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Research Paper

Catalytic oxidant scavenging by selenium-containing compounds: Reduction of selenoxides and *N*-chloramines by thiols and redox enzymes

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ABSTRACT

Myeloperoxidase produces strong oxidants during the immune response to destroy invading pathogens. However, these oxidants can also cause tissue damage, which contributes to the development of numerous inflammatory diseases. Selenium containing compounds, including selenomethionine (SeMet) and 1,4-anhydro-5-seleno-D-talitol (SeTal), react rapidly with different MPO-derived oxidants to form the respective selenoxides (SeMetO and SeTalO). This study investigates the susceptibility of these selenoxides to undergo reduction back to the parent compounds by intracellular reducing systems, including glutathione (GSH) and the glutathione reductase and thioredoxin reductase systems. GSH is shown to reduce SeMetO and SeTalO, with consequent formation of GSSG with apparent second order rate constants, k_2, in the range 10^3–10^4 M^{-1} s^{-1}. Glutathione reductase reduces both SeMetO and SeTalO at the expense of NADPH via formation of GSSG, whereas thioredoxin reductase acts only on SeMetO. The presence of SeMet and SeTal also increased the rate at which NADPH was consumed by the glutathione reductase system in the presence of *N*-chloramines. In contrast, the presence of SeMet and SeTal reduced the rate of NADPH consumption by the thioredoxin reductase system after addition of *N*-chloramines, consistent with the rapid formation of selenoxides, but only slow reduction by thioredoxin reductase. These results support a potential role of seleno compounds to act as catalytic scavengers of MPO-derived oxidants, particularly in the presence of glutathione reductase and NADPH, assuming that sufficient plasma levels of the parent selenoether can be achieved in vivo following supplementation.

1. Introduction

Neutrophils are key effector cells of the innate immune response, and are responsible for the release of bactericidal enzymes, including myeloperoxidase (MPO), and the generation of powerful oxidants to destroy invading pathogens [1,2]. MPO is a heme enzyme that catalyzes the reaction between hydrogen peroxide (H_2O_2) and halide and pseudohalide ions (chloride, Cl^-; bromide, Br^-; thiocyanate, SCN^-) to produce the corresponding hypohalous acids, hypochlorous acid (HOCl), hypobromous acid (HOBr) and hypothiocyanous acid (HOSCN) [1,3]. HOCl and HOBr react with amines to form the secondary oxidants *N*-halamines, which can retain the oxidizing capacity of the parent oxidants [1,4]. Although all of these species are effective in controlling or destroying pathogens, they are also capable of causing host tissue damage, and have been associated with many inflammatory diseases, including atherosclerosis, neurodegeneration, arthritis, kidney disease and some cancers [1,5]. Given the strong association between the generation of MPO oxidants in vivo and the development of inflammatory disease, there is significant interest in developing novel scavengers and / or inhibitors, which may be of therapeutic benefit

Abbreviations: DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); DTT, dithiothreitol; GlyCl, glycine chloramine; GPx, glutathione peroxidase; GSH, reduced glutathione; GSR, glutathione reductase; GSSG, oxidised glutathione; LysCl, *N*-α-acetyllysine chloramine; MPO, myeloperoxidase; MSCO, methylselenocysteine selenoxide; MsrA, methionine sulfoxide reductase A; MsrB2, methionine sulfoxide reductase B2; NADPH, nicotinamide adenine dinucleotide phosphate; NASMO, *N*-acetylselenomethionine selenoxide; PBS, phosphate-buffered saline; ONOOH, peroxynitrous acid; SeMet, selenomethionine; SeMetO, selenomethionine selenoxide; SePropO, seleno-bis-propionic acid selenoxide; SeTal, 1,4-anhydro-5-seleno-D-talitol; SeTalO, 1,4-anhydro-5-seleno-D-talitol selenoxide; Tau, taurine; TauCl, taurine chloramine; TCA, trichloroacetic acid; TFA, trifluoroacetic acid; TNB, 5-thio-2-nitrobenzoic acid; Trx, thioredoxin; TrxR, thioredoxin reductase

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(reviewed [6]).

Selenium-containing compounds, including selenomethionine (SeMet) and 1,4-anhydro-5-seleno-D-talitol (SeTal), are potential candidates to limit oxidative damage during inflammation *in vivo*, on account of their high reactivity with a number of key biological oxidants, including HOCl, HOBr, HOSCN, *N*-chloramines, H_2O_2 and peroxynitrous acid (ONOOH) [7-11]. The second-order rate constants for these reactions are some of the highest reported for biologically relevant reactions [7-11]. The reaction of SeMet with oxidants results primarily in the formation of selenomethionine selenoxide (SeMetO), which can be subsequently reduced by thiols such as glutathione (GSH) (Reaction 1) [7,11-15]. Other selenoxides formed on reaction of Se compounds with H_2O_2, can also be reduced by GSH, regenerating the parent Se compound and forming GSSG [16,17], which provides a pathway for potentially catalytic oxidant scavenging.

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$$SeMetO + 2GSH \rightarrow SeMet + GSSG + H_2O \quad (1)$$

Glutathione reductase (GSR) regenerates GSH from GSSG at the expense of NADPH, which potentially links oxidant removal with NADPH consumption and cellular metabolism [20]. Whilst evidence has been presented previously for selenoxide reduction by GSH [14,21], the mechanisms and rate constants for these reactions have not been reported. For rapid detoxification of oxidants and prevention of selenoxide accumulation, which would arrest the reduction cycle, it is critical for these rate constants to be of a high value and hence competitive with the initial reactions resulting in selenoxide formation.

SeMetO can also be reduced enzymatically, via the action of thioredoxin reductase (TrxR) and thioredoxin (Trx) (Reaction (2)) [21]. There is evidence that other Se compounds, including ebselen, selenocystamine and diselenides, can interact with TrxR [22,23], which allows catalytic reduction of peroxides and ONOOH [21–23]. This detoxification pathway may extend to MPO-derived oxidants, including HOCl and *N*-chloramines, that react rapidly with the Se compounds SeMet and SeTal, forming selenoxides [11].

$$SeMetO + NADPH \xrightarrow{TrxR} SeMet + NADP^+ + H_2O \quad (2)$$

The aim of this study was therefore to assess the catalytic oxidant scavenging potential of SeMet and SeTal by determining rate constants for the reduction of the corresponding selenoxides by GSH, and investigating the interaction between the selenoxides and the enzymatic reductants GSR and TrxR in the presence of NADPH. The study was subsequently extended to assess whether catalytic removal of *N*-chloramines could also be mediated by SeMet and SeTal in the presence of GSR and TrxR. The resulting data support the possibility of supplementation with Se compounds as way to reduce oxidative damage during chronic inflammatory conditions.

2. Materials and methods

2.1. Materials

All buffers, and solutions where indicated, were prepared using nanopure water filtered through a four-stage Milli-Q system (Millipore Waters, Australia). Solutions were prepared in 0.1 M, pH 7.4, phosphate buffer, unless otherwise specified. HOCl was prepared by dilution of a concentrated stock solution of NaOCl (12.5% w/v; Ajax FineChem Ltd, Australia), standardised at pH 12 by UV absorbance at 290 nm ($\varepsilon = 350\ M^{-1}\ cm^{-1}$ [24]). SeMet and methylselenocysteine were from Sigma-Aldrich (Castle Hill, NSW, Australia), and 1,4-anhydro-4-seleno-D-talitol (SeTal) was synthesised as described previously [25]. *N*-acetylselenomethionine was kindly prepared by Dr Lara Malins and seleno-bis-propionic acid was kindly provided by Prof. Indira Priyadarshini. Selenoxides (SeMetO, SeTalO, *N*-acetylselenomethionine selenoxide (NASMO), seleno-bis-propionic acid selenoxide (SePropO) and methylselenocysteine selenoxide (MSCO)) were prepared by mixing the parent Se compound (4 mM) with an equal volume of H_2O_2 (2 mM; Merck, Bayswater, VIC, Australia) with incubation for 10 min at 21 °C before dilution to the desired concentration in phosphate buffer (0.1 M, pH 7.4).

2.2. Thiol quantification

The consumption of GSH (4 μM) on addition of SeMetO and SeTalO (0–1 μM) was assessed by measuring the change in fluorescence ($\lambda_{ex} = 360$ nm; $\lambda_{em} = 530$ nm) of ThioGlo-1 using an M2e plate reader (Molecular Devices) as described previously [26]. The thiol concentrations were determined by comparison of the fluorescence intensity to a standard curve prepared using GSH (0–5 μM).

2.3. N-Chloramine formation and quantification

N-chloramines were prepared by reaction of taurine (Tau), glycine (Gly) or *N*-α-acetyllysine (Lys) (10 mM) with HOCl (2 mM). *N*-chloramine concentrations were determined by measuring 5-thio-2-nitrobenzoic acid (TNB) consumption at 412 nm as described previously [26], using an extinction coefficient $\varepsilon = 14{,}150\ cm^{-1}$ [27].

2.4. Stopped-flow kinetics

Stopped flow kinetics experiments were carried out using an Applied Photophysics SX.20MV stopped flow system maintained at 22 °C using a thermostatted water bath. The detection system consisted of an Xe light source (150 W; Osram GmbH, Munich, Germany) with wavelength selection achieved using a single monochromator (slit width, 0.5 mm; bandwidth, ± 1.2 nm) and photomultiplier detection. The system was controlled by a computer running Pro-Data SX (version 2.2.12; Applied Photophysics). Spectral data from 200 to 310 nm were obtained by acquiring kinetic traces at 10 nm intervals with the photomultiplier over this wavelength region. Second-order rate constants were typically obtained by global analysis of the kinetic traces using second order models with $\lambda > 240$ nm using ProKIV (Applied Photophysics, Version 1.0.1.4), although in some cases it was more appropriate to apply pseudo-first order analysis at selected wavelengths to derive the rate constants.

2.5. Enzymatic NADPH consumption

The reduction of each selenoxide (200 μM) by TrxR (25 nM; IMCO, Stockholm, Sweden) was assessed by measuring the change in absorbance of NADPH (700 μM; Roche, Castle Hill, NSW, Australia) at 340 nm every 30 s for 2 h using a M2e plate reader (Molecular Devices). The rate of consumption of NADPH (700 μM) was also used to assess the effect of SeMet and SeTal (20–200 μM) on the rate of *N*-chloramine (200 μM) reduction by TrxR (25 nM). An Applied Photophysics SX.20MV stopped flow system was used to quantify NADPH (500 μM) loss upon addition of each selenoxide (200 μM) to GSR (25 nM) and GSH (400 μM) in phosphate buffer (pH 7.4, 0.1 M). *N*-Chloramine (200 μM) reduction experiments were also performed by addition of SeMet or SeTal (20–200 μM) prior to exposure of the resulting solution to the NADPH/GSR/GSH mixture. For the GSR experiments, the loss of NADPH was measured by monitoring the absorbance of 340 nm over 60 s with a linear slope fitted to the initial decrease in absorbance, over 2–10 s, where maximum enzymatic activity was observed.