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A new technique for investigating lipid protein films

PETER FROMHERZ

Physikalisch-Chemisches Institut der Universität Marburg, Marburg/Lahn and Max-Planck-Institut für Physikalische Chemie, Göttingen (Germany)

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SUMMARY

A technique is described which permits independent investigation of (1) the adsorption of protein on lipid monolayers, and (2) the penetration of the adsorbed protein into the lipid film. Some results on the interaction of hemiglobin with arachidic acid and stearic acid methyl ester as model substances are presented, indicating that the adsorbed protein is not decoiled. The technique is suited as well to the investigation of the enzymatic activity of adsorbed enzymes as shown qualitatively with catalase.

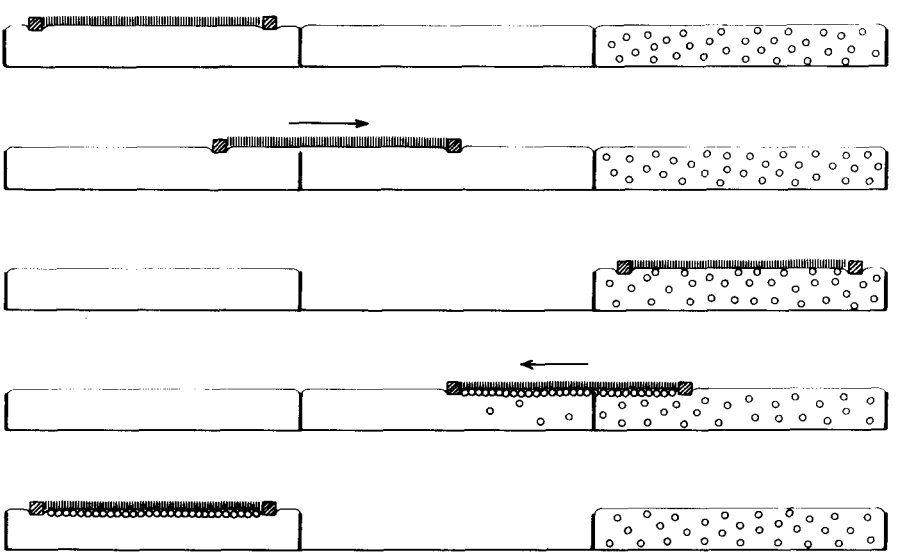

Fig. 1. Procedure in producing a lipid protein film.

In the past the interaction of proteins with lipid monolayers at the air–water interface has been investigated by measuring the surface pressure during the adsorption process[1-3]. Recently investigations with ^{14}C-labelled proteins were reported[4,5]. In this paper a technique is presented, which allows the investigation of the adsorption of a protein on a lipid monolayer independent of the investigation of its penetration into the monolayer. Simple lamellar structures of lipids and proteins are built up by transferring the lipid protein films onto a solid support[6,10].

The principle of the technique is shown in Fig.1. A lipid monolayer on the water surface of a multicompartment trough is enclosed between two barriers. By moving the two barriers simultaneously, the monolayer can be shifted over a protein solution without being disrupted. By adsorbing protein molecules on the highly compressed lipid film (surface pressure, *e.g.* 35 dynes/cm), a lipid protein film is produced. It can be separated from the underlying protein solution by shifting it over some protein-free compartments. Thus a lipid protein film is obtained which is isolated from a protein containing subphase and which is suited for further investigations as described below. The circular trough (Fig.2) is milled from a teflon plate. The two barriers resting on the edges of the trough can be moved individually or together by means of two concentric axles. A Wilhelmy balance[7] is shifted with the film and the surface pressure is controlled continuously. The walls separating the compartments are somewhat lower than the edges of the trough. By this

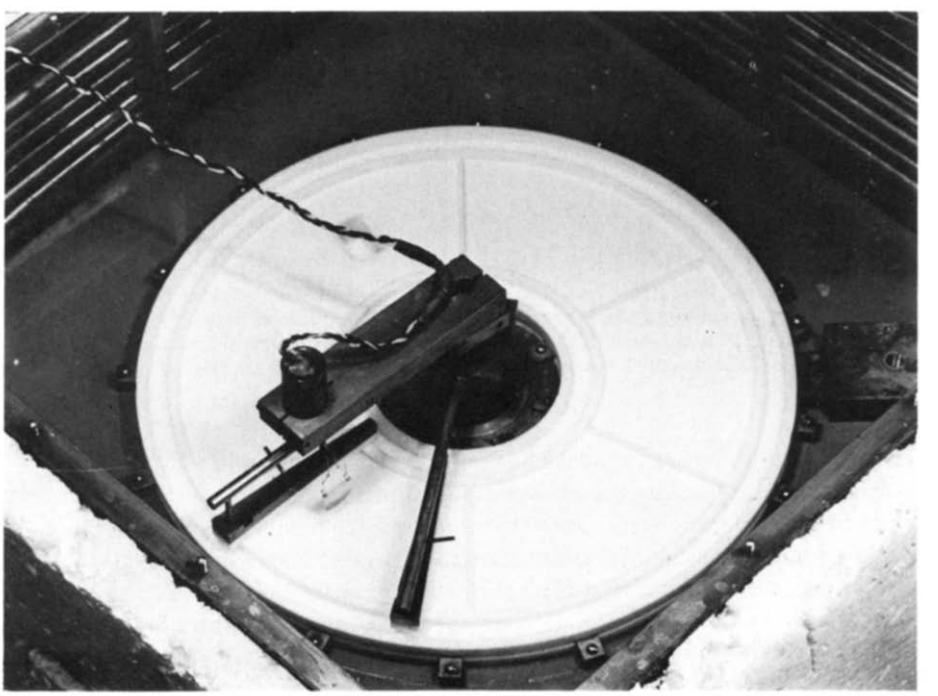

Fig.2. Multicompartment trough allowing the shift of monolayers over different subphases. The trough is made from teflon. The two barriers and the Wilhelmy plate balance are moved by two concentric axles.

means it is possible to transport the intact monolayer from one compartment to another and to fill and empty the different compartments independently.

The area between the barriers is measured with the help of a potentiometer. A feedback system allows investigations with constant surface pressure. The trough rests in a thermostated box. The implications of the technique are demonstrated by some examples as follows:

In order to investigate the adsorption kinetics of proteins on lipid monolayers, the lipid protein films are separated from the protein solutions at different time intervals. Then a hydrophobic glass slide is dipped in and out through the film, and thus a double layer of the lipid protein film is deposited on the slide. The number of the adsorbed protein molecules is determined by measuring the absorption spectrum of this transferred lipid protein film in a special spectrometer[7].

In Fig.3 four examples are presented showing the adsorption kinetics of hemiglobin. The absorbance of the absorption maximum of the Soret band of hemiglobin was measured, and the extinction coefficient for hemiglobin solutions ($6.24 \cdot 10^5$ $l \cdot mole^{-1} \cdot cm^{-1}$ ref. 8) were used for calculating the adsorbed quantity. Fig.3 shows that more hemiglobin

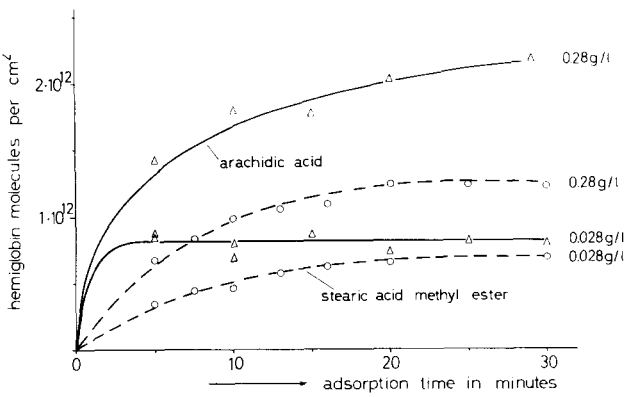

Fig.3. Number of adsorbed hemiglobin molecules per cm^2 *versus* time of adsorption. The initial surface pressure of the arachidic acid film and of the stearic acid methyl ester film is 35 dynes/cm. The hemiglobin concentration in the subphase is 0.28 and 0.028 g/l, respectively. Veronal buffer, pH 7; temperature, 25°. The human hemiglobin was delivered by Calbiochem AG Luzern.

is fixed at the carboxyl groups of arachidic acid than at the nonpolar methyl groups of stearic acid methyl ester. Using more concentrated protein solutions more protein molecules are adsorbed. A closely packed layer of hemiglobin molecules was not obtained. However, an equilibrium does not seem to be present since a desorption of the adsorbed protein molecules into a protein-free subphase was not observed. This apparent discrepancy is not yet well understood. In any case the adsorption rate is not determined by diffusion. Measurements at different pH's and with other lipids[6] show that electrically charged protein molecules are less adsorbed. Equal charges on the lipids and on the proteins hinder the adsorption, whereas opposite charges facilitate it. The quantity adsorbed on monolayers of stearic alcohol, lecithin and cephalin is low.

In order to investigate the penetration of proteins into a lipid monolayer a lipid protein film, isolated from the protein-containing subphase, can be expanded suddenly by a few percent and subsequently the area or the surface pressure can be kept constant. The adsorbed protein molecules penetrate the lipid monolayer which leads to an increase of the surface pressure or to an expansion of the film area.

In Fig. 4 some results are shown, obtained when $1 \cdot 10^{12}$ hemiglobin molecules per cm^2 were adsorbed on an arachidic acid film or a stearic acid methyl ester film, and the surface pressure was diminished suddenly from 35 to 10 dynes/cm and kept constant during the subsequent spontaneous expansion of the film. The penetration rate is not directly related to the adsorption rate as seen by comparing Fig. 4 with Fig. 3.

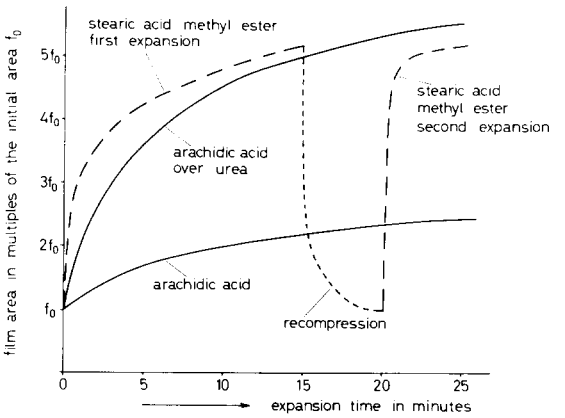

Fig.4. Area of lipid protein films isolated from the protein-containing subphase *versus* time after a sudden lowering of surface pressure from 35 to 10 dynes/cm. In all cases $1 \cdot 10^{12}$ hemiglobin molecules per cm^2 are adsorbed. Veronal buffer and 5 M urea solution, pH 7; temperature, 25°.

One may conclude that the protein molecules are more tightly bound to the carboxyl groups than to the ester groups and therefore cannot penetrate as easily. The penetration can be reversed by raising the surface pressure to 40 dynes/cm. The procedure can be repeated, and the expansion is found to be faster than in the first cycle. Thus the first slow expansion rate is not determined by the penetration step itself. The adsorbed protein is decoiled during penetration and the decoiling rate determines the rate of expansion. During the second expansion the molecules already decoiled can penetrate much faster.

This assumption is supported by the result obtained from shifting the lipid protein film over a 5 M solution of urea. In this case the penetration rate is strongly enhanced. The adsorbed protein molecules are denatured by the urea and therefore they penetrate to a greater extent. It can be concluded from these experiments that proteins adsorbed on a lipid film under a high surface pressure have retained a considerable part of their tertiary structure. By measuring the expansion and recompression at different surface pressures one can obtain surface pressure–area isotherms of the penetrated lipid protein films showing a notable hysteresis. In this way one can produce monolayers of proteins and lipids of different mixing ratio and at any desired degree of denaturation of the protein.

In order to obtain more information about the extent of denaturation of adsorbed proteins, it is useful to control the catalytic activity of adsorbed enzymes. For this purpose a lipid enzyme film is shifted over a compartment containing a solution of enzyme substrate, or the film is transferred onto a glass slide which is put into a substrate solution. For example, Fig. 5 shows the decomposition of H_2O_2 by catalase adsorbed on a monolayer of arachidic acid.

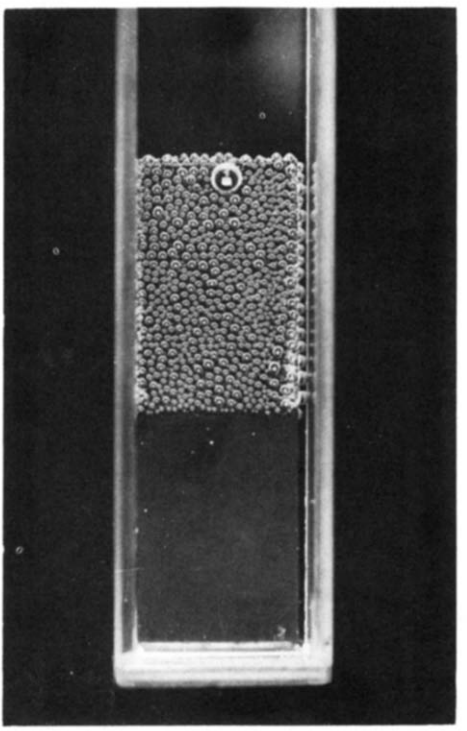

Fig. 5. Decomposition of H_2O_2 by catalase adsorbed on an arachidic acid film. The oxygen bubbles arise only in that part of the slide which is covered by the lipid enzyme film. The picture was taken 1 min after dipping the slide into a 0.03% solution of H_2O_2.

Monolayer assemblies, containing lipid protein films as described or films with two different proteins, can be constructed by superposing layers of different composition on a solid support. Thus it is possible to build simple organized lamellar systems of different lipids and proteins[6,10] as was done previously with different lipids alone[9]. The order of these lipid protein multilayers is controlled by energy transfer from fluorescent dye lipids to cytochrome *c* or hemiglobin.

In addition electron microscopic studies were performed to investigate the arrangement of the adsorbed protein molecules[11]. By building up lipid protein multilayers it becomes possible to apply different spectroscopical methods as infrared, optical rotatory dispersion and electron spin resonance. Moreover the shifting method seems to be suitable for the investigation of immunological problems and of other reactions at monolayers.

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