Formation of Water-Soluble Complexes between Phosphatidylcholine and Methionine, Carnosine, and Glutathione in the Presence of Sodium Hypochlorite

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The interaction of phosphatidylcholine monolayers with methionine, carnosine, and glutathione in the presence of sodium hypochlorite is studied. A certain proportion of these components in the solution is shown to lead to the formation of water-soluble lipid-peptide and lipid-peptide-sodium hypochlorite complexes.

Key Words: *lipid-peptide complexes; sodium hypochlorite*

A great number of diverse enzymes are known to be released from leukocytes during inflammation. One of them, myeloperoxidase [2], produces hypochlorite ions ClO^- (HPC), which exhibit strong oxidative properties. An adverse secondary process induced by ClO^- is the oxidation of intact proteins and lipids of the macroorganism, and therefore a search was undertaken for compounds able to bind the excess ClO^-. On the other hand, sodium hypochlorite is sometimes injected to patients for treatment of endo- and exotoxicoses of various origin [6] and the removal of excess preparation is also important. Mono-, di-, and tripeptides of various composition exhibit HPC-binding properties [7]. The effect of HPC on phospholipids is poorly understood, but it may be assumed to facilitate their oxidation. Since lipids are concentrated primarily in membranous structures of the organism, we believed it important to study the effect of ClO^- on phospholipid monolayers, which represent a good structural model of biomembranes. We also studied the effect of HPC-binding peptides and amino acids on HPC-lipid interaction. In the present study we used methionine, carnosine, and glutathione.

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MATERIALS AND METHODS

Phosphatidylcholine (PC, Nattermann), methionine and carnosine (Serva), and glutathione (Reanal) were used in the study. Surface tension (σ) was measured after Wilhelmy using a quartz helix and a glass plate [4]. The accuracy of measurements was ± 0.1 mN/m. A 20-ml round dish with a diameter of 4 cm served as cuvette. The measurements were performed as follows: a carefully washed wet Wilhelmy plate was suspended on a quartz helix over the cuvette filled with distilled water to make contact with the plate. The surface tension σ of water was calculated from the submersion of the plate. If σ corresponded to the tabulated value (72.2 ± 0.2 mN/m), a PC monolayer was created on the water surface. To this end, small amounts of a dilute solution of PC in chloroform were applied until σ attained the desired value. The recordings were made after several minutes necessary for evaporation of the solvent. Then 0.2 ml of solution containing NaClO, test peptide, or their mixture was injected into the water and the kinetics of σ was recorded. The solution was gently

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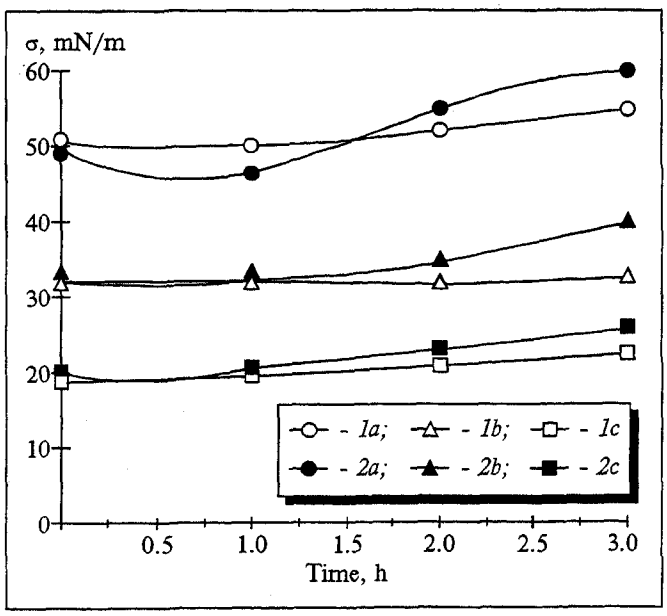


Fig. 1. Kinetic curves of accumulation of water-soluble products from PC monolayers of different density in the absence (*1a*, *1b*, and *1c*) and the presence (*2a*, *2b*, and *2c*) of 1.2×10^{-4} M sodium hypochlorite.

agitated during the experiment with a magnetic stirrer. The concentration of HPC in the injected solution was 1.2×10^{-2} mM, the final concentration in the subphase was 1.2×10^{-4} M.

RESULTS

Previously we studied PC peroxidation in monolayers on the surface of water [5]. This process is slow in the absence of oxidants but it considerably accelerates after addition of Fe^{2+} and H_2O_2 to the subphase. Although HPC is also an oxidant, its effect on the PC monolayer was negligible. This is clearly seen from Fig. 1, which represents three pairs of kinetic curves characterizing the changes in surface tension for monolayers of different density in the absence (curves *1a*, *1b*, and *1c*) and presence (curves *2a*, *2b*, and *2c*) of 1.2×10^{-4} M ClO^- in the subphase. The shape of these curves is analogous to the shape of those obtained earlier [5]. The first stage of oxidation yields products which reduce surface tension in the system. The surface tension then gradually increases due to the formation of water-soluble products which leave the monolayer. For a monolayer of all chosen densities (24, 36, and 50 mN/m) the σ rise is more pronounced in the presence of ClO^-. This difference may be attributed either to accelerated oxidation of PC by ClO^- ions or to increased solubility of some oxidation products in the subphase due to the increased charge resulting from bound ions. Limitations of the method used do not allow us to distinguish between these processes, but it is important for further discussion to note that the addition of sodium hypochlorite to the subphase only slightly affects the behavior of PC monolayers.

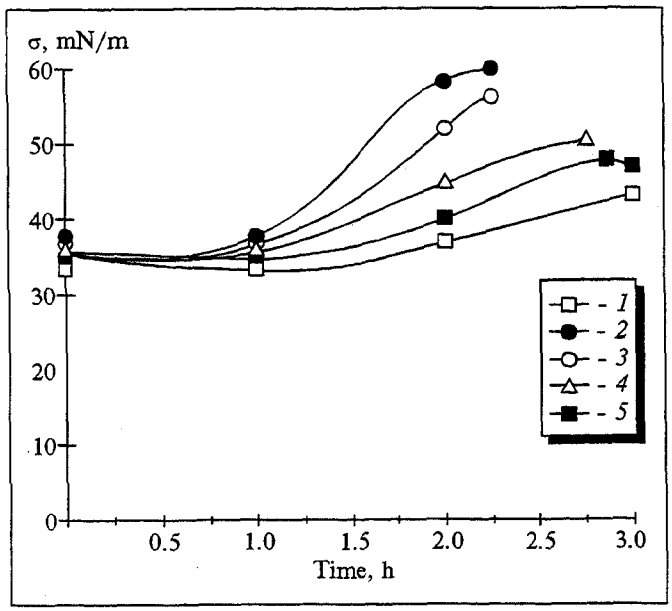
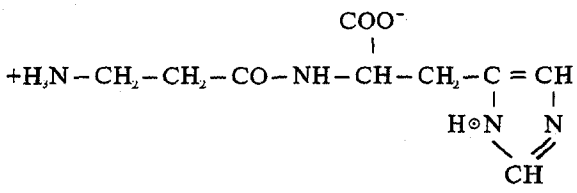


Fig. 2. Kinetics of σ of PC monolayers after addition of 1.2×10^{-4} M carnosine (*1*) and mixtures carnosine:HPC = 1:1 (2), 1:2 (3), and 1:4 (*4*). *5*) carnosine:HPC = 1:1 with 0.5 M NaCl in the subphase.

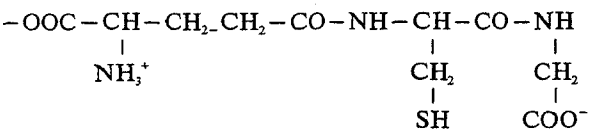
More pronounced effects were observed when polypeptides were added to the subphase. Figure 2 shows a series of kinetic curves describing monolayers after the addition of carnosine alone and in combination with HPC. Carnosine alone in a concentration of 1.2×10^{-4} M hastens the dissolution of a PC monolayer in comparison with distilled water (curve *1*). A dramatic increase of surface tension was observed after injection of a carnosine:HPC mixture (1:1 molar ratio). This was due to efflux of the substance from the monolayer (curve *2*). A lower content of carnosine in the system (carnosine:HPC=1:2 and 1:4 for the same initial concentration of HPC, 1.2×10^{-4} M) diminished this effect (curves *3* and *4*). Since HPC exhibits just a slight oxidative effect vis-a-vis PC and carnosine is not an oxidant by its chemical nature, the observed effects cannot be explained by the formation of water-soluble products of PC oxidation. If carnosine had exhibited the expected antioxidative effect, protecting PC from HPC action, the slight oxidative effect of HPC should have decayed even more, and the resultant kinetic curve would have approached the control one (PC layered on distilled water). Since the above effect was not observed, another mechanism has to be invoked to explain the experimental data. According to modern views on the possible rearrangements of a

lipid monolayer, the obtained results may be explained in terms of complexation of the phospholipid with carnosine and HPC. Under our experimental conditions the carnosine molecule (alanylhistidine) is a zwitterion possessing an additional weak positive charge in the histidine ring [3]:



The PC molecule also possesses both a positive and a negative charge near the polar head [3]. Since phospholipid molecules are arranged as a monolayer on the water surface, the polar parts of PC are submerged and their charges are accessible for charged groups of carnosine. It may be assumed that the formed complexes are electrostatic in nature. However, in the absence of ClO^- just a small number of such complexes may be formed (curve *1*). The high polarity and, consequently, high solubility of carnosine apparently interfere with the formation of such complexes. Another picture is observed in the presence of HPC in the system. Indeed, published data demonstrate ion-peptide binding [1]. Carnosine, methionine, and glutathione tested here were previously shown to interact with ClO^- [7]. The mixture carnosine:HPC=1:1 presumably contains complexes of various composition. Due to partial neutralization of charges in carnosine, these complexes may interact with the polar head of the PC molecule. This affects the hydrophilic-lipophilic balance of the carnosine-loaded lipid molecule: the size and polarity of the hydrophilic head increase, and hence the resultant complex becomes water-soluble. This is responsible for the marked depletion of the monolayer after injection of the carnosine:HPC=1:1 mixture. The lower effect of the carnosine:HPC=1:2 and 1:4 mixtures (curves *3* and *4*) is due to a reduced content of dipeptide-HPC complexes in proportion to the carnosine content in the subphase.

Absolutely analogous dependences were obtained in experiments where glutathione alone and in combination with HPC was injected into the subphase (Fig. 3). This is not surprising, since glutathione (γ-glutamyl-cysteinyl-glycine) also represents a hydrophilic charged molecule [3]:



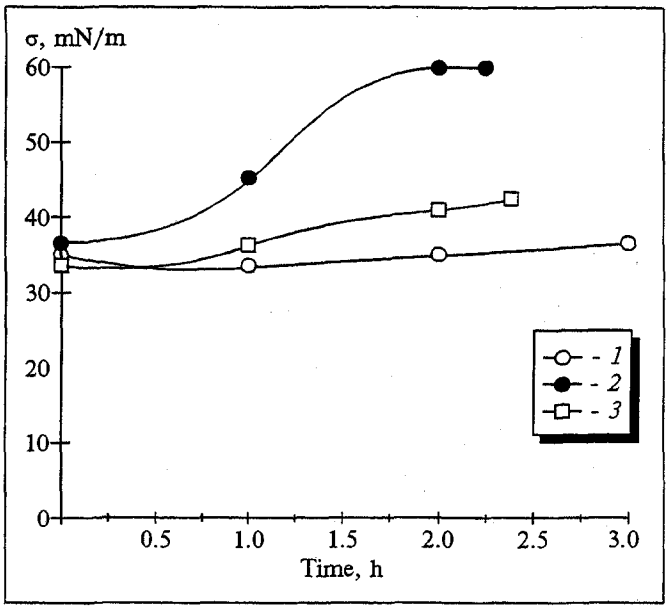
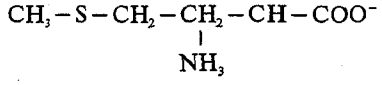


Fig. 3. Kinetics of σ of PC monolayers after addition of 1.2×10^{-4} M glutathione (*1*) and mixtures glutathione:HPC=1:1 (*2*), and 1:2 (*3*) to the subphase.

and all speculations regarding carnosine pertain equally to glutathione.

A different picture was obtained for methionine (Fig. 4). Methionine is considerably less hydrophilic and less soluble in water [3]:



Therefore, even without HPC methionine may interact with PC, and the resultant complex passes

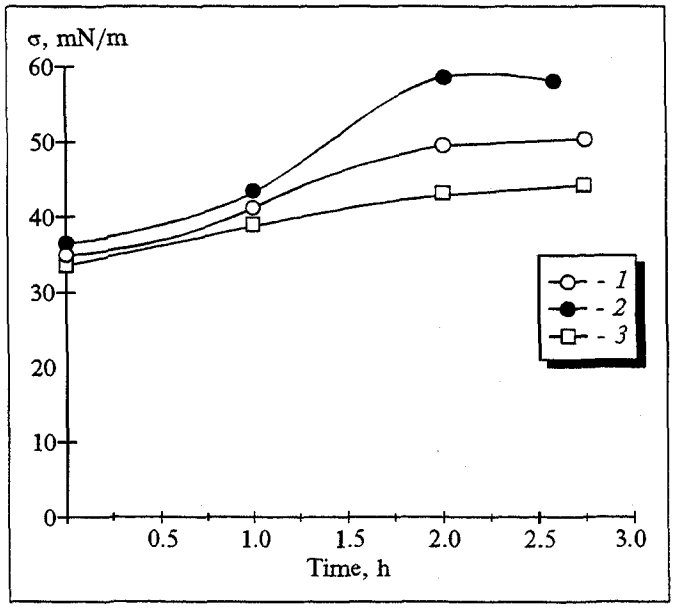


Fig. 4. Kinetics of σ of PC monolayers after addition of 1.2×10^{-4} M methionine (*1*) and mixtures methionine:HPC=1:1 (*2*), and 1:2 (*3*) to the subphase.

from the monolayer to the subphase (curve *1*). However, the observed effect is insignificant. Partial neutralization, which is expected to take place in the mixture methionine:HPC=1:1, just slightly enhanced the effect (curve *2*). And finally, the observed effect decayed when the mixture 1:2 with a reduced content of methionine-HPC complexes was used (curve *3*).

The electrostatic nature of interactions in the complexes was verified for all test substances by adding NaCl in a high concentration (0.5 M) to the subphase. No marked efflux of the phospholipid from the monolayer was observed for any of the compounds, and the obtained kinetic curves were close to the control ones. As an example, Fig. 2 demonstrates the kinetic curve for the complex carnosine:HPC=1:1 with 0.5 M NaCl as a subphase (curve *5*).

Thus, in the present study we demonstrated the ability of PC molecules to form water-soluble complexes with peptides in the presence (and in some cases in the absence) of HPC. This effect may be assumed to play a certain biological role. HPC-induced defects in biomembranes may be caused by a different mechanism apart from the established one mediated through the oxidation of PC. Phospholipid molecules in the presence of ClO^- form complexes with peptide substances and leave the membrane, which results in defects in the membrane monolayers.

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