Free Radical Biology and Medicine 113 (2017) 551–563

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Mechanisms of myeloperoxidase catalyzed oxidation of H_2S by H_2O_2 or O_2 to produce potent protein Cys-polysulfide-inducing species

Dorottya Garai[a,1], Bessie B. Ríos-González[b,1], Paul G. Furtmüller[c], Jon M. Fukuto[d], Ming Xian[e], Juan López-Garriga[b], Christian C. Obinger[c], Péter Nagy[a,*]

[a] Department of Molecular Immunology and Toxicology, National Institute of Oncology, Ráth György utca 7-9, Budapest 1122, Hungary
[b] Department of Chemistry, University of Puerto Rico, Mayagüez Campus, P.O. Box 9019, Mayagüez, PR 00681-9019, United States
[c] Division of Biochemistry, Department of Chemistry, Vienna Institute of BioTechnology, BOKU-University of Natural Resources and Life Sciences, Muthgasse 18, A-1190 Vienna, Austria
[d] Department of Chemistry, Sonoma State University, Rohnert Park, CA 94928, United States
[e] Department of Chemistry, Washington State University, Pullman, WA 99164, United States

ARTICLE INFO

Keywords:
Myeloperoxidase
Hydrogen sulfide
Sulfane sulfur species
Persulfidation
Sulfhydration
Redox signaling
Lactoperoxidase

ABSTRACT

The interaction of heme proteins with hydrogen sulfide is gaining attention as an important element in sulfide-mediated protection against oxidative stress and in regulation of redox signaling. In our previous study we reported the efficient reversible inhibition of myeloperoxidase (MPO) activity by sulfide and the kinetics of the reactions of sulfide with ferric MPO, Compound I and Compound II. Here we provide several lines of evidence that a central intermediate species in the turnover of MPO by sulfide is the Compound III state. Compound III is formed in the reactions of sulfide with ferric or ferrous MPO in the presence of oxygen or via the reductions of Compound I or Compound II by sulfide. The regeneration of active ferric MPO from Compound III is slow - representing the rate-limiting step during turnover - but facilitated by ascorbate or superoxide dismutase. These catalytic cycles produce inorganic sulfane sulfur species, which were shown to promote protein Cys persulfidation. Based on compiling experimental data we propose that in contrast to hemoglobin, myoglobin, catalase or lactoperoxidase the formation of a sulfheme derivative in the oxidative interactions of sulfide with MPO is not a major pathway. Using the Met243Val mutant we demonstrated that the sulfonium ion linkage of the Met243 sulfur to the heme pyrrole ring A, which is a unique feature of MPO, is pivotal in the catalytic oxidation of sulfide via Compound III. The proposed novel MPO-catalyzed sulfide oxidation model does not require the initial presence of hydrogen peroxide, only oxygen to provide a slow flux of sulfane sulfur species generation, which could be important in sulfide-mediated endogenous signaling. Furthermore, peroxide-induced formation of sulfane sulfur species by MPO may have a role in protection of regulatory or functional Cys residues during (for example neutrophil induced) inflammatory oxidative stress.

1. Introduction

The diverse biological actions of the small signaling molecule hydrogen sulfide[2] have triggered increasing attention in the last two decades on evolutionary, physiological as well as pathophysiological grounds [1–5]. An important element of the underlying molecular mechanisms of the biological actions of sulfide is its interactions with heme proteins [6]. For example, the oxidative stress alleviating effects of sulfide were associated with i) inhibition of ROS producing heme protein functions (e.g. myeloperoxidase [7]) or ii) with reduction of highly oxidizing heme protein redox intermediate species [6,7]. In addition, it is becoming increasingly appreciated that a number of

Abbreviations: 3MST, 3-Mercaptopyruvate sulfurtransferase; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); BSA, bovine serum albumin; CBS, cystathione β-synthase; CHO, chinese hamster ovary; CSE, cystathione γ-lyase; DTNB, 5-5'-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; EPR, electron paramagnetic resonance; HSA, human serum albumin; IAB, EZ-Link Iodoacetyl-PEG2- biotin; MPO, myeloperoxidase enzyme; NADPH, Nicotinamide adenine dinucleotide phosphate; PVDF, polyvinylidene fluoride; ROS, reactive oxigen species; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; SOD, superoxide dismutase; SSP4, 3',6'-Di(O-thiosalicyl)fluorescein or Sulfane Sulfur Probe 4; TCEP, Tris(2-carboxyethyl)phosphin; tRNA, transfer RNA; TTBS, tris-buffered saline and Tween 20 buffer; UV-vis, ultraviolet-visible; WT, wild type

* Corresponding author.
E-mail address: peter.nagy@oncol.hu (P. Nagy).

[1] These authors contributed equally to this study.

[2] From now on we will use the term sulfide to refer to the sum of its different protonated forms that exist in solution, i.e. H_2S, HS^- and S^{2-}.

http://dx.doi.org/10.1016/j.freeradbiomed.2017.10.384
Received 20 June 2017; Received in revised form 12 October 2017; Accepted 26 October 2017
Available online 31 October 2017

important biological actions of sulfide are apparently due to sulfide-derived oxidation products, which contain zero-valent sulfur (often referred to as sulfane sulfur species[3]) including inorganic polysulfide and protein per/polysulfide species [6,8–11]. Consistent with the idea that poly sulfur species are biologically relevant and important signaling/effector species, sulfide exists at relatively low endogenous concentrations [12], while intracellular Cys per- and polysulfides were recently demonstrated to be highly abundant (including low molecular weight [13] and protein persulfides [14]). Protein persulfides can be generated post translationally via the reactions of sulfide with oxidized Cys residues (such as disulfides [15,16] or sulfenic acids [17]), oxidation of protein thiols by inorganic sulfane sulfur species [18,19] or enzymatically without the direct involvement of H_2S by 3-mercaptopyruvate sulfurtransferase (3MST) [20], cystathione β-synthase (CBS) [13] or cystathione γ-lyase (CSE) [21]. Furthermore, we recently demonstrated that protein Cys persulfides can be produced translationally by generating and incorporating Cys-SSH into nascent polypeptides by cysteinyl tRNA synthetase [22]. Protein persulfide speciation is highly regulated by selective and specific NADPH driven reduction mechanisms orchestrated by the thioredoxin and glutathione systems [14]. These recent mechanistic insights gave credence to the previous proposal [6,8,11] that persulfide formation on regulatory or functional protein Cys residues is an important element of sulfide signaling.

In this contribution we found a link between post-translational oxidative generation of protein persulfides and the interactions of sulfide with the heme enzyme human myeloperoxidase (MPO).

Based on a thorough kinetic study we previously reported efficient reversible inhibition of MPO peroxidase activity by sulfide and demonstrated that sulfide can also serve as a substrate for MPO in the presence of added H_2O_2 [7]. In that report we provided evidence for efficient interactions between different MPO enzyme forms and sulfide including coordination of sulfide to ferric MPO, reduction of ferric MPO by excess sulfide to ferrous MPO or fast oxidation of sulfide in its reactions with MPO Compound I and Compound II. Based on our pre-steady-state and steady-state enzyme kinetic studies we proposed a mechanism to explain both the turnover of MPO with sulfide using H_2O_2 and the reversible inhibition of MPO peroxidase activity in the presence of sulfide.

In this work we aimed at providing further insights into the reactions of MPO with sulfide by 1) investigating sulfide oxidation products and 2) further characterizing MPO intermediate species that form during turnover. We demonstrate that MPO can catalytically produce sulfane sulfur species even in the absence of added H_2O_2 by utilizing oxygen in a concerted action with superoxide dismutase (SOD) or ascorbate via the involvement of its Compound III (ferric-superoxide/ferrous-dioxygen complex) state. These MPO-induced sulfane sulfur species can oxidize protein Cys residues to their corresponding per/polysulfide derivatives. Therefore, we propose that this novel mechanism could play an important role in sulfur biology by providing a slow flux of sulfane sulfur production via MPO catalyzed sulfide oxidation by oxygen that can lead - among other biological actions - to protein persulfidation.

On the other hand, activated neutrophils excrete large amounts of H_2O_2 and MPO at sites of inflammation, which were associated with a number of inflammatory diseases via the production of promiscuous hypohalous acids and radical species [23,24]. Interestingly, sulfide was found to be protective in a number of these pathologies (see Ref. [7] for more info). We propose that this can potentially be due to inhibiting MPO-mediated oxidant production and/or due to sulfane sulfur production (via MPO catalyzed sulfide oxidation by neutrofil-produced H_2O_2). The latter can induce temporary per/polysulfidation on functional and/or regulatory protein Cys to protect these oxidant sensitive residues from irreversible excessive oxidation [9].

[3] "Sulfane sulfur species" is used herein as a collective term for oxidized sulfide derivatives, which, contain sulfur(s) at the zero valent oxidation state. Hence, sulfane sulfur, as we view it, regarding its oxidation state is equivalent to sulfur atom (a sulfur, containing 6 valence electrons).

2. Materials and methods

2.1. Materials

All reagents were purchased from Sigma-Aldrich unless otherwise indicated. Sulfide stock solutions were prepared as described previously [12]. Briefly, $Na_2S \times 9H_2O$ crystals were thoroughly washed and dissolved in ultrapure water. To determine the concentration of the stock solution, appropriate dilutions were made and their absorbances were measured at 230 nm ($\varepsilon_{230} = 7700\ M^{-1}\ cm^{-1}$). The purity of the solution was checked by measuring the absorbance at 412 nm after the addition of 5-5'-dithiobis(2-nitrobenzoic acid) (DTNB) ($\varepsilon_{412} = 14{,}100\ M^{-1}\ cm^{-1}$) (for details see Ref. [16]). Stock solutions were stored on ice in tightly closed and covered plastic containers to avoid oxidation and working solutions were prepared right before use by dilution with the appropriate assay buffer. Experiments were carried out in 100 mM (KH_2PO_4/K_2HPO_4) phosphate buffer at pH 7.4 unless otherwise specified.

Human leukocyte myeloperoxidase enzyme with a purity index ($A_{428\ nm}/A_{280\ nm}$) of at least 0.85 was purchased from Planta Natural Products (http://www.planta.at) The concentration of MPO, hydrogen peroxide (at pH 7.4) and hypochlorite (at pH 12) were determined spectrophotometrically using extinction coefficients of $\varepsilon_{428} = 91{,}000\ M^{-1}\ cm^{-1}$ per heme, $\varepsilon_{240} = 43.6\ M^{-1}\ cm^{-1}$ and $\varepsilon_{292} = 350\ M^{-1}\ cm^{-1}$, respectively.

2.2. Production of the Met243Val MPO mutant

Transfection, selection, CHO cell culture procedures, and protein purification protocols were described previously [25,26]. The Met243Val mutant had a purity index (A_{412}/A_{280}) of about 0.7 and the concentration was determined by using an extinction coefficient (ε) of $84{,}000\ M^{-1}\ cm^{-1}$ at 412 nm [27].

2.3. UV-vis measurements

The interactions of H_2S with ferrous MPO and with ferric MPO under anaerobic conditions were measured with an Agilent 8453 spectrophotometer using sealed quartz cuvettes with septum (1 cm path length). Reagents were added in 1 μL aliquots to the samples (in the cuvette) using gastight syringes. All measurements were performed at 25 °C and pH 7.0 in 0.1 M KH_2PO_4/Na_2HPO_4 buffer. Ferric MPO, buffer, $Na_2S \times 9H_2O$, sodium dithionite ($Na_2S_2O_4$), and protein solutions were prepared anaerobically by degassing for 30 min and flushing the headspace with nitrogen for at least 20 min. To investigate the interaction of ferric MPO with H_2S, 115 μM of H_2S was added to 2.5 μM of ferric MPO. Following a 36 min incubation time, samples were purged with O_2 for 30 s. For the ferrous MPO experiments, to accomplish the reduction of 2.7 μM MPO a fivefold excess of sodium dithionite was used from a freshly prepared anaerobic stock solution. When the reduction was complete, 115 μM of H_2S was added to start the reactions. Pure O_2 was introduced by purging the solutions for 30 s. All reactions were followed by monitoring the absorbance changes in the Soret band and in the visible region of MPO, collecting time resolved polychromatic data from 300 to 800 nm.

Spectral changes upon the addition of sulfide and SOD (from bovine erythrocytes) to ferric MPO derivatives were measured on a Zeiss Specord S10 photometer. In a typical experiment 2 μM MPO was mixed with 500 μM sulfide and after 500 s 3 μM SOD was added.

Sulfide-mediated reversible and irreversible inhibition of MPO and of the MPO Met243Val mutant, respectively was investigated by measuring the peroxidase activity using 2,2'-azino-bis(3-ethylbenzothiazoline-6-