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Interaction of hypochlorous acid and myeloperoxidase with phosphatidylserine in the presence of ammonium ions

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ABSTRACT

The close association of the heme enzyme myeloperoxidase to phosphatidylserine epitopes on the surface of non-vital polymorphonuclear leukocytes (PMNs) and other apoptotic cells at inflammatory sites favours modifications of this phospholipid by myeloperoxidase products. As detected by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, ammonium ions inhibit in a concentration-dependent manner the hypochlorous acid-mediated formation of aldehyde and nitrile products from 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS). Concomitantly, the formation of monochloramine (NH_2Cl) raises with increasing NH_4^+ concentrations. A transchlorination from monochlorinated O-phospho-L-serine to NH_4^+ with the formation of NH_2Cl occurs only when extraordinary high NH_4^+ concentrations are applied. Due to the low rate of 0.044 M^{-1} s^{-1} for this process, a transhalogenation reaction from transient chlorinated intermediates of the serine moiety to NH_4^+ can be ruled out as an important process contributing to the HOCl-mediated formation of NH_2Cl. A significant formation of NH_2Cl by myeloperoxidase interacting with DPPS in the presence of ammonium ions takes only place at acidic pH values around 5, a scenario that may occur in phagosomes of macrophages after the uptake of apoptotic PMNs.

1. Introduction

Polymorphonuclear leukocytes (PMNs) are among the first cells that are recruited in large amounts at inflammatory sites [1]. Here they release various hydrolytic and bactericidal proteins which contribute to pathogen inactivation, the degradation of damaged cell and tissue material as well as to the regulation of the inflammatory process [2–5].

The heme enzyme myeloperoxidase (MPO, donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) is stored in the azurophilic granules of PMNs, but is never found on the surface of vital cells. However, MPO becomes expressed on the surface of apoptotic and necrotic PMNs where it is closely associated with phosphatidylserine (PS) containing epitopes [6]. MPO released from stimulated PMNs binds also to PS domains on the surface of other apoptotic cells such as spermatozoa [7].

The serine head group of this phospholipid resembles in its structure an α-amino acid. The MPO product hypochlorous acid (HOCl) is known to convert α-amino acids via chlorinated amine derivatives to aldehyde and nitrile species. The aldehyde species is derived from the decay of the *N*-monochloramine derivative, while the nitrile product results from the *N*-dichloramine intermediate [8–10]. Phosphatidylglycolaldehyde was detected as a product from PS upon its reaction with HOCl or the MPO–hydrogen peroxide–chloride system [11]. Recently we found aldehyde and nitrile species as final products and *N*-mono- and *N*-dichloramine derivatives as well as a chlorimine species as transient intermediates in these reactions [12].

Although the close proximity between MPO and phosphatidylserine on the surface of apoptotic cells favours modifications of this phospholipid, other molecules being a target for HOCl may interfere with PS alterations. Ammonia that is in equilibrium with ammonium ions is known to react with HOCl under the formation of the small, lipophilic and volatile molecule monochloramine (NH_2Cl) [13,14]. Moreover, a transfer of the Cl^+ moiety from various *N*-monochloramines to other molecules bearing amino groups has been reported [13,15–17]. It remains unknown to what extent ammonium ions may compete with PS for MPO products and how transient chlorinated products of phosphatidylserine are involved in the transchlorination to ammonia/ammonium ions or other targets.

Here we investigated effects of ammonium ions on the HOCl- and myeloperoxidase-induced modification of phosphatidylserine. Ammonium ions decreased the product yield of phosphatidylserine metabolites as revealed by mass spectrometry measurements and caused the formation of NH_2Cl. With MPO, the formation of NH_2Cl is

Abbreviations: DHB, 2,5-dihydroxybenzoic acid; DPPS, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine; MALDI-TOF, matrix-assisted laser desorption/ionisation time-of-flight; MPO, myeloperoxidase; PMNs, polymorphonuclear leukocytes; PS, phosphatidylserine.

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favoured under acidic conditions. Kinetic data showed that transchlorination reaction from *N*-monochloramine intermediates to ammonia is only of minor importance.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS) was purchased from Avanti Polar Lipids, Alabaster, Alabama. Myeloperoxidase was obtained from Planta Natural Products, Vienna, Austria. Its concentration was determined using $\varepsilon_{430} = 91{,}000\ M^{-1}\ cm^{-1}$ per heme [18]. All other chemicals were purchased in highest commercially available purity from Fluka, Buchs, Switzerland and Sigma, Taufkirchen, Germany and used without further purification. Concentrations of the stock solutions of HOCl and H_2O_2 were determined immediately prior to use using $\varepsilon_{290} = 350\ M^{-1}\ cm^{-1}$ for -OCl [19] and $\varepsilon_{240} = 39.4\ M^{-1}\ cm^{-1}$ for H_2O_2 [20]. These solutions were essentially stable within one hour and were used in this time.

2.2. Treatment of DPPS liposomes with HOCl

Liposomes were prepared from DPPS suspensions followed by ultra-sonification (10 min, Sonifier 250, Bransson Ultrasonics, Danbury, Connecticut). They were prepared in 50 mM phosphate buffer (pH 7.4 to 6) or 50 mM citrate–phosphate buffer (pH 6 and lower). Liposomes (0.2 mM, final lipid concentration) were incubated with equimolar amounts or a two-fold molar excess of HOCl for 5 min at room temperature. At the end of the incubation, lipids were extracted as described by Bligh and Dyer [21].

2.3. Incubation of DPPS with the MPO–H_2O_2–Cl^-system

Sixty μM of DPPS liposomes were incubated with 140 nM myeloperoxidase in the presence of 0.14 M NaCl. The incubations were performed in 50 mM phosphate buffer (pH 7.4 to 6) or 50 mM citrate–phosphate buffer (pH 6 and lower). Ten portions of 88 μM hydrogen peroxide were added within 30 min (880 μM, final concentration) followed by the extraction of lipids. These experiments were performed in these presence of varying concentrations of ammonium chloride (0–140 μM).

2.4. Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry

All mass spectrometry measurements were done on a Bruker Autoflex (Bruker Daltonics GmbH, Leipzig, Germany) supplied with a 337 nm nitrogen laser. As a matrix preparation 0.5 M 2,5-dihydroxybenzoic acid (DHB) containing 0.1% trifluoroacetic acid was usually used. All spectra were obtained in the positive ion mode which gave by far the best results concerning traceability, reproducibility and signal-to-noise ratio [22].

2.5. Determination of monochloramine

Monochloramine (NH_2Cl) was quantified in PS-containing solutions by a nitroprusside-catalysed reaction with salicylate to yield a coloured indophenol derivative [23]. This modified Berthelot reaction allows the detection of NH_2Cl even in the presence of *N*-monochloramines or other chlorinated products of PS since this reaction occurs only when the nitrogen atom of the chloramine bears two exchangeable hydrogen atoms. Briefly, the sample solution (0.4 ml) was mixed with a solution (0.1 ml) containing 360 mM salicylate and 220 mM tartrate. Then, a freshly prepared sodium nitroprusside solution (3 mM, 10 μl) was added. Following the addition of 20 μl 1.2 M NaOH and dilution with distilled water to 1 ml, the sample was incubated for 1 h at room temperature. The absorbance was red at 703 nm, the spectral maximum of the indophenol species. The concentration of this coloured complex was determined using $\varepsilon_{703} = 11{,}500\ M^{-1}\ cm^{-1}$ [23].

A freshly prepared solution of NH_2Cl was used for calibration purposes to verify the above described method. Monochloramine was produced adding HOCl in small drops to an ice-cold equimolar concentrated solution of ammonium chloride. Its concentration was determined using $\varepsilon_{243} = 461\ M^{-1}\ cm^{-1}$ [24].

2.6. UV–visible (UV–vis) spectroscopy

O-Phospho-L-serine (2 mM) was incubated with HOCl at different molar ratios at 22 °C and UV–vis spectra were recorded. After 120 s various amounts of NH_4Cl (50–350 mM) were added in order to examine the transchlorination reaction from transient chlorinated intermediates of O-phospho-L-serine to ammonia. Incubations were performed in 0.14 M NaCl, 50 mM phosphate buffer, pH 7.4. Absorbance spectra as well as changes at selected wavelengths were monitored under temperature control using a Varian Cary 50 UV–VIS spectrophotometer (Varian, Mulgrave, Australia).

3. Results

3.1. Inhibition of HOCl-mediated phosphatidylserine modification by ammonium ions

The incubation of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS) with HOCl leads to a loss of DPPS and the appearance of two new final products, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoacetonitrile and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoacetaldehyde as revealed by MALDI-TOF mass spectrometry using the DHB matrix. The non-reacted DPPS (molecular weight in solution as monoanion: 734.5 Da) showed four peaks at 736.5 Da, 758.5 Da, 780.5 Da and 802.5 Da corresponding to the +2H, +H+Na, +2Na, and +3Na–H adducts, respectively, in the positive MALDI-TOF mass spectrum. These peaks disappeared and new signals arose after the reaction of DPPS with HOCl at pH 7.4 at *m/z* 710.5 Da and 732.5 Da for the nitrile species and at *m/z* 713.5 Da and 735.5 Da for the aldehyde product (Fig. 1A, traces a and b). The first peak corresponded to a M + H + Na adduct, while the second one was a M + 2Na adduct. Peak assignment was performed as done before [12] and applying data from mass spectrometric analysis of products formed in the reaction of HOCl with phosphatidylethanolamine [25]. The peak at 727 Da corresponded to a matrix peak.

Ammonium ions inhibited the formation of these new products when they were present in the incubation cocktail at the moment of the HOCl addition (Fig. 1A, trace c). At 70 μM NH_4^+ about 60 % of non-reacted DPPS was detected in the mass spectrum. Parallel to the recovery of non-reacted DPPS with increasing ammonium ion concentration, there was a decrease in the nitrile product (Fig. 1B). The aldehyde product also decreased with increasing NH_4^+, but only after an initial increase at 30 μM NH_4^+. At 140 μM, the highest applied NH_4^+ concentration, only small amounts of DPPS modification products were found. Apparently, ammonium ions compete with phosphatidylserine for HOCl.

We next analysed our samples for the presence of monochloramine (NH_2Cl), the product of the reaction between ammonia and HOCl [13,14]. The formation of NH_2Cl (Fig. 1C) increased with the amount of NH_4^+ in the incubation cocktail. It coincides well with the disappearance of phosphatidylserine products.

Similar data about effects of ammonium ions on HOCl-mediated modification of DPPS were obtained at pH 5 (data not shown).

The addition of ammonium ions (70 μM) after the start of the reaction between HOCl and DPPS inhibited partially the nitrile and aldehyde formation. The degree of inhibition decreased with the time