INTERACTION OF MYELOPEROXIDASE WITH MIXED LECITHIN AND CHOLESTEROL MONOLAYERS

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The writers found myeloperoxidase (MPO) previously in the lens of the eye and in the retina by enzymic and immunologic tests [2]. This enzyme is present in large quantities in neutrophilic granulocytes [4]. The functions performed by MPO in the lens have not been studied. The question arises how does MPO enter the lens, or more specifically, is it synthesized inside the lens or does it diffuse there from the aqueous humor. The lens, as we know, is a laminated system consisting of dense membranes lying close together. The lipid composition of the lens is distinguished by a high concentration of cholesterol; its concentration in the nucleus of the lens, molar ratio cholesterol:lecithin 4:2, moreover, is higher than in the cortical regions (in which it is 1:2) [5].

This paper describes an attempt to determine, on a model system, whether MPO molecules can be adsorbed on lipid monolayers, and can diffuse through a system of membranes in a direction perpendicular to their plane. The process of diffusion through a single membrane can be divided conventionally into three stages: adsorption of the substance on the phase boundary water — layer of charged lipid heads, passage through the hydrophobic middle part of the membrane, and desorption from the opposite side of the membrane. The second phase is essentially the dissolving of the diffusing substance in the hydrophobic region of the membrane.

We accordingly studied: 1) the ability of MPO molecules to be adsorbed on the phase boundary water—lipid monolayer, and as the lipid we used lecithin, cholesterol, and a mixture of both; 2) solubility of MPO in 1-octanol and octane. Octanol is widely used as a substance which closely reproduces the hydrophilic—lipophilic properties of the lipid layer as a whole. The nonpolar solvent octane can be regarded as a model of the middle part of the membrane.

EXPERIMENTAL METHOD

Lecithin was obtained from "Fluka" (West Germany) and cholesterol from "Sigma" (USA). MPO was isolated from hog leukocytes by the method described in [1].

To measure the surface tension δ Wilhelmy's method was used, in A. A. Trapeznikov's modification [3]. The accuracy of measurement of was ± 0.1 mN/m. The order of the operations was as follows. A clean wet glass Wilhelmy plate was suspended above a 20-ml cuvette on a quartz spiral. The cuvette was filled with distilled water until it touched the plate. The value of water was calculated from the depth of emersion of the plate in water. If it agreed with the value given in tables (72.3 ± 0.1 mN/m) the experiment was continued. To create a monolayer from lecithin and cholesterol, a solution of these substances in chloroform was carefully applied to the surface of the water in the cuvette. After a few minutes, necessary to allow evaporation of the solvent, the pressure created by the lipid monolayer was estimated from the force of repulsion of the measuring plate.

It was calculated as $\pi = \delta$ (clean water) $- \delta$ (water with monolayer).

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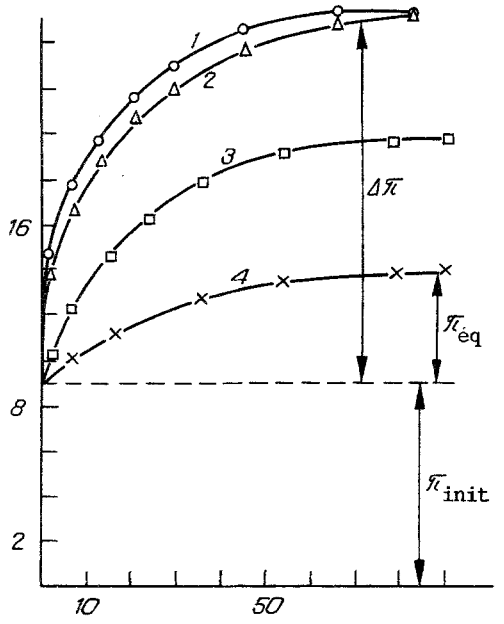

Fig. 1

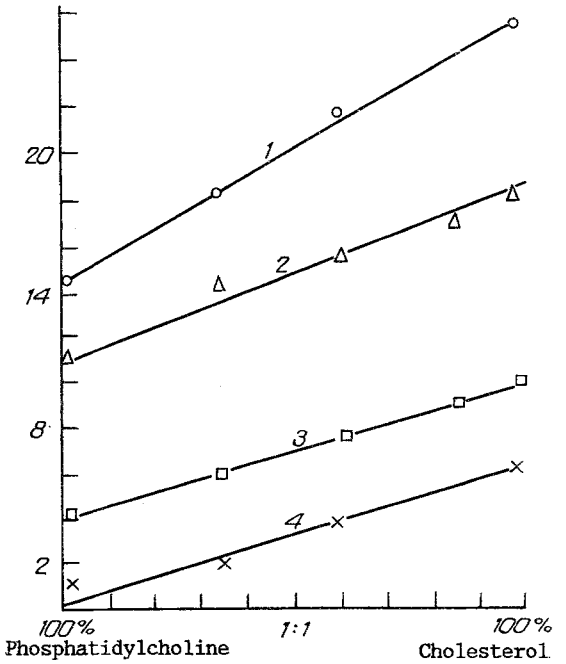

Fig. 2

Fig. 1. Kinetics of growth of surface pressure after injection of different quantities of MPO beneath cholesterol monolayer. π_{init} = 0.9 mN/m. MPO concentration (in mg/ml): $5 \cdot 10^{-3}$ (1), $1 \cdot 10^{-3}$ (2), $0.5 \cdot 10^{-3}$ (3), $0.2 \cdot 10^{-3}$ (4). Abscissa, time (in min); ordinate, values of π (in mN/m).

Fig. 2. Dependence of maximal equilibrium surface pressure ($\Delta\pi$, mN/m — ordinate), of MPO on relative content of lecithin and cholesterol in monolayer. At π = 4 mN/m (1), 9 mN/m (2), 20 mN/m (3), and 32 mN/m (4).

Since a solution with a low concentration of lipids was used, it was possible to establish the chosen pressure π with an accuracy of ±0.3 mN/m. Next, 0.1 ml of a solution of MPO of the necessary concentration was added to the volume of water under the monolayer by means of a syringe and the kinetics of growth of the surface pressure caused by insertion of MPO molecules into the lipid monolayer was measured. The highest MPO concentration in the solutions used for injection was 1 mg/ml. Throughout the experiment, careful mixing of the solution was carried out by means of a magnetic mixer.

To measure the partition coefficient of MPO between water and octane or 1-octanol, vigorous shaking of the solution of the enzyme was carried out with an equal volume of the solvent. The protein concentration before and after shaking and the reaching of an equilibrium state was judged from the height of the maximum of absorption of MPO at 427 nm. The standard for comparison during measurement of the spectra after shaking was an equal volume of water, which had been shaken with the organic solvent in the same way as the MPO solution. The spectra were measured on a Hitachi-323 spectrophotometer (Japan).

EXPERIMENTAL RESULTS

A series of kinetic curves for the case when the initial pressure of the cholesterol monolayer was 9.0 ± 0.3 mN/m is illustrated in Fig. 1. It can be seen that when small quantities of MPO were injected beneath the monolayer, the increase in surface pressure was proportional to the quantity of protein injected, but if large quantities were injected, it no longer depended in this manner.

Equilibrium in the last case already corresponds to the state when, in the initial monolayer of the lipid with a given initial pressure π_{init} it the largest possible number of MPO molecules was inserted. It is this value of $\Delta\pi$ that will subsequently be regarded as a measure of the capacity of the lipid monolayer, and it is taken to be proportional to the number of MPO inserted into it.

There are no data in the literature on the surface pressure of the lipid monolayer in membranes of the lens. The process of insertion of MPO was therefore studied for a wide range of pressures of the lipid monolayer covering the whole region of biological values in membranes. According to data published in [6], this interval is 7-32 mN/m. The experimental data are given in Fig. 2. Maximal equilibrium values of $\Delta\pi$, characteristic of MPO molecules adsorbed beneath the monolayer, are plotted along the ordinate. The composition of the monolayer is indicated on the abscissa. The initial pressure of a monolayer consisting of cholesterol and lecithin is shown above the corresponding straight lines. Naturally, with an increase in density of the lipid monolayer, the quantity of enzyme inserted will be reduced. However, two facts which were important were observed. Until the highest values of π_{init} characteristic of biological membranes, the values of $\Delta\pi$ did not equal zero. This means that for any density of packing of the lipids in the membranes of the lens, a certain number of MPO molecules will be concentrated on the phase boundaries. The most interesting fact is that with an increase in the relative quantity of cholesterol in the monolayer, whatever the initial pressure, the quantity of enzyme inserted into the monolayer will be proportional to the cholesterol concentration in it.

We do not know how the adsorbed MPO molecules are arranged on the phase boundary. They may be present in the aqueous phase, merely in contact with the layer of charged lipid heads, or they may separate the lipid monolayer and occupy sites at the same level as the lipid molecules. In the first case, the insolubility of MPO in lipids may be an obstacle to diffusion of the protein into the lens nucleus. Accordingly, the partition coefficient of MPO in water:1-octanol and water:octane systems were estimated ($K_1 = C_{water}/C_{1\text{-octanol}}$ and $K_2 = C_{water}/C_{octane}$, where C denotes the MPO concentration in the corresponding solvent).

K_1 was found to be 1.4 and K_2 4.0. Thus MPO is readily soluble in 1-octanol and quite soluble in octane. As a result of this, the ability of MPO molecules to move from the periphery of the lens toward the nucleus is not in doubt.

It must also be pointed out that besides MPO dissolved in the lipid phases, some of the protein will also accumulate on the phase boundaries. It can even be postulated that the MPO content in the nucleus will be higher than in the cortical regions, due to the higher cholesterol concentration in membranes of the lens nucleus [5].

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