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Modification of phosphatidylserine by hypochlorous acid

Joerg Flemmig, Holger Spalteholz, Katja Schubert, Sandra Meier, Juergen Arnhold*

Institute for Medical Physics and Biophysics, Medical Department, University of Leipzig, Haertelstr. 16-18, D-04107 Leipzig, Germany

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ABSTRACT

The binding of the heme enzyme myeloperoxidase to phosphatidylserine epitopes on the surface of non-vital polymorphonuclear leukocytes and other cells at inflammatory sites favours modifications of this phospholipid by myeloperoxidase products. As detected by MALDI-TOF mass spectrometry hypochlorous acid and the myeloperoxidase–hydrogen peroxide–chloride system convert 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine into 1,2-dipalmitoyl-*sn*-glycero-3-phosphoacetaldehyde and 1,2-dipalmitoyl-*sn*-glycero-3-phosphonitrile. A transient chlorimine derivative was detected using 4-chloro-α-cyanocinnamic acid as matrix in mass spectrometry only at short incubation times and supplying HOCl in two-fold excess. The decay of transient chlorinated products was followed by changes in absorbance spectra using O-phospho-L-serine to model the behavior of the serine head group in phosphatidylserine. *N*-Chlorimine and *N*-monochloramine derivatives decayed with half-life times of 1.5 and 57 min, respectively, at 22 °C and pH 7.4. *N*-Dichloramines decayed within few seconds under these conditions.

1. Introduction

The heme protein myeloperoxidase (MPO), the most abundant protein in polymorphonuclear leukocytes (PMNs), is well known for its ability to produce hypochlorous acid (HOCl) in the presence of hydrogen peroxide and chloride (Klebanoff, 1991). Myeloperoxidase contributes with these reactive species to inactivation and killing of foreign microorganisms as well as to regulation of inflammatory processes (Brennan et al., 2001; Milla et al., 2004).

Upon release from internal stores of PMNs at inflammatory sites, MPO is known to become attached to the surface of apoptotic cells on phosphatidylserine containing epitopes (Leßig et al., 2007; Flemmig et al., 2008). The serine head group of this phospholipid resembles in its structure an α-amino acid. Reactions of different α-amino acids with HOCl have been known for a long time (Raschig, 1907; Langheld, 1909; Dakin, 1916). Hypochlorous acid converts 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 (Stelmaszyńska and Zgliczynski, 1978; Hazen et al., 1998; Joo and Mitch, 2007).

Little is known about the reaction between HOCl and phosphatidylserine. Hypochlorous acid converts phosphatidylserine to an unstable chloramine which decays with a half-life time of about 10 min at 37 °C under formation of phosphatidylglycolaldehyde (Kawai et al., 2006). The later product was also found as a product of the reaction of the MPO–H_2O_2–Cl^- system with phosphatidylserine (Kawai et al., 2006). The formation of further products in the reaction of HOCl with phosphatidylserine remains unknown.

As the close proximity between MPO and phosphatidylserine on the surface of apoptotic cells favours modifications of this phospholipid, we addressed here the question which intermediate and final products will be formed in the reaction of phosphatidylserine with HOCl. Reaction was investigated at neutral and also at acidic pH values, because of the decrease of pH in the forming phagolysosomes during phagocytosis of apoptotic cells by macrophages.

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. O-Phospho-L-serine was obtained from Sigma–Aldrich, Taufkirchen, Germany. 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 stock solutions of HOCl and H_2O_2 were determined before application using $\varepsilon_{290} = 350\ M^{-1}\ cm^{-1}$ for ^-OCl (Morris, 1966) and $\varepsilon_{240} = 39.4\ M^{-1}\ cm^{-1}$ for H_2O_2 (Beers and Sizer, 1952). These solutions were essentially stable within 1 h and were used in this time.

* Corresponding author. Tel.: +49 341 9715705, fax: +49 341 9715 709.
E-mail address: juergen.arnhold@medizin.uni-leipzig.de (J. Arnhold).

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2.2. Treatment of DPPS liposomes with HOCl

Liposomes were obtained from DPPS suspensions followed by ultra-sonification (10 min, Sonifier 250, Bransson Ultrasonics, Danbury, CT). They were prepared in 50 mM phosphate buffer (pH 7.4–6) or 50 mM citrate–phosphate buffer (pH 6 and lower). Liposomes (0.2 mM, final lipid concentration) were incubated with equimolar amounts or two-fold molar excess of HOCl for 5 min at room temperature. At the end of incubation, lipids were extracted as described in Bligh and Dyer (1959).

2.3. Treatment of DPPS liposomes 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. Incubations were performed in 50 mM phosphate buffer, pH 7.4, or 50 mM citrate–phosphate buffer, pH 5.0. Ten portions of 88 μM hydrogen peroxide were added within 30 min (880 μM, final concentration) followed by extraction of lipids as illustrated in Bligh and Dyer (1959).

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

All mass spectrometry measurements were taken on a Bruker Autoflex (Bruker Daltonics GmbH, Leipzig, Germany) supplied with a 337 nm nitrogen laser. As a matrix 0.5 M 2,5-dihydroxybenzoic acid (DHB) containing 0.1% trifluoroacetic acid was normally used. In some cases, a 30 mM solution of 4-chloro-α-cyanocinnamic acid (ClCCA) in acetonitrile/1.5% trifluoroacetic acid (70/30, v/v) (Jaskolla et al., 2009) was also used as matrix. All spectra were obtained in the positive ion mode which gave by far the best results concerning traceability, reproducibility and signal-to-noise ratio (Petković et al., 2001).

2.5. UV–vis spectroscopy

O-Phospho-L-serine (2 mM) was incubated with HOCl at different molar ratios and at various temperatures (10–37 °C). Incubations were performed in 50 mM phosphate buffer, pH 7.4, or 50 mM citrate–phosphate buffer, pH 5.0 or 6.0. Absorbance spectra as well as changes at 252 or 225 nm were monitored under temperature control using a Varian Cary 50 UV–vis spectrophotometer (Varian, Mulgrave, Australia).

3. Results

3.1. Reaction of HOCl with phosphatidylserine

In the first set of experiments, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS) was incubated with the reagent HOCl in order to study final products formed in this reaction with the help of mass spectrometry. A phosphatidylserine species with saturated acyl chains was chosen to avoid any interferences of hypochlorous acid with olefinic double bonds. In the later reaction, the

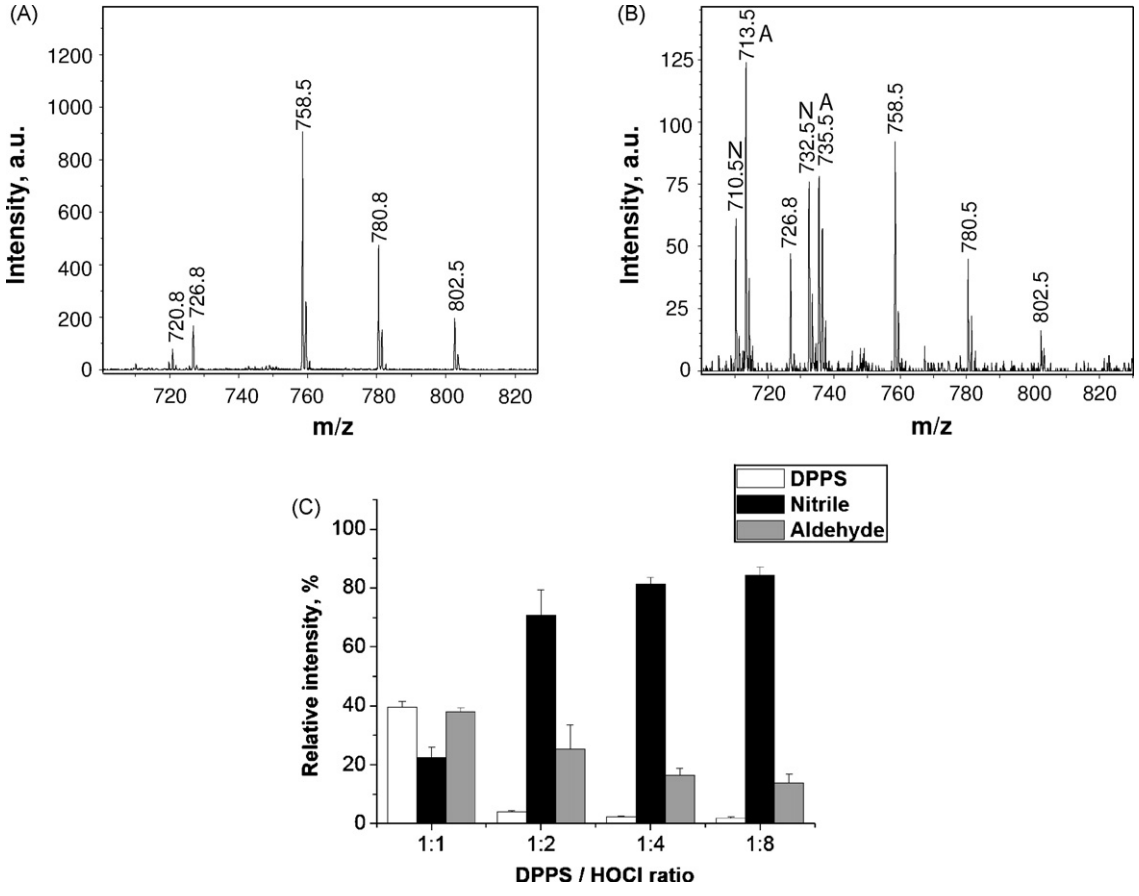

Fig. 1. (A) Positive ion MALDI-TOF mass spectrum of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS) obtained in a DHB matrix. (B) MALDI-TOF mass spectrum of DPPS (0.2 mM, final concentration) after its reaction with an equimolar amount of HOCl at pH 7.4 and room temperature for 5 min. Reaction medium was 0.14 M NaCl, 10 mM phosphate. (C) Relative impacts of total peak heights for non-reacted DPPS (*m*/*z*: 758.5, 780.5, and 802.5), nitrile (N) product (*m*/*z*: 710.5 and 732.5), and aldehyde (A) product (*m*/*z*: 713.5 and 735.5) in dependence on the HOCl to DPPS ratio. The sum of all signals was set to 100%. DPPS concentration and reaction conditions are as in (B). Means and s.d. of three independent experiments are given.