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EFFECT OF LIPID COMPOSITION ON SENSITIVITY OF LIPID MEMBRANES TO TRITON X-100

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SUMMARY

The effect of lipid composition on the sensitivity of liposomes (multilamellar liposomes) and sonicated liposomes, which were composed mostly of single-compartment liposomes, toward Triton X-100, a detergent, was examined. Monomeric molecules of Triton X-100 seem to penetrate into liposomal bilayers, since Triton X-100 could modify the permeability of liposomes at concentrations below its critical micellar concentration. Cholesterol incorporation into egg lecithin liposomes had a negligible effect on the sensitivity to Triton X-100. On the other hand, cholesterol incorporation drastically reduced the Triton-induced permeability change of multilamellar liposomes prepared with dipalmitoyl and dimyristoyl phosphatidylcholines. Liposomes prepared with dipalmitoyl phosphatidylcholine or dimyristoyl phosphatidylcholine showed a strong resistance to the action of the detergent when prepared with 50 mol% of cholesterol, releasing at most 10 % of trapped glucose. Ultrasonicated liposomes of dipalmitoyl phosphatidylcholine were, however, still sensitive to Triton X-100, releasing almost 100 % of the trapped glucose even when 50 mol% of cholesterol was incorporated. This fact indicates that only the outermost bilayers in multilamellar liposomes composed of dipalmitoyl phosphatidylcholine and cholesterol may be sensitive to the Triton X-100.

In cases of multilamellar liposomes with less than 33 mol% of cholesterol, sensitivities of liposomes to the detergent decreased in the following order; dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylcholine, egg lecithin and rat liver phosphatidylcholine liposomes. The same order of sensitivity to Triton was also observed in the system of sonicated liposomes. Membranes in the gel state are most sensitive to the detergent, followed by membranes around the phase transition. Egg and rat liver phosphatidylcholine membranes, both of which are liquid-crystalline, were the least sensitive to the detergent.

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INTRODUCTION

The non-ionic detergent Triton X-100, an alkylphenoxy, polyethoxy surfactant was shown to be capable of totally disrupting lyposomes, mitochondria and erythrocytes [1–3]. This detergent has been routinely used for solubilizing membrane components. Weissmann et al. [4] reported that Triton X-100 modified the permeability of glucose and ions through liposomes derived from egg lecithin, dicetyl phosphate and cholesterol in the molar ratio of 1 : 0.25 : 0.125. According to Kinsky et al. [5], liposomes derived from mixtures of beef brain sphingomyelin, dicetyl phosphate and cholesterol in the molar ratio of 1 : 0.1 : 0.75 showed a resistance to the action of Triton X-100, while egg lecithin liposomes (egg lecithin/dicetyl phosphate/cholesterol, 1 : 0.1 : 0.75) were completely damaged by treatment with 1 % of Triton. Dipalmitoyl phosphatidylcholine liposomes with 50 mol% of cholesterol also resisted the detergent [6]. In a previous paper [7], we reported that liposomal sensitivities to exogenous lysophosphatidylcholine, which can be regarded as a non-ionic detergent, were strongly influenced by the fluidity of the membranes. Very little has been systematically studied on the relationship of the sensitivity to a non-ionic detergent, Triton X-100, and the lipid composition of the membrane. In this paper, we examine the effects of lipid composition on the sensitivity of liposomal membranes to the action of Triton X-100.

MATERIALS AND METHODS

Total lipids of erythrocyte membranes and phosphatidylcholines of egg yolk and rat liver were prepared in our laboratory as described previously [6, 7]. Other lipids used for preparing liposomes were obtained from the same commercial sources as described in previous papers [6–8]. Triton X-100 (Rohm and Haas) was used without further purification. Monomolecular weight of Triton X-100 was tentatively postulated to be 628.

Multilamellar liposomes with cholesterol were prepared by the method of Kinsky et al. [5]. Saturated phosphatidylcholine liposomes were prepared and assayed by the method described in a previous paper [6]. Sonicated liposomes were prepared by the following procedures; undialyzed liposome preparations obtained by mechanically shaking as described previously were sonicated for 1–120 min with a Sonore 150 microprobe, Umeda Electronic, Tokyo (operating at 100 mA output) without cooling. The product after 30–120 min sonication was shown to be mostly single-compartment liposomes with a diameter of 250 Å under the electron microscope. The almost clear suspension was dialyzed against more than 200 ml of the salt solution for 2 h.

Release of trapped glucose was enzymatically assayed by the method developed by Kinsky et al. [5]. The amount of glucose trapped in liposomes was obtained by the "organic solvent method" [5]. Light scatter of liposomes was assayed by reading absorbances at 340 nm of the samples suspended in the buffered saline containing no enzymes and no cofactors. The shift-down of the incubation temperature was usually performed by dipping the cuvettes into a water bath (1 °C). Triton X-100 was dissolved in veronal-buffered saline. The critical micellar concentration of Triton X-100 was determined by the method described by Bonsen et al. [9]. The spectral shift

induced by the incorporation of rhodamine 6G into Triton micelles was measured in the same solution as that used for the assay of liposomal leakage.

RESULTS

Effects of concentration of Triton X-100 on multilamellar liposomes with egg lecithin

Aliquots of liposome preparations (2.5 μl) which contained egg lecithin, dicetyl phosphate (molar ratio, 1 : 0.1) and different amounts of cholesterol were incubated with varying concentrations of Triton X-100 at room temperature for 30 min. This time interval was chosen because the amount of glucose released within 30 min provided an approximate measure of the extent to which glucose is released (Fig. 1). At the critical threshold concentrations (90–120 μg/ml), Triton X-100 produced extensive glucose release from the liposomes (Fig. 2). There was little difference in sensitivity to the detergent between liposomes without cholesterol and those prepared with 40 mol% of cholesterol, while liposomes with 50 mol% of cholesterol were slightly less sensitive. Liposomes first incubated with Triton X-100 at room temperature for 30 min were further incubated at 1 °C for 10 min. Glucose permeability of liposomes incubated with Triton X-100 was slightly enhanced by shift-down of the incubation temperature, since the concentration of Triton X-100 required for half-maximum glucose release clearly shifted to lower concentration by this procedure (108 μg/ml to 80 μg/ml, 113 μg/ml to 89 μg/ml and 120 μg/ml to 110 μg/ml in liposomes without cholesterol and liposomes with 40 and 50 mol% of cholesterol, respectively). The concentration of detergent required to damage the liposomes was much below the critical micellar concentration of Triton X-100 (200 μg/ml) calculated from Fig. 5C.

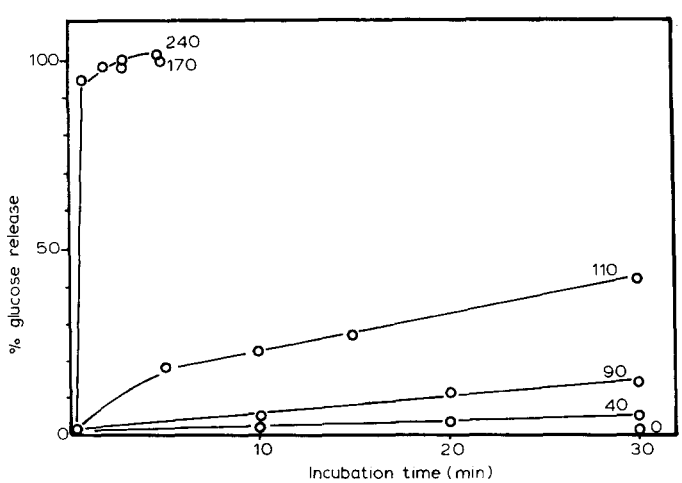

Fig. 1. Kinetics of glucose release from egg lecithin liposomes by Triton X-100. Liposomes (egg lecithin, dicetyl phosphate and cholesterol; 1 : 0.1 : 0.75) were incubated with varying concentrations of Triton X-100 (μg/ml), as indicated in the figure, at room temperature.

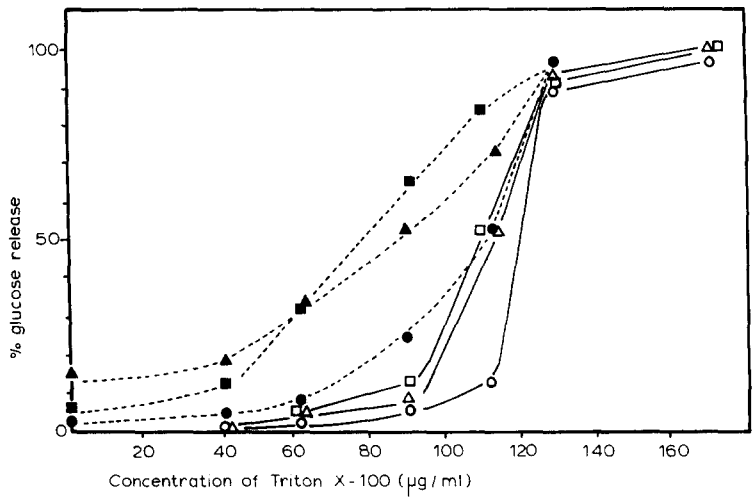

Fig. 2. Effect of the concentration of Triton X-100 on liposomal damage. Liposomes were prepared from the mixtures of egg lecithin, dicetyl phosphate and varying amounts of cholesterol. Liposomes without cholesterol were incubated with various amounts of the detergent, as indicated on the abscissa, for 30 min at room temperature (△). The reaction mixtures were further incubated for 10 min at 1 °C (▲). The same experiments were performed by using liposomes with 40 (□, ■) and 50 (○, ●) mol% of cholesterol. The glucose release at room temperature is indicated by open symbols and that after the shift-down of temperature to 1 °C by closed symbols.

Effect of cholesterol incorporation into multilamellar liposomes of dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine on their sensitivities to Triton X-100

Liposomes were prepared with dipalmitoyl phosphatidylcholine or dimyristoyl

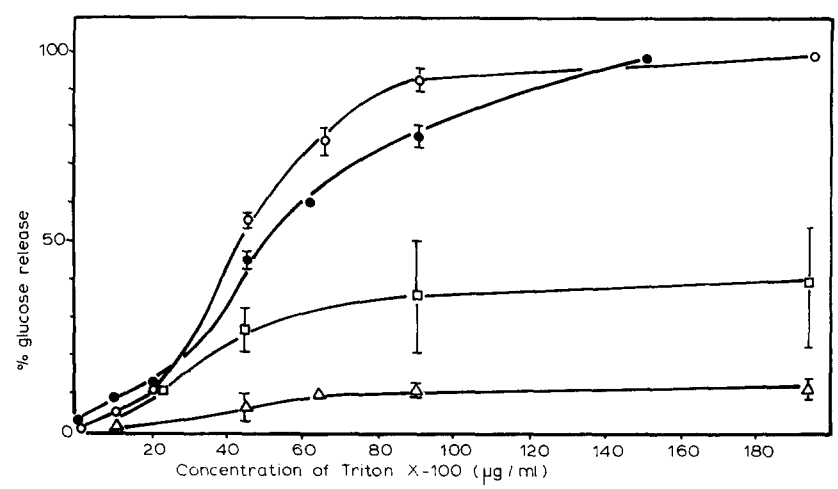

Fig. 3. Effect of Triton X-100 on liposomes of dipalmitoyl phosphatidylcholine. The experimental conditions were the same as described in the legend to Fig. 2, except that dipalmitoyl phosphatidylcholine was used instead of egg lecithin. Cholesterol contents in liposomes were 0 (○), 23 (●), 33 (□) and 50 (△) mol%.

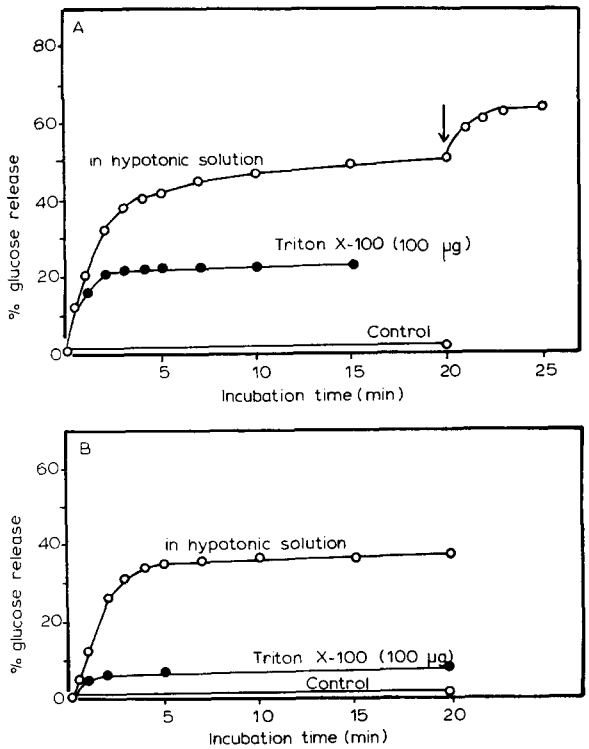

Fig. 4. Time course of glucose release from multilamellar liposomes of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 0.5) (A) and from liposomes of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 1) (B), in hypotonic solution or in the presence of Triton X-100. Liposomes (2.5 μl) were incubated in a mixture of distilled water (250 μl) and isotonic solution containing enzymes and cofactors (250 μl) at room temperature (○). The arrow indicates the addition of 100 μg of Triton X-100. Liposomes were also incubated with Triton X-100 (100 μg) from the beginning of the incubation (●).

phosphatidylcholine, dicetyl phosphate and varying amounts of cholesterol. Since dimyristoyl phosphatidylcholine liposomes are temperature sensitive when prepared with less than 23 mol% of cholesterol [6], liposomes with more than 30 mol% of cholesterol were examined in this experiment. Incorporation of more than 33 mol% of cholesterol definitely suppressed the effect of Triton X-100 on dipalmitoyl phosphatidylcholine liposomes (Fig. 3). Dipalmitoyl phosphatidylcholine liposomes with 50 mol% of cholesterol released little glucose at concentrations as high as 200 μg/ml of Triton X-100. Enhancement of glucose release by the shift-down of the temperature was almost negligible in the case of dipalmitoyl phosphatidylcholine liposomes.

Fig. 4 shows the time course of glucose release from liposomes of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 0.5) and of glucose release from liposomes of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 1) by Triton X-100. In the preparations described in Fig. 4, Triton X-100 (200 μg/ml) could release only 10–20 % of the glucose, while 40–50 % of the glucose could be released from liposomes in the hypotonic solution. In the

hypotonic solution, about 50 % of the trapped glucose remained in liposomes. Further addition of Triton X-100 (200 μg/ml) to liposomes treated osmotically released an additional 12–15 % of glucose. These facts indicate that 12–15 % of the glucose might localize in the compartment that is resistant to osmotic treatment but sensitive to Triton X-100.

It should be noted, however, that even multilamellar dipalmitoyl phosphatidylcholine liposomes with 50 mol% of cholesterol released about 50 % of the trapped glucose when incubated with Triton X-100 at a concentration of 10 mg/ml for 30 min (data not shown). Liposomes prepared from beef brain sphingomyelin and cholesterol (1 : 0.75) were reported [5] to resist the action of Triton X-100. The sphingomyelin liposomes also released about 50 % of the glucose when incubated with a large amount of Triton X-100 (10 mg/ml) [5]. Almost the same suppressive effect of cholesterol incorporation on sensitivity to Triton was observed in multilamellar dimyristoyl phosphatidylcholine liposomes.

Effect of fatty acid composition on the sensitivity of multilamellar liposomes to Triton X-100

Fig. 5 summarizes the effect of fatty acid composition on the sensitivities of liposomes containing 33 mol% of cholesterol to Triton X-100. To compare the sensitivities to be detergent of liposomes having different fatty acyl chains, liposomes with more than 33 mol% of cholesterol were more suitable than liposomes with less than 33 mol% of cholesterol, because liposomes tested in the experiment were not temperature sensitive when prepared with more than 33 mol% of cholesterol [6]. By using these liposomes, we could compare the sensitivity of liposome to the detergent at a certain temperature (25 °C). Dipalmitoyl phosphatidylcholine liposomes were the most sensitive to the detergent, followed by dimyristoyl phosphatidylcholine, egg

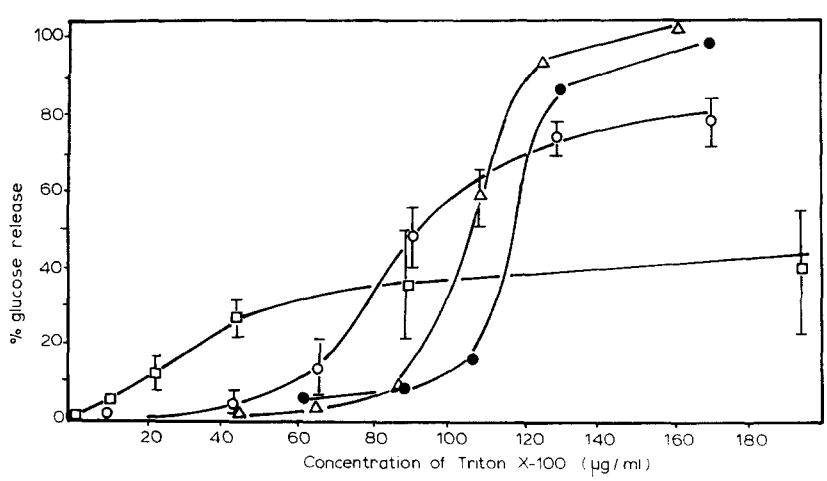

Fig. 5. Effect of fatty acid composition of phospholipids on susceptibility of liposomes to Triton X-100. Liposomes were prepared with various phosphatidylcholines and 33 mol% cholesterol. Liposomes of rat liver phosphatidylcholine (●), egg lecithin (△), dimyristoyl phosphatidylcholine (○) and dipalmitoyl phosphatidylcholine (□) were incubated with varying amounts of Triton X-100 for 30 min at room temperature (25 °C).

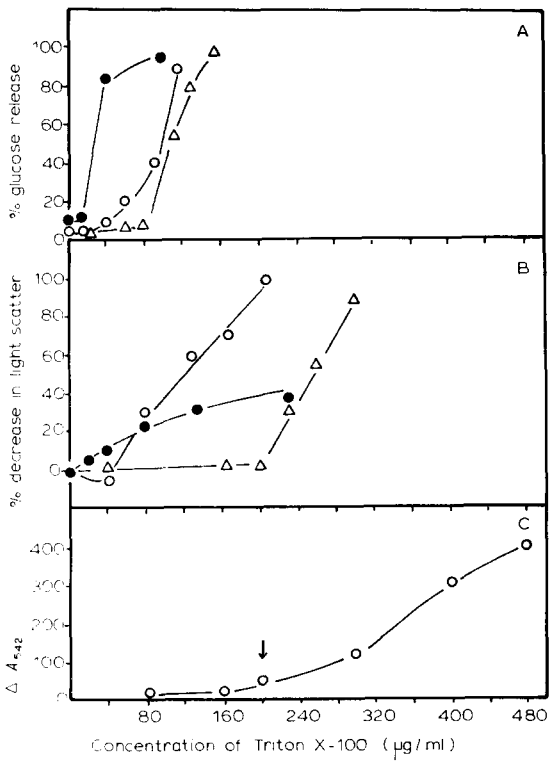

Fig. 6. (A) Effect of the concentration of Triton X-100 on liposomal damage. Liposomes were prepared from various phosphatidylcholines without cholesterol. Liposomes of egg lecithin (△) and dipalmitoyl phosphatidylcholine (●) were incubated with Triton X-100 for 30 min at room temperature. Liposomes of dimyristoyl phosphatidylcholine (○) were incubated under the same conditions, except that the incubation temperature was 15 °C. (B) Change in light scatter (ΔA_{340}) of liposomes described in Fig. 5A, due to the action of the detergent. Liposomes were incubated for 30 min with Triton X-100 under the same conditions as described in Fig. 5A. In the experiments, enzymes and cofactors were omitted from the reaction mixtures. (C) Change in absorbance at 542 nm of a rhodamine 6G solution with increasing concentrations of Triton X-100, measured at pH 7.5 in the same solution used for the experiment described in Fig. 5A. The arrow indicates the critical micelle concentration.

lecithin and rat liver phosphatidylcholine liposomes. Dipalmitoyl phosphatidylcholine liposomes, however, could not release all of the trapped glucose even at the highest concentration of Triton X-100 (200 μg/ml), while liposomes of egg lecithin and rat liver phosphatidylcholine leaked 100 % of trapped glucose eventually.

Sensitivities of multilamellar liposomes without cholesterol to Triton X-100 showed almost the same tendency (Fig. 6). The concentrations of Triton X-100 required for the half-maximum glucose release from dipalmitoyl phosphatidylcholine, dimyristoyl phosphatidylcholine and egg lecithin liposomes were as follows; 25 μg/ml for dipalmitoyl phosphatidylcholine, 95 μg/ml for dimyristoyl phosphatidylcholine and 108 μg/ml for egg lecithin liposomes. The result on dimyristoyl phosphatidylcholine liposomes was not really comparable with the others, since the experiments on the liposomes of dimyristoyl phosphatidylcholine had to be performed below 15 °C.

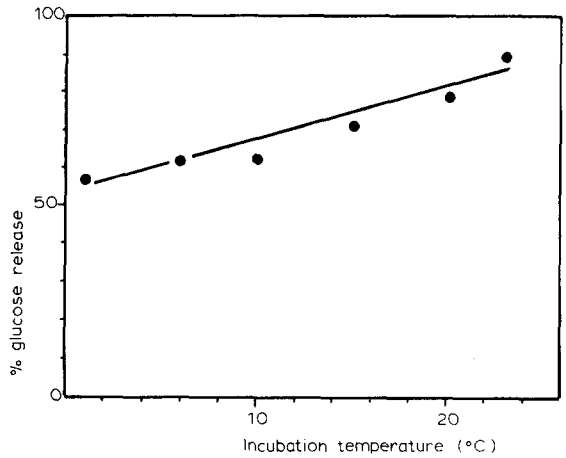

Fig. 7. Effect of the incubation temperature on Triton X-100-induced damage of liposomes prepared from mixtures of dipalmitoyl phosphatidylcholine and dicetyl phosphate (1 : 0.1). Liposomes were incubated for 30 min at varying temperatures with Triton X-100 (70 μg/ml).

In the cases of liposomes containing more than 40 mol% of cholesterol, the order of the sensitivities of liposomes to the detergent was reversed. As described above, sensitivities of egg lecithin liposomes were not so strongly influenced by cholesterol incorporation, while those of dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine liposomes were drastically depressed by cholesterol incorporation. Therefore, liposomes made with mixtures of egg lecithin and cholesterol (1 : 1) were most sensitive to the Triton X-100, followed by dimyristoyl phosphatidylcholine. Liposomes of dipalmitoyl phosphatidylcholine and cholesterol (1 : 1) showed a strong resistance to the action of the detergent.

Interaction of Triton X-100 with multilamellar liposomes prepared from dipalmitoyl phosphatidylcholine and dicetyl phosphate

Dipalmitoyl phosphatidylcholine liposomes without cholesterol were extremely sensitive to Triton X-100 at room temperature (Fig. 3). The sensitivity of the liposomes was independent of the incubation temperature (Fig. 7). Though the packing of the lipid bilayers in the liposomes should be quite tight at low temperature, the detergent molecules seem to penetrate into the bilayers.

Effects of Triton X-100 concentration on the modification of permeability and change of light scatter of multilamellar liposomes

Egg lecithin, dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine liposomes without cholesterol were treated with varying concentrations of Triton X-100. Egg lecithin and dipalmitoyl phosphatidylcholine liposomes were incubated with the detergent at room temperature, while dimyristoyl phosphatidylcholine liposomes were treated at 15 °C. In the case of egg lecithin liposomes, permeability change by the detergent was observed first, followed by the change in light scatter (Fig. 6). Permeability changes of the liposomes were induced by Triton X-100 at a concentration of 90 μg/ml. On the other hand, light scatter of the liposomes was

affected by the detergent at a higher concentration, that is, at more than 200 μg/ml. The concentration of Triton X-100 at which light scatter of the liposomes was affected roughly corresponded to the critical micellar concentration of the detergent, indicating that egg lecithin might be solubilized into Triton X-100 micelles above this concentration. Dimyristoyl phosphatidylcholine liposomes almost completely lost their light scatter on treatment with Triton X-100. The concentration of detergent needed to change light scatter was almost the same as that required for the permeability change. The changes in light scatter and permeability of dipalmitoyl phosphatidylcholine liposomes also occurred almost simultaneously when the liposomes were incubated with the detergent. Light scatter of dipalmitoyl phosphatidylcholine liposomes was not affected so dramatically as those of egg lecithin and dimyristoyl phosphatidylcholine liposomes, though it was definitely changed. The fact that decrease in light scatter reached a plateau at about 40 % may suggest that the size of mixed micelles of dipalmitoyl phosphatidylcholine and Triton X-100 is larger than that of dimyristoyl phosphatidylcholine or egg lecithin and Triton X-100. The change in light scatter of dipalmitoyl phosphatidylcholine and in dimyristoyl phosphatidylcholine liposomes was caused by Triton X-100 below its critical micellar concentration. It is possible to assume that the intense sensitivities of dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine liposomes to the detergent may be due to the ease of fragmentation by the detergent. Egg lecithin liposomes may resist the fragmentation effect of Triton X-100. Cholesterol incorporation may inhibit fragmentation induced by the detergent, since the change of light scatter decreased generally when liposomes were prepared with cholesterol.

Effect of sonication on the sensitivity of dipalmitoyl phosphatidylcholine liposomes to Triton X-100

Table I indicates the effect of sonication on the amount of trapped glucose,

TABLE I

EFFECT OF SONICATION OF DIPALMITOYL PHOSPHATIDYLCHOLINE LIPOSOME ON THE AMOUNT OF TRAPPED GLUCOSE, OSMOTIC FRAGILITY AND SENSITIVITY TO TRITON X-100

Liposomes were prepared from dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol in the ratios shown in the table by sonicating for various times. Liposomes thus prepared were incubated with 97 μg of Triton X-100 for 15 min at 21 °C or incubated in the same hypotonic solution as described in the legend for Fig. 4.

Liposomes	Time of sonication (min)	Trapped glucose (mol glucose/mol phosphorus)	Glucose release (%)	
			By Triton X-100	In hypotonic solution
1 : 0.1 : 1	0	5.52	7.9, 12.0	37.4
	1	0.57	19.0	–
	5	0.42	47.2	2.1
	30	0.31	64.5	–
	120	0.23	86.0	–
1 : 0.1 : 0.5	0	4.56	23.6, 30.1, 55.6	51.9
	1	0.55	35.4, 43.1, 57.0	5.2
	5	0.43	70.7	–
	30	0.21	100	–

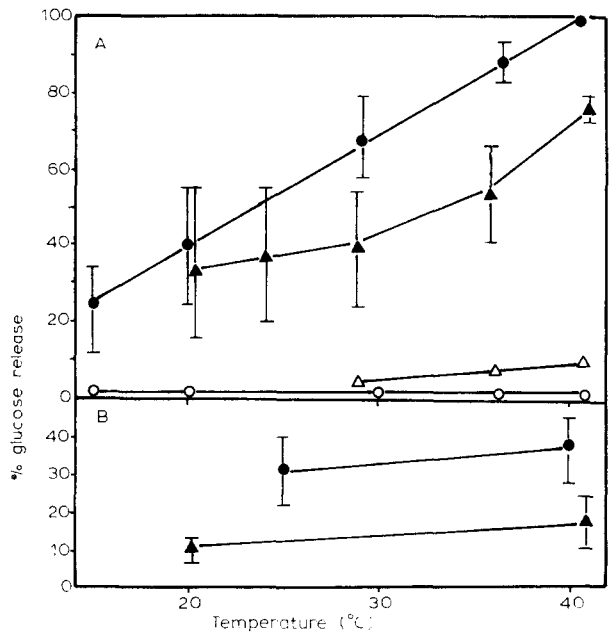

Fig. 8. Temperature dependence of the sensitivity of liposomes (dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol, 1 : 0.1 : 0.5) (A) and of liposomes (dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol, 1 : 0.1 : 1) (B), to Triton X-100 (200 μg/ml). Nonsonicated liposomes (▲) and liposomes prepared by sonication for 5 min (●) were incubated with 100 μg of Triton X-100 for 15 min at varying temperatures. Glucose release without any addition to nonsonicated liposomes (△) and to sonicated liposomes (○) are also plotted.

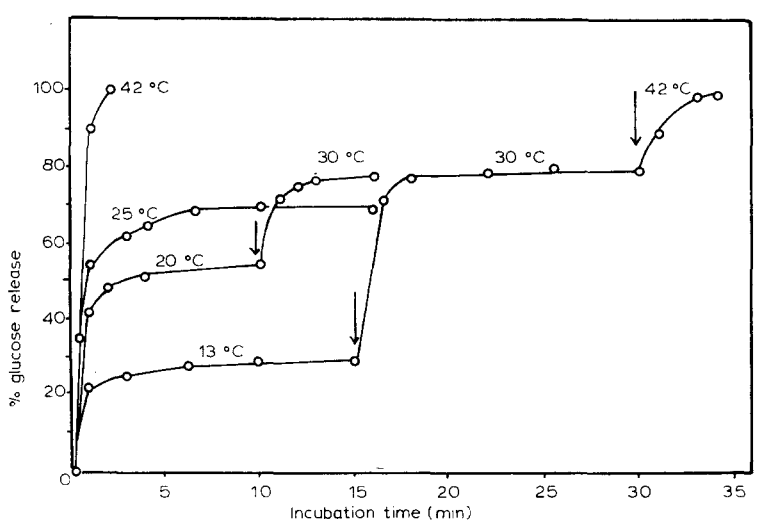

Fig. 9. Time course of glucose release from liposomes (dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol, 1 : 0.1 : 0.5) by Triton X-100 (100 μg) at various temperatures indicated in the figure. Liposomes were prepared by sonication for 1 min. Arrows indicate the time of the shift-up of temperature.

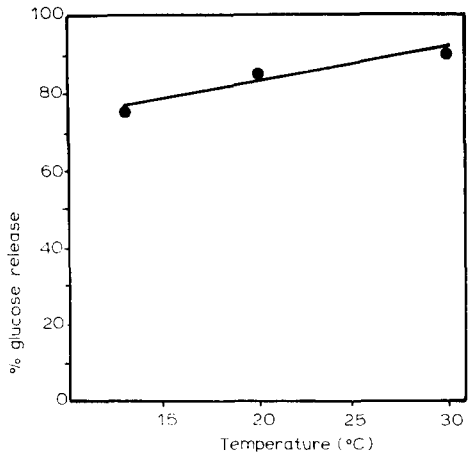

Fig. 10. Effect of incubation temperature on Triton X-100-induced damage of sonicated liposome (dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol, 1 : 0.1 : 1). Liposomes were incubated with Triton X-100 (100 μg) for 15 min at various temperatures.

osmotic fragility and sensitivity to Triton X-100 of dipalmitoyl phosphatidylcholine liposomes. With an increase of sonication time, liposomes trapped less glucose. In agreement with the observation by Alhanaty and Livne [10], sonicated liposomes were osmotically resistant. Sensitivity of liposomes to Triton was increased by sonication, in spite of considerable variations among preparations (especially in liposomes which were sonicated within 1 min). The reaction of Triton X-100 with liposomes of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 0.5) were quite temperature sensitive (Figs. 8 and 9). Unsonicated multilamellar liposomes released more glucose at 42 °C than at lower temperatures, when treated with Triton. Preparations obtained by sonication for 1 min were more sensitive to temperature. Even the preparation which showed 10 % glucose release at incubation in the presence of Triton at 13 °C released 100 % glucose when the incubation temperature was altered to 42 °C. The time course of the experiment is shown in Fig. 9. Multilamellar liposomes of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 1) were rather insensitive to temperature in the reaction with Triton X-100 (Fig. 8B).

The data shown in Fig. 9 indicate heterogeneous distribution of trapped glucose in the liposome preparations. In these preparations, 25 % (at 13 °C), 50 % (at 20 °C), 70 % (at 25 °C), 80 % (at 30 °C) and 100 % (at 42 °C) of trapped glucose were released by a given amount of Triton X-100 (200 μg/ml). This temperature dependence of liposomes was not shown in liposomes that were sonicated for more than 60 min (Fig. 10), whether the liposomes contained cholesterol or not.

Sensitivity of sonicated liposomes (single-compartment liposome) to Triton X-100

Fig. 11 shows that sonicated liposomes of dipalmitoyl phosphatidylcholine and dicetyl phosphate (1 : 0.1) were more sensitive to Triton than nonsonicated multilamellar liposomes composed of the same constituents. The ratio of Triton to phospholipid (egg lecithin) in the mixed micelles was reported to be about 2 : 1 [11].

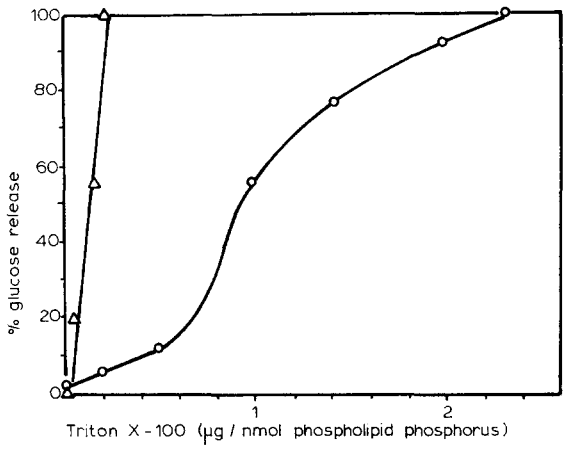

Fig. 11. Effect of sonication on the sensitivity of liposomes composed of dipalmitoyl phosphatidylcholine and dicetyl phosphate (1 : 0.1) to Triton X-100. Sonicated liposomes (△) and non sonicated liposomes (○) were incubated with various amounts of Triton X-100 at 21 °C.

The same ratio was observed in the mixed micelles of Triton and dipalmitoyl phosphatidylcholine. Assuming that the molecular weight of Triton X-100 is 628, the amount (about 2.2 μg) required to damage a certain amount of dipalmitoyl phosphatidylcholine liposomes (1 nmol of phospholipid phosphorus) is estimated to be 3.5 nmol. The ratio of Triton to phospholipid was, therefore, about 3.5. In the case of sonicated liposomes, on the other hand, the ratio was estimated to be 0.3.

Light scatter of sonicated liposomes was increased by Triton X-100 when the liposomes contained cholesterol. The increase in light scatter was much slower than glucose release from liposomes (Fig. 12).

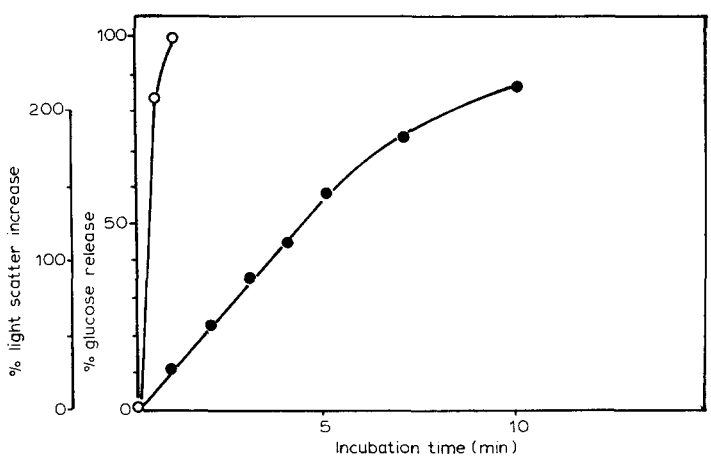

Fig. 12. Effect of Triton X-100 on the glucose permeability and on light scatter of liposomes. Sonicated liposomes prepared from dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 0.5) were incubated with Triton X-100 (100 μg) at 21 °C for various times. Glucose release (○) and the increase in light scatter of liposomes (●) were assayed.

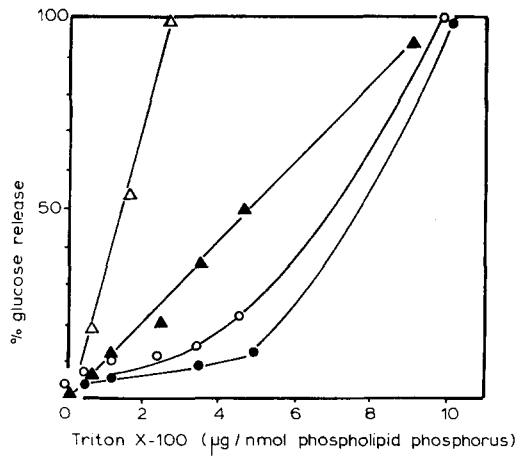

Fig. 13. Effect of Triton X-100 on sonicated liposomes prepared from various components. Liposomes composed of dipalmitoyl phosphatidylcholine and dicetyl phosphate (1 : 0.1) (△), of dipalmitoyl phosphatidylcholine, dicetyl phosphate and cholesterol (1 : 0.1 : 1) (▲), of egg lecithin and dicetyl phosphate (1 : 0.1) (○) and of egg lecithin, dicetyl phosphate and cholesterol (1 : 0.1 : 1) (●) were prepared by sonication for 30–120 min. Liposomes thus prepared were incubated with various amounts of Triton X-100 for 15 min at 20 °C.

TABLE II

EFFECT OF SONICATION OF EGG LECITHIN LIPOSOMES ON THE AMOUNT OF TRAPPED GLUCOSE, OSMOTIC FRAGILITY AND SENSITIVITY TO LYSOPHOSPHATIDYLCHOLINE

Liposomes were prepared from egg lecithin, dicetyl phosphate and cholesterol in the ratios shown in the table. Release of glucose from liposomes in the isotonic solution and in the hypotonic solution was assayed. Release of glucose from liposomes by cold shock was obtained by incubating liposomes for 10 min at 21 °C, followed by the incubation at 0 °C for 5 min. Liposomes were incubated in the presence of excess lysophosphatidylcholine (500 μg) at 21 °C for 10 min and then the incubation temperature was shifted down from 21 °C to 0 °C. The whole reaction mixture was further incubated for 5 min.

Liposomes	Time of sonication (min)	Trapped glucose (mol glucose/ mol phosphorus)	Glucose release %				
			Control (21 °C)	Release in hypotonic solution (21 °C)	Release by cold shock (21 → 0 °C)	Release in the presence of lyso-phosphatidyl-choline	
						21 °C	21 → 0 °C
1 : 0.1 : 1	0	3.76	3.6	26.1	5.1	4.4	29.5
	30	0.23	3.8	6.1	3.8	3.0	3.3
1 : 0.1	0	2.55	11.2	45.6	35.5	10.0	80.9
	30	0.17	2.4	4.6	7.3	2.0	7.9

Sensitivities of sonicated liposomes of various constituents to Triton were compared at 23 °C (Fig. 13). Dipalmitoyl phosphatidylcholine liposomes were more sensitive than egg lecithin liposomes, whether cholesterol was incorporated or not. In dipalmitoyl phosphatidylcholine liposomes, cholesterol depressed sensitivity to Triton X-100, while the sensitivity of egg lecithin liposomes was not significantly influenced by cholesterol incorporation.

Effect of sonication on the properties of egg lecithin liposomes

Sonicated liposomes of egg lecithin were less fragile osmotically than nonsonicated liposomes (Table II). Nonsonicated liposomes were sensitive to cold shock, releasing 35 % of the glucose, while sonicated liposomes were resistant to the same treatment, releasing a negligible amount of the marker. Lysophosphatidylcholine could not attack sonicated liposomes under the conditions that damaged multilamellar liposomes. The mode of action of lysophosphatidylcholine to lipid bilayers might be quite different from that of Triton X-100.

Effect of Triton X-100 on liposomes of erythrocyte lipids

Multilamellar liposomes prepared from lipids of sheep and rat erythrocytes were treated with Triton X-100 (Fig. 14). In agreement with the observation by Kinsky et al. [5], these liposomes were quite sensitive to the detergent, releasing 100 % of the glucose. These liposomes showed almost the same sensitivity to the detergent as egg lecithin liposomes. More pronounced effect of shift-down of temperature was observed in liposomes of lipids extracted from sheep erythrocyte membrane than in liposomes of rat erythrocyte lipids.

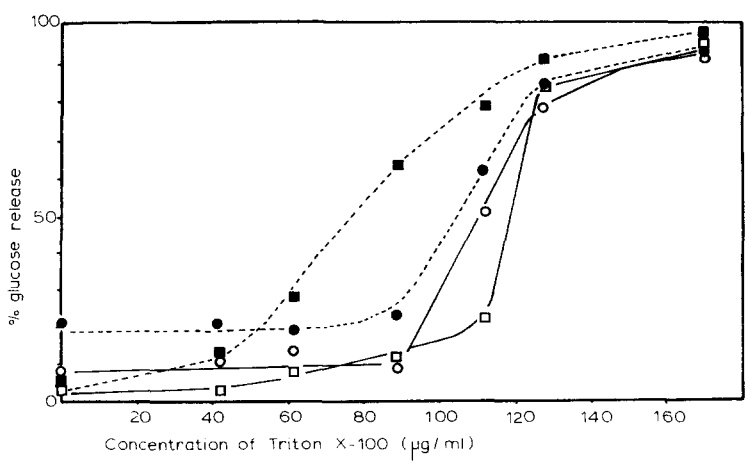

Fig. 14. Sensitivities of multilamellar liposomes derived from erythrocyte lipids to Triton X-100. Liposomes prepared from lipids of sheep erythrocyte membranes (□, ■) and rat erythrocytes (○, ●) were incubated with Triton X-100 at room temperature for 30 min (open symbols) and for another 10 min at 1 °C (closed symbols).

DISCUSSION

On the basis of the results obtained here, damage of lipid bilayers by Triton X-100 was influenced by the composition of the membrane and by the size of vesicle. Liposomes of rat liver phosphatidylcholine and egg lecithin, both of which have more than 40 % of unsaturated fatty acids, were resistant to the action of the detergent. In the case of multilamellar egg lecithin liposomes, small amount of Triton might be incorporated into the bilayers without any influence on the function of the membrane as a permeability barrier. Further incorporation of the detergent into the bilayers caused a drastic loss of function as a barrier. At that point, however, no morphological change could be observed on the liposomes under the electron microscope. Additional Triton, which formed micelles above its critical micellar concentration, decreased the light scatter of the multilamellar liposomes, indicating that the micelles of the detergent might incorporate lipid bilayers to form mixed micelles. In the case of dimyristoyl phosphatidylcholine liposomes, change in light scatter induced by Triton occurred simultaneously with the permeability change. The permeability change by Triton, therefore, may result from a drastic change of molecular organization of bilayers. Once penetrated by Triton, the bilayers of dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine, both of which are in the gel state under the present condition, might easily be fragmented releasing trapped glucose. Liposomes having unsaturated fatty acyl residues, which are liquid-crystalline, may resist the occurrence of fragmentation by Triton. As a result, sensitivities of unsaturated phosphatidylcholine liposomes to the detergent might be less than those of saturated phosphatidylcholines. The same tendency of the sensitivity of liposomes to Triton X-100 was also observed in sonicated liposomes.

In this connection, it is interesting to note that *E. coli* whose membrane has low contents of unsaturated fatty acid was reported to be much more sensitive to an ionic detergent, sodium dodecyl phosphate, than *E. coli* cells having more than 30 % of unsaturated fatty acid [12]. These differences in the sensitivity of living cells to a detergent may result from the fatty acid compositions of the cell membranes. Fatty acid compositions of cell membrane seem to be one of the most important factors determining the sensitivity of cells to detergent.

Introduction of cholesterol generally suppressed the sensitivities of liposomes to various lytic reagents such as lysophosphatidylcholine [7] and some basic proteins [13]. The suppressive effect of cholesterol on the sensitivity to the detergent was not so apparent in egg lecithin liposomes, while dramatic effect could be observed in multilamellar liposomes of dimyristoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine. The poor permeability change by limited amounts of the detergent in multilamellar dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine liposomes with 50 mol% of cholesterol may not be due to the failure of the Triton to penetrate into the bilayers, since sonicated liposomes were quite sensitive to the detergent. Bilayers of dimyristoyl phosphatidylcholine or dipalmitoyl phosphatidylcholine with more than 40 mol% of cholesterol may resist a radical change in the molecular organization, and then Triton X-100 may not give the effect on inner lamellae of liposomes.

A stimulating effect of the shift-down of the incubation temperature was observed on the sensitivity of multilamellar egg lecithin liposomes to Triton X-100.

The shift-down of the incubation temperature from room temperature to 1 °C decreased the concentration of the detergent required to damage the liposomes. The effect was, however, rather small when compared with that observed in lysophosphatidylcholine-induced liposomal damage [7]. In the latter, the damage could not be observed even at the highest concentration of lysophosphatidylcholine, unless the temperature was shifted down. In the case of Triton X-100, the shift-down of temperature was not absolutely necessary to damage the liposomes. Sonicated liposomes were insensitive to lysophosphatidylcholine even when temperature was shifted down. For the lipid bilayers to be attacked by lysophosphatidylcholine, the existence of some kind of defect may be required in the membrane. The fact that nonsonicated liposomes are sensitive to lysophosphatidylcholine as well as to cold shock, osmotic shock and temperature [14] may suggest that there are some defects on these multilamellar liposomal membranes.

The amounts of Triton X-100 required were much larger than the amounts of lysophosphatidylcholine required, i.e. 105 μg/ml of Triton X-100 and 10 μg/ml of lysophosphatidylcholine were required for the half-maximal release of marker glucose from multilamellar liposomes (egg lecithin/dicetyl phosphate/cholesterol, 1 : 0.1 : 0.5). The critical micellar concentrations of Triton X-100 and lysophosphatidylcholine were measured to be 200 μg/ml and 5 μg/ml, respectively. Triton X-100 apparently interacts with lipid bilayers as a form of monomer, while lysophosphatidylcholine may interact with the bilayers as a form of micelle. These may be responsible for the difference observed between Triton X-100 and lysophosphatidylcholine.

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