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Leukocytic Myeloperoxidase-Mediated Formation of Bromohydrins and Lysophospholipids from Unsaturated Phosphatidylcholines

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Abstract—Using MALDI-TOF mass spectrometry, we have shown that leukocytic myeloperoxidase (MPO) in the presence of its substrates (H_2O_2 and Br^-) does not induce any changes in saturated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine. Incubation of liposomes prepared from mono-unsaturated phosphatidylcholine (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) with the (MPO + H_2O_2 + Br^-) system resulted in formation of bromohydrins as the main products. 1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lysophosphatidylcholine) was the main product of the reaction of polyunsaturated phosphatidylcholine (1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine) with the (MPO + H_2O_2 + Br^-) system. The formation of lysophospholipids as well as of bromohydrins was not observed when the enzyme or one of its substrates (H_2O_2 or Br^-) was absent from the incubation medium, or if an inhibitor of MPO (sodium azide) or hypobromite scavengers (taurine or methionine) were added. Thus, it can be postulated that the formation of bromohydrins as well as lysophospholipids by the (MPO + H_2O_2 + Br^-) system results from reactions of hypobromite formed during MPO catalysis with double bonds of acyl chains of phosphatidylcholine. Such destructive processes may take place *in vivo* in membrane- or lipoprotein-associated unsaturated lipids in centers of inflammation.

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Leukocytic myeloperoxidase (MPO) (donor:hydrogen peroxide oxidoreductase, EC 1.11.1.7) is a component of monocytes, particularly neutrophils. Under activation of these cells the enzyme is excreted into the extracellular medium and catalyzes oxidation of halogen ions (Cl^-, Br^-, and I^-) by hydrogen peroxide to corresponding hypohalous acids (HOCl, HOBr, and HOI) in the following reaction:

$$X^- + H_2O_2 + H^+ \rightarrow HOX + H_2O,$$
$$HOX \leftrightarrow H^+ + OX^-, \quad (1)$$

where X is halogen [1]. The overall equation (1) describes the so-called MPO chlorination cycle. This name is given to the cycle because chloride is the major halogen oxidized in accordance with reaction (1) in human blood. Its concentration in plasma (100-140 mM) is at minimum three orders higher than that of bromide (20-100 μM) and 5-6 orders higher than that of iodide (0.1-0.5 μM) [2]. The formed hypochlorous acid (HOCl) and its ionized form, hypochlorite (OCl^-), are potent oxidizers playing an important role in detoxification of the organism and functioning as antiviral and antibacterial agents in centers of inflammation [3]. HOCl/OCl^- reacts with many biologically important molecules (nucleic

Abbreviations: DMPC) 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; EPO) eosinophil peroxidase; HOCl/OCl^-) hypochlorite; HOBr/OBr^-) hypobromite; lyso-PC) 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine; MALDI-TOF) matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MPO) myeloperoxidase; PAPC) 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine; POPC) 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

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acids, proteins, carbohydrates, lipids, vitamins, etc.) [4-8].

Earlier we studied reactions between hypochlorite and lipids and revealed that HOCl/OCl^- interacts with unsaturated phospholipids by two main mechanisms. The first is the free-radical mechanism characterized by accumulation of lipid peroxidation products [4, 9-16]. The second is molecular. According to this mechanism, chlorohydrin isomers, as well as their oxygen- and chlorine-containing metabolites, are formed as main products resulting from attachment of HOCl to a double bond [17-21]. Also, in the case of polyunsaturated phospholipids we have revealed the formation of their lyso-derivatives [21, 22].

As mentioned above, not only chloride, but also bromide is present in human blood. Although bromide concentration is 3-4 orders lower than that of chloride [2, 23], it has been shown that the rate constant of its oxidation by MPO (compound I) under physiological pH is approximately 40-50 times higher than the analogous rate constant of chloride oxidation [24]. Considering this fact, one can suppose that bromide can compete with chloride as a substrate for MPO under *in vivo* conditions. In other words, hypobromite formation (HOBr/OBr^-) in human blood resulting from MPO-dependent bromide oxidation is a tangible process.

Carr with coworkers have detected the formation of bromohydrins under the action of HOBr/OBr^- on unsaturated phosphatidylethanolamine [25], as well as under incubation of micelles from free unsaturated fatty acids in the presence of the (MPO + H_2O_2 + Br^-) system [26]. However, it is worth noting that MPO-dependent formation of bromohydrins from free unsaturated fatty acids formed into micelles can substantially differ from that in the case of unsaturated phospholipids. The use of phosphatidylethanolamine for the study of HOBr/OBr^- reaction (*per se*, as well as formed from MPO-catalysis) with unsaturated bonds of acyl chains is complicated by the circumstance that HOBr/OBr^- primarily reacts with amino group of phosphatidylethanolamine. The resulting bromamines can brominate the double bond with the formation of the same bromohydrins [25]. Phosphatidylcholine is devoid of this weakness because its phosphocholine group does not react with hypochlorite [18, 20, 21]. This fact raises the hope that phosphatidylcholine can be used in the study of the interaction mechanism between hypohalous acids formed in the (MPO + H_2O_2 + halide) system and double bonds of unsaturated acyl chains of phospholipids.

In the present study, we have tried to reveal whether leukocytic MPO can modify various unsaturated phosphatidylcholines as substrates in the presence of Br^-, whether this modification is associated with the formation of hypobromite, and what might be the nature of products formed. We therefore used MALDI-TOF mass spectrometry, which does not require any preliminary derivatization of the substances under study, is relatively simple, and is highly sensitive and specific, thus enabling detection of phospholipids [27, 28] and their derivatives [20-22] directly in extracts.

MATERIALS AND METHODS

Chemicals. 1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (PAPC), and 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-PC) were from Avanti Polar Lipids (USA); myeloperoxidase from human polymorphonuclear leukocytes was from Planta GmbH (Austria); NaBr from Sigma (USA); 2,5-dihydroxybenzoate from Aldrich (USA); H_2O_2, methionine, taurine, sodium azide, trifluoroacetic acid, as well as all the other chemicals and solvents were from Fluka (Switzerland).

Preparation of phospholipid liposomes. Multilayer liposomes were prepared from DMPC, POPC, or PAPC in 50 mM phosphate buffer, pH 5.0, by dispersing for 30 sec of dry film formed from phospholipids as a result of vacuum-desiccation of their chloroform solutions.

Phospholipids were stored in chloroform solutions at −70°C with periodic monitoring of contents of autoxidation and hydrolysis products. Only phospholipids without any signs of decomposition were used in experiments.

Incubation of phospholipid liposomes with the (MPO + H_2O_2 + Br^-) system. Liposomes formed from various phosphatidylcholines (at final concentration 30 μg/ml) were incubated in medium containing the necessary amount of NaBr and 50 mM phosphate buffer, pH 5.0, at room temperature for 40 min in the presence of MPO (final concentration 0.14 μM). The reaction was initiated by addition of H_2O_2. The agent was added by 3 μM doses, 3 min apart, during all the incubation period. A series of control experiments was carried out in absence of enzyme or one of its substrates (H_2O_2 or Br^-), as well as in the presence of MPO inhibitor, sodium azide (2 mM), or hypobromite scavengers—methionine (2 mM) or taurine (2 mM). Two different buffer solutions, 50 mM phosphate/citrate in pH region of 3.3-7.4 and 50 mM borate buffer at pH > 7.4, were used in the study of pH dependence of bromohydrin formation. After finishing the incubation, lipid material was extracted with chloroform–methanol (2 : 1 v/v) and used for MALDI-TOF-analysis.

Analysis of lipids by MALDI-TOF mass spectrometry. Aliquots (10-50 μl) of organic extract of phospholipids or their reaction products were evaporated under vacuum. Matrix solution was added to the sample (5 μl of 0.5 M 2,5-dihydroxybenzoic acid solution in methanol containing 0.1% trifluoroacetic acid). One microliter of the mixture was applied onto a sample plate of the mass spec-

trometer and rapidly dried with a warm air stream. This procedure provides homogenous crystallization of matrix with lipid, enhancing the sensitivity of the method and reproducibility of results [27, 28].

MALDI-TOF-measurements were conducted in positive ionization (cationization) mode on a Voyager Biospectrometry workstation device (PerSeptive Biosystems, USA), equipped with pulse laser with wavelength of 337 nm. The voltage was 20,000 V. The signal was accumulated as an average from 128 single laser pulses. The range of relative molecular masses was 450-1500 daltons.

The mass spectrum of phosphatidylcholine (spectra *1*, *3*, and *5* in Fig. 1) presents a set of narrow bands, one of which with maximum intensity corresponds to the relative mass (M_r) of a molecule composed of major isotopes (^{12}C, ^{1}H, ^{16}O, ^{14}N, and ^{31}P) plus the mass of the ion ionizing the molecule. In our case (positive ionizing), H^+ or Na^+ can play the role of cation; hence, the position of the band in the mass spectrum would correspond to $[M_r + H]^+$ and $[M_r + Na]^+$. On the right from the band with maximum intensity a stub is visible of attenuated bands corresponding to cationized phospholipid molecules containing not only major, but also minor isotopes (mainly ^{13}C and ^{2}H) [27].

When calculating the relative signal intensity of phosphatidylcholines and products of their reaction, the signal intensity of DMPC was used as internal standard, which does not change during the incubation of saturated phosphatidylcholine with MPO (Fig. 1, spectra *1* and *2*).

RESULTS

Formation of bromohydrins. Mass spectra of liposome extracts produced from various phosphatidylcholines are given in Fig. 1 (spectra *1*, *3*, and *5*). It is seen that the spectra of all phosphatidylcholines contain two components differing by 22 mass units and corresponding to ions resulting from cationization of phospholipid molecules by hydrogen $[M_r + 1]^+$ and sodium $[M_r + 23]^+$. Also, in all the spectra a peak with m/z 551.1 is present, which is assigned to a matrix derivative, as shown earlier [22, 28].

Figure 1 also shows the mass spectra of phosphatidylcholine liposome extracts incubated in the presence of the (MPO + H_2O_2 + Br^-) system (spectra *2*, *4*, and *6*). It is seen that 40-min incubation of DMPC liposomes does not result in appearance of additional peaks in the mass spectrum (Fig. 1, spectra *1* and *2*). This means that DMPC, a saturated phospholipid, is not changed when incubated with this system.

When liposomes were formed from monounsaturated POPC, their incubation for 40 min in the presence of the (MPO + H_2O_2 + Br^-) system resulted in new signals in the mass spectrum, the main being peaks with m/z 856.5/858.5 and 878.5/880.5 assigned to H^+- and Na^+-adducts of the formed monobromohydrin, respectively (Fig. 1, spectrum *4*). The splitting of peaks is associated with the fact that bromine is present as a mixture of two isotopes, ^{79}Br and ^{81}Br.

Kinetic curves for the sum intensities of the signals $[M_r + H]^+$ and $[M_r + Na]^+$ for the initial POPC and its monobromohydrin are given in Fig. 2. It is seen that in the course of incubation of liposomes with the (MPO + H_2O_2 + Br^-) system the content of native phospholipid decreases in the reaction mixture (Fig. 2, curve *1*), with formation of monobromohydrin instead of phospholipid as the main product (Fig. 2, curve *2*). Apart from this, the presence of a minor signal with m/z 838.5/840.5 can be seen on the mass spectrum (Fig. 1, spectrum *4*) identified earlier in our work as H^+-adduct of bromo-derivative resulting from a substitution of H by Br in fatty acid chains [29].

The dependence of the residual part of the POPC (curve *1*) and the formed monobromohydrin (curve *2*) after 40 min of incubation in the presence of (MPO + H_2O_2 + Br^-) on bromide concentration is given in Fig. 3a. It is obvious that a significant amount (more than 50% of the initial POPC) of POPC monobromohydrin is accumulated already at low NaBr concentrations (50-100 μM) corresponding to bromide levels in human blood plasma [2, 23].

It has been shown that incubation of POPC liposomes in the presence of the (MPO + H_2O_2 + Br^-) system at alkaline pH (>7.5) is not accompanied by phospholipid destruction (Fig. 4, curve *1*). Monobromohydrin is also not formed under these conditions (Fig. 4, curve *2*). The amount of initial POPC decreases with decrease in pH into the acidic region. In parallel, the amount of monobromohydrin, the major reaction product formed, increases. Optimum pH value for this reaction is 4.5-5.0.

Formation of bromoderivatives was also registered in our experiments with liposomes composed of PAPC and containing a residue of polyunsaturated arachidonic acid. Figure 1 displays the mass spectrum (spectrum *6*) of extract obtained after 40-min incubation of PAPC liposomes with the (MPO + H_2O_2 + Br^-) system. The spectrum is characterized by the presence of fuzzy bands of low intensity with molecular masses higher than the mass of initial phosphatidylcholine, among which the signals at m/z 878.5/880.5 and 900.5/902.5 can be distinguished and assigned to H^+ and Na^+ adducts of PAPC monobromohydrin, respectively. The signals assigned to H^+ and Na^+ adducts of PAPC di- and tribromohydrins, respectively, are seen starting from m/z 974.5 and 996.5, 1070.4 and 1092.4. Fine structure of these signals is more complex due to the inclusion into the molecule of two or three bromine atoms. Starting from m/z 1166.4 even the signal from the H^+ adduct of tetrabromohydrin can be distinguished. Apart from this, peaks shifted by 18 units compared with the corresponding H^+ adduct of bromohydrin to lower molecular masses (starting from m/z 860.5,

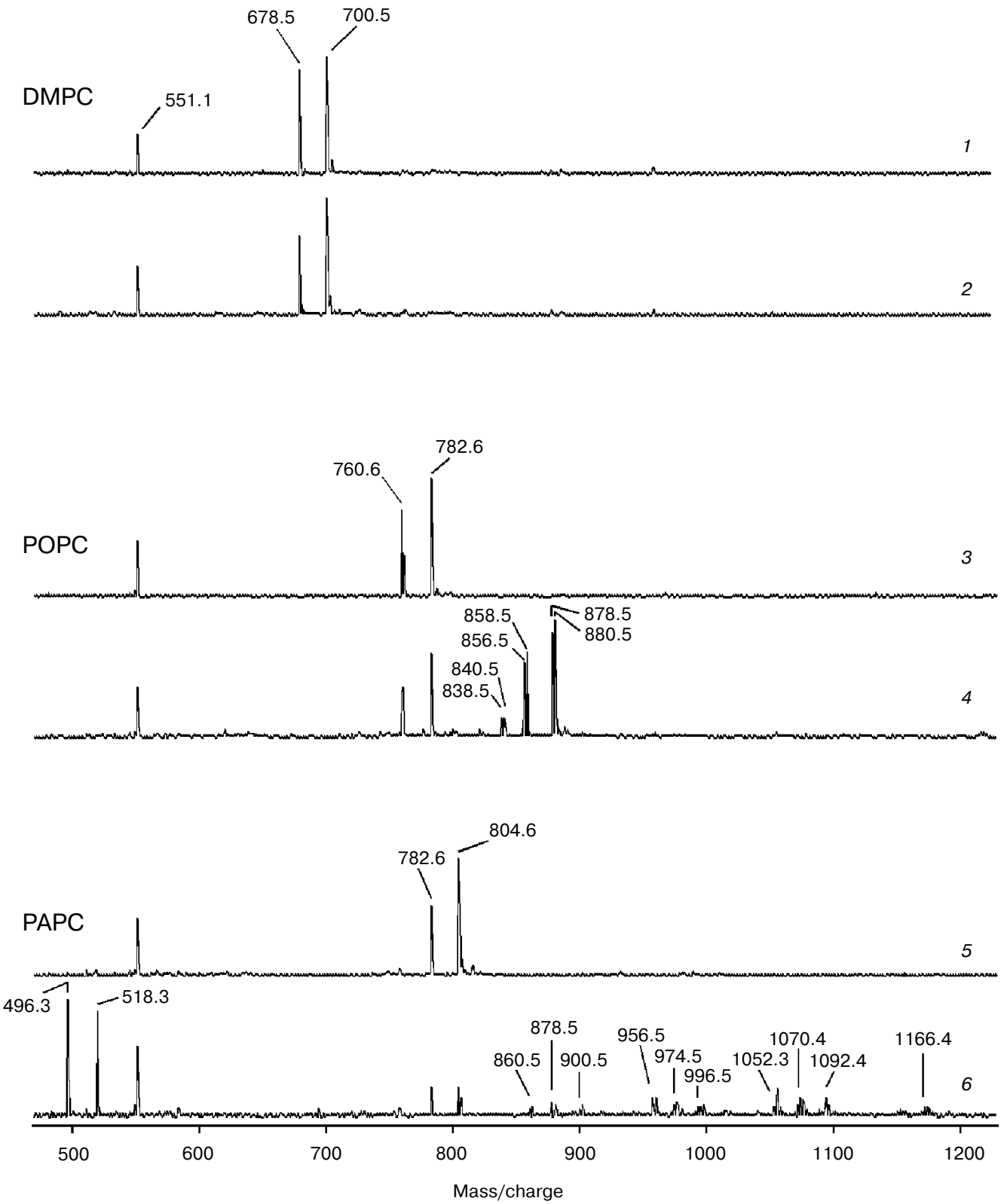

Fig. 1. Typical mass spectra of lipid extract from DMPC, POPC, and PAPC liposomes before (*1*, *3*, and *5*) and after (*2*, *4*, and *6*) incubation in the presence of the (MPO + H_2O_2 + Br^-) system. The incubation medium contained 100 μM NaBr in 50 mM phosphate buffer, pH 5.0. Liposome concentration was 0.03 mg/ml and MPO concentration was 0.14 μM. The reaction was initiated by addition of H_2O_2 (3 μM portions at 3 min intervals) into the reaction mixture. Incubation duration was 40 min, temperature 23°C.

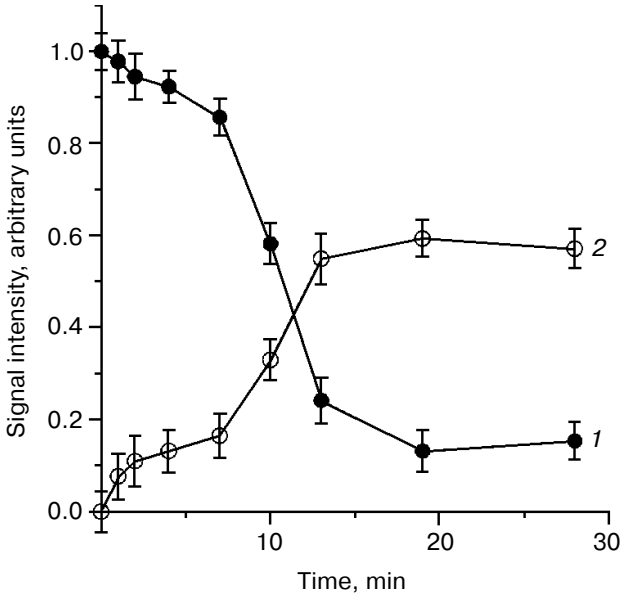

Fig. 2. Kinetics of POPC decrement (*1*) and POPC monobromohydrin increment (*2*) during the incubation of POPC liposomes in the presence of the (MPO + H_2O_2 + Br^-) system. Incubation conditions as in the legend to Fig. 1. The sum of signal intensities of $[M_r + H]^+$ and $[M_r + Na]^+$ with m/z 760.6 and 782.6 in the POPC mass spectrum (see Fig. 1, spectra *3* and *4*) before the incubation is taken as 1.

956.5, and 1052.3) and identified earlier in our work as H^+ adducts of bromo-derivatives resulting from the exchange H for Br in the fatty acid chain [29] are seen in the mass spectrum (Fig. 1, spectrum *4*). On one hand, this pattern of mass spectra is due to the presence of a variety of products resulting from the interaction of hypobromite, formed upon MPO catalysis, with four double bonds of PAPC. On the other hand, mass spectra of Br-containing products are broadened due to the approximately equally frequent occurrence of isotopes ^{79}Br (50.5%) and ^{81}Br (49.5%) in nature.

Formation of lysophosphatidylcholine. It is seen from Fig. 1 that the mass spectrum of lipid extract contains two intense bands in the region of molecular masses lower than the initial PAPC (spectrum *6*) after 40-min incubation of PAPC liposomes with the (MPO + H_2O_2 + Br^-) system. These peaks with m/z 496.3 and 518.3 were assigned to 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine cationized by hydrogen and sodium, respectively.

Figure 5 displays the dependence of the portion of residual PAPC (curve *1*) and formed lyso-PC (curve *2*) after 40-min incubation in the presence of the (MPO + H_2O_2 + Br^-) system on bromide concentration. The content of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine formed apparently increases with increasing concentration of NaBr (up to 100 μM). Further elevation of

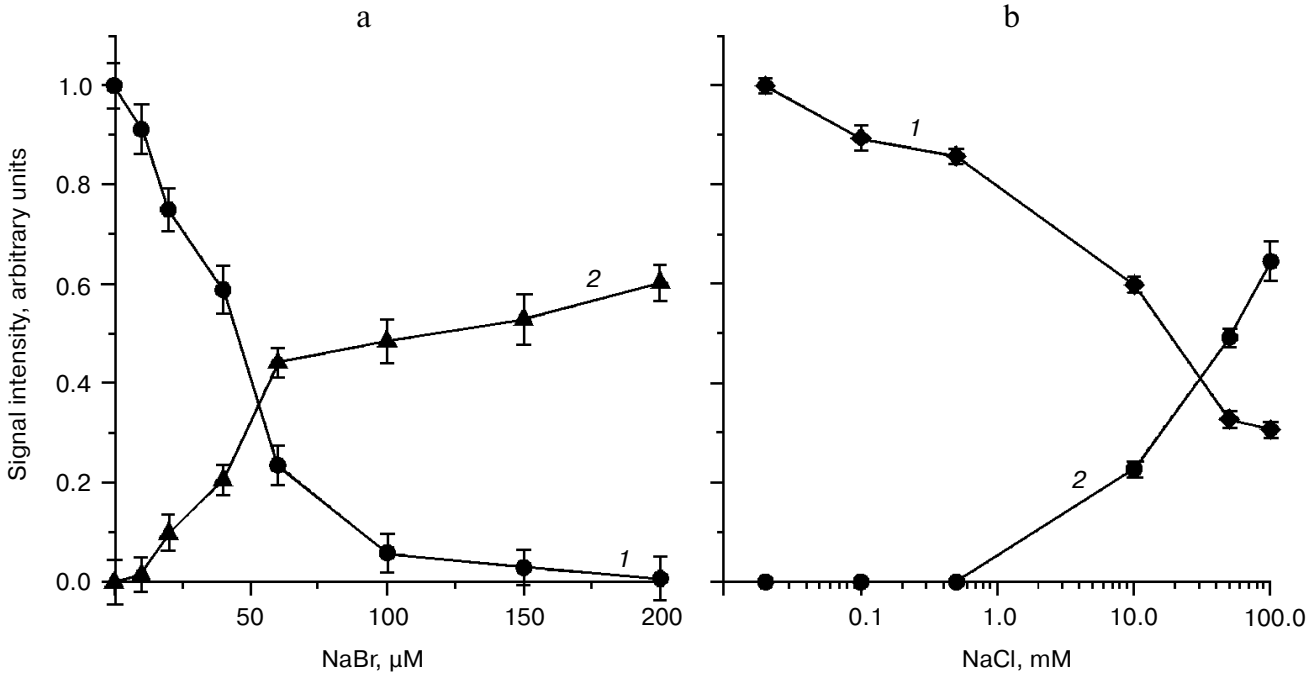

Fig. 3. Dependence of contents of POPC (*1*) and its monobromo- or monochlorohydrin (*2*) after 40-min incubation of POPC liposomes in the presence of (MPO + H_2O_2 + Br^-) (a) or (MPO + H_2O_2 + Cl^-) (b) on concentration of NaBr (a) or NaCl (b), respectively. Incubation conditions as in the legend to Fig. 1. The sum of signal intensities of $[M_r + H]^+$ and $[M_r + Na]^+$ with m/z 760.6 and 782.6 in POPC mass spectrum (see Fig. 1, spectrum *3*) before the incubation is taken as 1.

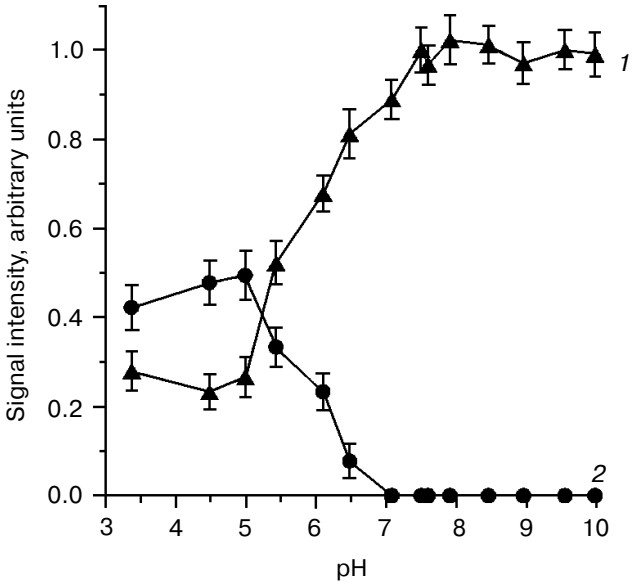

Fig. 4. The pH dependence of contents of POPC (*1*) and its monobromohydrin (*2*) after 40-min incubation of POPC liposomes in the presence of the (MPO + H_2O_2 + Br^-) system. The incubation medium contained 100 μM NaBr in either 50 mM phosphate/citrate buffer, pH range 3.3-7.4, or 50 mM borate buffer, pH > 7.4. Concentrations of liposomes and MPO were 0.03 mg/ml and 0.14 μM, respectively. The reaction was initiated by addition of H_2O_2 (3 μM portions in 3-min intervals) into the reaction mixture (at 23°C). The sum of signal intensities of $[M_r + H]^+$ and $[M_r + Na]^+$ with m/z 760.6 and 782.6 in POPC mass spectrum (see Fig. 1, spectra *3* and *4*) before the incubation is taken as 1.

bromide concentration does not result in growth of the lyso-PC yield. Nevertheless, in the case of polyunsaturated PAPC the formed lyso-PC is the main reaction product. Its fraction comprises about 40% of the initial phospholipid.

Effect of MPO inhibitor and hypobromite scavengers. MPO oxidizes bromide in the presence of hydrogen peroxide to form hypobromite according with reaction (1). We tried to elucidate whether the formation of the detected products (mainly bromohydrins and lysophospholipid in the case of mono- and polyunsaturated phosphatidylcholine, respectively) is associated with the enzyme functioning and is due to the interaction of hypobromite with the studied phosphatidylcholines. With this aim, we incubated MPO with phospholipid liposomes in the absence of one of the substrates (H_2O_2 or Br^-) or in the presence of sodium azide (an MPO inhibitor) as well as with hypobromite scavengers (taurine or methionine) in the incubation medium. After 40-min incubation, the lipid material was extracted and analyzed by mass spectrometry. The results of the experiments are shown in Fig. 6. When the enzyme, bromide, and hydrogen peroxide were present in the incubation medium together with liposomes, the spectrum contained intense bands with m/z 856.5/858.5 and 878.5/880.5 suggesting the formation of monobromohydrins (Fig. 6, spectrum *1*). In the absence of MPO or at least one of its substrates (H_2O_2 or Br^-), we did not reveal the above-listed signals in the mass spectrum (Fig. 6, spectra *2*, *3* and *4*, respectively). These signals were also absent when sodium azide (Fig. 6, spectrum *5*) and methionine or taurine (Fig. 6, spectra *6* and *7*, respectively) were added into the incubation medium. Only bands assigned to the initial POPC (with m/z 760.6 and 782.6) were present in all these cases.

Analogous results were obtained when the effects of MPO inhibitor and hypobromite scavengers on the products formed during the incubation of polyunsaturated PAPC with the (MPO + H_2O_2 + Br^-) system were studied (Fig. 7). As seen from spectrum *1* (Fig. 7), the main reaction product is lyso-PC (signals at m/z 496.3 and 518.3). Also, low-intensity signals of bromo-derivatives with molecular mass higher than in the initial PAPC are seen. The control spectrum of synthetic lyso-PC is also given in Fig. 7 (spectrum *8*) for comparison. When the reaction was carried out in absence of MPO (Fig. 7, spectrum *2*) or at least one of its substrates, H_2O_2 (Fig. 7, spectrum *3*) or Br^- (Fig. 7, spectrum *4*), we did not observe the appearance of peaks with m/z 496.3 and 518.3 or peaks corresponding to bromohydrins and other bromoderivatives in the mass spectrum. Addition into the incubation medium of sodium azide (Fig. 7, spectrum *5*) and methionine or taurine (Fig. 7, spectra *6* and *7*, respectively) also com-

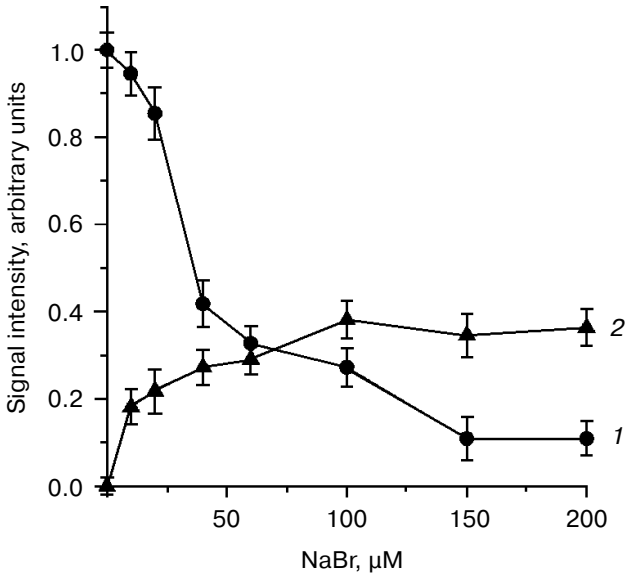

Fig. 5. Dependence of contents of PAPC (*1*) and lyso-PC (*2*) after 40-min incubation of PAPC liposomes in the presence of the (MPO + H_2O_2 + Br^-) system on NaBr concentration. Incubation conditions were as in the legend to Fig. 1. The sum of signal intensities of $[M_r + H]^+$ and $[M_r + Na]^+$ with m/z 782.6 and 804.6 in the PAPC mass spectrum (see Fig. 1, spectra *5* and *6*) before the incubation is taken as 1.

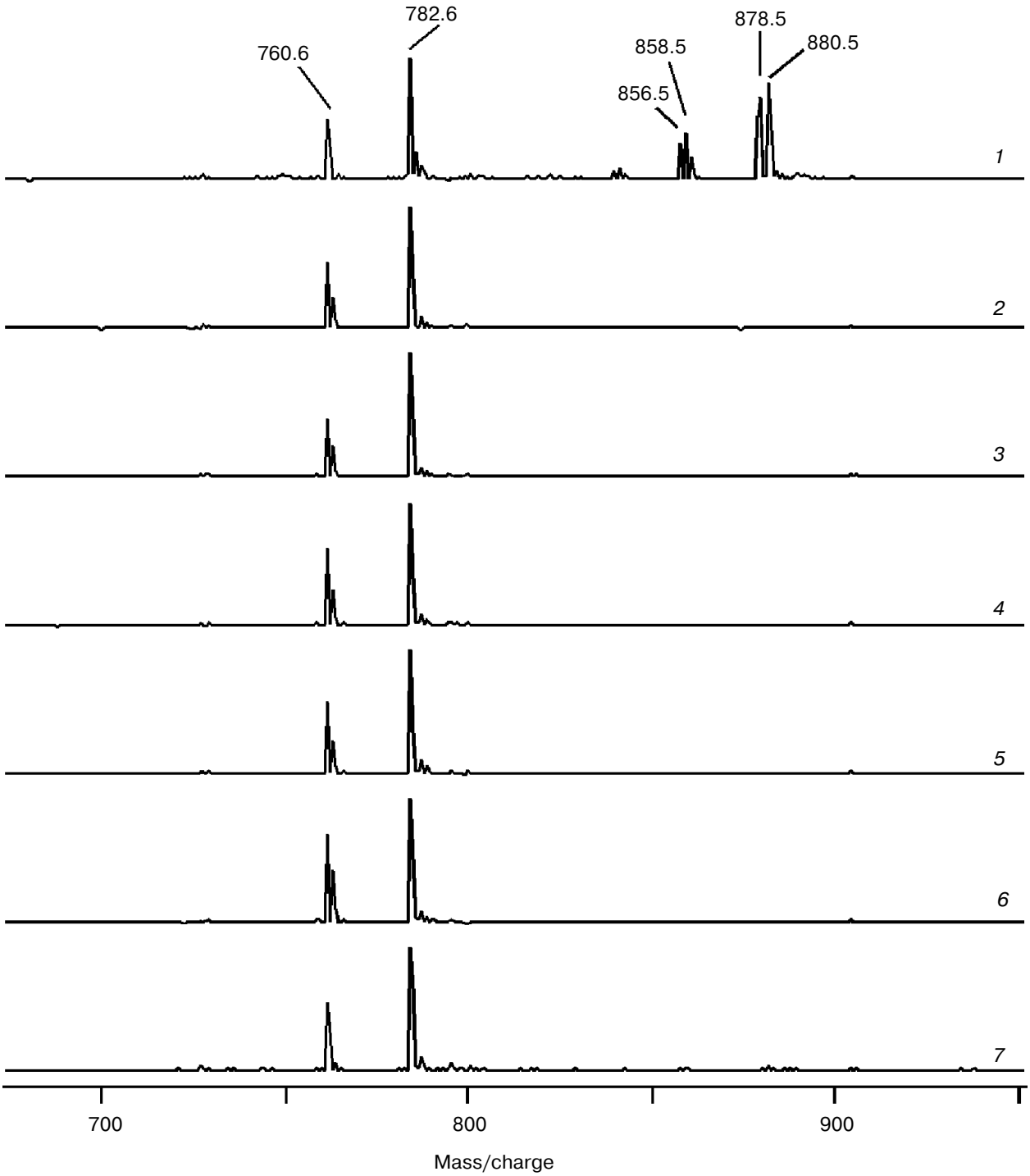

Fig. 6. Mass spectra of lipid extract from POPC liposomes after 40-min incubation in the presence of the (MPO + H_2O_2 + Br^-) system. Incubation conditions were as in the legend to Fig. 1. *1*) Liposomes + MPO + H_2O_2 + Br^-; *2*) same as (*1*) without MPO; *3*) same as (*1*) without H_2O_2; *4*) same as (*1*) without Br^-; *5*) same as (*1*) with 2 mM sodium azide; *6*) same as (*1*) with 2 mM methionine; *7*) same as (*1*) with 2 mM taurine.

pletely prevented the appearance of any products of PAPC destruction.

The results indicate that the formation of bromoderivatives (mainly bromohydrins), as well as lysophosphatidylcholine, happens due to the reaction of unsaturated phospholipids with hypobromite resulting from two-electron oxidation of bromide in reaction (1) catalyzed by myeloperoxidase.

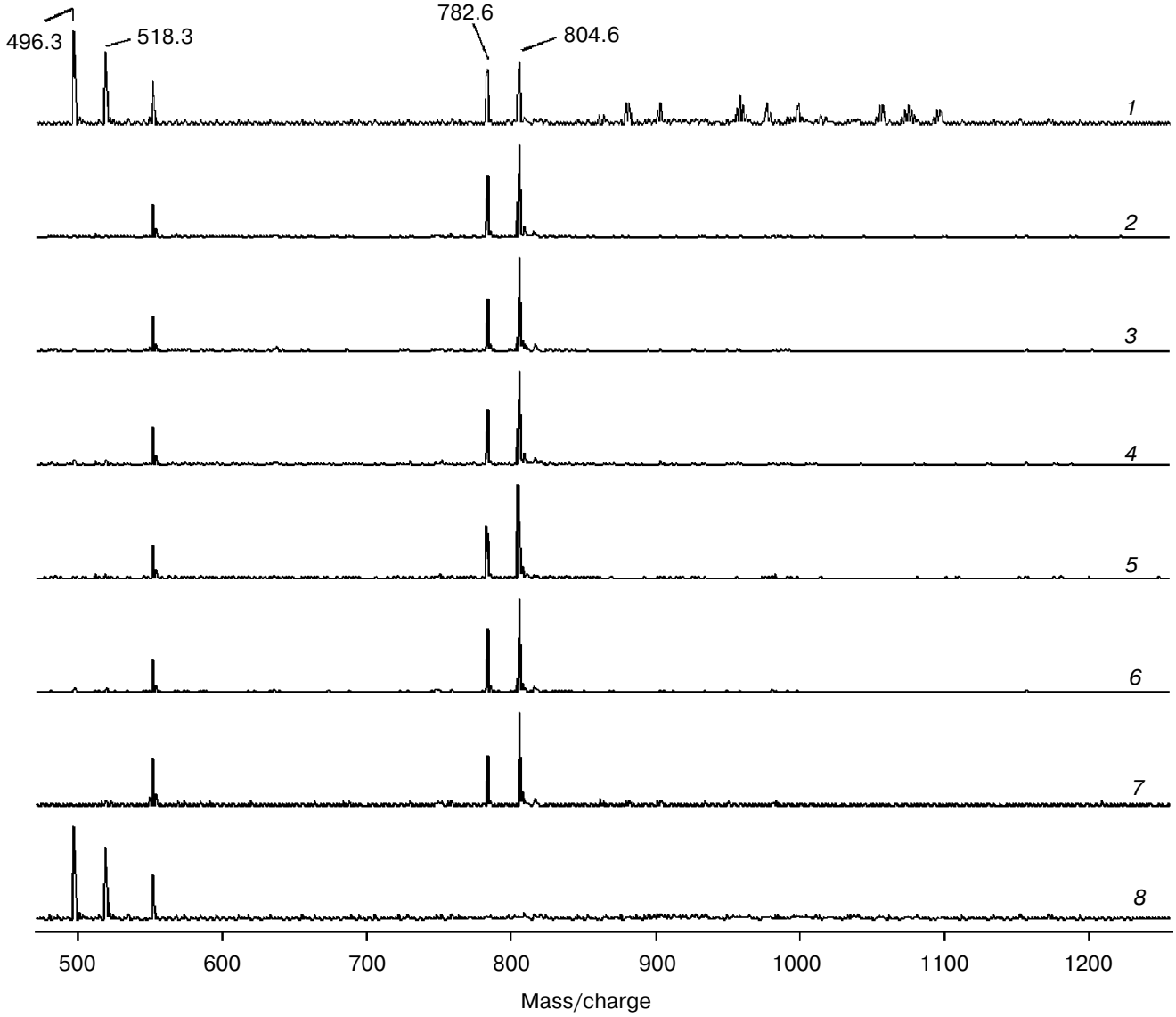

Fig. 7. Mass spectra of lipid extract from PAPC liposomes after 40-min incubation in the presence of the (MPO + H_2O_2 + Br^-) system. Incubation conditions were as in the legend to Fig. 1. *1*) Liposomes + MPO + H_2O_2 + Br^-; *2*) same as (*1*) without MPO; *3*) same as (*1*) without H_2O_2; *4*) same as (*1*) without Br^-; *5*) same as (*1*) with 2 mM sodium azide; *6*) same as (*1*) with 2 mM methionine; *7*) same as (*1*) with 2 mM taurine; *8*) control spectrum of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine.

DISCUSSION

Our data provide evidence for the possible oxidation of bromide (taken at physiological concentrations) by leukocytic MPO to HOBr/OBr^-. The latter modifies unsaturated phospholipids. In fact, the incubation of POPC in the presence of the (MPO + H_2O_2 + Br^-) system leads to formation of monobromohydrins as main products with molecular mass exceeding that of POPC by 96 Dalton.

When comparing our data with those of earlier studies [25, 26], it is worth noting that hypobromite produced in the (MPO + H_2O_2 + Br^-) system reacts to form bromohydrins not only with free unsaturated fatty acids [26], but also with homologous fatty-acid chains of phosphatidylcholines (Figs. 1-3).

An feature of our study is that a signal corresponding to the molecular mass 18 dalton less than that of bromohydrins was observed in the mass spectrum (Fig. 1, spectrum *4*). According to previous data obtained with NaBr containing isotopes ^{79}Br or ^{81}Br as a substrate for myeloperoxidase [29], we imagine formation of the main bromoderivatives of unsaturated phosphatidylcholines as follows. Initially, the HOBr molecule combines electrophilically with an unsaturated acyl chain of POPC at its double bond. The positive part of the HOBr molecule,

namely the Br atom, attacks the double bond to form a π-complex, which quickly rearranges into a carbocation [29]. There are two possible ways of stabilization of this unstable intermediate: addition of a nucleophile (in this case, $^{-}$OH) or removal of a proton. In the first case, bromohydrin isomers are formed. In the second case, the substitution of an H atom by a Br atom takes place. As a result, a water molecule is formed, so the molecular mass of the product is 18 dalton less than that of bromohydrin.

Unlike earlier studies [25, 26], we have first demonstrated the appearance of lysophospholipids in the (polyunsaturated phosphatidylcholine + MPO + H_2O_2 + Br^-) system (Fig. 1, spectrum *6*). Their formation can be explained as follows. Incorporation of electron-acceptor substituents, such as bromine and hydroxyl, into an acyl hydrocarbon chain on synthesis of bromohydrins and other derivatives should cause electron density deficit on the carbon atom of the carbonyl group and, as a result, further weaken the relatively weak ester bond due to a negative inductive effect. This effect is known [30] to depend on amount and efficacy of electron-acceptor substituents and relatively quickly decays along with increase in carbon atom quantity in the saturated chain. Thus, appearance of bromohydrin in the place of double bond at the fifth position in the case of PAPC and possible formation of up to four such groups in the chain are critical for synthesis of lysophospholipid. In the case of POPC, the proximal double bond is at the ninth position, at which only one bromohydrin group can be formed, so the probability of the phospholipid decay with formation of lyso-derivative is greatly reduced.

Inhibitory analysis using the MPO inhibitor sodium azide and HOBr scavengers taurine and methionine, as well as control experiments without one of MPO substrates (H_2O_2 or Br^-) or the enzyme itself, have shown that both bromohydrin and lysophospholipid formation in the presence of the (MPO + H_2O_2 + Br^-) system results from the interaction of hypobromite produced from MPO catalysis with saturated phosphatidylcholines.

The results suggest that a phospholipase-independent MPO-mediated pathway of lysophospholipid production can exist in living organisms. Most probably, MPO, via catalytic synthesis of HOBr/OBr^- (as well as HOCl/OCl^- [21, 22]), leads to formation of halohydrins from polyunsaturated phospholipids followed by spontaneous hydrolysis of ester bond and production of lysophospholipids. This reaction might be of great biological worth, because it is known that lyso-PC plays a regulatory role in various pathophysiological processes, in particular, exhibits a series of features suggesting that it is an atherothrombotic molecule [31]. It stimulates production of reactive oxygen species by endothelial cells via activation of NADPH-oxidase [32], is a chemoattractant for monocytes and T-lymphocytes, induces expression of growth factor and adhesion molecules in endothelial cells [33] and migration of smooth muscle cells [34], inhibits motility of endothelial cells and NO-dependent vessel relaxation [35, 36], and is a mitogen for macrophages and smooth muscle cells [37, 38].

Despite firm beliefs that under conditions close to conditions *in vivo* chloride is a preferential substrate for leukocytic MPO [24, 39], our results demonstrate the fundamental possibility of this enzyme to catalyze oxidation of bromide to form hypobromite at concentrations capable of modifying unsaturated phospholipids. Note that modification of unsaturated phospholipids in the presence of the (MPO + H_2O_2 + Br^-) system is observed already at micromolar concentrations of Br^- (Figs. 3a and 5), whereas in the case of the system (MPO + H_2O_2 + Cl^-) chlorohydrins begin to appear only at chloride concentrations of 1 mM and more (Fig. 3b).

Our data are qualitatively similar to those reported in [25], where the authors registered enhanced reactivity of HOBr compared with HOCl towards phospholipid double bonds. Nonetheless, we suspect that the data reported here are insufficient for positive comparative kinetic analysis of bromo- and chlorohydrin production rates. This analysis is complicated because the hypohalogenites produced can not only be consumed in the reaction medium by double bonds, but also interact with either MPO itself or hydrogen peroxide. Finally, they are able to react with unsaturated lipids by a free-radical mechanism evoking lipid peroxidation and then react with formed products of lipid oxidation (see [4, 9-16]).

Moreover, along with MPO, eosinophil peroxidase (EPO) is also found in human blood; in centers of inflammation this enzyme is secreted by eosinophils into the extracellular medium, in which it catalyzes oxidation of halides by analogy with MPO according to reaction (1). It is notable that the bimolecular rate constant of bromide oxidation with EPO compound I as a minimum 6000 times exceeds that of chloride oxidation [39]. Taking into account this fact and the data of our study, one can conclude that production of hypobromite and products of its reaction with lipids and with other biologically important molecules in centers of inflammation seems to be real process, and the so-called chlorination cycle of MPO should be correctly named the halogenation cycle, if not the bromination cycle.

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