Effect of Cholesterol on Ca^{2+}-Induced Aggregation of Liposomes and Calcium Diphosphatidate Membrane Traversal†

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ABSTRACT: Sonicated cholesterol–phosphatidylcholine (PC) liposomes containing 4 mol % phosphatidic acid (PA) aggregate in 10 mM Ca^{2+}, slowly at low molar fractions of cholesterol (up to 30%) and 15 times faster at higher concentrations; the inflection point is at ca. 35 mol % bilayer cholesterol. *O*-[[(Methoxyethoxy)ethoxy]ethyl]cholesterol (OH-blocked cholesterol) does not give this rate enhancement. If PC is replaced by diether PC (CO groups abolished), cholesterol does not accelerate aggregation at concentrations in the bilayer below 50 mol %. No change in Ca^{2+}-induced aggregation rates was observed if the ester CO groups of the bridge-forming PA only were replaced by CH_2 (diether PA) in liposomes containing PC and cholesterol. PA-mediated Ca^{2+} membrane traversal seems to be accelerated by the addition of cholesterol to the PC–PA membrane, but analysis shows that the effect is due to the bilayer condensation effect of cholesterol resulting in an increase in the surface concentration of PA and that membrane cholesterol in fact slightly reduces the rate of $Ca(PA)_2$ traversal; OH-blocked cholesterol, however, increases this rate 3-fold. It appears that lipid OH and CO groups interact, directly or with the mediation of water, in establishing the structure of the membrane "hydrogen belts", i.e., the strata containing those hydrogen-bond donors and acceptors. Cholesterol hydroxyl above 33 mol % (saturation of a 2:1 PC/cholesterol complex?) causes a restructuring of the hydrogen belts that facilitates membrane–water–membrane dehydration, the prerequisite for liposome aggregation by *trans*-$Ca(PA)_2$ formation. On the other hand, the formation of the dehydrated *cis*-$Ca(PA)_2$ complex that precedes Ca^{2+} membrane traversal is not accelerated by presence of the cholesterol hydroxyl group.

Aggregation and fusion of membranes mediate many biological events such as secretion, endocytosis, exocytosis, fertilization, muscle development, and intracellular transport (Poste & Nicolson, 1978). Membrane fusion is also involved in the infectious entry of enveloped viruses into cells (White et al., 1983). Calcium ion and its interaction with acidic phospholipids (phosphatidate, phosphatidylserine, or phosphatidylglycerol) have been implicated in a critical regulatory role in these membrane fusion phenomena (Poste & Allison, 1973; Douglas, 1975; Papahadjopoulos et al., 1979; Düzgünes et al., 1980). The fusion of unilamellar liposomes among themselves (Nir et al., 1983; Bentz et al., 1983a,b) or to planar bilayers (Düzgünes & Ohki, 1981; Cohen et al., 1982) involves an aggregation step followed by the fusion event. Although aggregation and fusion can be considered as independent phenomena, aggregation is a necessary prerequisite for membrane fusion, and it is achieved, in all likelihood, by the formation of "*trans*-" (i.e., intervesicle) $Ca(PL)_2$[1] bridges followed by dehydration of the membrane–water–membrane interfacial system (Papahadjopoulos et al., 1978; Portis et al., 1979).

In contrast to vesicle aggregation, traversal of calcium through a bilayer requires the formation of a "*cis*-" (i.e., intramembrane) $Ca(PL)_2$ complex, which must also be dehydrated to allow penetration of the lipid layer. Among the phospholipids, phosphatidic acid is known to be a calcium-specific ionophore (Serhan et al., 1981, 1982; Chauhan & Brockerhoff, 1984). In the diether analogue of phosphatidic acid, however, the ability to transport calcium through a bilayer is diminished by more than 90% (Chauhan & Brockerhoff, 1984). Kinetic data showed that two phosphatidic acid molecules bind one Ca^{2+} (Chauhan & Brockerhoff, 1984; Reusch, 1985). A molecular model shows that in this *cis*-$Ca(PA)_2$ complex both Ca^{2+} and the two phosphatidate head groups are dehydrated with calcium captive in a cage formed by the phosphate and carbonyl oxygens of the two lipid molecules.

The effect of cholesterol on phospholipid membranes has been extensively investigated (Demel & DeKruyff, 1976; Bloch, 1983). Structural parameters change slowly with increasing cholesterol content up to about 32 mol %, and a relatively abrupt structural alteration as revealed by hydrodynamic parameters occurs above this cholesterol content (Newman & Huang, 1975). Cholesterol interacts with phospholipids to decrease the area occupied per phospholipid molecule (Lecuyer & Dervichian, 1969); it also controls the fluidity of bilayers (Demel & DeKruyff, 1976); it induces membrane aggregation by nascent calcium phosphate (Ohki & Leonards, 1984), and it activates the low-pH-triggered membrane fusion activity of Semliki forest virus (Kielian & Helenius, 1984). In our study, we investigate both Ca-induced vesicle aggregation and phosphatidic acid mediated bilayer traversal by calcium ion and find that cholesterol has a large influence on aggregation [*trans*-$Ca(PA)_2$ formation] but little on calcium traversal [*cis*-$Ca(PA)_2$ formation].

Experimental Procedures

Chemicals. Egg phosphatidylcholine (PC) was isolated and purified by the method of Singleton et al. (1965). Diether phosphatidylcholine (diether PC), i.e., 1-hexadecanyl-2-ole-

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[1] Abbreviations: PA, phosphatidic acid or phosphatidate; PC, phosphatidylcholine; PL, phospholipid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Tris, tris(hydroxymethyl)aminomethane.

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yl-*sn*-glycerophosphocholine, was synthesized (Brockerhoff & Ayengar, 1979). Phosphatidic acid (PA) was prepared from egg PC (Davidson & Long, 1958). The PA analogue containing no CO groups, diether PA, was prepared from 1-palmityl-*sn*-glycerol (chimyl alcohol) as described previously (Chauhan & Brockerhoff, 1984). Cholesterol was recrystallized 3 times from ethanol. 3-*O*-[[(Methoxyethoxy)ethoxy]ethyl]cholesterol (OH-blocked cholesterol) was synthesized (Fong et al., 1977). All lipids were pure as judged by thin-layer chromatography. Arsenazo III dye was purchased from Sigma and purified (Weissmann et al., 1976).

Preparation of Liposomes. Chloroform–methanol (1:1 v/v) solutions containing 50 μmol of lipid were dried under vacuum at 70 °C for 1 h while flushed with N_2 every 10 min. The lipid film was dispersed by shaking in 2 mL of buffer containing 72.5 mM NaCl, 72.5 mM KCl, and 5 mM HEPES, adjusted to pH 7.45 with Tris for aggregation experiments, and in 2 mL of the same buffer containing 40 μmol of purified Arsenazo III for Ca^{2+} traversal experiments. For preparation of unilamellar liposomes, the dispersed lipids were sonicated under argon with a Branson tip sonicator to clearing. Metal particles from the sonicator tip and multilamellar liposomes were removed from the preparation by centrifugation at 100000*g* for 30 min. Liposomes with trapped dye to be used for Ca^{2+} transport experiments were isolated by Sepharose 4B column chromatography (Weissmann et al., 1976). There was no leakage of the dye from the liposomes as shown by the reproducibility of experiments the next day. Phospholipid phosphorus and cholesterol concentration in the liposomes was estimated by the methods of Marinetti (1962) and Zlatkis et al. (1953), respectively.

Assay of Vesicle Aggregation. Ca^{2+}-induced vesicle aggregation was monitored turbidimetrically; 30 μL of 0.68 M $CaCl_2$ was added to 2 mL of the stirred liposomal suspension in buffer containing 2 μmol of lipid in a cuvette to give a final Ca^{2+} concentration of 10 mM. The time-dependent turbidity change was recorded continuously at 400 nm with a Gilford spectrophotometer, and the initial slope of change in optical density (OD) per minute was measured.

Assay of Ca^{2+} Traversal. To a cuvette containing 3.0 μmol of liposomal lipid, with trapped dye, in buffer (total volume of 2.0 mL) was added 30 μL of 0.203 M $CaCl_2$, and the increase of OD at 650 nm was recorded continuously. At a $CaCl_2$ concentration of 10 mM, which had been used in a previous study (Chauhan & Brockerhoff, 1984), aggregation of those liposomes containing cholesterol was so fast as to interfere with the measurement of the dye intensity; therefore, the $CaCl_2$ concentration was reduced to 3 mM to minimize OD changes due to aggregation. The increase of OD by light scattering due to the coagulation of liposomes was then determined in control experiments under identical conditions except for the omission of the dye and subtracted. Ca–Arsenazo III complex formation was quantitated from the initial slope of OD with use of a molar extinction coefficient (Serhan et al., 1982) of 2.06×10^4 M^{-1} cm^{-1}.

Surface Factor. At equal molar percentage of PA, its amount in the outer liposome surface will vary between PC/PA liposomes and liposomes containing PC, PA, and cholesterol or OH-blocked cholesterol, for two reasons: (A) the condensation caused by cholesterol in the liposome bilayer will tend to increase the surface concentration of PA in PC/PA/cholesterol liposomes, and (B) the increase in volume, leading to a reduction in surface curvature in cholesterol-containing liposomes (Johnson 1973; Gent & Prestegard, 1974), will result in less PA being available on the outer surface (because there is less of that surface) in PC/PA/cholesterol liposomes as compared to PC/PA liposomes. As for (A), replacing 50 mol % PC with cholesterol leads to a shrinkage of the membrane by a factor of 1.8 (Johnson, 1973; Huang & Mason, 1978; Ramsammy & Brockerhoff, 1982); the bilayer concentration of PA is therefore increased by this factor, which then must be squared and reversed because Ca traversal is a direct second-order function of PA concentration (Chauhan & Brockerhoff, 1984). As for (B), the size of outside area over total surface is 72% for PC liposomes and 64% for PC/cholesterol liposomes (Ramsammy & Brockerhoff, 1982); the ratio therefore is 1.13. Consequently, the surface factor to be applied is $(1/1.8)^2 \times 1.13$, i.e., 0.35, for liposomes containing 50 mol % cholesterol or OH-blocked cholesterol. The values for 20 and 35 mol % were obtained by interpolation between 0 and 50 mol % cholesterol or OH-blocked cholesterol.

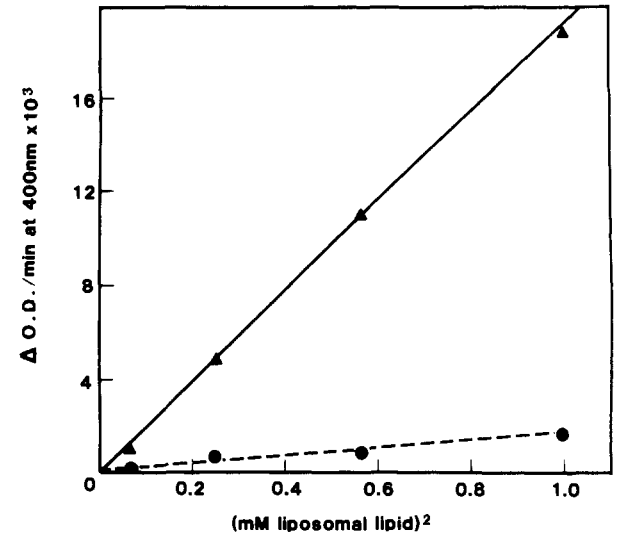

FIGURE 1: Effect of liposome concentration on Ca^{2+}-induced aggregation. Final $CaCl_2$ concentration was 10 mM. PC/PA/cholesterol (46:4:50) (▲); PC/PA (96:4) (●).

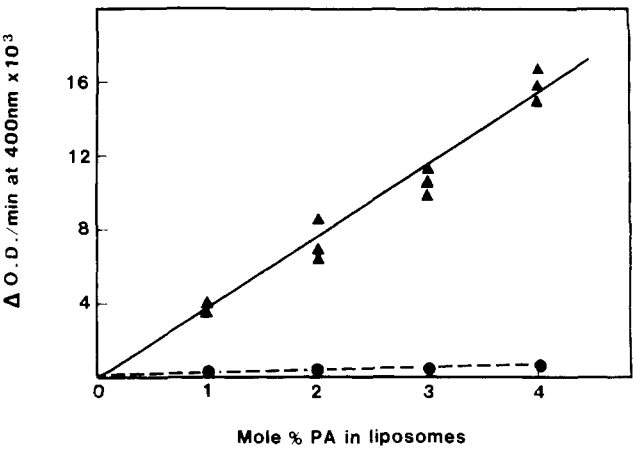

FIGURE 2: Effect of PA concentration in liposomes on rates of Ca^{2+}-induced aggregation. $CaCl_2$ added was 10 mM to liposomes containing 2.0 μmol of total lipid. Cholesterol when present in liposomes was at 50 mol % concentration. PC/PA/cholesterol liposomes (▲); PC/PA liposomes (●).

RESULTS

The experiments shown in Figures 1–3 establish the compatibility with previous studies, most of which have been carried out with phosphatidylserine, rather than PC/PA, vesicles.

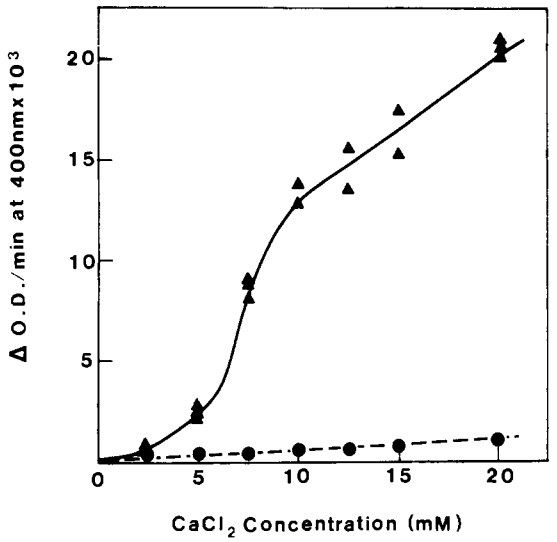

FIGURE 3: Effect of $CaCl_2$ concentration on aggregation of liposomes containing PC and PA with or without cholesterol. PC/PA/cholesterol (46:4:50) (▲); PC/PA (96/4) (●).

Table I: Effect of Cholesterol and OH-Blocked Cholesterol on $CaCl_2$-Induced Aggregation of Lipid Vesicles[a]

composition of liposomes	$CaCl_2$-induced aggregation (ΔOD/min × 10^3)
PC/PA (96:4)	1.08 ± 0.29
PC/PA/cholesterol (46:4:50)	15.07 ± 2.46
PC/PA/OH-blocked cholesterol (46:4:50)	1.00 ± 0.29
PC/PA/OH-blocked cholesterol (39:4:57)	0.92
PC/PA/OH-blocked cholesterol (31:4:65)	0.80

[a] Sonicated liposomes (2.0 μmol of lipid) composed of egg PC, PA, and cholesterol or OH-blocked cholesterol were suspended in buffer containing 72.5 mM NaCl, 72.5 mM KCl, and 5 mM HEPES, adjusted to pH 7.45 with Tris. Absorbance changes at 400 mM were recorded upon addition of 10 mM $CaCl_2$. Values are mean ± SD of three experiments.

Effect of Vesicle Concentration on Aggregation. Figure 1 shows that the initial rate of vesicle aggregation is proportional to the square of vesicle concentration. In the simplest interpretation, the initial rate of change in optical density of the liposome solution measures the rate of aggregation between two vesicles. This result has been obtained by others (Portis et al., 1979; Wilschut et al., 1981) with phosphatidylserine liposomes.

Effect of PA Concentration on Aggregation. The rate of aggregation increases in a linear fashion with PA concentration (Figure 2). Such a dependence might be expected if in the reaction liposome$^{(1)}$ → liposome$^{(1)}$–Ca^+ → liposome$^{(1)}$–Ca–liposome$^{(2)}$ the second step is rate-limiting and a straight function of the PA concentration on liposome$^{(2)}$; but, we do not wish to commit ourselves to that interpretation.

Ca^{2+} Dependence of Aggregation. The response to Ca^{2+} concentration is complex (Figure 3). The shape of the curve is very similar to that obtained for the Ca dependence of phosphatidylserine vesicle aggregation (Lansman & Haynes, 1975); previous discussions should be consulted (Lansman & Haynes, 1975; Portis et al., 1979).

Figure 4 and Tables I and II present experiments on the aggregation of acidic liposomes containing cholesterol.

Effect of Cholesterol and OH-Blocked Cholesterol on Ca^{2+}-Induced Aggregation. If, in PC/PA liposomes, 50 mol % cholesterol is incorporated (PA being kept constant at 4 mol %), the rate of Ca-induced aggregation increases 15-fold (Table I). [[(Methoxyethoxy)ethoxy]ethyl]cholesterol

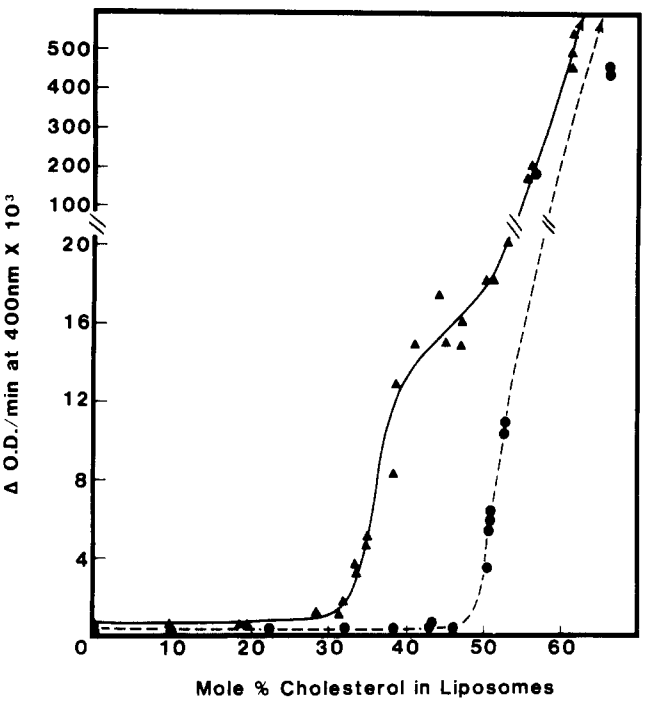

FIGURE 4: Effect of concentration of cholesterol on Ca^{2+}-induced aggregation in liposomes. Liposomes contained 4 mol % PA and PC/cholesterol (▲) or diether PC/cholesterol (●). Final [Ca^{2+}] was 10 mM, and absorbance changes were recorded at 400 nM.

Table II: Rates of $CaCl_2$-Induced Aggregation of Lipid Vesicles Containing Egg PC + 4 mol % Diester PA (Egg) or Egg PC + 4 mol % Diether PA with or without Cholesterol[a]

% cholesterol in liposomes	$CaCl_2$-induced aggregation of liposomes containing (ΔOD/min × 10^3)	
	diester PA	diether PA
	0.74	0.65
40	16.7	14.7
50	16.6	18.4

[a] Final Ca^{2+} concentration was 10 mM, and absorbance changes at 400 nm were recorded.

(blocked cholesterol) does not give this effect even at concentrations higher than 50 mol %. Figure 4 shows that cholesterol is without effect up to a concentration, in the bilayer, of ca. 30 mol %; then, aggregation rates rise with an inflection point at ca. 35 mol %. The cholesterol effect, as it is dependent on the free OH group of the sterol, so is also dependent on the CO groups of the phospholipid in the bilayer; if diether PC (which lacks these groups) replaces PC, the cholesterol effect does not occur (Figure 4). At cholesterol concentrations above 50 mol %, both PC and diether PC liposomes aggregate very rapidly, probably because the entire bilayer structure becomes destabilized at that point (Gershfeld, 1978). Table II, finally, shows that for aggregation to occur there is no need for CO groups on the PA molecule, as long as the matrix phospholipid, PC, possesses them. This is in contrast to the formation of the *cis*-$Ca(PA)_2$, where the phosphatidate CO groups are essential (Chauhan & Brockerhoff, 1984).

Effect of Cholesterol on $Ca(PA)_2$ Membrane Traversal. Measured rates (Figure 5) seem to show that Ca-traversal rates double for PC/PA liposomes in the presence of 50 mol % cholesterol. However, addition of cholesterol to a liposomal bilayer increases the actual surface concentration of a bilayer phospholipid (at constant molar percentage) because of the so-called condensation effect (Lecuyer & Dervichian, 1969; Demel et al., 1972); on the other hand, cholesterol, by en-

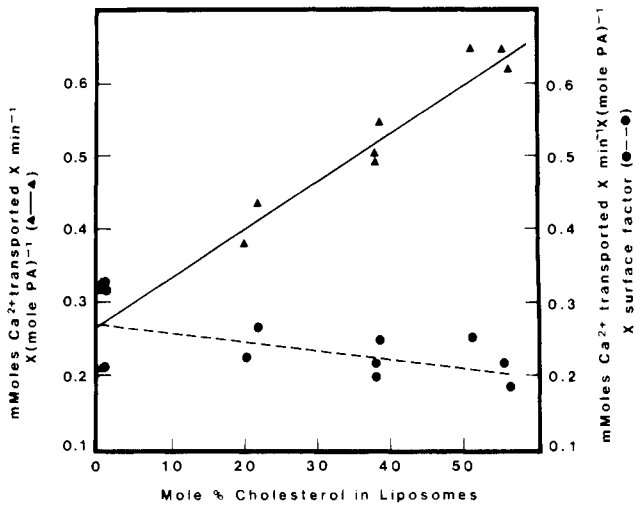

FIGURE 5: Traversal rates of Ca^{2+} in liposomes containing 10% PA and different amounts of cholesterol and egg PC. Final [Ca^{2+}] was 3 mM; mmol of Ca^{2+} transported min^{-1} (mol of PA)$^{-1}$ (▲); mmol of Ca^{2+} transported min^{-1} (mol of PA)$^{-1}$ × surface factor (●). Surface factor represents correction factor for the change in surface concentration of PA due to the change in surface area caused by the condensing effect of cholesterol. See Experimental Procedures.

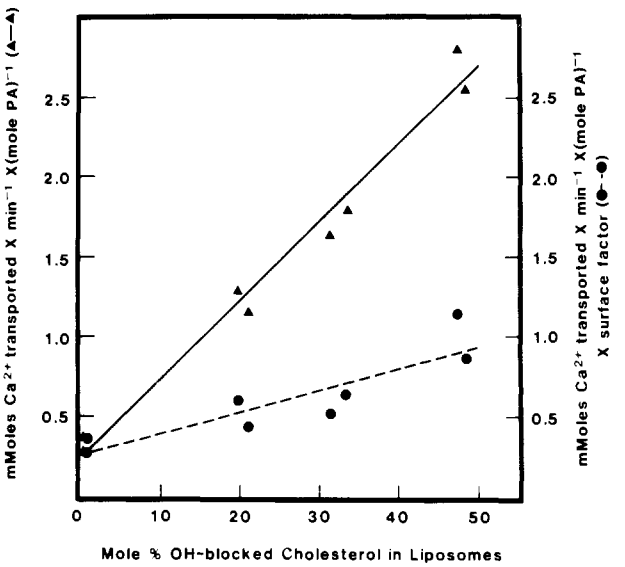

FIGURE 6: Dependence of rates of Ca^{2+} translocation on concentrations of *O*-[[(methoxyethoxy)ethoxy]ethyl]cholesterol (OH-blocked cholesterol) in liposomes containing egg PC + 10 mol % PA. Final [Ca^{2+}] was 3 mM; mmol of Ca^{2+} transported min^{-1} (mol of PA)$^{-1}$ (▲); mmol of Ca^{2+} transported min^{-1} (mol of PA)$^{-1}$ × surface factor (●).

larging liposomes and thus reducing the curvature of the bilayer, diminishes the percentage of PA molecules in the outer monolayer of the liposome, which are, in our assays, the PA molecules available for complexing Ca^{2+}. When both these factors are considered, it is seen (Figure 5) that cholesterol does *not* accelerate $Ca(PA)_2$ traversal and even reduces it somewhat. The OH-blocked cholesterol, however, stimulates Ca^{2+} traversal, if only moderately (Figure 6).

Discussion

Two processes are compared here: (A) the aggregation of PA-containing lipid vesicles induced by Ca^{2+} and (B) the PA-mediated traversal of Ca^{2+} through a lipid bilayer. Process A involves the formation of *trans*-$Ca(PA)_2$ as a bridge between two vesicles. Process B involves the formation of a *cis*-$Ca(PA)_2$ complex which is lipid-soluble. Process A is heavily stimulated by the presence of cholesterol in the bilayer; process B is not.

Aggregation. For the formation of *trans*-$Ca(PA)_2$, each of the two anions must be anchored in a different liposome for the bridge to be formed between them. The structure of the anion is not too critical; probably, any strong and lipophilic anion will do. Phosphatidylserine is known, from many studies, to be an efficient anchor. The similarity of response to calcium gradients for liposomes of different phospholipid composition suggests that in all cases it is the phosphate group, O_3PO^-, that supplies the anionic site that receives the calcium ion and that no other part of the lipid is involved in bonding, neither the head group (e.g., serine) nor any component of the hydrogen belt (see below); this is born out by the ability of diether PA to replace diester PA as the bridge.

An involvement of the hydrogen belt (Brockerhoff, 1974; 1977), i.e., that stratum of the bilayer which contains lipid hydrogen bond acceptors (the CO groups of phospholipids) and donors (OH of cholesterol and sphingolipids), is, however, definitely indicated in the action of cholesterol. For bridging one vesicle to another, the availability of lipid anionic sites and Ca^{2+} is not enough; there must also occur a dehydration of the opposed bilayer surfaces, a loosening of bound water (Portis et al., 1979). This modification of the surfaces is promoted by cholesterol. Up to a cholesterol:phospholipid ratio of 1:2, the sterol can be accommodated by the phospholipid bilayer without a critical change in surface hydration or hydrogen-belt structure. Then, the change takes place. Many properties of a bilayer change at this particular ratio, e.g., permeabilities, transition temperatures and energies, and hydrodynamic properties (Newman & Huang, 1975; Gershfeld, 1978), and the existence of a 1:2 sterol–phospholipid "complex" has been suggested (Hinz & Sturtevant, 1972). However this may be, the free OH group of the sterol is required for the cholesterol effect: if it is blocked (though the body of cholesterol residing in the membrane is left intact), the effect is also blocked. But not only the sterol OH group is necessary: removal of the hydrogen bond accepting CO groups of the matrix phospholipid also blocks the cholesterol effect.

The aggregation of two lipid vesicles, then, may proceed as follows: an excess of cholesterol over the 1:2 sterol:phospholipid ratio causes a restructuring of the region containing the OH and CO groups, the hydrogen belt, of the bilayer, which leads to a dehydration of the bilayer surface. Then, opposed bilayers can approach near enough to allow bridging from lipid phosphate over Ca^{2+} to an opposed lipid phosphate. The structure of the bridging anchors is not critical; they must only be lipophilic and perhaps also sterically uncluttered.

Calcium Traversal. Calcium can cross lipid bilayers as dehydrated $Ca(PA)_2$ complex (Chauhan & Brockerhoff, 1984). Only phosphatidic acid among phospholipids can serve as such an ionophore (Serhan et al., 1982). The formation of the cis complex (intrabilayer complex) cannot be much affected by such changes in hydrogen-belt structure as those caused by excessive cholesterol: the phenomenon of a cholesterol-induced rate increase (so large for vesicle aggregation) does not appear even at 55 mol % of the sterol. Rather, there seems to be a moderate decrease of calcium traversal (Figure 5). Other evidence (A. Chauhan et al., unpublished results) shows that this decrease is real and may be a reflection of cholesterol interfering with the formation of the $Ca(PA)_2$ complex. This speculation is supported by the result of the addition of OH-blocked cholesterol: this compound causes an increase in Ca traversal. We offer the following explanation for these effects: Ca^{2+} ion can be complexed in a cage not only of two phosphatidates but also of two other phospholipids, as long as one is acidic. [Evidence for the existence of such complexes has been obtained in our laboratory—unpublished

results—and in a study by R. N. Reusch (1985).] In our system, then, there exists $Ca(PA)_2$ as well as Ca(PA·PC), of which only the first is hydrophobic enough to traverse the bilayer and thus be measured in our experiments. When cholesterol is present in the bilayer, it will hydrogen bond, directly or with the mediation of water, with PA as well as PC. This will cause a proportional reduction in the concentration of $Ca(PA)_2$. (The degree will depend on the relative affinity of cholesterol for PA and PC.) On the other hand, the presence of the OH-blocked choiesterol, which cannot hydrogen bond, has no effect except that of reducing the concentration of PC in the bilayer (PA is kept constant); this leads to a decrease of Ca(PA·PC) and an increase in $Ca(PA)_2$, i.e., to higher Ca-traversal rates, as we find them. In PC/PA/cholesterol bilayers the two effects nearly cancel each other.

The CO groups of PA, unnecessary for bridge formation in aggregation, are essential for the formation of *cis*-$Ca(PA)_2$ because they are part of the calcium cage, i.e., the eight-cornered coordination complex (Chauhan & Brockerhoff, 1984).

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Registry No. Ca, 7440-70-2; diether PC, 65268-57-7; 3-*O*-[[(methoxyethoxy)ethoxy]ethyl]cholesterol, 72593-64-7; cholesterol, 57-88-5.

REFERENCES

Bentz, J., Düzgünes, N., & Nir, S. (1983a) *Biochemistry 22*, 3320–3330.

Bentz, J., Nir, S., & Wilschut, J. (1983b) *Colloids Surf. 6*, 33–66.

Bloch, K. E. (1983) *Crit. Rev. Biochem. 14*, 47–92.

Brockerhoff, H. (1974) *Lipids 9*, 645–650.

Brockerhoff, H. (1977) in *Bioorganic Chemistry* (Van Tamelen, E. E., Ed.) Vol. III, pp 1–20, Academic, New York.

Brockerhoff, H., & Ayengar, N. K. N. (1979) *Lipids 14*, 88–89.

Chauhan, V. P. S., & Brockerhoff, H. (1984) *Life Sci. 35*, 1395–1399.

Cohen, F. S., Akabas, M. H., & Finkelstein, A. (1982) *Science (Washington, D.C.) 217*, 458–460.

Davidson, F. M., & Long, C. (1958) *Biochem. J. 69*, 458–466.

Demel, R. A., & DeKruyff, B. (1976) *Biochim. Biophys. Acta 457*, 109–132.

Demel, R. A., Bruckdorfer, K. R., & Van Deenen, L. L. M. (1972) *Biochim. Biophys. Acta 255*, 311–320.

Douglas, W. W. (1975) in *Calcium Transport in Contraction and Secretion* (Carafoli, E., Clements, F., Drabikowski, W., & Margareth, A., Eds.) pp 167–174, North-Holland, Amsterdam.

Düzgünes, N., & Ohki, S. (1981) *Biochim. Biophys. Acta 640*, 734–747.

Düzgünes, N., Hong, K., & Papahadjopoulos, D. (1980) in *Calcium Binding Proteins: Structure and Function* (Siegel, F. L., Carafoli, E., Krestinger, R. H., MacLennan, D. H., & Wasserman, R. H., Eds.) pp 17–22, Elsevier/North-Holland, New York.

Fong, J. W., Tirri, L. J., Deshmukh, D. S., & Brockerhoff, H. (1977) *Lipids 12*, 857–862.

Gent, M. P. N., & Prestegard, J. H. (1974) *Biochemistry 13*, 4027–4033.

Gershfeld, N. L. (1978) *Biophys. J. 22*, 469–488.

Hinz, H. J., & Sturtevant, J. M. (1972) *J. Biol. Chem. 247*, 3697–3700.

Huang, C., & Mason, J. T. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 308–310.

Johnson, S. M. (1973) *Biochim. Biophys. Acta 307*, 27–41.

Kielian, M. C., & Helenius, A. (1984) *J. Virol. 52*, 281–283.

Lansman, J., & Haynes, D. H. (1975) *Biochim. Biophys. Acta 394*, 335–347.

Lecuyer, H., & Dervichian, D. G. (1969) *J. Mol. Biol. 45*, 39–57.

Marinetti, G. V. (1962) *J. Lipid Res. 3*, 1–20.

Newman, G. C., & Huang, C. (1975) *Biochemistry 14*, 3363–3369.

Nir, S., Bentz, J., Wilschut, J., & Düzgünes, N. (1983) *Prog. Surf. Sci. 13*, 1–124.

Ohki, S., & Leonards, K. S. (1984) *Biochemistry 23*, 5578–5581.

Papahadjopoulos, D., Portis, A., & Pangborn, W. (1978) *Ann. N.Y. Acad. Sci. 308*, 50–66.

Papahadjopoulos, D., Poste, G., & Vail, W. J. (1979) *Methods Membr. Biol. 10*, 1–121.

Portis, A., Newton, C., Pangborn, W., & Papahadjopoulos, D. (1979) *Biochemistry 18*, 780–790.

Poste, G., & Allison, A. C. (1973) *Biochim. Biophys. Acta 300*, 421–465.

Poste, G., & Nicolson, G. L., Eds. (1978) *Cell Surf. Rev. 5*, 1–862.

Ramsammy, L. S., & Brockerhoff, H. (1982) *J. Biol. Chem. 257*, 3570–3574.

Reusch, R. N. (1985) *Chem. Phys. Lipids 37*, 53–67.

Serhan, C., Anderson, P., Goodman, E., Dunham, P., & Weissmann, G. (1981) *J. Biol. Chem. 256*, 2736–2741.

Serhan, C. N., Anderson, P., Goodman, E., Dunham, P. B., & Weissmann, G. (1982) *J. Biol. Chem. 257*, 4746–4752.

Singleton, W. S., Gray, M. S., Brown, M. L., & White, J. L. (1965) *J. Am. Oil Chem. Soc. 42*, 53–56.

Weissmann, G., Collins, T., Evers, A., & Dunham, P. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 510–514.

White, J., Kielian, M., & Helenius, A. (1983) *Q. Rev. Biophys. 16*, 151–195.

Wilschut, J., Düzgunes, N., & Papahadjopoulos, D. (1981) *Biochemistry 20*, 3126–3133.

Zlatkis, A., Zak, B., & Boyle, A. J. (1953) *J. Lab. Clin. Med. 41*, 486–492.