Biochimica et Biophysica Acta, 363 (1974) 361–372

BBA 76725

EFFECT OF EXOGENOUS LYSOLECITHIN ON LIPOSOMAL MEMBRANES ITS RELATION TO MEMBRANE FLUIDITY

KEIZO INOUE and TAKAYUKI KITAGAWA

Department of Chemistry, National Institute of Health, Kamiosaki, 2-10-35, Shinagawa-ku, Tokyo (Japan)

(Received April 19th, 1974)

SUMMARY

The effect of exogenous lysolecithin on liposomal membranes has been studied. Lysolecithin did not have any significant influence on the glucose permeability of egg lecithin liposomes at any temperature range tested, unless the incubation temperature was shifted down from 21 °C to 1 °C. With the shift-down of the temperature, lysolecithin-induced damage of liposomes occurred. When liposomes were prepared with dimyristoyl lecithin, they were sensitive to lysolecithin in the temperature range from 40 °C to 21 °C without the necessity for a shift-down of temperature. Sensitivity of dimyristoyl lecithin liposomes to lysolecithin was shown to be completely dependent on the temperature. Below 10 °C, the damage of the liposomes by lysolecithin was negligible. With liposomes containing the equimolar mixture of dimyristoyl lecithin and egg lecithin, lysolecithin had the largest effect on the permeability of glucose at around 15 °C. Generally, cholesterol incorporation suppressed the sensitivity of liposomes to lysolecithin.

The results obtained in this study indicate that lysolecithin-induced liposomal damage requires certain states of fluidity in lipid bilayers. It seems likely that the first interaction of liposome with lysolecithin and the following expression of permeability change may require different states of fluidity of lipid bilayer.

INTRODUCTION

Lysolecithin, which is well-known for its highly lytic property and activity to induce fusion of various cell types [1, 2], belongs to a group of substances described by Haydon and Taylor [3] as wedge-shaped molecules. It is the generally accepted concept that the sequence of events leading to lysis of cells is first an adsorption of lysolecithin onto the membrane followed by its penetration [4]. This results in a disorganization of the structural arrays, a change of permeability, a disturbance in osmotic equilibrium and finally a loss of cellular contents. It seems conceivable that the biological activities of lysolecithin such as fusion enhancement are related to the sequence of events leading to lysis. Lucy [5] proposed that lysolecithin might cause cell fusion by producing localized micellar structures in the plasma membrane.

Though very little is known about the receptor for lysolecithin on the membranes, it is assumed that lysolecithin interacts primarily with the lipid constituents of the membranes. Bangham and Horne [6] observed electron microscopically some disorganization of lecithin–cholesterol spherules by lysolecithin. These results were further confirmed by Howell et al. [7]. More recently, an interesting study has been undertaken on the lysis of red blood cells and artificial bimolecular leaflets by lysolecithin [4]. It was observed that the action of various lysolecithin derivatives toward red cells and lipid bilayers showed reasonable similarity. These facts may suggest that the interaction of the lytic reagents with the lipid constituents of the membrane plays an important role in the process of membrane disruption. An NMR study by Chapman et al. [8] revealed that hydrophobic region of lysolecithin might interact with some membrane materials (probably lipids).

The purpose of this paper is to obtain further direct evidence for the interaction of lysolecithin with lipid membranes (i.e. liposomes) and to examine the mechanism by which lysolecithin interacts with lipid bilayers to produce permeability changes.

MATERIALS AND METHODS

Chemicals and enzymes were obtained from the following companies: Oriental Yeast Company, Tokyo (hexokinase, glucose-6-phosphate dehydrogenase and NADP); Sigma Chemical Company, St. Louis, Mo., U.S.A. (cholesterol and β,γ-dipalmitoyl-DL-α-lecithin); Calbiochem., San Diego, Calif., U.S.A. (β,γ-dimyristoyl-L-α-lecithin); K. and K. Laboratories, Inc., Plainview, N. J. (dicetyl phosphate); Clarkson Chemical Company, Inc., Williamsport, Pa., U.S.A. (Unisil); Woelm, Eschwege, G.F.R. (Aluminum Oxide Neutral).

Lecithin of egg yolk was prepared by chromatography on Aluminum Oxide Neutral and Unisil. Lysolecithin was obtained from egg lecithin by treatment with phospholipase A from snake venom (*Naja naja*). All lipid preparations showed a single spot by silica gel thin-layer chromatography. Lipids were extracted by the procedure of Folch and Lees [9] from rat erythrocytes or from the membranes of sheep red blood cells prepared by the method of Dodge et al. [10].

Liposomes were prepared by the method of Kinsky et al. [11] with the slight modification as described previously [12]. In brief, appropriate quantities of the lipids (2 μmoles of phospholipid phosphorus) were added to a 10-ml flask or 10-ml tube. After removal of the organic solvent with a rotary evaporator, the flask or tube was kept in desicator in vacuo for more than 1 h. The dried lipid film was swollen in 0.2 ml of 0.3 M glucose by agitating with a Vortex mixer. The liposome preparation was dialyzed at room temperature against isotonic salt solution for more than 1.5 h. The amount of trapped glucose and the leakage of trapped glucose were assayed using enzymes as described previously [12]. The reaction was usually started by the addition of the proper amount of lysolecithin which was dissolved in the veronal-buffered saline. Addition of lysolecithin did not have an appreciable influence on the enzyme system. The reaction of liposome with lysolecithin was determined at varying temperatures by the following procedure. Appropriate aliquots of the liposomes (about 2.5 μl) were added to cuvettes containing 250 μl of Tris-buffered isotonic solution of enzymes and cofactors and about 240 μl of veronal-buffered saline. The cuvettes were

kept at an appropriate temperature for about 20 min before adding lysolecithin. After absorbance was measured at 340 nm, lysolecithin was added and the cuvettes were incubated for various times at a desired temperature. Absorbance was measured again to determine the increase of the amount of glucose released. The shiftdown of the incubation temperature was usually performed by dipping the cuvettes in a water bath which was kept at a desirable temperature (usually 1 °C).

The critical micellar concentration of lysolecithin was determined by the method described by Bonsen et al. [13]. The spectral shift induced by the incorporation of a dye (Rhodamine 6G) into lysolecithin micelles was measured in the same solution as that used for assay of liposomal leakage.

RESULTS

Effect of the shift-down of the incubation temperature on permeability change of egg lecithin liposomes induced by lysolecithin

Aliquots of liposome preparations (2.5 μl) which contained egg lecithin, dicetyl phosphate and cholesterol in the molar ratios of 1 : 0.1 : 0.5 were incubated with 21 μg/500 μl of lysolecithin at room temperature. As shown in Fig. 1, no detectable difference was observed within 10 min in the release of trapped glucose between control liposomes (spontaneous release) and liposomes incubated with lyso-

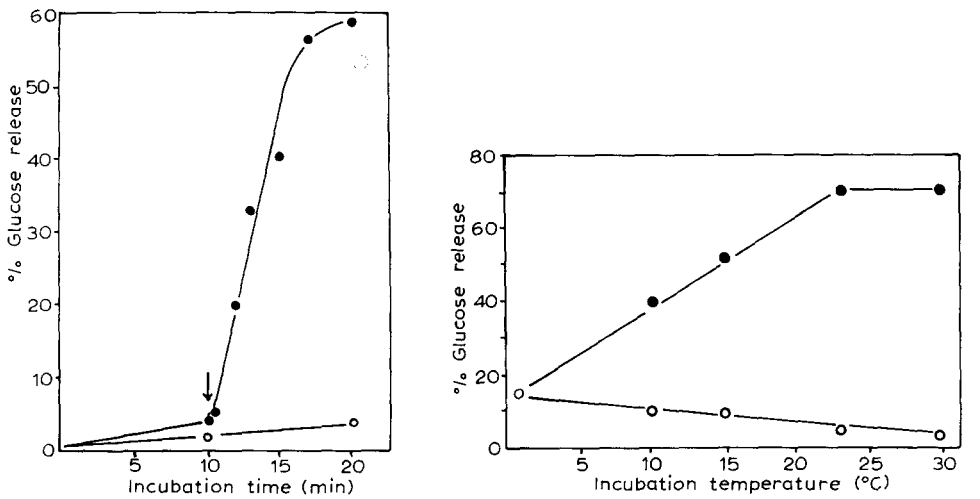


Fig. 1. Effect of the shift-down of the temperature on lysolecithin-induced leakage of trapped glucose from liposomes. Liposomes were prepared from egg lecithin, dicetyl phosphate and cholesterol with molar ratios of 1 : 0.1 : 0.5. After addition of lysolecithin (21 μg), the cuvette was incubated for 10 min at room temperature and then further incubated for 10 min at 1 °C (●–●). The arrow indicates the time at which the temperature was shifted down. Spontaneous release (glucose release without lysolecithin) was also measured under the same conditions (○–○).

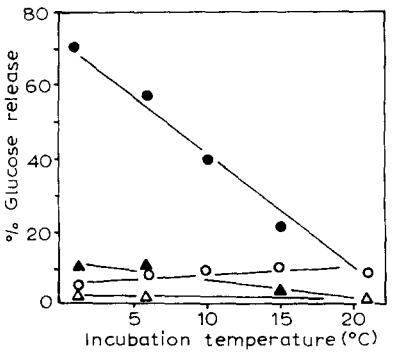
Fig. 2. Effect of the pre-incubation temperature on lysolecithin-induced leakage of glucose. Liposomes (egg lecithin, dicetyl phosphate and cholesterol, 1 : 0.1 : 0.5) were incubated with 21 μg of lysolecithin for 10 min at various temperatures. After pre-incubation, the cuvettes were incubated for another 10 min at the same temperature as pre-incubation (○–○) or at 1 °C (●–●).

lecithin at room temperature. However, immediately after the temperature was shifted down to 1 °C, glucose permeability of liposomes incubated with lysolecithin was enhanced dramatically, while the spontaneous release of glucose did not change significantly.

When the liposomes were incubated with lysolecithin at 1 °C without pre-incubation at a higher temperature, leakage of glucose marker induced by lysolecithin was very little (Fig. 2). Therefore, to determine the maximum condition of the pre-incubation, the liposomes were pre-incubated for 10 min with a certain amount of lysolecithin (21 μg) at varying temperatures. Without the shift-down of the temperature, no detectable leakage of glucose was observed at any temperature range between 30 °C and 1 °C (Fig. 2). With the elevating of the pre-incubation temperature, liposomal damage was increased, which was detectable on the shift-down of the temperature.

The temperature at which liposomes pre-treated with lysolecithin at 21 °C were further incubated is also important. With the fall in the incubation temperature, the glucose release was enhanced (Fig. 3).

Experiments were next performed to follow the time course of the reaction between liposomal membranes and lysolecithin at 21 °C. The liposomes were first incubated with lysolecithin at 21 °C for varying times, then the glucose release from the liposomes was checked after a further 10 min incubation at 1 °C. The initial



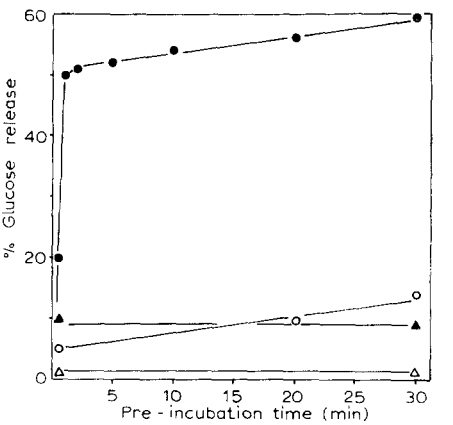


Fig. 3. Effect of the temperature at which liposomes pre-treated with lysolecithin at 21 °C were further incubated. Liposomes (egg lecithin, dicetyl phosphate and cholesterol, 1 : 0.1 : 0.5) were pre-incubated with lysolecithin (21 μg) for 10 min at 21 °C. They were then further incubated for 10 min at temperature indicated. Glucose release after the pre-incubation with lysolecithin ○–○; glucose release after pre-incubation and subsequent incubation at varying lower temperature, ●–●; glucose release after the preincubation at 21 °C without lysoPC, △–△; glucose release after the pre-incubation at 21 °C and further incubation at varying lower temperatures without lysoPC, ▲–▲.

Fig. 4. Effect of pre-treatment time on the lysolecithin-induced damage of egg lecithin liposome. Liposomes (egg lecithin, dicetyl phosphate and cholesterol, 1 : 0.1 : 0.5) were incubated with lysolecithin (21 μg) for various times at 21 °C, ○–○. Following the pre-incubation, liposomes were further incubated at 1 °C for 10 min, ●–●. Glucose release at 21 °C without lysolecithin was also plotted, △–△. Liposomes pre-incubated at 21 °C without lysolecithin were further incubated for 10 min at 1 °C, ▲–▲.

reaction at 21 °C seemed to be very rapid, reaching a plateau within 1 or 2 min (Fig. 4).

Fig. 5 shows the effects of lysolecithin concentration on glucose release from these liposomes. At any concentration of lysolecithin, the glucose release was negligible without the shift-down of temperature. The concentration which is required for damage of the liposomes closely corresponded to the critical micellar concentration of lysolecithin (Fig. 5b). These observations may suggest that monomeric lysolecithin molecule cannot effectively influence the permeability of the lipid membranes.

Effect of cholesterol incorporation into egg lecithin liposome on their sensitivities to lysolecithin

Liposomes were prepared with egg lecithin, dicetyl phosphate (molar ratio, 1 : 0.1) and varying amounts of cholesterol. Release of glucose from the liposomes

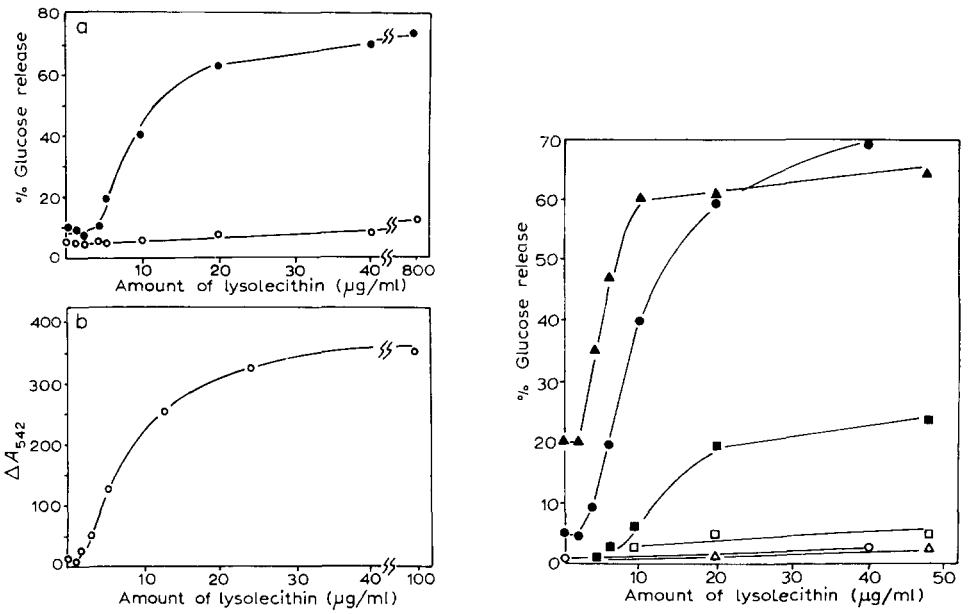


Fig. 5. (a) Effect of the concentration of lysolecithin on liposomal damage. Liposomes were prepared from the mixtures of egg lecithin, dicetyl phosphate and cholesterol (1 : 0.1 : 0.5). Liposomes were incubated with varying amounts of lysolecithin as indicated on the absicissa for 30 min at room temperature, ○-○. The reaction mixtures were further incubated for 10 min at 1 °C, ●-●. Additional details are described in the text. (b) Change in absorbance at 542 nm of a Rhodamine 6G solution with increasing concentration of lysolecithin, measured at pH 7.5 in the same solution used for the experiment summarized in Fig. 5a.

Fig. 6. Effect of lysolecithin on liposomes with varying amounts of cholesterol. Liposomes of egg lecithin without cholesterol were incubated with varying amounts of lysolecithin for 30 min at room temperature, △-△. The pre-incubated liposomes were further incubated for 10 min at 1 °C, ▲-▲. The same experiments were performed by using liposomes with 40 mole% of cholesterol. The release of glucose at room temperature and after the shift-down of temperature to 1 °C are indicated by ○-○ and ●-● respectively. The results with liposomes containing 50 mole% of cholesterol were also expressed by □-□ (without the shift-down of the temperature), and ■-■ (with shift-down of the temperature).

was determined as a function of the amount of lysolecithin added (Fig. 6). There was little difference in sensitivities to lysolecithin between liposome without cholesterol and that prepared with 40 mole% of cholesterol, although marker release in the absence of lysolecithin was significant in the liposomes devoid of cholesterol when the incubation temperature was shifted down to 1 °C. The fragility of egg lecithin liposome at low temperature was overcome by incorporation of cholesterol as described previously [12]. Liposomes with 50 mole% of cholesterol were rather insensitive to lysolecithin even at concentrations as high as 50 μg/ml.

Effect of lysolecithin on liposome with dimyristoyl lecithin

Fig. 7 describes the lysolecithin-induced glucose release from liposomes prepared from dimyristoyl lecithin. In contrast to egg lecithin liposomes, the shift-down of the incubation temperature was not essential for the sensitivity of these liposomes to lysolecithin. Damage of the dimyristoyl lecithin liposomes was detectable at room temperature and furthermore, enhancement of glucose release by the shift-down of the temperature was much less.

It was also indicated in Fig. 7 that cholesterol incorporation reduced lysolecithin-induced permeability change of liposomes with dimyristoyl lecithin. Incorporation of more than 33 mole% of cholesterol suppressed the effect of lysolecithin on dimyristoyl lecithin liposomes. Since such liposomes with less than 25 mole% of cholesterol are sensitive to temperature [12], their reactivities with lysolecithin were

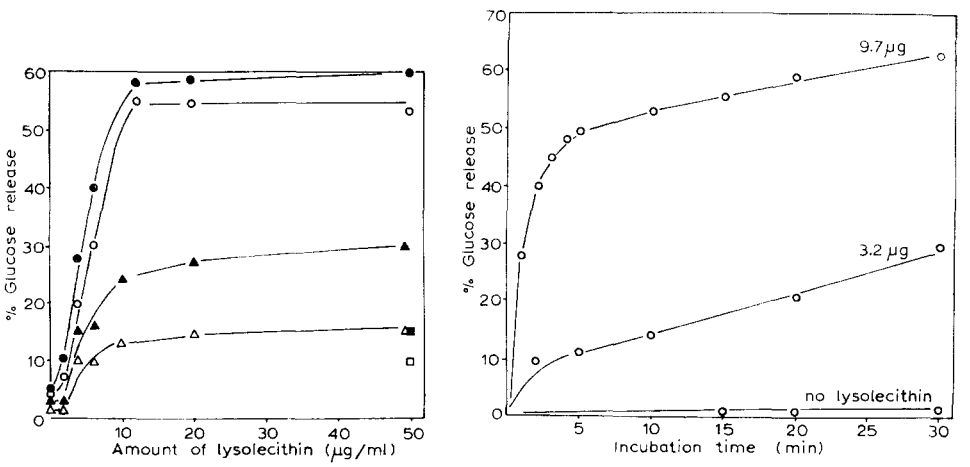


Fig. 7. Effect of lysolecithin on liposomes of dimyristoyl lecithin. The experimental condition was the same as that described for the legend of Fig. 6 except that dimyristoyl lecithin liposomes were used instead of egg lecithin liposomes. The compositions of liposomes were as follows: dimyristoyl lecithin, dicetyl phosphate and cholesterol (1 : 0.1 : 0.4), ○–○ (without the shift-down of temperature) and ●–● (with the shift-down of temperature); dimyristoyl lecithin, dicetyl phosphate and cholesterol (1 : 0.1 : 0.5), △–△ (without the shift-down) and ▲–▲ (with the shift-down); dimyristoyllecithin, dicetyl phosphate and cholesterol (1 : 0.1 : 0.75), □ (without the shift-down) and ■ (with the shift-down).

Fig. 8. Kinetics of glucose release from dimyristoyl lecithin liposomes by lysolecithin. Liposomes (dimyristoyl lecithin, dicetyl phosphate and cholesterol; 1 : 0.1 : 0.4) were incubated with varying amounts of lysolecithin as indicated in the figure for various times at room temperature.

not determined. In all liposomes tested, the amount of lysolecithin required for induction of glucose release was close to that needed for egg lecithin liposomes (4–6 μg/ml). Again, it seems likely that lysolecithin interacts with the liposomes in the form of micellar structure.

Time course of glucose release from dimyristoyl lecithin liposomes by the action of lysolecithin at room temperature was also rapid (Fig. 8). With 9.7 μg/ml of lysolecithin, liposomes prepared with dimyristoyl lecithin, dicetyl phosphate and cholesterol in molar ratios of 1 : 0.1 : 0.4 showed almost 70 % maximum glucose release within 2 min at room temperature.

Effect of the incubation temperature on lysolecithin-induced damage of liposomes containing dimyristoyl lecithin

Liposomes with dimyristoyl lecithin, dicetyl phosphate and cholesterol (molar ratios, 1 : 0.1 : 0.4) were incubated for 30 min with excess lysolecithin (21 μg) at different temperatures (Fig. 9). In the absence of lysolecithin, the liposomes released little glucose at any temperature. Damage of the liposomes by lysolecithin was markedly dependent on temperature. Below 10 °C, the reaction was not detectable, while glucose release was observed at higher incubation temperatures. At about 21 °C, the reaction reached maximum, and over the temperature range from 21–40 °C, the amount of glucose released in the presence of lysolecithin was almost constant.

Liposomes made from dimyristoyl lecithin, egg lecithin, dicetyl phosphate in molar ratios of 1 : 1 : 0.2 showed a different temperature dependence of lysolecithin-induced damage from liposomes with dimyristoyl lecithin and dicetyl phosphate

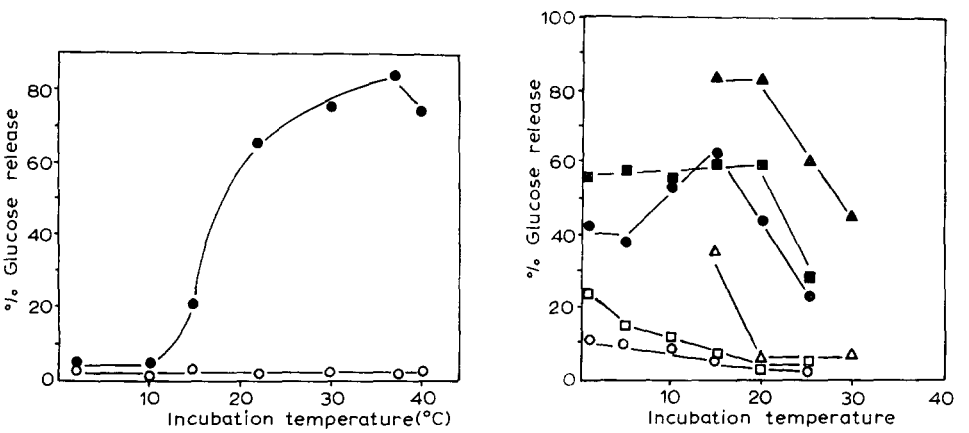


Fig. 9. Effect of the incubation temperature on lysolecithin-induced damage of liposomes prepared from dimyristoyl lecithin. Liposomes described in Fig. 8 were incubated for 30 min at varying temperatures with lysolecithin (21 μg), ●–●; and without lysolecithin, ○–○.

Fig. 10. Effect of incubation temperature on lysolecithin-induced damage of liposomes with mixtures of dimyristoyl lecithin and egg lecithin. The experimental conditions were identical to those described in the legend to Fig. 9. The results with liposome containing equimolar mixture of dimyristoyl lecithin and egg lecithin prepared without cholesterol are expressed by triangle. Results with liposomes with 23 mole% and 30 mole% of cholesterol are expressed by rectangle and circle respectively. Open symbols indicate the results obtained without lysolecithin; closed symbols describe the results with lysolecithin (21 μg).

(Fig. 10). The reactivity of lysolecithin with liposomes containing 30 mole% of cholesterol was maximum at about 15 °C. With increase of temperature above 15 °C, the amount of glucose release from the liposomes was apparently decreased. At 25 °C, only 20 % of trapped glucose was released within 30 min. At lower temperature range below 15 °C, decreased permeability change due to lysolecithin was observed. The temperature dependence of lysolecithin-induced glucose leakage was also observed in both liposomes prepared without cholesterol and with cholesterol in the molar ratio 0.3 to phospholipid. Since spontaneous release of glucose occurred from liposomes with less than 25 mole% of cholesterol in the low temperature range [12], the exact temperature dependence of lysolecithin-induced permeability change could not be obtained.

Effect of fatty acid composition on the amount of glucose released from liposomes in the presence of lysolecithin

To determine how the nature of fatty acid substituents influences the temperature dependence of lysolecithin-induced permeability change, an experiment was performed with liposomes prepared from mixtures of dipalmitoyllecithin, egg lecithin and 23 mole% of cholesterol. Liposomes thus prepared were incubated with lysolecithin at 20 °C. As already described, egg lecithin liposomes were not damaged by lysolecithin at this temperature, unless the temperature was shifted down. Glucose permeability of liposomes prepared with dipalmitoyl lecithin was not influenced by

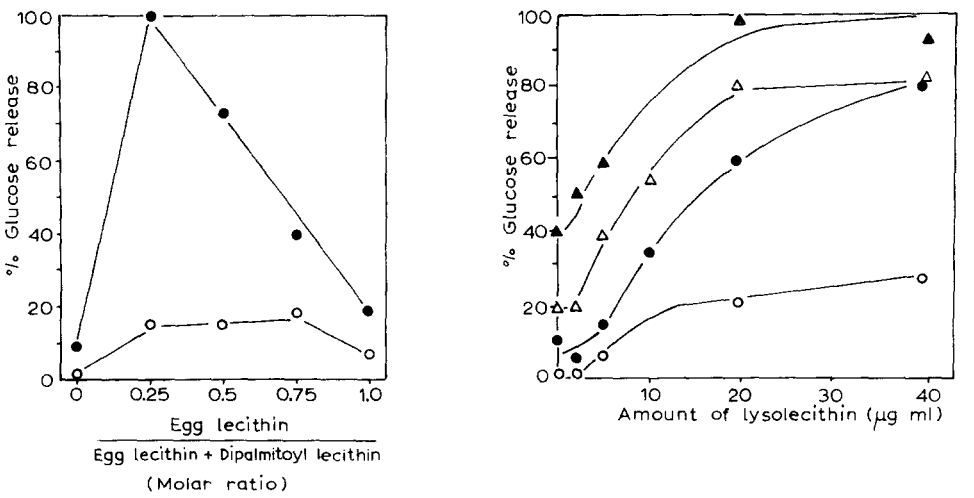


Fig. 11. Effect of fatty acid compositions of phospholipids on susceptibility of liposome against lysolecithin. Liposomes with 10 mole% of dicetyl phosphate and 23 mole% of cholesterol were prepared with various mixtures of dipalmitoyl lecithin and egg lecithin as indicated. Glucose release from liposomes incubated with lysolecithin (21 μg) for 30 min at room temperature is expressed by ●–●. Control glucose release (without lysolecithin) is described by ○–○.

Fig. 12. Sensitivities of liposomes derived from erythrocytes lipids against lysolecithin. Liposomes were prepared from lipids of sheep erythrocytes membrane (circles) and lipids of rat erythrocytes (triangles). The results obtained by incubating at room temperature without the shift-down of temperature are expressed by open symbols while those obtained with the shift-down of temperature are expressed by closed symbols.

lysolecithin at any temperature range tested (1 °C–25 °C) whenever liposomes were prepared with or without cholesterol. However, liposomes containing both dipalmitoyl and egg lecithins in the molar ratio of 1 : 1 or 3 : 1 were extremely sensitive to lysolecithin (Fig. 11). These liposomes released much higher amounts of trapped glucose when incubated with lysolecithin at room temperature than liposomes containing only dipalmitoyl lecithin or egg lecithin.

Effect of lysolecithin on liposomes prepared with lipids extracted from erythrocytes

Liposomes with lipids extracted from sheep blood cell ghosts were poorly sensitive to lysolecithin at room temperature, while they showed the significant enhancement of the sensitivity to lysolecithin when the temperature was shifted down from room temperature to 1 °C (Fig. 12). In contrast, liposomes with lipids extracted from rat red blood cells were sensitive to lysolecithin even at room temperature. It was further shown that the effect of the shift-down of the temperature was rather small in the liposomes prepared from total lipids of rat blood cells.

DISCUSSION

Since Bangham et al. [14] found that closed vesicles consisting of liquid crystals of phospholipids were formed spontaneously when phospholipids were swollen in salt solution, it has become evident that many properties of natural membranes can be duplicated in these simple lipid bilayer systems (liposomes) [11, 15–22]. Such liposomes provide a means to study the mechanism of membrane lysis by various reagents.

In the present investigation, it was found that liposomal membranes interacted with lysolecithin to cause the release of trapped glucose under some conditions. This constitutes direct evidence that the interaction of exogeneous lysolecithin and lipid bilayer leaflet leads to permeability changes. As already noted, Reman et al. [4] reported that lysolecithin influenced the stability of bilayer lipid membranes generated from the total lipids of beef erythrocytes since the survival time of bimolecular lipid films was greatly affected by lysolecithin. Bangham and Horne [6] observed by electron-microscope that adding lysolecithin to a dispersion composed of equimolar quantities of egg lecithin and cholesterol resulted in the release of many discs of approximately uniform diameter which consisted of two lamellae enclosing a water compartment. In our experiments, egg lecithin liposomes were not significantly damaged by simple exposure to lysolecithin. Damage occurred, however, if the lysolecithin treatment was followed by a shift-down of the incubation temperature. On the basis of studies by Reman et al. [4] and many others, hemolysis by lysolecithin can be considered to be built up from at least the following 5 consecutive reactions: (1) An adsorption of the molecules to the membranes; (2) Penetration of the molecules into membranes; (3) Induction of a change in the molecular organization by penetrated lysolecithin molecules; (4) A radical change in permeability of the membrane and a disturbance of the osmotic equilibrium; (5) Leak of hemoglobin.

In the case of the interaction between lysolecithin and liposomes, almost the same process could be considered. Since lysolecithin in the micellar form seems to interact with liposomes, it is feasible to consider that micelles of lysolecithin may

fuse with liposomal membranes instead of the penetration into the membranes.

The lack of detectable permeability change by lysolecithin in egg lecithin liposomes without the shift-down of the incubation temperature might be explained by the suppression of some steps. The importance of the temperature at which liposomes were reacted with lysolecithin suggests that the membrane fluidity is related to susceptibility of liposomes toward lysolecithin. It seems likely that the fluidity of liposomal membranes derived from egg lecithin is enough to interact with lysolecithin above 21 °C (step 1 or step 2). At these temperature ranges, however, the condition of fluidity in the membranes may inhibit events which occur at step 3 or 4. At lower temperature, molecular motion should be inhibited. As a result, lysolecithin, which has already interacted with the bilayer at higher temperature, may lead to a structural alteration and/or permeability change of the membranes. If the optimum temperature is different for the occurrence of events at step 1 and/or 2 and for that at step 3 and/or 4, the lack of permeability change without the shift-down of the temperature can be explained.

There is a possibility that the incubation temperature may affect micellar structure of lysolecithin. Therefore, the experiments were performed with liposomes which have different temperature sensitivities [12]. Liposomes with dimyristoyl lecithin, dicetyl phosphate and cholesterol in molar ratios of 1 : 0.1 : 0.4 were shown to be damaged by lysolecithin without the shift-down of the temperature. The liposome was susceptible toward lysolecithin at the temperature ranges from 20–40 °C. Below 20 °C, their sensitivities to lysolecithin were decreased. These experiments indicated that the temperature dependence of lysolecithin-induced liposomal damage did not result from the change in micellar structure of lysolecithin. In addition, it was suggested that the state of molecular motion in the liposomes at the temperature range from 20–40 °C should be suitable both for interacting with lysolecithin (step 1 and 2) and for expressing the permeability change (step 3 and 4). Molecular motion in dimyristoyl lecithin liposome may be quite different from liposome composed of egg lecithin which has unsaturated fatty acid residues. When liposomes were prepared with the equimolar mixture of dimyristoyl lecithin and egg lecithin, which should have a different phase transition temperature, the optimum temperature at which liposomes reacted with lysolecithin was shifted to the lower temperature range, that is, around 15 °C instead of 25 °C. Above 15 °C, the reactivity was decreased with the elevation of the temperature, which indicated that the fluidity might be enough to resist events occurring at step 3 or 4. The different behaviour between saturated lecithin and egg lecithin liposome in their reactivity with lysolecithin may be relevant to the difference observed in their temperature dependent permeabilities [12]. It was previously reported that liposomal membranes prepared from saturated lecithin showed more plastic or less flexible properties than egg lecithin liposomes [12].

On the mode of action of the lysoderivatives to liposomes, Uemura et al. [23] obtained interesting results. In extensive studies on passive and active sensitization by lipid antigens [23–26], it was observed that dinitrophenyalted lysophosphatidylethanolamine could produce glucose release from liposomes of egg lecithin, dicetyl phosphate and cholesterol (1 : 0.1 : 0.75) above a critical threshold concentration of 20 nmoles/ml. The concentration was surprisingly close to that of lysolecithin required for the damage of the liposomes used in the present study. However, dinitrophenylated lysophosphatidylethanolamine gave extensive glucose release from egg

lecithin liposomes without the shift-down of the temperature, while egg lysolecithin in the present study produced appreciable marker loss from the same liposomes only when the incubation temperature was shifted down. The difference between the reactivity of dinitrophenylated lysophosphatidylethanolamine and that of egg lysolecithin can not be well explained at the present time. Dinitrophenylated lysophosphatidylethanolamine was effective, as well as S- and R-form lipopolysaccharides [24] treated mildly with alkali, under conditions of passive sensitization. In the passive sensitization procedure, the liposomes are formed initially in the absence of any antigens and then incubated with the liposome. The liposome incubated with a small amount of lysophosphatidylethanolamine derivatives were shown to be damaged by the corresponding antibody and complement. The above observations indicate that these molecules can interact with the liposomal membranes without any appreciable change of permeability. It seems likely that the molecule might interact with the membranes as a monomer in the passive sensitization procedure since the concentration used was very small.

Introduction of cholesterol generally suppressed the sensitivities of liposomes to lysolecithin. The condensing effect of cholesterol might interfere with the penetration of lysolecithin molecules or fusion of lysolecithin micelles. Our preliminary results (Kitagawa, T. and Inoue, K., unpublished) indicated that endogenous lysolecithin could not introduce loss of capacity of permeability barrier when liposomes were prepared with a higher amount of cholesterol. Therefore, it is also possible that the presence of cholesterol inhibits the occurrence of drastic changes in this organization of lipid bilayer (step 3 or 4).

Liposomes with lipids extracted from natural erythrocytes showed somewhat different behavior against lysolecithin from liposomes prepared with individual lipid components. Liposomes made from total lipids of sheep red blood cells membrane released a significant amount of trapped glucose when they were first incubated with lysolecithin at room temperature and then incubated further at 1 °C or 10 °C, even though the molar ratio of cholesterol to phospholipid in the total lipid fraction is close to 1. Liposomes with lipids from rat red blood cell were sensitive to lysolecithin even at room temperature without the shift-down of the temperature. It is unknown why liposomes derived from total lipids of erythrocytes behave in a different way from the liposomes derived from various pure lecithins. Phospholipid compositions in the erythrocyte lipid may give some effects on lysolecithin sensitivity of the liposomes. A comparative study of the action of the various lysoderivatives on several model membranes is now in progress.

The effect of lysolecithin on the natural membranes is generally rather independent of the temperature with some exception. In our preliminary experiments on chick erythrocytes, the shift-down of temperature stimulated lysolecithin-induced hemolysis only when Ca^{2+} coexisted in the reaction mixtures. The discrepancy between natural and model membranes might be explained by the fact that natural membranes are composed not only of lipids but also proteins and other constituents. The existence of protein in membranes was reported to inhibit the molecular motion of lipids [27]. The lipid bilayer in the membranes where molecular motion is restricted by proteins, may be susceptible to the action of lysolecithin. The alternative possibility is that some protein in the membrane may play a more important role as a receptor for lysolecithin than the lipid bilayer still remains to be clarified.

ACKNOWLEDGEMENTS

We wish to thank Dr S. Nojima, Director, Department of Chemistry, National Institute of Health for his encouragement and helpful suggestions throughout this study. We also thank Professor Dr S. C. Kinsky in Washington University, St. Louis, Mo. for his valuable comments and discussions during preparation of the manuscript.

REFERENCES

1 Poole, A. R., Howell, J. I. and Lucy, J. A. (1970) Nature 227, 810–813
2 Croce, C. M., Sawicki, W., Kritchevsky, D. and Koprowski, H. (1971) Exp. Cell. Res. 67, 427–435
3 Haydon, D. A. and Taylor, J. (1963) J. Theor. Biol. 4, 281–296
4 Reman, F. C., Demel, R. A., de Gier, J., van Deenen, L. L. M., Eibl, H. and Westphal, O. (1969) Chem. Phys. Lipids 3, 221–233
5 Lucy, J. A. (1970) Nature 227, 814–817
6 Bangham, A. D. and Horne, R. W. (1964) J. Mol. Biol. 8, 660–668
7 Howell, J. I., Fisher, D., Goodall, A. H., Verrinder, M. and Lucy, J. A. (1973) Biochim. Biophys. Acta 332, 1–10
8 Chapman, D., Kamat, V. B., de Gier, J. and Penkett, S. A. (1968) J. Mol. Biol. 31, 101–114
9 Folch, J. and Lees, M. (1951) J. Biol. Chem. 191, 807–817
10 Dodge, J. T., Mitchell, C. and Hanahan, D. J. (1963) Arch. Biochem. Biophys. 100, 119–130
11 Kinsky, S. C., Haxby, J. A., Zopf, D. A., Alving, C. R. and Kinsky, C. B. (1969) Biochemistry 8, 4149–4158
12 Inoue, K. (1974) Biochim. Biophys. Acta, 339, 390–402
13 Bonsen, P. P. M., de Haas, G. H., Pieterson, W. A. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 270, 364–382
14 Bangham, A. D., Standish, M. and Watkins, J. C. (1965) J. Mol. Biol. 13, 238–252
15 Sessa, G. and Weissmann, G. (1968) J. Lipid Res. 9, 310–318
16 Kinsky, S. C. (1970) Annu. Rev. Pharmacol. 10, 119–142
17 Bangham, A. D. (1972) Annu. Rev. Biochem. 41, 753–776
18 Kinsky, S. C. (1972) Biochim. Biophys. Acta 265, 1–23
19 Inoue, K., Kataoka, T. and Kinsky, S. C. (1971) Biochemistry 10, 2574–2581
20 de Kruyff, B., Demel, R. A. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 255, 331–347
21 Haest, C. W. M., de Gier, J., van Es, G. A., Verkleij, A. J. and van Deenen, L. L. M. (1972) Biochim. Biophys. Acta 288, 43–53
22 Imai, M. and Inoue, K., Biochim. Biophys. Acta, in the press
23 Uemura, K. and Kinsky, S. C. (1972) Biochemistry 11, 4085–4094
24 Kataoka, T., Inoue, K., Luderitz, O. and Kinsky, S. C. (1971) Eur. J. Biochem. 21, 80–85
25 Kataoka, T., Inoue, K., Galanos, C. and Kinsky, S. C. (1971) Eur. J. Biochem. 24, 123–127
26 Six, H. R., Uemura, K. and Kinsky, S. C. (1973) Biochemistry 12, 4003–4011
27 Dupont, Y., Gabriel, A., Chabre, M., Gulik-Krzywicki, T. and Schechter, E. (1972) Nature 238, 331–333