Monolayers of Lysolecithins and Analogs

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Received June 7, 1968

INTRODUCTION

Lysolecithins are known to be important intermediates in lecithin metabolism (1). Small amounts of lysocompounds are found in natural membranes, and given local concentrations may alter the permeability of these membrane regions. This view is supported by recent bilayer studies which showed that introduction of lysolecithin in the film-forming solution caused a significant drop in the specific electrical resistance (2). Introduction of lysolecithin into the lipid bilayer surrounding medium causes disruption of the black film, as is observed with red cells (3). However, not all lysolecithins were equally effective in disrupting artificial and red cell membranes. Lysolecithins containing fatty acid constituents such as stearate, myristate, and oleate affect the red cell membrane in this order, at concentrations varying from 10^{-2} to 10^{-1} mM. In comparison with these compounds, much higher concentrations of lysolecithins containing linoleate and decanoate were needed. These experiments indicate that the lytic activity of lysolecithin is highly dependent on chain length and unsaturation of the fatty acid constituents. These observations with regard to the lytic activity of different lysolecithin species made it of interest to study the effect of chain length and unsaturation on the surface properties of lysolecithin compounds. Such studies may also be important with respect to the work of Shah and Schulman (4), Colacicco and Rapport (5), and Dawson (6) on the formation of lysolecithins in the aqueous interface by the action of phospholipase A. In addition, the effect of the free hydroxyl group (using deoxylysolecithins), the change in phosphate-fatty acid ester linkage distance, and the change in phosphate-quaternary ammonium group distance were studied.

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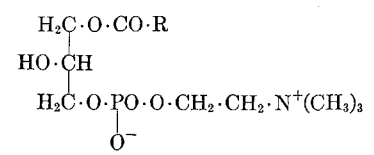
MATERIALS AND METHODS

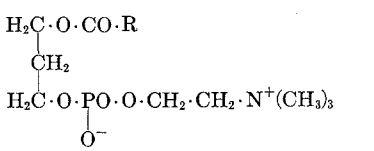
The following lysolecithins (formula I) were formed by hydrolysis of synthetic lecithins with snake venom phospholipase A (EC 3.1.1.4) (7).



[I]

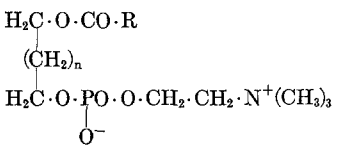
1-stearoyl-*sn*-glycero-3-phosphorylcholine ((stearoyl)lysolecithin) (I*a*); 1-oleoyl-*sn*-glycero-3-phosphoryl-choline ((oleoyl)lysolecithin) (I*b*); 1-linoleoyl-*sn*-glycero-3-phosphorylcholine ((linoleoyl)lysolecithin) (I*c*); 1-palmitoyl-*sn*-glycero-3-phosphorylcholine ((palmitoyl)lysolecithin) (I*d*); 1-myristoyl-*sn*-glycero-3-phosphorylcholine ((myristoyl) lysolecithin) (I*e*); 1-decanoyl-*sn*-glycero-3-phosphorylcholine ((decanoyl)lysolecithin) (I*f*).

The following lysolecithin analogs were synthesized according to methods published elsewhere (8, 9). Synthetic deoxylysolecithins (formula II):



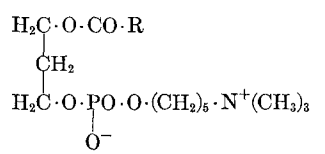
[II]

1-lauroyl propane diol-3-phosphorylcholine ((lauroyl)deoxylysolecithin) (II*a*); 1-palmitoyl propane diol-3-phosphorylcholine ((palmitoyl)deoxylysolecithin) (II*b*); 1-stearoyl propane diol-3-phosphorylcholine ((stearoyl)deoxylysolecithin) (II*c*); 1-arachidoyl propane diol-3-phosphorylcholine ((arachidoyl)deoxylysolecithin) (II*d*); 1-behenoyl propane diol-3-phosphorylcholine ((behenoyl)-deoxylysolecithin) (II*e*); 1-cerotoyl propane diol-3-phosphorylcholine ((cerotoyl)deoxylysolecithin) (II*f*). Synthetic $\omega\omega'$ alkane diol phosphorylcholines (formula III):



[III]

1-palmitoyl butane diol-4-phosphorylcholine (III*a*); 1-lauroyl pentane diol-5-phosphorylcholine (III*b*); 1-myristoyl pentane diol-5-phosphorylcholine (III*c*); 1-palmitoyl pentane diol-5-phosphorylcholine (III*d*); 1-palmitoyl hexane diol-6-phosphorylcholine (III*e*). Synthetic propane diol phosphoryl trimethylamino $\omega\omega'$ alkane diols (formula IV):



[IV]

1-palmitoyl propane diol-3-phosphoryl-5-trimethylamino pentane-1-ol (IV*a*).

Force-area measurements were performed at the air-water interface with a conventional Langmuir-Adam surface balance, using a paraffin-coated quartz trough. The trough was filled with salt-free unbuffered water that had been distilled from alkaline permanganate and then redistilled in a quartz still. The pH of this water, in equilibrium with laboratory air, was about 5.4. The aqueous surface was swept clean with a Teflon bar. The experiments were performed at room temperature (22°C). Known amounts of the pure lipids in solvent were delivered on the surface with an Agla micro syringe and the solvent was allowed to evaporate for 3 min before compressing the spread film. Chloroform or chloroform-methanol (85%–15%) was used as a solvent for all the lipids. The time required to carry out a complete force-area curve was 10–30 min. The recorded collapse pressures were stable for at least 15 min and checked with the Wilhelmy plate method.

RESULTS

The force-area characteristics of some (1-acyl) lysolecithins are compiled in Fig. 1. (Stearoyl)lysolecithin and (oleoyl)lysolecithin form stable monolayers with collapse pressures of 35 dynes/cm. Below the collapse pressure there was no hysteresis found for these lysolecithins. Under similar conditions, the collapse pressure of (1-stearoyl-2-oleoyl)lecithin was found to be 43 dynes/cm. Differences in surface area between (stearoyl lysolecithin and (oleoyl)lysolecithin are extremely small, whereas between (distearoyl) lecithin and (dioleoyl)lecithin a difference of 27–40 A^2/molecule was noted, this being dependent on the surface pressures (10). If we compare the force-area plots of (1-stearoyl-2-oleoyl)lecithin and (stearoyl)lysolecithin or (oleoyl)lysolecithin, a shift to smaller areas of approximately 15 A^2/molecule can be noticed (Fig. 1).

The surface properties of lysolecithins are dependent on chain length and unsaturation of the fatty acid chain. A decrease in chain length of 2 CH_2 units reduced the collapse pressure by 22.6 dynes/cm; compare (stearoyl) lysolecithin with (palmitoyl) lysolecithin.[2] (Myristoyl) lysolecithin barely forms monolayers whereas (decanoyl) lysolecithin becomes completely water soluble. The presence of a second double bond as in (linoleoyl) lysolecithin causes a decrease in collapse pressure of 25.2 dynes/cm when compared with the oleoyl analog. Contrary to the results for diacyl lecithin compounds, there is hardly any shift in surface area among (1-acyl)lysolecithins with different chain lengths and degrees of unsaturation (Fig. 1; compare also reference 10, Figs. 1 and 2). Chain interactions are apparently

[2] D(palmitoyl) lysolecithin (3-palmitoyl-*sn*-glycero-1-phosphorylcholine) as well as mixtures of D(palmitoyl)lysolecithin and L(palmitoyl)-lysolecithin showed the same interfacial behavior as L(palmitoyl)lysolecithin.

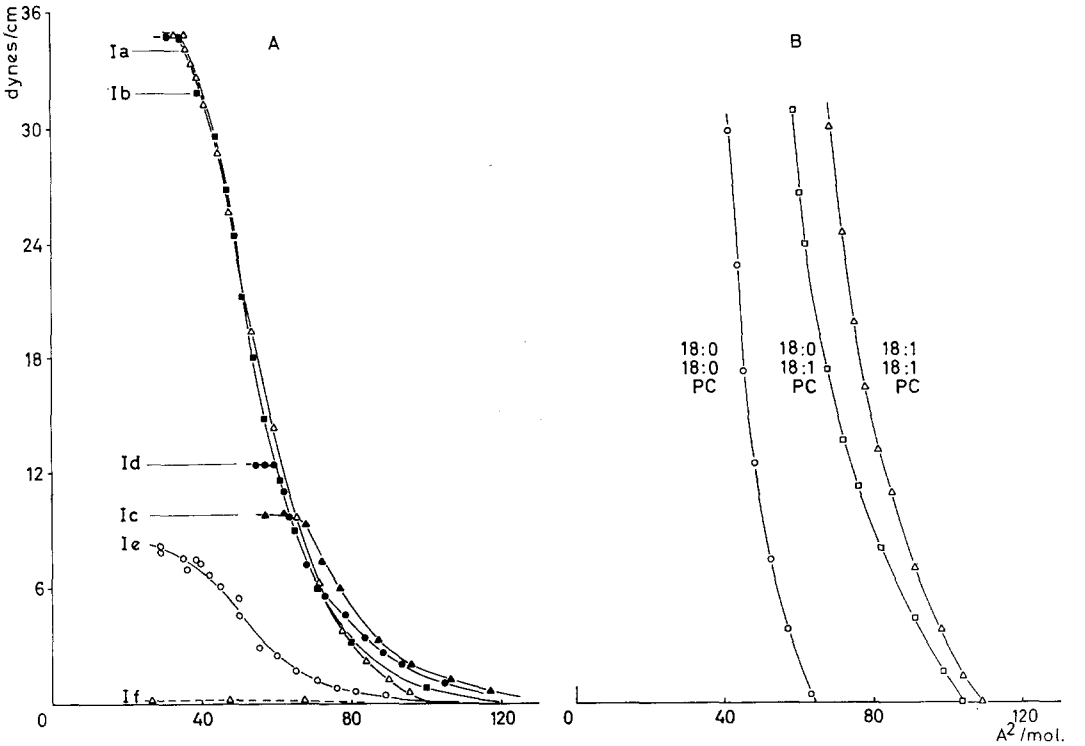


FIG. 1A. Force-area characteristics of various (1-acyl) lysolecithins (Formula I) on unbuffered water, containing as fatty acid constituent stearate (I*a*); oleate (I*b*); linoleate (I*c*); palmitate (I*d*); myristate (I*e*); decanoate (I*f*).

FIG. 1B. Force-area characteristics of (distearoyl)lecithin (18:0/18:0 PC); (oleoyl-stearoyl)lecithin (18:0/18:1 PC); and (dioleoyl)lecithin (18:1/18:1 PC). Reproduced from reference 10.

very small in monolayers of lysolecithins; this is supported by the observation that no condensation effect could be observed between (oleoyl) lysolecithin and cholesterol (10). The zwitter ionic phosphate choline moiety is obviously more important in limiting the surface area than the fatty acid chain.

Deoxylysolecithins (devoid of an OH group at C2) exhibit a similar lytic activity to that of (1-acyl)lysolecithins (11). The force-area plots of deoxylysolecithins with different fatty acid chain length are visualized in Fig. 2. (Lauroyl) deoxylysolecithin is nearly water soluble whereas (palmitoyl) deoxylysolecithin forms monolayers comparable with those of (palmitoyl)lysolecithin. When compared with the (1-acyl) lysolecithins, the differences in area and collapse pressure are small. The areas per molecule of the deoxylysolecithins with a fatty acid chain length of 16–22 carbon atoms, are rather close at surface pressures between 6 and 17 dynes/cm. The area per molecule is apparently determined by the polar moiety, as was found for the lysolecithins studied. At higher surface pressures (arachidoyl) and (behenoyl) deoxylysolecithins show a transition to smaller areas per molecule, whereas (cerotoyl) deoxylysolecithin has already condensed at low surface pressures. In deoxylysolecithins with a chain length longer than 18 carbon atoms, chain interactions apparently become important causing a shift to a more condensed type of film. In this respect, reorientation of the phosphate-choline body, perpendicular to the interface, can also play a part. The collapse pressures of the deoxylysolecithins studied show a maximum for the stearoyl compound. At present, it is difficult to visualize how and why these liquid monolayers reveal this maximum. It is, however, interesting to note that the lytic activity

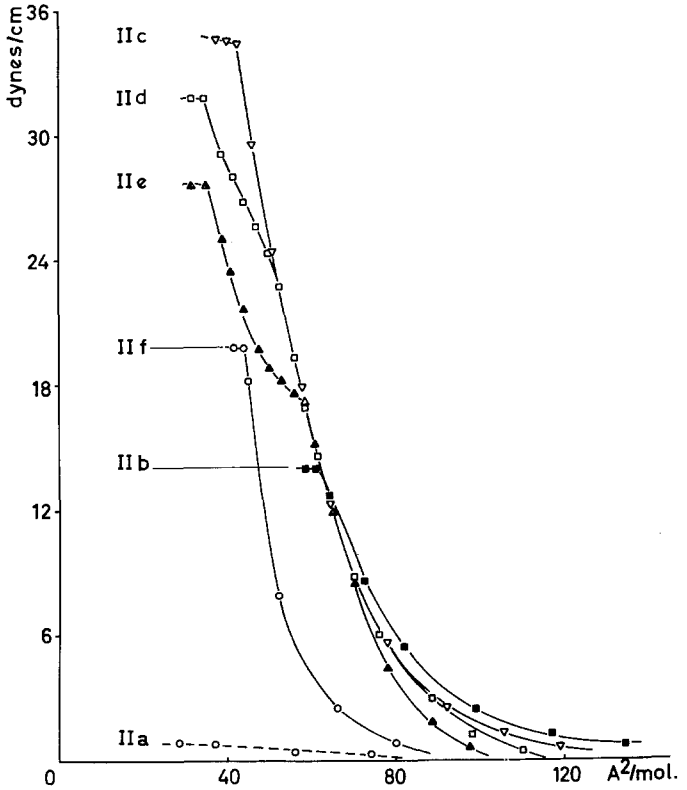


FIG. 2. Force-area characteristics of various deoxylysolecithins (Formula II) on unbuffered water, containing as fatty acid constituent laurate (II*a*); palmitate (II*b*); stearate (II*c*); arachidate (II*d*); behenate (II*e*); cerotate (II*f*).

of these derivatives revealed a maximum for the stearoyl compound. Compounds with longer and shorter chain lengths appeared to be less disruptive towards red cell membranes.

The 1-acyl pentane diol-5-phosphorylcholines (Fig. 3) showed the same dependency of surface properties on chain length as already demonstrated for lysolecithins and deoxylysolecithins. Elongation of the fatty acid chain from 12 to 16 carbon atoms provides a more stable monolayer at the air-water interface with higher collapse pressures.

The effect of replacing the propane diol body of the deoxylyso compound by butane diol, pentane diol, and hexane diol, respectively, is visualized in Fig. 4. Elongation of the $\omega\omega'$ alkane diol part with the fatty acid chain length kept constant results in an increase in area per molecule as well as in collapse pressure. The change in collapse pressure is presumed to be due to extension of the polar parts of the molecule. Increasing the fatty acid chain of (palmitoyl) deoxylysolecithin with 2 CH_2 units to (stearoyl) deoxylysolecithin increases the collapse pressure by 20.6 dynes/cm (Fig. 2). However, the increase of the propane diol body of 1-palmitoyl propane diol-3-phosphorylcholine with 2 CH_2 units to 1-palmitoyl pentane diol-5-phosphorylcholine, increases the collapse pressure by only 13 dynes/cm. The change in area per molecule is thought to be brought about by the increase in distance between the polar fatty acid ester linkage and the phosphate choline moiety.

Elongation of the phosphate-quaternary ammonium distance of 2 carbon atoms (1-palmitoyl propane diol-3-phosphorylcholine) to 5 carbon atoms (1-palmitoyl-propane diol-3-phosphoryl-5-trimethylamino pentane-1-ol) has only a slight effect on the collapse pressure (1.8 dynes/cm), whereas

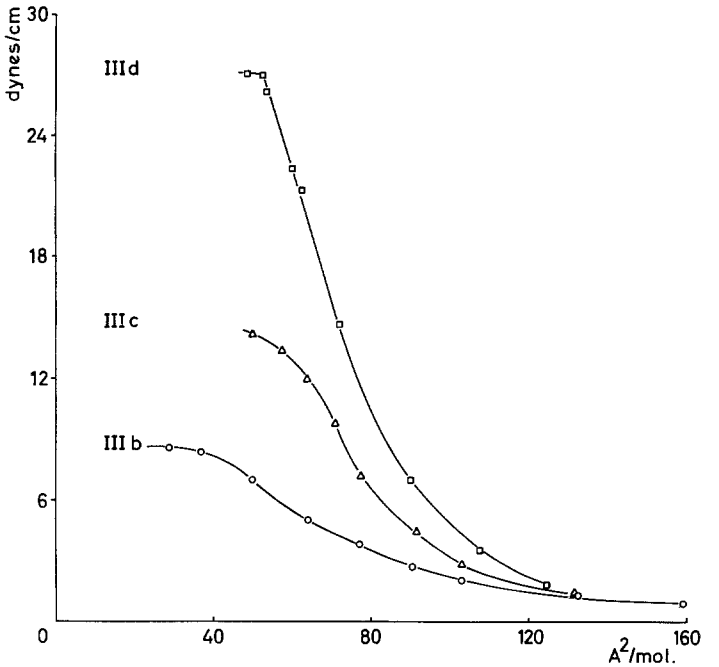


FIG. 3. Force-area characteristics of various $\omega\omega'$ pentane diol phosphorylcholines (Formula III) on unbuffered water containing as fatty acid constituent laurate (III*b*); myristate (III*c*); palmitate (III*d*).

the increase in area per molecule is about the same as for elongation of the propane diol moiety with the same number of carbon atoms.

DISCUSSION

The importance of the lysolecithins as lecithin precursors and membrane constituents has already been mentioned in the Introduction. Measurements of the interfacial characteristics of defined, pure lysocompounds can provide some clue to understanding the behavior of these physiologically important compounds. However, care has to be exercised in extrapolating these results to biological phenomena. In all cases studied, the lysolecithins and deoxylysolecithins revealed a much bigger area per molecule than would be expected if fatty acid chains were to limit the surface area. It is not surprising that, owing to small chain interactions, all monomolecular films were liquid, as estimated by spreading talc powder on the surface. All lysolecithins, which orientated at the air-water interface, were lytic towards red cell membranes and bimolecular lipid films. Even (myristoyl) lysolecithin, which forms poor monolayers, appeared to be a very disruptive agent (3). The deoxylysolecithins revealed an increasing lytic activity with increasing film stability. The maximum activity is found for (stearoyl)deoxylysolecithin. Longer chain derivatives with lower collapse pressures are less, or not at all, lytic towards biological or artificial membranes. It seems likely that a certain ratio between the polar and the apolar moiety of the amphipathic molecule is necessary for it to be an effective lytic agent.

To study and interpret phospholipid-protein interactions such as the action of phospholipase A (EC 3.1.1.4) on monomolecular films of lecithins, a knowledge of the interfacial behavior of the lysolecithin species is important. Dawson (6) studied the hydrolysis of monomolecular layers of P^{32}-labeled phospholipid films, following the changes in surface radioactivity. He observed that the lysolecithins formed from P^{32} lecithin of *Saccharomyces cerevisiae* were about 80%–85% dissolved at a surface pressure of 30 dynes/cm. Shah and Schulman (4), using some natural and synthetic

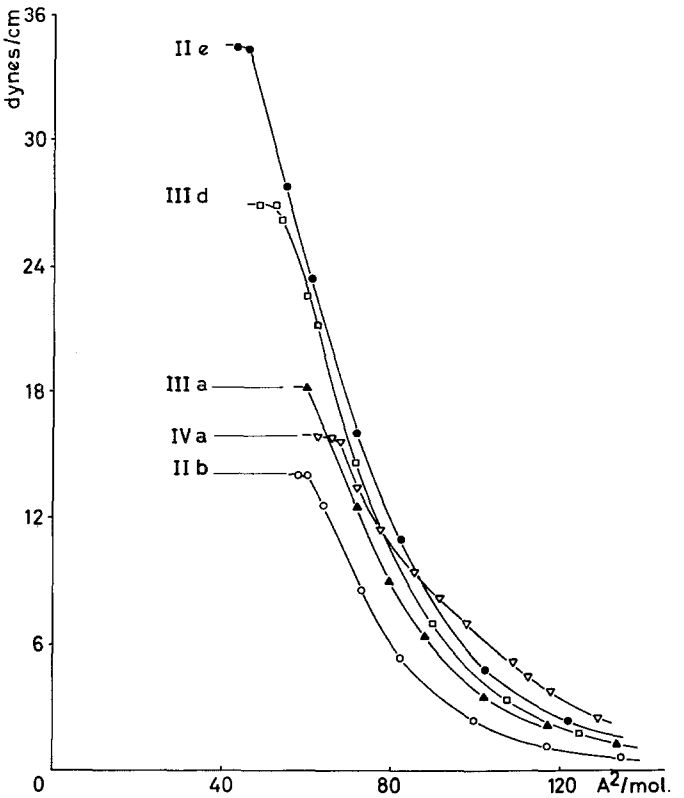


FIG. 4. Force-area characteristics of various palmitoyl $\omega\omega'$ alkane diol phosphorylcholines (Formulas II and III): 1-palmitoyl propane diol-3-phosphorylcholine (II*b*); 1-palmitoyl butane diol-4-phosphorylcholine (III*a*); 1-palmitoyl pentane diol-5-phosphorylcholine (III*d*); 1-palmitoyl hexane diol-6-phosphorylcholine (III*e*); and 1-palmitoyl propane diol-3-phosphoryl-5-trimethylamino pentane-1-ol (Formula IV) (IV*a*).

lecithins, showed a decrease in surface pressure after phospholipase A treatment of soya bean lecithin and (dioleoyl)lecithin at pressures of >20 and >30 dynes/cm, respectively. On the other hand, Colacicco and Rapport (5) estimated the action of phospholipase A on egg lecithin and bovinal heart phosphatidalcholine monolayers, assuming that at a surface pressure of 12 dynes/cm, the lysolecithin formed remained in the film.

As demonstrated in the present study, the interfacial behavior of lysolecithin depends greatly on the nature of the fatty acid constituent. At a surface pressure of 30 dynes/cm, the lysolecithins containing as paraffinic chains, palmitate, myristate, or linoleate will dissolve in the subphase, but those containing stearate or oleate may remain in the film. At a pressure of 12 dynes/cm, several lysolecithin species will remain in the interface, although the (palmitoyl) lysolecithin is near its collapse pressure and a considerable part may leave the film after long incubation times. The nature of the lysolecithins involved and the different surface pressures applied in previous studies (4–6) may account for part of the differences in the results reported. However, it is not yet proved that lysolecithins would leave a mixed film containing unchanged lecithin substrate and fatty acid in the same state in which they leave a pure monolayer. In addition, the presence of protein in the subphase might affect the release of lysolecithin from the film especially as phospholipase A is usually combined with its products in incubation mixtures. Further studies, however, are required to elucidate

the behavior of individual species in mixed films of different lysolecithins.

SUMMARY

1) The interfacial characteristics of lysolecithins are highly dependent on chain length and unsaturation of the fatty acid chain. (Stearoyl) and (oleoyl) lysolecithins form stable monolayers with high collapse pressures (35 dynes/cm) whereas (palmitoyl) (myristoyl), and (linoleoyl) lysolecithin monolayers show low collapse pressures ($\leqq$12.4 dynes/cm).

2) Deoxylysolecithins show an interfacial behavior similar to that of lysolecithins. The lysolecithins and deoxylysolecithins studied revealed a much higher area per molecule as would be expected if fatty acid chains were to limit the surface area. (Stearoyl) and (oleoyl) lysolecithin occupy an area which is 15 A^2/molecule smaller than that of (stearoyl-oleoyl) lecithin.

3) The effect of the free hydroxyl group, the elongation of the alkane diol part of palmitoyl $\omega\omega'$ alkane diol phosphorylcholines, and the elongation of the zwitter ionic phosphate-quaternary ammonium distance were studied.

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