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THE INVOLVEMENT OF THE LIPID PHASE TRANSITION IN THE PLASMA-INDUCED DISSOLUTION OF MULTILAMELLAR PHOSPHATIDYLCHOLINE VESICLES

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Summary

Unsonicated liposomes prepared from dimyristoyl phosphatidylcholine were nearly completely dissolved during a 3 h incubation with rat plasma at or close to the phase-transition temperature of 24°C. At 37 or 15°C virtually no liposomal disintegration was observed even after 24 h of incubation. The liposomal solubilization, which was monitored by turbidity measurements or by determination of phospholipid sedimentability, was accompanied by the formation of a phospholipid-protein complex similar or identical to the one we previously reported to be formed from sonicated liposomes of egg phosphatidylcholine (Scherphof, G., Roerdink, F., Waite, M. and Parks, J. (1978) Biochim. Biophys. Acta 542, 296—307). Unsonicated multilamellar liposomes made of egg phosphatidylcholine were completely resistant to the dissolving potency of plasma when incubated at 37°C. Liposomes from equimolar mixtures of dimyristoyl and dipalmitoyl phosphatidylcholine were only degraded by plasma in the temperature range between 30 and 35°C at which temperature this cocrystallizing phospholipid mixture undergoes a phase transition. However, even at these temperatures the rate of dissolution of this mixture was significantly lower than of dimyristoyl phosphatidylcholine at 24°C. In the dissolving process of this mixture a slight preference for the lower-melting component was observed.

The ability of cholesterol to completely abolish the susceptibility of dimyristoyl phosphatidylcholine liposomes to plasma at a 1 : 2 molar ratio of cholesterol to phospholipid substantiates the essential role of the phase transition in the process of liposome solubilization.

When liposomes of the monotectic mixtures dimyristoyl and distearoyl phosphatidylcholine or dilauroyl and distearoyl phosphatidylcholine were incubated with plasma at temperatures in between those at which the constituent

lipids undergo a phase change in the mixture, the liposomes were slowly dissolved. Under those conditions a selective removal of the lipids in the liquid-crystalline phase was observed.

It is concluded that for the plasma-induced dissolution of unsonicated liposomes, which is most probably achieved by interaction with (apo)lipoproteins, the presence of phase boundaries is required in much the same way as was first reported for phospholipases by Op den Kamp, J.A.F., de Gier, J. and Van Deenen, L.L.M. (1974) Biochim. Biophys. Acta 345, 253—256).

Introduction

In previous papers we reported on the plasma-induced release of liposome-entrapped solutes [1] and, more recently, on the involvement of high density lipoproteins in the mechanism of the concurrent liposomal destruction [2]. The transfer of egg phosphatidylcholine we observed from liposomes to a lipoprotein particle later turned out to be much less pronounced when (small-size) multilamellar rather than unilamellar vesicles were used (Scherphof, G. and Damen, J., unpublished results). By contrast, Tall et al. [3] recently reported the rapid and complete dissolution of unsonicated (large-size) multilamellar vesicles of dimyristoyl phosphatidylcholine at 27°C by purified high density lipoproteins. In an attempt to clarify this apparent discrepancy we undertook a comparative study of dimyristoyl and egg phosphatidylcholine vesicles with respect to their stability in plasma. Temperature rather than specificity of phospholipids or differences in experimental system turned out to be decisive in the stability of the liposomes. Observations by Gotto and coworkers demonstrated that dimyristoyl phosphatidylcholine vesicles can also be degraded by the very low density apolipoprotein C-III [4]. Initially, these workers suggested that the apoprotein would interact with the liposomes only if the lipids were in the fluid phase, above the phase-transition temperature [5]. More recently they demonstrated for another apolipoprotein, A-I from high density lipoprotein, that optimal dissolution takes place not above but within the phase transition [6]. This observation is in good agreement with our present findings. On the other hand, we demonstrated that the occurrence of a phase transition is not an absolute prerequisite for liposomal destruction to occur in these systems. In that respect our observations are consistent with those of Op den Kamp et al. [7,8] and our own [9,10], on phospholipase A_2 attack of phospholipid vesicles.

Materials and Methods

All phospholipids used were commercial preparations from Sigma or Calbiochem and were shown to be satisfactorily pure by thin-layer chromatography.

Liposomes were prepared in 150 mM NaCl/5 mM Tris-HCl, pH 7.4, by vortexing above the phase-transition temperature of the highest-melting lipid involved. Radiolabeling of phosphatidylcholines was achieved according to the method described by Stoffel [11], involving demethylation of the unlabeled lipid followed by remethylation with [^{14}C]methyliodide.

Lipids were extracted according to Bligh and Dyer [12]. Fatty acids were

analyzed as described previously [13]. Phospholipid phosphorus was assayed according to Chen et al. [14] after lipid destruction as described by Ames and Dubin [15].

Radioactivity was determined by liquid scintillation counting with Plasmasol as a scintillation mixture [16].

Plasma was obtained from heparinized blood of fasted female Wistar rats.

Results

The dissolution of turbid suspensions of phospholipids can conveniently be monitored by light-absorption measurements as demonstrated by Tall and Small [17]. The effect of rat plasma on the turbidity of a hand-dispersed preparation of dimyristoyl phosphatidylcholine liposomes at various temperatures is shown in Fig. 1A. At the phase-transition temperature of 24°C [18] a rapid decrease in turbidity is observed, leading to nearly complete transparency in approx. 3—4 h. At temperatures far above the phase transition (33°C; 37°C) the turbidity of the suspension decreases only slowly, the total decrease after 22 h amounting to not more than 10—20%. At temperatures considerably below the phase transition (15°C) hardly any change in turbidity occurs even during 22 h of incubation. Closer to the phase transition (21°C, 27°C) appreciable dissolution of the liposomes is observed, at 27°C occasionally leading to nearly complete transparency after 22 h. Fig. 1B, presenting the initial rates of turbidity change at the various temperatures, demonstrates even more conspicuously the strongly preferred solubilization of liposomes at the phase transition. Consistent with these results is the complete lack of effect of plasma at 37°C on a suspension of hand-dispersed liposomes made of egg phosphatidylcholine (Fig. 1A) which has a transition temperature well below 0°C [19].

In Fig. 2 the reliability of turbidity change as a parameter of liposomal

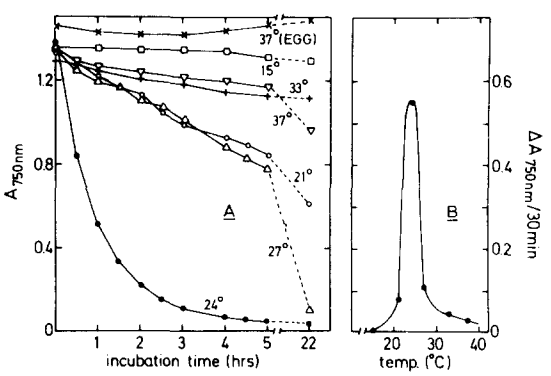
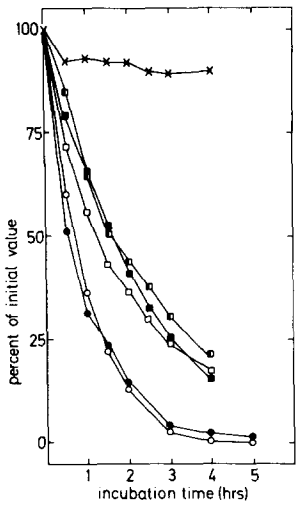


Fig. 1. Temperature-dependent solubilization of dimyristoyl phosphatidylcholine liposomes by plasma. 2.2 μmol dimyristoyl phosphatidylcholine liposomes in 1.5 ml NaCl/Tris-HCl were mixed with 1.5 ml rat plasma. Such mixtures were incubated at the temperatures indicated and the light absorbance at 750 nm was monitored. (A) Time course of A_{750nm} during 22 h. □, 15°C; ○, 21°C; ●, 24°C; △, 27°C; +, 33°C; ▽, 37°C; X, egg phosphatidylcholine in 50% plasma at 37°C (1.4 μmol/ml incubation mixture). (B) Initial rates of absorbance changes.



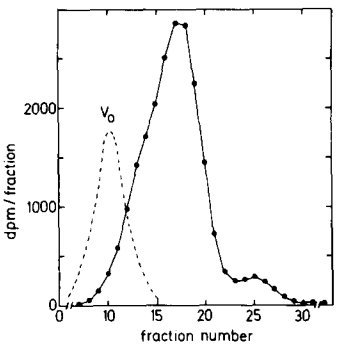


Fig. 2. Comparison of rates and parameters of dissolution in plasma of pure and mixed phospholipid liposomes. 7.5 μmol liposomes consisting of ^{14}C-labeled dimyristoyl phosphatidylcholine or of an equimolar mixture of ^{14}C-labeled dimyristoyl and unlabeled dipalmitoyl phosphatidylcholine in 5 ml NaCl/Tris-HCl were incubated at 24 and 33°C, respectively, with an equal volume of rat plasma. At the times indicated A_{750nm} was read and a sample of 0.5 ml was centrifuged (15 min, 10 000 × *g*). The pellets were assayed for phosphate and/or radioactivity. ○, A_{750nm}, dimyristoyl liposomes; ●, pellet radioactivity, dimyristoyl liposomes; ■, A_{750nm}, mixed liposomes; □, pellet radioactivity, mixed liposomes; ◧, pellet phosphorus, mixed liposomes; X, specific radioactivity pellet, mixed liposomes.

Fig. 3. Formation of a lipoprotein complex during incubation of dimyristoyl phosphatidylcholine liposomes with rat plasma. 5 μmol lipid in 0.5 ml NaCl/Tris-HCl containing $3.8 \cdot 10^4$ dpm were incubated at 24°C with 1.5 ml plasma. After 3.5 h the mixture was centrifuged for 15 min at 10 000 × *g* and 1.5 ml of the supernatant, which contained 77% of the radioactivity, was chromatographed on Ultrogel AcA34 with NaCl/Tris as eluting buffer. Fractions of 3.0 ml were assayed for radioactivity. - - - - - -, where liposomes elute from the column (V_0).

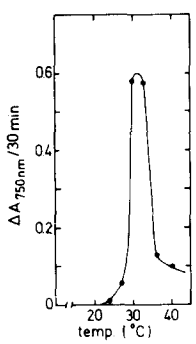
breakdown is demonstrated. Suspensions of liposomes prepared from either radioactive dimyristoyl phosphatidylcholine alone or from an equimolar mixture of this lipid with the dipalmitoyl analogue were incubated with plasma at 24 or at 33°C, respectively. At various time intervals light absorbance was measured while at the same time a sample was centrifuged to sediment remaining liposomes. The percentages of total radioactivity or of phosphorus which were recovered in the pellet (liposomes) closely followed the absorbance values. The consistent discrepancy between phosphorus and radioactivity measurements for the mixture, as expressed by the drop in specific radioactivity, points to a slight preference for the dimyristoyl species to be taken into solution (cf. the results presented in Table I).

Fig. 3 demonstrates the formation during plasma-induced dissolution of radioactive dimyristoyl phosphatidylcholine liposomes, of a radioactive particle which, according to its elution behavior on Ultrogel, is similar to the particle we previously demonstrated to arise from egg phosphatidylcholine and high density lipoproteins [2]. Also with this saturated phosphatidylcholine some radioactivity was found associated with the albumin peak, confirming the capacity of this protein to bind phosphatidylcholine as we reported before [1].

The involvement of the phase transition in the process of liposome breakdown is emphasized by the experiment described in Fig. 4. An equimolar mixture of dimyristoyl and dipalmitoyl phosphatidylcholine was dispersed to form a liposome preparation which was incubated with plasma at various temperatures between the transition temperatures of the pure components, 24 and 42°C, respectively [18]. As was also shown in Fig. 2, rapid dissolution of these liposomes occurred at 30—33°C. At 24 and 42°C, however, little or no change in turbidity was observed at all, indicating very limited complex formation at these temperatures. It is generally accepted that dimyristoyl and dipalmitoyl phosphatidylcholine form a cocrystallizing mixture, i.e. when mixed, they undergo one main phase transition, at approx. 33°C, involving both species simultaneously [20]. Thus, also with this mixture it is obvious that plasma-induced dissolution of the liposomes occurs only at temperatures at which the phospholipid is in the process of undergoing the transition.

The involvement of the phase transition in plasma-induced liposomal disintegration is substantiated by our observation that cholesterol, when incorporated into the bilayers of dimyristoyl phosphatidylcholine liposomes, drastically decreases the rate of plasma-induced solubilization of the liposomes (Fig. 5). A molar ratio of cholesterol to phospholipid of 1 : 4 reduced the initial rate of dissolution by approx. 75%, while at a 1 : 2 ratio the liposomes even remained completely stable in presence of plasma as indicated by the total lack of turbidity change over a period of several hours.

In view of the findings by Op den Kamp et al. [8] on the susceptibility of phospholipids to phospholipase A_2 under conditions of phase separation we thought it of interest to investigate also the interaction of (apo)lipoproteins in



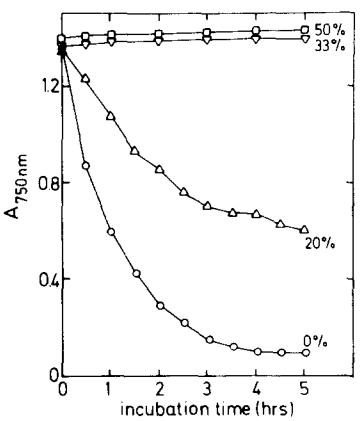


Fig. 4. Temperature-dependent solubilization of liposomes consisting of an equimolar mixture of dimyristoyl and dipalmitoyl phosphatidylcholine. 2.2 μmol of an equimolar mixture of the two phospholipids in 1.5 ml NaCl/Tris-HCl were incubated with 1.5 ml rat plasma at 24, 27, 30, 33, 36 or 40°C. The decrease in A_{750nm} during the first 30 min of incubation was plotted vs. the temperature of incubation.

Fig. 5. Effect of cholesterol on plasma-induced solubilization of dimyristoyl phosphatidylcholine liposomes. Liposomes were prepared in NaCl/Tris-HCl from dimyristoyl phosphatidylcholine containing 0, 20, 33 or 50 mol% cholesterol. 1.9 μmol total lipid in 1.3 ml was incubated at 24°C with 1.3 ml rat plasma and A_{750nm} was monitored during the incubation.

plasma with hand-dispersed liposomes of equimolar, binary monotectic mixtures of phosphatidylcholines. In such mixtures the two components undergo separate phase transitions. The lower-melting at approximately the same temperature as the pure compound, the higher-melting at a lower temperature than the pure compound [8,21,22]. This implies that in such mixtures at intermediate temperatures a fluid and a solid phase are present, the former consisting of pure low-melting phospholipid and the latter mainly of high-melting phospholipid. The question, then, to be answered was: is the coexistence of such domains of different fluidity in the liposomal surface as favorable a condition for plasma lipoprotein attack as the coexistence of two phases during a phase transition such as in Fig. 4? And if so, is there a preference for any one of the two constituent phospholipids in the formation of the lipoprotein complex? Op den Kamp et al. [8] had found that under those conditions the phospholipase A_2 shows a clear preference for the lower-melting component whereas Gotto and coworkers, although initially claiming that apolipoprotein C-III interacts with fluid lipid domains only, more recently arrived at the conclusion that apoprotein is unable to react selectively with the lower-melting component [5].

Fig. 6 shows the effect of plasma on the turbidity of a liposome preparation consisting of dimyristoyl and distearoyl phosphatidylcholine. The rate as well as the extent of turbidity decrease is considerably lower than for pure dimyristoyl phosphatidylcholine or for the dimyristoyl/dipalmitoyl mixture (cf. Fig. 2). Moreover, there is a considerable discrepancy between turbidity change and actual solubilization of the vesicles. After 12 h of incubation turbidity is at 82% of its initial value whereas only 63% of liposomal phosphorus can be sedimented by that time. An even much larger discrepancy is found when we compare turbidity or phosphate assay with radioactivity measurements. The radioactive dimyristoyl phospholipid apparently is solubilized

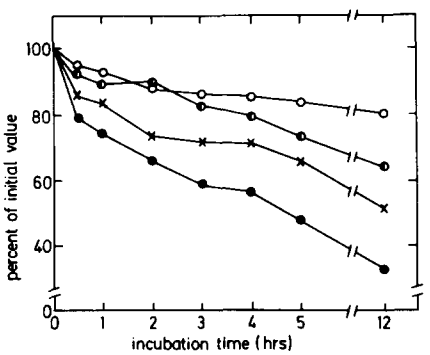


Fig. 6. Solubilization by plasma of dimyristoyl/distearoyl phosphatidylcholine liposomes. Liposomes were prepared in NaCl/Tris-HCl from an equimolar mixture of ^{14}C-labeled dimyristoyl phosphatidylcholine and unlabeled distearoyl phosphatidylcholine. 5 ml of this preparation, containing 7.5 μmol of phospholipid and $2 \cdot 10^5$ dpm ^{14}C radioactivity, were incubated with 5 ml rat plasma at 37°C. At the times indicated A_{750nm} was read and 1.0 ml of the incubation mixture was centrifuged (15 min, 10 000 × *g*). The pellet was washed once with NaCl/Tris buffer and then extracted. The extract was assayed for phosphorus and radioactivity. The supernatant was assayed for radioactivity only. The results are presented as percentage of the values measured at the start of the incubation. ○, A_{750nm}; ●, pellet radioactivity; ◐, pellet phosphorus; X, specific radioactivity of pellet.

TABLE I

MOLECULAR SPECIES ANALYSIS OF PHOSPHOLIPIDS SOLUBILIZED BY PLASMA FROM VARIOUS BINARY PHOSPHATIDYLCHOLINE MIXTURES

Liposomes of indicated compositions were prepared at a concentration of 5 μmol/ml NaCl/Tris-HCl, pH 7.4. 2.5 ml of each preparation was diluted with 2.5 ml freshly isolated rat plasma and incubated for the time and at the temperature indicated. At the end of the incubation the liposomes were sedimented (25 min, 10 000 × *g*). Pellet and supernatant were extracted with chloroform/methanol [12] and the extracts were chromatographed on thin layers of silicagel HF (Merck) with chloroform/methanol/water (65 : 35 : 2) as a solvent. The phosphatidylcholine spot was removed and transesterified for gas chromatographic analysis [13]. A sample of plasma was treated similarly to obtain the fatty acid composition of plasma phosphatidylcholine. The supernatant figures were corrected for the contribution by plasma. The incubation mixtures were also assayed for turbidity (A_{750nm}) and of mixtures A–C the pellets were assayed for phospholipid phosphorus. Mixtures D and E contained ^{14}C-labeled dimyristoyl phosphatidylcholine and pellets as well as supernatants of these incubation mixtures were assayed for radioactivity. 12 : 0, 14 : 0, 16 : 0, and 18 : 0 stand for dilauroyl, dimyristoyl, dipalmitoyl and distearoyl phosphatidylcholine, respectively. n.d., not determined.

Mixture	Molecular species	Molar ratio species	Incubation		$-\Delta A_{750nm}$ (%)	Lipid released (%)		Molar ratio species in	
			Temp. (°C)	Time (min)		P	^{14}C	Supernatant	Pellet
A	12 : 0/18 : 0	47.5/52.5	24	360	22	44	—	97.0/ 3.0	16.2/83.8
B	14 : 0/18 : 0	51.1/48.9	38	300	21	30	—	62.4/37.6	41.8/58.2
C	14 : 0/16 : 0	48.7/51.3	33	135	56	63	—	47.7/52.3	n.d.
D	14 : 0/16 : 0	55.7/44.3	30	135	48	—	51	55.0/45.0	49.9/50.1
E	14 : 0/16 : 0	64.5/35.5	30	210	51	—	57	63.6/36.4	61.9/38.1

much faster than the distearoyl species. This is clearly reflected in the gradual drop in specific radioactivity of the liposomal pellet during the incubation. Obviously, the lower-melting component is preferentially transformed to the lipoprotein particle. As we had no labeled dilauroyl or distearoyl phosphatidylcholine available a similar experiment as in Fig. 6 could not be done with a mixture of these lipids. Instead, we made use of fatty acid analyses to obtain information on this mixture. Simultaneously, we applied this experimental approach to the same mixtures as were used in Figs. 2 and 6 in order to check the results described there. The results of these experiments are summarized in Table I. The two mixtures showing monotectic behavior, dilauroyl/distearoyl phosphatidylcholine and dimyristoyl/distearoyl phosphatidylcholine, are designated A and B, respectively. The cocrystallizing mixture dimyristoyl/dipalmitoyl phosphatidylcholine is designated mixture C. In mixture A the transition temperatures of the two components are approx. 0°C and approx. 40°C, respectively [8,21]. This mixture was incubated at 24°C. For mixture B the transitions of the individual components reportedly occur at approx. 22°C and approx. 47°C, respectively [21,22]. This mixture, in our experiments, was incubated at 37°C. The cocrystallizing mixture C was incubated at 33°C. After 135 min of incubation with plasma this mixture showed a decrease in turbidity of approx. 50%, corresponding fairly well with the proportion of liposomal phospholipid which, by that time, had been solubilized (compare also Fig. 2). For mixture A it took 6 h to obtain an only 20% decrease in turbidity. Yet, at that time nearly half of the liposomal phospholipid was recovered in the supernatant. Upon examination of the fatty acid compositions of solubilized phospholipid (supernatant) and remaining liposomes (pellet) it appeared that nearly all of the solubilized phospholipid consisted of dilauroyl phosphatidylcholine, whereas this species made up only 16% of the phospholipid in the non-solubilized liposomes. For mixture B a similar tendency was observed, although less pronounced. Also with this mixture the phospholipid-dissolving activity of plasma displayed a preference for the component with the lower phase-transition temperature thus confirming the results described in Fig. 6. Mixture C on the other hand, showed only very little if any preferential solubilization of the dimyristoyl species. Although the ratio of the two lipid species in the pellet was slightly lowered as compared to the ratio in the starting material, this decrease was not compensated for by the ratio in the supernatant, which remained unchanged as compared to the starting material. This might be due to the inaccuracy introduced in the supernatant figures by the correction that had to be made for the presence of endogenous plasma phosphatidylcholine. A similar tendency, i.e: a slightly preferential removal of the dimyristoyl species from the sedimented liposomes not being reflected in the composition of the supernatant, was found when two other dimyristoyl/dipalmitoyl mixtures (D and E) were incubated at 30°C. These results are consistent with those presented in Fig. 2 where we found a slightly higher rate of release of the dimyristoyl phosphatidylcholine than of total phospholipid phosphorus.

Discussion

The observations described in this paper emphasize that the interaction of proteins with phospholipid can be strongly influenced by the existence of

phase boundaries. It has been known for several years that the permeability of liposomes towards various solutes drastically increases at the phase transition of the lipids [23—26]. Interactions of lipolytic enzymes with their substrates were also greatly enhanced when the substrate was in the process of undergoing a phase transition [8—11,27]. A similar observation was recently reported on the formation of lipoprotein complexes from multilamellar liposomes and purified apolipoprotein A-I [6]. Formerly it was thought that proteins preferentially interact with lipids which are in the liquid-crystalline phase. This led a number of investigators of lipid-protein interactions to do their experiments above the phase-transition temperature of the lipid involved, i.e. under suboptimal conditions as we know now [4,5,28—30]. On the other hand, it should be recognized that the requirement for phase-transition conditions does not necessarily apply for all lipid-protein interactions. For some proteins or peptides preferential interaction with the lipid in the gel phase has been reported [31,32].

Our experiments have been done with non-sonicated liposomes. Previously, we reported on the dissolution of sonicated egg phosphatidylcholine vesicles at 37°C, i.e. far above the phase-transition temperature of this lipid [2]. It is conceivable that the strong phase-transition dependence we describe in the present paper is most pronounced for relatively large liposomes, in much the same way as we found for the action of phospholipase A_2 [10]. Among the structural irregularities favoring the action of this enzyme we found the radius of curvature of the substrate vesicle very important. Morrisett et al. [33] recently reported that the reactivity of single-bilayer vesicles of egg phosphatidylcholine was considerably greater than that of multi-bilayer vesicles at the incubation temperature of 25°C.

The experiments showing the effect of cholesterol on the solubilizing potency of plasma add to an extensive list of observations on the stabilizing effect of cholesterol on phospholipid membranes [29,30,34—37] and provide only circumstantial evidence of the involvement of the phase transition in the process of solubilization. It has been shown on several occasions that the incorporation of cholesterol in phospholipid vesicles diminishes their susceptibility to protein interaction irrespective of the requirement for coexisting fluid and solid domains [29,30,38]. When a protein requires the phospholipid to be in the fluid, liquid-crystalline phase cholesterol will mainly act to condense the lipid surface so as to prevent interaction. In the case of plasma-induced degradation as well as in phospholipase-catalyzed breakdown of liposomes cholesterol probably acts by withdrawing phospholipid molecules from the process of phase transition, as is clearly demonstrated by differential scanning calorimetry, ultimately abolishing the transition altogether at a 1 : 2 ratio of cholesterol to phospholipid [8,39].

The susceptibility of monotectic mixtures to the dissolving potency of plasma, although to a lesser extent than pure lipid or a cocrystallizing mixture, shows that for dissolution to occur it is not necessary to have coexisting phases under equilibrium conditions. In the dilauroyl/distearoyl mixture at 24°C, for instance, there will be little if any exchange of lipid between liquid-crystalline and gel phase. We presume that the attacking protein(s) get access to the lipid surface at the boundaries between the two phases. At those sites irregularities will exist in the liposomal surface facilitating the access of the protein(s). Once

having gained access the protein(s) apparently pull(s) out molecules from the liquid-crystalline phase only. Table I shows that with this mixture only the dilauroyl phospholipid is solubilized while the remaining liposomes representing the gel phase contain only 16% of this species. The relatively low transition temperature of the distearoyl lipid in this mixture, approx. 45 vs. 56°C for the pure compound [21,22,39], is possibly accounted for by freezing point depression caused by this 16% 'contamination' with dilauroyl phosphatidylcholine.

From the phase diagram of the dimyristoyl/distearoyl mixture [20] it follows that at the incubation temperature some 80% of the gel phase consists of the distearoyl lipid and that the liquid-crystalline phase contains approx. 25% of this species. Based upon these figures it can be calculated that, at 38°C, of an equimolar mixture of these lipids 54.5% is in the liquid-crystalline phase. Our experiments show that after 5 h of incubation in plasma such liposomes have released approx. 25% of their phospholipid (Fig. 6). Presuming that only the liquid-crystalline phase is susceptible to solubilization it can be calculated that the liposomes remaining after 5 h should have a fatty acid composition of 40.6% myristic and 59.4% stearic acid. This fits remarkably well with the experimental figures of 41.8 and 58.2%, suggesting that our presumption that only the liquid-crystalline lipid is solubilized was right. The fatty acid analysis of the supernatant is not entirely consistent with this view as only some 62% myristic acid was found whereas the liquid-crystalline phase should contain approx. 73% of this acid. This discrepancy can possibly be ascribed to experimental error. As mentioned before we consistently find low values for myristic acid in the supernatant fractions (mixtures C—E), probably as a result of the corrections that have to be made for plasma phospholipid.

Little preference for solubilization of the lower-melting compound is observed with the dimyristoyl/dipalmitoyl mixture. Various considerations could contribute to an explanation of this phenomenon. First of all, these two phospholipids are nearly completely miscible in the gel phase which causes the compositions of the gel and liquid-crystalline phases in equilibrium to be not extremely different, as follows from the phase diagram [21]. Obviously, this would make it experimentally more difficult than for the other mixtures to pick up differences in composition between supernatant, remaining liposomes and starting material. Secondly, in this cocrystallizing mixture rapid exchange of both lipid species between the two phases can occur, which might prevent the attacking protein(s) to discriminate accurately between the two phases. The features of the dimyristoyl/dipalmitoyl mixture are probably also reflected in the observation that eventually always complete dissolution of such liposomes is obtained, similar to what we see with pure dimyristoyl phosphatidylcholine. The monotectic mixtures, at intermediate temperatures, never give rise to more than 50% solubilization even after prolonged incubation.

The diversity in rates at which the various types of liposomes used in this study are solubilized is possibly also related to the rate of exchange of individual molecules between the gel and the liquid-crystalline phase. When there is rapid exchange the boundaries of the phase clusters conceivably are subject to rapid changes in form and size. If we assume that the attacking protein(s) enter the lipid surface at sites where the coexistence of two phases causes a structural

irregularity it follows that the dissolving rate decreases with the rate at which fluctuations occur in the cluster boundaries. Moreover, in the monotectic mixtures the total length of phase boundary may be considerably smaller than for cocrystallizing or pure lipid due to larger cluster size and a concomitant smaller number of clusters, i.e. low cluster density in the terminology of Freire and Biltonen [40]. Apparently, increased lateral compressibility, as was demonstrated to occur at the phase transition by Phillips et al. [41], is not an absolute prerequisite for penetration of protein and subsequent solubilization of the lipid. However, enhanced lateral compressibility may contribute to the high rates of dissolution observed with dimyristoyl liposomes at 24°C and with dimyristoyl/dipalmitoyl liposomes at 33°C.

The discrepancy between turbidity measurements and actual solubilization of the dimyristoyl/distearoyl liposomes (Fig. 6) may be the result of rearrangement of non-dissolved (gel-phase) phospholipid to form new vesicles, with different light-scattering characteristics. Obviously, when the liquid-crystalline lipids in the outer layer of a liposome have been taken into solution, the amount of remaining gel-phase lipid will be insufficient to cover the original liposome surface, and membranous fragments thus formed will probably recombine to form a new population of vesicles. The lack of agreement between turbidity change and phospholipid solubilization was also observed with the dilauroyl/distearoyl mixture.

Interesting practical implications of the phenomena described in this paper are suggested in a recent paper by Yatvin et al. [42]. Abruptly increased plasma-induced permeability of liposomes towards an entrapped drug at the phase transition would allow specific release of the drug at body areas with (artificially) elevated temperature, such as tumors heated to a few degrees above body temperature by microwave irradiation.

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