow, 119435, Russia
[e] Pirogov Russian National Research Medical University, 1 Ostrovityanova Str., Moscow, 117997, Russia
[f] Belarusian State University of Informatics and Radioelectronics, 6 P. Brovka Str., Minsk, 220013, Belarus
[g] Institute of Materials Science, Kaunas University of Technology, K. Donelaičio g. 73, Kaunas, 44249, Lithuania
[h] Polytechnic School, Arizona State University, Arizona State University Polytechnicm, 7001 East Williams Field Road, Mesa, AZ, 85212, USA

ARTICLE INFO

Keywords:
Myeloperoxidase
Monomeric myeloperoxidase
HOCl
Platelet
Ceruloplasmin
Surface-enhanced Raman scattering spectroscopy
Immunothrombosis
Inflammation
Neutrophil

ABSTRACT

Myeloperoxidase (MPO), an oxidant-producing enzyme of neutrophils, has been shown to prime platelet activity promoting immunothrombosis. Native MPO is a homodimer, consisting of two identical protomers (monomer) connected by a single disulfide bond. But in inflammatory foci, MPO can be found both in the form of a monomer and in the form of a dimer. Beside MPO can also be in complexes with other molecules and be modified by oxidants, which ultimately affect its physicochemical properties and functions. Here we compared the effects of various forms of MPO as well as MPO in complex with ceruloplasmin (CP), a physiological inhibitor of MPO, on the platelet activity. Monomeric MPO (hemi-MPO) was obtained by treating the dimeric MPO by reductive alkylation. MPO was modified with HOCl in a molar ratio of 1:100 (MPO-HOCl). Using surface-enhanced Raman scattering (SERS) spectroscopy we showed that peaks at about 510 and 526 cm^{-1} corresponded to disulfide bond was recognizable in the SERS-spectra of dimeric MPO, absent in the spectrum of hemi-MPO and less intense in the spectra of MPO-HOCl, which indicates the partial decomposition of dimeric MPO with a disulfide bond cleavage under the HOCl modification. It was shown hemi-MPO to a lesser extent than dimeric MPO bound to platelets and enhanced their agonist-induced aggregation and platelet-neutrophil aggregate formation. MPO modified by HOCl and MPO in complex with CP did not bind to platelets and have no effect on platelet activity. Thus, the modification of MPO by HOCl, its presence in monomeric form as well as in complex with CP reduces MPO effect on platelet function and consequently decreases the risk of thrombosis in inflammatory foci.

1. Introduction

Myeloperoxidase (MPO; donor: hydrogen peroxide oxidoreductase, EC 1.11.2.2) is a heme-containing peroxidase expressed mainly in neutrophils (2–5% of total cellular proteins or 2–4 μg per 10^6 cells) and to a lesser degree in monocytes [1,2]. Normally stored in azurophilic granules of fully differentiated neutrophils (mature granulocytes) MPO is a dimer (~145 kDa) consisting of two identical heme-containing protomers (~75 kDa) linked through a disulfide bond [3] and released into extracellular fluid in the setting of inflammatory process during neutrophil degranulation, inappropriate trafficking, neutrophil extracellular traps (NETs) formation or necrosis [4]. MPO is detected in inflammation foci both in the form of dimer and a monomer one [5–8]. The appearance of monomeric MPO under *in vivo* conditions is possible as a result of incomplete processing to the mature enzyme [6–8]. *In vitro* monomeric MPO, termed "hemi-MPO" can be obtained if reduction and alkylation break a disulfide bond linking two identical protomers in native MPO [9]. It should be noted, that the recombinant human MPO expressed in the Chinese hamster ovary cell line, which is used in many experimental studies, is also monomeric [10].

Another important reason for changing MPO structure can be its

* Corresponding author. Department of Biophysics, Faculty of Physics, Belarusian State University, 4 Nezavisimosti Avenue, Minsk 220030, Belarus.
E-mail address: irinagorudko@gmail.com (I.V. Gorudko).

https://doi.org/10.1016/j.abb.2022.109353
Received 23 May 2022; Received in revised form 28 June 2022; Accepted 11 July 2022
Available online 16 July 2022
0003-9861/