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Original article

Mesna (2-mercaptoethane sodium sulfonate) functions as a regulator of myeloperoxidase☆

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ABSTRACT

Myeloperoxidase (MPO), an abundant protein in neutrophils, monocytes, and macrophages, is thought to play a critical role in the pathogenesis of various disorders ranging from cardiovascular diseases to cancer. We show that mesna (2-mercaptoethanesulfonic acid sodium salt), a detoxifying agent, which inhibits side effects of oxazaphosphorine chemotherapy, functions as a potent inhibitor of MPO; modulating its catalytic activity and function. Using rapid kinetic methods, we examined the interactions of mesna with MPO compounds I and II and ferric forms in the presence and absence of chloride (Cl^-), the preferred substrate of MPO. Our results suggest that low mesna concentrations dramatically influenced the build-up, duration, and decay of steady-state levels of Compound I and Compound II, which is the rate-limiting intermediate in the classic peroxidase cycle. Whereas, higher mesna concentrations facilitate the porphyrin-to-adjacent amino acid electron transfer allowing the formation of an unstable transient intermediate, Compound I*, that displays a characteristic spectrum similar to Compound I. In the absence of plasma level of chloride, mesna not only accelerated the formation and decay of Compound II but also reduced its stability in a dose depend manner. Mesna competes with Cl^-, inhibiting MPO's chlorinating activity with an IC_{50} of 5 μM, and switches the reaction from a $2e^-$ to a $1e^-$ pathway allowing the enzyme to function only with catalase-like activity. A kinetic model which shows the dual regulation through which mesna interacts with MPO and regulates its downstream inflammatory pathways is presented further validating the repurposing of mesna as an anti-inflammatory drug.

1. Introduction

Myeloperoxidase (MPO) is a dimeric hemoprotein with two identical subunits. Each subunit is comprised of light and heavy chains with molecular masses of 15 and 60 kDa, respectively [1,2]. MPO utilizes hydrogen peroxide (H_2O_2) as the electron acceptor in the catalysis of oxidative reactions, which have a critical role in the generation of inflammatory injury specifically in cardiovascular diseases [3–5]. Under physiological conditions, MPO catalyzes the formation of antimicrobial species such as cytotoxic hypochlorous acid (HOCl), through the oxidation of chloride (Cl^-) as the substrate [3,5]. Indeed, MPO is the only human enzyme known to selectively generate reactive chlorinating species under physiological concentration of halides [6]. MPO final products can serve as markers to identify sites of MPO-mediated oxidative damage [7–10]. The mechanism that governs the typical MPO catalytic activity is represented in Eq. (1)–(5):

$$\text{MPO-Fe(III)} + H_2O_2 \rightarrow \text{MPO-Fe(IV)} = O^{\bullet+\pi}{}_{Por} + H_2O \quad (1)$$

Compound I.

$$\text{Compound I} + Cl^- + H^+ \rightarrow \text{MPO-Fe(III)} + HOCl \quad (2)$$

$$\text{Compound I} + AH_2 \rightarrow \text{MPO-Fe(IV)} = O + {}^{\bullet}AH \quad (3)$$

Compound II.

Abbreviations: Cl^-, Chloride; MPO, Myeloperoxidase; H_2O_2, Hydrogen peroxide; e^-, Electron

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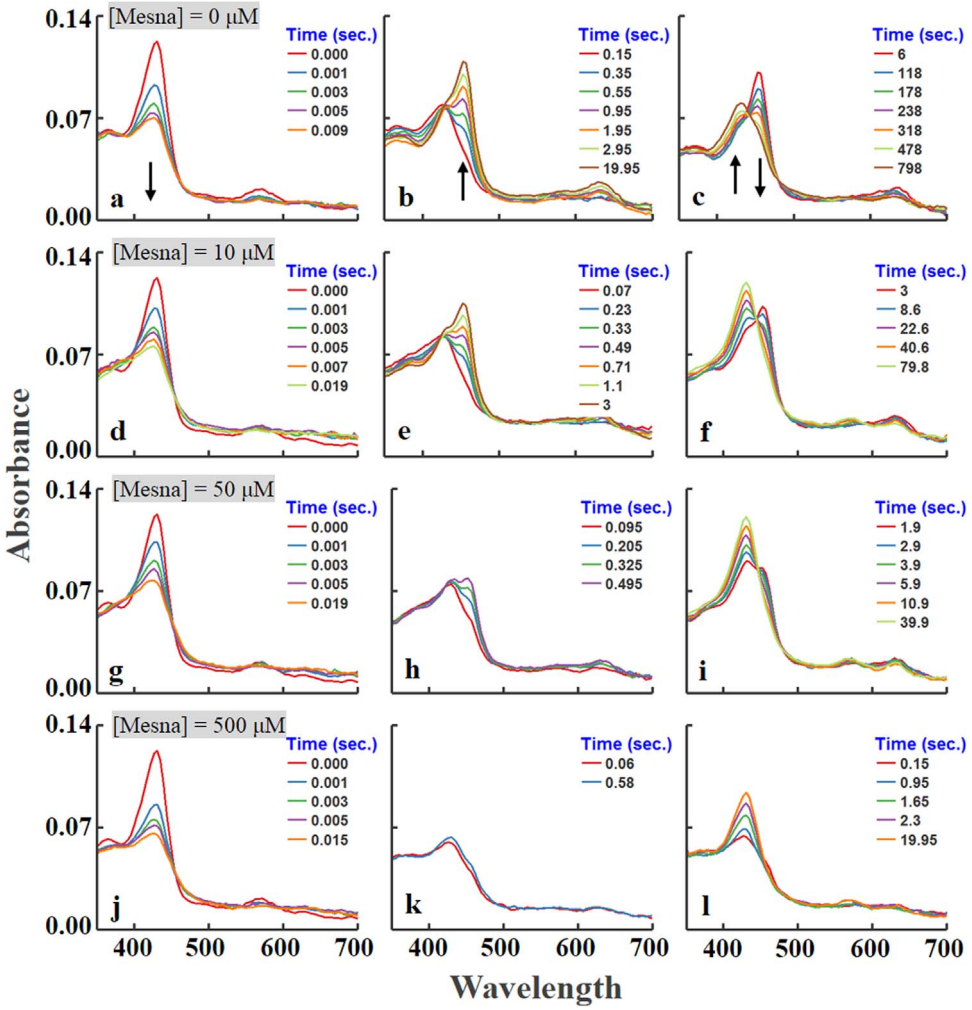

Fig. 1. Diode array rapid scanning spectra for the intermediates and for the reaction of increasing concentrations of mesna with MPO-Fe(III) at three sequential time frames. Experiments were carried out by rapid mixing a solution containing sodium phosphate buffer (200 mM, pH 7.4) supplemented with 1 μm (final) MPO-Fe(III) and increasing concentrations of mesna (0, 10, 50, and 500, final) was rapidly mixed with an equal volume of buffer containing fixed concentrations of H_2O_2 (20 μM, final) at 10 °C. The final concentration of mesna in mixtures is indicated. Arrows indicate the direction of spectral change over time as each intermediate advanced to the next. These data are representative of three independent experiments.

Compound I → MPO-Fe(IV) = $O^{\bullet\ +\pi}$ami → Compound II (4)

Compound I*.

Compound II + AH_2 → MPO-Fe(III) + $^{\bullet}AH$ (5)

Firstly, H_2O_2 typically reacts rapidly with MPO-Fe(III) and generates a ferryl porphyrin π cation radical (MPO-Fe(IV) = $O^{\bullet\ +\pi}$por) intermediate named Compound I (Eq. (1)) [11,12]. Compound I is capable of oxidizing either Cl^- through a $2e^-$ transition, generating MPO-Fe(III) and HOCl (Eq. (2)) or it can oxidize multiple organic and inorganic molecules (AH_2) by two successive sequential $1e^-$ transitions generating their corresponding free radicals ($^{\bullet}AH$) and the peroxidase intermediates Compound II (MPO-Fe(IV) = O) and MPO-Fe(III), respectively (Eqs. (3) and (5)) [11–15]. Compound II is a longer lasting intermediate whose decay to MPO-Fe(III) is considered to be the rate-limiting step during steady-state catalysis [12,15]. In some cases, the chlorinating activity of MPO can be disturbed through the formation of Compound I* that can be characterized by the electron hopping from the porphyrin ring site to a specific amino acid residue located near the heme molecule (Eq. (4)), and displays electron-acceptor properties [16–18]. Acceleration in Compound II formation and decay has been examined using series of organic and inorganic substrates as well as physiological reductants like nitric oxide and superoxide [11–14,19,20]. Chloride binds close enough to the heme iron of the peroxidase to influence both its reduction potential and binding affinity to small molecules, such as NO and superoxide, when these molecules bind to the heme center of the enzyme [21–23].

Mesna, (2-mercaptoethanesulfonic acid sodium salt), is an adjuvant drug utilized to reduce the risk of hemorrhagic cystitis in patients receiving treatment with chemotherapy such as isofamide or cyclophosphoamide, in general oxazaphosphorine chemotherapy [24,25]. Mesna is a cytoprotectant, which works by binding to the break down products of ifosfamide or cyclophosphoamide through a Michael addition to form a less harmful substance and reduce the risk of hemorrhage [24,25]. It is administered either orally or intravenously (IV) injected, most notably at the same time as the chemotherapy treatment [26,27]. When given orally, it's at 40% of the ifosfamide (1.2 g/m^2) dose at 2 and 6 h after each dose of ifosfamide or IV at 20% of the ifosfamide (1.2 g/m^2) dose once at the time of ifosfamide administration and 20% of the ifosfamide dose 4 and 8 h after each dose of ifosfamide, or as a single injection followed by 2 oral doses [26,27]. Alternatively, 10% of the dose can be given before the ifosfamide infusion and the remainder continued during and for 12–24 h after the ifosfamide infusion has stopped [26,27].

Myeloperoxidase-mediated reactive oxidants promote oxidative tissue damage in a variety of inflammatory diseases, ranging from various cancers, cardiovascular disorders and infertility [4,9,28]. Mesna, an agent known for its antioxidant properties and reducing the undesired side effects of chemotherapeutics, displays minimum to