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Interaction of double-chained cationic surfactants, dimethyldialkylammoniums, with erythrocyte membranes: stabilization of the cationic vesicles by phosphatidylcholines with unsaturated fatty acyl chains

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Abstract

We studied the interaction of double-chained cationic surfactants, dimethyldialkylammoniums, $(CH_3)_2N^+(C_nH_{2n+1})_2$, with the lipid bilayer of guinea-pig erythrocytes by observing the haemolysis, aggregation and shape change in the erythrocytes. In the presence of sonicated dispersions of the five dimethyldialkylammoniums tested (n = 10, 12, 14, 16 and 18), haemolysis was induced dose dependently, and at 0.1 mM or higher concentrations, haemolysis was induced more rapidly by dimethyldialkylammoniums with shorter alkyl chains. The cationic surfactants with longer alkyl chains, such as dimethyldipalmitylammonium, induced aggregation of the erythrocytes before haemolysis fully progressed. The vesicles of these long-chain dimethyldialkylammoniums in the presence of phosphatidylcholines with unsaturated fatty acyl chains markedly reduced the haemolysis rates. Furthermore, in the presence of phosphatidylcholines with unsaturated acyl chains the formation of tightly aggregated structures of several erythrocytes was observed. These findings, and analysis by spin label 5-doxylstearic acid, indicate that phosphatidylcholines enriched with unsaturated acyl chains stabilize the cationic vesicles of long-chain dimethyldialkylammoniums and the interaction with the lipid bilayer of erythrocyte membranes as cationic vesicles became prominent.

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Introduction

Various kinds of double-chained cationic surfactants have been shown to form bilayer vesicles and have been applied to gene delivery (Hong et al 1997; Katsel et al 2000). Among them, dimethyldialkylammoniums, $(CH_3)_2N^+(C_nH_{2n+1})_2$, have been used as antistatic agents (Polefka 1999) and those with long alkyl chains have also been reported to form vesicles (Bhattacharya & Haldar 1996; Hong et al 1997; Feitosa et al 2000). However, dimethyldialkylammoniums with relatively shorter alkyl chains such as dimethyldidecylammonium (C10) seem to form micelles instead of vesicles (Kitagawa et al 2001). The interaction of these double-chained cationic surfactants with lipid bilayers of biological membranes is not clearly defined. Therefore, we examined this interaction using erythrocytes as a model biological membrane system, since erythrocytes have been used as suitable models to study the interaction of amphiphiles and liposomes with biological membranes (Sheetz & Singer 1974; Isomaa et al 1987; Li & Hui 1997; Hägerstrand et al 1999; Dubničková et al 2000). We observed haemolysis, aggregation and shape change

in the erythrocytes induced by five sonicated dispersions of dimethyldialkylammoniums – dimethyldidecylammonium (C10), dimethyldilaurylammonium (C12), dimethyldimyristylammonium (C14), dimethyldipalmitylammonium (C16) and dimethyldistearylammonium (C18), which have different alkyl chain lengths (n = 10, 12, 14, 16, and 18, respectively). Since phospholipids seem to affect the stability of the cationic vesicles (Farhood et al 1995), we also examined the interaction of the cationic surfactants in the presence of various phosphatidylcholines as vesicle constituents. We furthermore examined the molecular assemblies of the cationic surfactants in the presence of phosphatidylcholines by observing electron spin resonance (ESR) spectra using 5-doxylstearic acid (5-NS) as a spin label, since the spin label produces different spectra shapes when present in the micelles, bilayer vesicles or solid aggregates (Ueno & Kashiwagi 2000; Kitagawa et al 2001). Using the same spin label, we also examined the fluidity change of the lipid bilayer of the erythrocytes by the interaction with the cationic vesicles.

From these studies we tried to clarify the stabilizing effects of various phosphatidylcholines in dimethyldialkylammonium vesicles and the change in the interaction of the cationic surfactants with biological membranes due to their presence.

Materials and Methods

Materials

Dimethyldialkylammonium bromide salts were purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan). L-α-Phosphatidylcholine dimyristoyl (DMPC), L-α-phosphatidylcholine dipalmitoyl (DPPC), L-α-phosphatidylcholine dielaidoyl (DEPC), L-α-phosphatidylcholine dioleoyl (DOPC) and L-α-phosphatidylcholine dilinoleoyl (DLPC) were from Sigma Chemical Co. (St Louis, MO) and 5-doxylstearic acid (5-NS) was from Aldrich (Milwaukee, WI). Egg yolk phosphatidylcholine and all other reagents were from Wako Pure Chemical Industries, Ltd (Osaka, Japan).

Preparation of sonicated dispersions of dimethyldialkylammoniums and sonicated vesicles consisting of dimethyldialkylammoniums and phosphatidylcholines

Bromide salts of dimethyldialkylammoniums in the presence or absence of phosphatidylcholines were dissolved in chloroform, and the solvent was evaporated under a nitrogen stream. The dried surfactant-containing films were prepared by removing the solvent under vacuum evaporation. The cationic surfactants and lipids were hydrated and suspended by vortex mixing in phosphate-buffered saline (PBS) (150 mM NaCl, 10 mM phosphate buffer, pH 7.4). The suspension was then sonicated with a probe-type sonicator for 5 min at an output power of 80 W at 50°C. When phosphatidylcholines with unsaturated acyl chains were added as vesicle constituents, the suspension was sonicated at 30°C under a stream of nitrogen.

Measurement of haemolysis and observation of aggregation and shape change of erythrocytes

Guinea-pig blood was withdrawn from the abdominal aorta under ketamine anaesthesia into ACD anticoagulant solution (2.2% sodium citrate, 0.8% citric acid and 2.2% glucose). Blood was centrifuged at 2000 *g* for 5 min at 4°C to remove plasma and the buffy coat, and the erythrocyte suspension was prepared as described previously (Kitagawa et al 2001). The erythrocytes in the pellet were washed with PBS three times by centrifugation at 2000 *g* for 5 min and collected by centrifugation at 4500 *g* for 8 min. The erythrocytes were suspended in PBS at a haematocrit value of about 20% and incubated with the sonicated dispersions of the surfactants (or surfactant and lipid mixtures) at 37°C. A sample of this mixture was taken out after 1 min and 60 min of incubation. The extent of haemolysis was obtained by measuring the haemoglobin release in the supernatant after centrifugation at 2000 *g* for 2 min. Haemoglobin was determined spectrophotometrically at 541 nm according to the cyanmethaemoglobin method (Crosby et al 1954). The percentage of haemolysis was determined by comparing the haemoglobin release with that of control samples completely haemolysed with distilled water. Shape change and aggregation of the erythrocytes were observed using Olympus phase-contrast microscopy equipment (Tokyo, Japan).

Measurement of electron spin resonance (ESR) spectra

Measurement of ESR spectra was carried out using 5-NS as a spin label. The sonicated dispersions of the surfactants (or surfactant and lipid mixtures) prepared in PBS as described above were incubated with 5-NS 25 μM at 37°C for 2 min and transferred to duplicate

20-μL capillaries. One end of the capillaries was sealed with Hematoseal (Terumo, Tokyo, Japan) and inserted into ESR tubes. The ESR spectra were measured at 37°C with a TE-200 (X-band) spectrometer (JEOL, Tokyo, Japan) with a 100-kHz field modulation frequency and 0.1 mT modulation amplitude at an output power of 8 mW. The order parameter of 5-NS was calculated from the ESR spectra as described previously (Kitagawa et al 1990).

The ESR spectra of 5-NS in the erythrocytes were measured as follows. Erythrocytes were incubated with cationic vesicles consisting of dimethyldialkylammoniums and phosphatidylcholines at 37°C for 30 min. The erythrocytes were then centrifuged and washed with PBS at 4°C three times and resuspended at a haematocrit value of about 20%. The erythrocyte suspension was incubated with 5-NS 25 μM at 37°C for 2 min and transferred to duplicate 20-μL capillaries. Measurement of the ESR spectra was carried out using the same methods as described above.

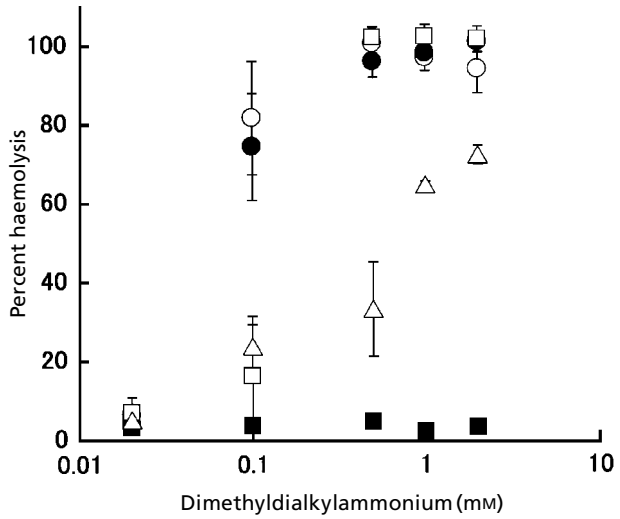

Figure 1 Dose-dependent haemolysis in guinea-pig erythrocytes induced by dimethyldialkylammoniums (C10, ○; C12, ●; C14, □; C16, ■; C18, △) after incubation for 1 min at 37°C. Data are means±s.d. of four experiments.

Statistical analysis

Bonferroni's *t*-test for multiple comparisons or Student's *t*-test was used to analyse the difference between the sets of data. A *P*-value less than 0.05 was considered statistically significant.

Results

Haemolysis by dispersions of dimethyldialkylammoniums

We examined the haemolytic effects of sonicated dispersions of dimethyldialkylammoniums on guinea-pig erythrocytes. Among them, C12, C14 and C16 seem to form bilayer vesicles, but C10 forms micelles and C18 is present as solid aggregates (Kitagawa et al 2001). As shown in Figure 1, dimethyldialkylammoniums dose-dependently induced haemolysis. Dimethyldialkylammoniums with relatively short alkyl chains, such as C10, induced haemolysis more rapidly at 0.1 mM or higher concentrations than those with longer alkyl chains. The haemolysis rate at relatively high concentrations was slowest for C16 (Figure 1) for the low haemolysis percentage after a 1-min incubation ($P < 0.01$ at 0.5 mM, $P < 0.001$ at higher concentrations). The concentrations of dimethyldialkylammoniums required to induce 50% haemolysis after 1 min for C10, C12, C14 and C18 were about 0.05 mM, 0.06 mM, 0.17 mM and 0.7 mM, respectively; the corresponding concentration for C16 was >2 mM.

Shape change and aggregation of erythrocytes by dispersions of dimethyldialkylammoniums

As with other amphiphiles, these double-chained cationic surfactants seem to induce a shape change in the erythrocytes at low concentrations. Erythrocytes contain negatively-charged sialic acid (Eylar et al 1962). Therefore, when the cationic surfactants form vesicles at higher concentrations, they seem to aggregate erythrocytes before haemolysis is completed. To confirm this, we next examined the aggregation and shape change of the erythrocytes at relatively low surfactant concentrations that did not fully induce haemolysis. As with single-chained cationic surfactants (Isomaa et al 1987), dimethyldialkylammoniums induced spiculate (echinocytic) shapes, at 0.02 mM in the initial stage and then changed to invaginated (stomatocytic) shapes (data not shown). This finding is consistent with the recent report on C12 by Dubničková et al (2000), which suggested the transbilayer movement of the surfactant molecules from the outer to the inner leaflets. On the other hand, at higher concentrations, dimethyldialkylammoniums with longer alkyl chains induced marked aggregation of the erythrocytes just after the addition of the cationic surfactants, when they induced only slight haemolysis.

Table 1 Haemolysis of guinea-pig erythrocytes induced by vesicles consisting of 1 mM dimethyldialkylammonium in the presence of 1 mM egg-yolk phosphatidylcholine, or in its absence, after 1 min or 60 min incubation at 37°C.

Dimethyldialkylammonium	Incubation time (min)	Percent haemolysis	
		Egg-yolk PC present	Egg-yolk PC absent
C10	1	91.5±9.9	97.1±3.3
C12	1	57.1±8.4*	98.3±1.5
C14	1	3.9±0.6**	102.4±3.0
C16	1	0.8±0.3**	2.1±0.2
C18	1	1.0±1.0**	64.7±0.9
C10	60	94.3±5.7	99.2±1.5
C12	60	99.1±4.0	98.0±2.1
C14	60	100.7±1.5	101.6±2.1
C16	60	4.1±3.7**	101.9±1.9
C18	60	5.6±0.9**	100.5±3.7

C10, dimethyldidecylammonium; C12, dimethyldilaurylammonium; C14, dimethyldimyristylammonium; C16, dimethyldipalmitylammonium; C18, dimethyldistearylammonium. Data are means±s.d. of three experiments. *$P < 0.01$, **$P < 0.001$ compared with the value in the absence of egg-yolk phosphatidylcholine (egg-yolk PC).

Table 2 Haemolysis induced in guinea-pig erythrocytes by vesicles consisting of 1 mM C16 and 1 mM phosphatidylcholine after 1 min or 60 min incubation at 37°C.

Phosphatidylcholine	Percent haemolysis	
	After 1 min	After 60 min
None	2.1±0.2	101.9±1.9
DMPC	3.8±0.3*	99.5±3.7
DPPC	13.9±0.9**	99.6±4.0
DEPC	0.6±0.2*	1.5±0.2**
DOPC	0.5±0.2*	2.8±0.2**
DLPC	0.4±0.1**	1.2±0.3**

DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine, DEPC, dielaidoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DLPC, dilinoleoylphosphatidylcholine. Data are means±s.d. of three experiments. *$P < 0.01$, **$P < 0.001$ compared with the value in the absence of phosphatidylcholines.

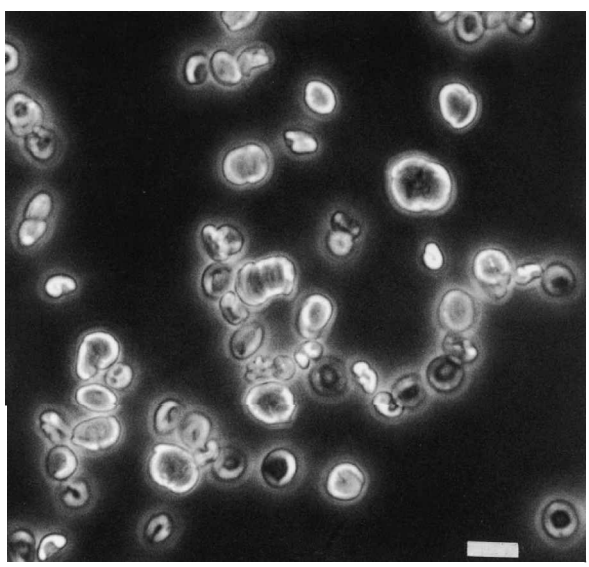

Figure 2 Formation of tightly aggregated structures of guinea-pig erythrocytes induced by 60 min incubation with vesicles consisting of 1 mM C16 and 1 mM egg-yolk phosphatidylcholine. Shape change and aggregation of the erythrocytes were observed by phase-contrast microscopy. The bar is 10 μm.

Haemolysis by vesicles consisting of dimethyldialkylammoniums and phosphatidylcholines

Double-chained cationic surfactants are usually applied to gene delivery by the formation of bilayer vesicles with phospholipids (Farhood et al 1995; Katsel & Greenstein 2000; Campbell et al 2001). Stability of cationic vesicles might be achieved by the addition of phosphatidylcholines which are known to form stable bilayers. Therefore, we next examined the effects, on haemolysis, of vesicles consisting of 1 mM dimethyldialkylammoniums and 1 mM egg-yolk phosphatidylcholine. Table 1 shows that dimethyldidecylammonium (C10; n = 10) immediately induced haemolysis in most of the

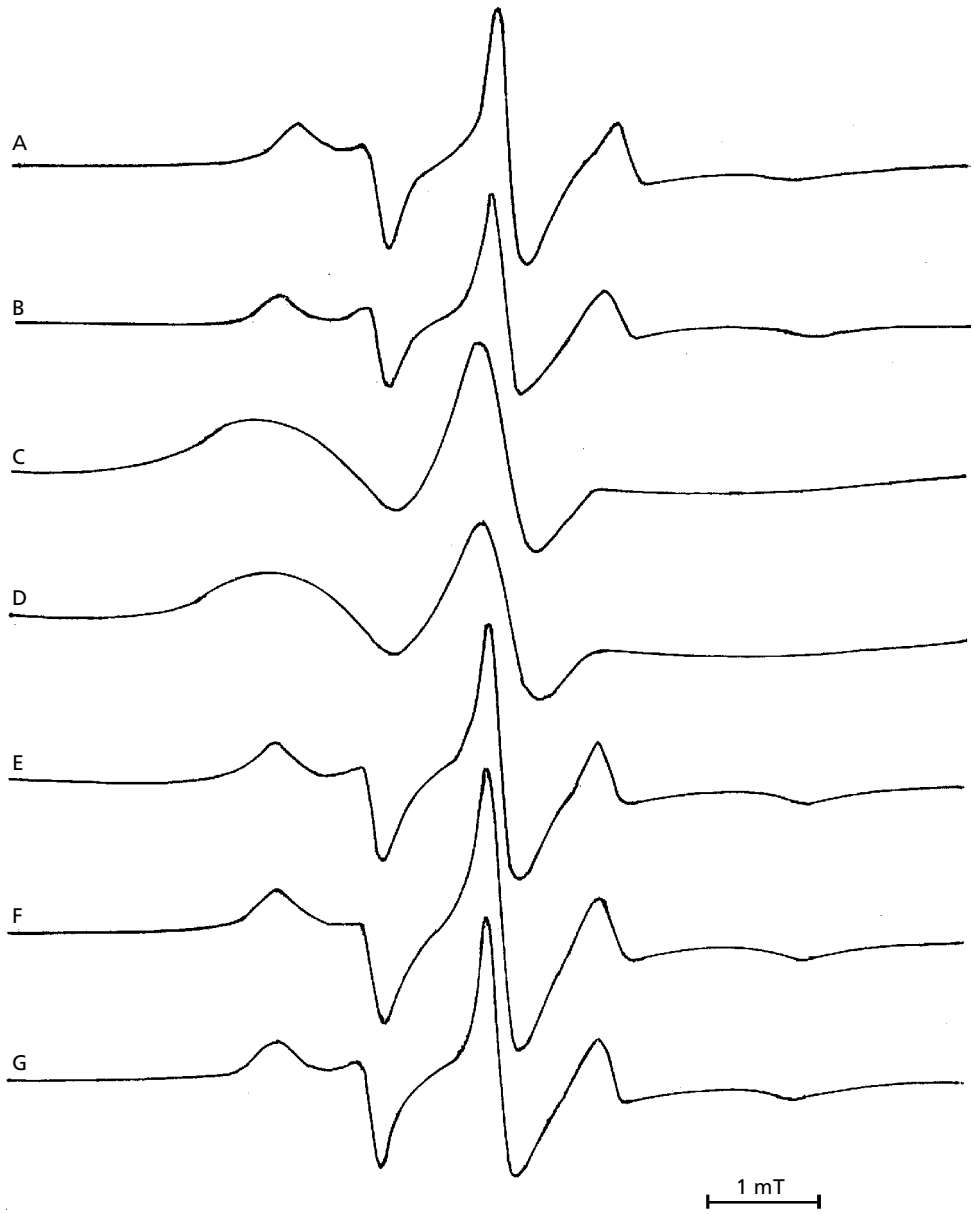

Figure 3 ESR spectra of 5-NS in C16 vesicles in the presence or absence of 1 mM phosphatidylcholine. A. None (absence of phosphatidylcholine); B. egg-yolk phosphatidylcholine; C. DMPC (L-α-phosphatidylcholine dimyristoyl); D. DPPC (L-α-phosphatidylcholine dipalmitoyl); E. DEPC (L-α-phosphatidylcholine dielaidoyl); F. DOPC (L-α-phosphatidylcholine dioleoyl); G. DLPC (L-α-phosphatidylcholine dilinoleoyl).

erythrocytes just as in the absence of egg-yolk phosphatidylcholine at the same surfactant concentration. The haemolysis rate decreased with increasing dimethyldialkylammonium alkyl chain length. Long-chain dimethyldialkylammoniums (n = 16, 18) only slightly induced haemolysis in the presence of egg-yolk phosphatidylcholine.

To examine the effects of phosphatidylcholine acyl chains on the haemolytic activity of the cationic vesicles, we next examined the haemolysis induced by vesicles consisting of 1 mM C16 and 1 mM of one of the phosphatidylcholines with different acyl chains. Table 2 shows that, for dielaidoylphosphatidylcholine (DEPC), dioleoylphosphatidylcholine (DOPC) and dilinoleoylphosphatidylcholine (DLPC), the presence of phosphatidylcholines with unsaturated fatty acyl chains reduced

the haemolytic activity of the cationic vesicles just as with egg-yolk phosphatidylcholine, which is enriched in oleic acid and linoleic acid in its acyl chains (Singleton et al 1965). However, the presence of phosphatidylcholines with saturated acyl chains, dimyristoylphosphatidylcholine (DMPC) and dipalmitoylphosphatidylcholine (DPPC), did not reduce the rate of haemolysis; DPPC slightly stimulated haemolysis.

Tightly aggregated structure formation of erythrocytes by long-chain dimethyldialkylammonium vesicles in the presence of phosphatidylcholines

In the presence of long-chain dimethyldialkylammonium vesicles, a mass formation of aggregated erythrocytes was observed before haemolysis was fully induced as described above. Vesicles consisting of equimolar ratios of C16 and egg-yolk phosphatidylcholine, which induced only slight haemolysis, as shown above, formed characteristic tightly aggregated structures of several erythrocytes (Figure 2). Similar structures were observed in the presence of the vesicles consisting of 1 mM C18 and 1 mM egg-yolk phosphatidylcholine and those consisting of 1 mM C16 and 1 mM phosphatidylcholines with unsaturated fatty acyl chains (data not shown).

ESR spectra of dimethyldialkylammonium vesicles in the presence of phosphatidylcholines

To reveal the molecular assemblies of dimethyldialkylammoniums in the presence of phosphatidylcholines we observed the ESR spectra of 5-NS. 5-NS is known to provide different shapes of spectra and different values of parameters for its motional freedom when it is present in the micelles, bilayer vesicles and solid aggregates (Ueno & Kashiwagi 2000; Kitagawa et al 2001). All the dimethyldialkylammoniums tested seemed to form bilayer vesicles in the presence of egg-yolk phosphatidylcholine, according to their anisotropic ESR signals (Figure 3B – C16). The bilayer vesicles consisting of dimethyldialkylammoniums with longer alkyl chains appeared to be more rigid in the presence of egg-yolk phosphatidylcholine according to the order parameters shown in Table 3.

Equimolar mixtures of the cationic surfactant C16 and either DMPC or DPPC, which have saturated acyl chains and did not have a significant effect on the haemolytic activity of C16, seemed to be present as solid aggregates at 37°C, according to their broad ESR spectra of 5-NS (Figures 3C and 3D). As with egg-yolk phosphatidylcholine, phosphatidylcholines with unsaturated acyl chains, which markedly reduced the haemolytic activity of C16, seem to form stable bilayer vesicles with C16, according to their anisotropic ESR spectra shown in Figures 3E–3G and the order parameters obtained from the spectra listed in Table 3.

Table 3 Order parameter of 5-NS in vesicles consisting of 1 mM dimethyldialkylammonium in the presence of 1 mM phosphatidylcholine, or in its absence, at 37°C.

Dimethyldialkylammonium	Phosphatidylcholine	Order parameter
None[a]	Egg-yolk PC	0.578±0.004
C10	Egg-yolk PC	0.513±0.004
C12	None	0.416±0.002[b]
	Egg-yolk PC	0.525±0.005*
C14	None	0.446±0.003[b]
	Egg-yolk PC	0.535±0.001*
C16	None	0.464±0.002[b]
	Egg-yolk PC	0.546±0.003*
	DEPC	0.549±0.003*
	DOPC	0.540±0.001*
	DLPC	0.535±0.004*
C18	Egg-yolk PC	0.557±0.003

C10, dimethyldidecylammonium; C12, dimethyldilaurylammonium; C14, dimethyldimyristylammonium; C16, dimethyldipalmitylammonium; C18, dimethyldistearylammonium; DEPC, dielaidoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DLPC, dilinoleoylphosphatidylcholine. Data are means±s.d. of four experiments. [a]1 mM egg-yolk phosphatidylcholine (egg-yolk PC) vesicles without dimethyldialkylammonium. [b]Data cited from Kitagawa et al (2001). *$P < 0.001$ compared with the value in the absence of phosphatidylcholines.

Table 4 Order parameter of 5-NS in guinea-pig erythrocytes after incubation with either the cationic vesicles consisting of 1 mM C16 and 1 mM phosphatidylcholine or with those consisting of 1 mM egg-yolk phosphatidylcholine (egg-yolk PC) at 37°C.

Phosphatidylcholine	Order parameter
None (control)	0.656±0.003
Egg yolk PC	0.567±0.004*
DEPC	0.570±0.002*
DOPC	0.556±0.002*
DLPC	0.543±0.002*
Egg yolk PC without C16	0.660±0.003

DEPC, dielaidoylphosphatidylcholine; DOPC, dioleoylphosphatidylcholine; DLPC, dilinoleoylphosphatidylcholine. Data are means±s.d. of four experiments. *$P < 0.001$ compared with the control value (value without any vesicles).

Fluidity change in the erythrocyte membrane produced by the cationic vesicles

We next examined the fluidity change in the erythrocyte membrane by incubating with the vesicles consisting of 1 mM C16 and 1 mM phosphatidylcholine with unsaturated fatty acyl chains which showed tightly aggregated structures using 5-NS as a spin label. As shown in Table 4, there were marked decreases in the order parameter observed with the cationic vesicles in the presence of phosphatidylcholines with unsaturated fatty acyl chains, which indicated an increase in the fluidity of the lipid bilayer of the erythrocyte membranes.

Discussion

Dimethyldialkylammoniums induced shape change in erythrocytes and at higher concentrations induced haemolysis due to penetration of the monomers into the erythrocyte membrane and interaction with the membrane components, as has been suggested for single-chained surfactants (Isomaa et al 1987). As we indicated previously (Kitagawa et al 2001), C10 forms micelles, and C12, C14 and C16 form bilayer vesicles. C18 is present as solid aggregates. More surfactant molecules appear to be present as monomers for dimethyldialkylammoniums that have shorter alkyl chains, which are present in equilibrium with the surfactants in the vesicles (or micelles). Furthermore, the vesicles of the surfactants with shorter alkyl chains appear to be unstable due to their loose molecular packing and therefore may easily become monomers. These factors seem to account for the more marked haemolytic activity of cationic surfactants with shorter alkyl chains.

The presence of egg-yolk phosphatidylcholine, or other phosphatidylcholines with unsaturated acyl chains in the cationic vesicles, reduced the haemolytic activity of long-chain dimethyldialkylammoniums. The formation of stable bilayers is important for the cationic vesicles to interact with the cellular membranes as vesicles and is essential to their use in drug delivery (Campbell et al 2001). Our ESR analysis suggested that these cationic surfactants are mostly present as bilayer vesicles and the molecular packing of the bilayer vesicles is increased by phosphatidylcholines with unsaturated acyl chains depending on their alkyl chain lengths. The presence of unsaturated fatty acyl chains in phosphatidylcholines seems to be essential for the formation of stable bilayer vesicles with equimolar dimethyldialkylammoniums, at least at 37°C. This is in contrast to the vesicle formation with DPPC of another double-chained cationic surfactant, DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium), which itself has *cis*-double bonds in its acyl chains (Campbell et al 2001). We also observed the ESR spectrum, which suggested the bilayer vesicle formation by an equimolar ratio of DPPC and DOTAP (data not shown).

The partition of the double-chained cationic surfactants into the erythrocyte membrane also seems to have been decreased by the presence of phosphatidylcholine vesicles. As well as the formation of the more stable vesicles mentioned above, this also seems to account for the reduction in the haemolytic activity of dimethyldialkylammoniums in the presence of phosphatidylcholines with unsaturated fatty acyl chains.

The formation of tightly aggregated structures of several erythrocytes was observed on incubation with the vesicles of long-chain dimethyldialkylammoniums in the presence of phosphatidylcholines with unsaturated fatty acyl chains. Li & Hui (1997) reported that rabbit erythrocyte fusion was induced by cationic vesicles consisting of DOTAP and phosphatidylethanolamine. Some erythrocytes may have been induced to fusion in this study, although it has been reported that fusion is induced much less by vesicles containing phosphatidylcholines than by those containing phosphatidylethanolamines (Wrobel & Collins 1995). Since an increase in erythrocyte membrane fluidity was observed, the presence of unsaturated fatty acyl chains in the vesicles may change the interaction with biological membranes due to their fluid nature (Wilshut et al 1984).

Conclusions

This study revealed that phosphatidylcholines enriched with unsaturated acyl chains stabilized the cationic vesicles of long-chain dimethyldialkylammoniums and changed the interaction of the double-chained cationic surfactants with erythrocytes. In their presence, the cationic surfactants seemed to interact with the erythrocytes as vesicles and induced the formation of tightly aggregated structures of several erythrocytes. Since an increase in erythrocyte membrane fluidity was observed, the unsaturated fatty acyl chains in the vesicles may also be important for the formation of the structures. As fusion of the cationic vesicles with erythrocytes and between erythrocytes could be induced, a new study is now in progress.

References

Bhattacharya, S., Haldar, S. (1996) The effects of cholesterol inclusion on the vesicular membranes of cationic lipids. *Biochim. Biophys. Acta* **1283:** 21–30

Campbell, R. B., Balasubramanian, S. V., Straubinger, R. M. (2001) Phospholipid–cationic lipid interactions: influences on membrane and vesicle properties. *Biochim. Biophys. Acta* **1512**: 27–39

Crosby, W. H., Munn, J. I., Furth, F. W. (1954) Standardizing a method for clinical hemoglobinometry. *US Armed Forces Med. J.* **5**: 693–703

Dubničková, M., Bobrowska-Hägerstrand, M., Söderström, T., Iglič, A., Hägerstrand, H. (2000) Gemini (dimeric) surfactant perturbation of the human erythrocyte. *Acta Biochimica Polonica* **47**: 651–660

Eylar, E. H., Madoff, M. A., Brody, O. V., Oncley, J. L. (1962) The contribution of sialic acid to the surface charge of the erythrocyte. *J. Biol. Chem.* **237**: 1992–2000

Farhood, H., Serbina, N., Huang, L. (1995) The role of dioleoylphosphatidylethanolamine in cationic liposome mediated gene transfer. *Biochim. Biophys. Acta* **1235**: 289–295

Feitosa, E., Barreleiro, P. C. A., Olofsson, G. (2000) Phase transition in dioctadecyldimethylammonium bromide and chloride vesicles prepared by different methods. *Chem. Phys. Lipids* **105**: 201–213

Hägerstrand, H., Danieluk, M., Bobrowska-Hägerstrand, M., Pector, V., Ruysschaert, J.-M., Kralj-Iglič, V., Iglič, A. (1999) Liposomes composed of a double-chain cationic amphiphile (Vectamidine) induce their own encapsulation into human erythrocytes. *Biochim. Biophys. Acta* **1421**: 125–130

Hong, K., Zheng, W., Baker, A., Papahadjopoulos, D. (1997) Stabilization of cationic liposome–plasmid DNA complexes by polyamines and poly(ethylene glycol)–phospholipid conjugates for efficient in vivo gene delivery. *FEBS Lett.* **400**: 233–237

Isomaa, B., Hägerstrand, H., Paatero, G. (1987) Shape transformations induced by amphiphiles in erythrocytes. *Biochim. Biophys. Acta* **899**: 93–103

Katsel, P L., Greenstein, R. J. (2000) Eukaryotic gene transfer with liposomes: effect of differences in lipid structure. *Biotechnol. Annu. Rev.* **5**: 197–220

Kitagawa, S., Kametani, F., Tsuchiya, K., Sakurai, H. (1990) ESR analysis with long-chain alkyl spin labels in bovine blood platelets. Relationship between the increase in membrane fluidity by alcohols and phenolic compounds and their inhibitory effects on aggregation. *Biochim. Biophys. Acta* **1027**: 123–129

Kitagawa, S., Kasamaki, M., Hiyama, F. (2001) Effects of double-chained cationic surfactants *n*-dimethyldialkylammoniums on skin permeation of benzoic acid through excised guinea pig dorsal skin; comparison of their enhancement effects with hemolytic effects on erythrocytes. *Chem. Pharm. Bull.* **49**: 1155–1158

Li, L. H., Hui, S. W. (1997) The effect of lipid molecular packing stress on cationic liposome-induced rabbit erythrocyte fusion. *Biochim. Biophys. Acta* **1323**: 105–116

Polefka, T. G. (1999) Surfactant interactions with skin. *Surfactant Sci. Ser.* **82**: 433–468

Sheetz, M. P., Singer, S. J. (1974) Biological membranes as bilayer couples. A molecular mechanism of drug–erythrocyte interactions. *Proc. Natl Acad. Sci. USA* **71**: 4457–4461

Singleton, W. S., Gray, M. S., Brown, M. L., White, J. L. (1965) Chromatographically homogeneous lecithin from egg phospholipids. *J. Am. Oil. Chem. Soc.* **42**: 53–56

Ueno, M., Kashiwagi, H. (2000) Interaction of liposomes with detergents. *Nihon Yukagakkaishi* **49**: 1131–1139

Wilshut, J., Düzgünes, N., Hoekstra, D., Papahadjopoulos, D. (1985) Modulation of membrane fusion by membrane fluidity: temperature dependence of divalent cation induced fusion of phosphatidylserine vesicles. *Biochemistry* **24**: 8–14

Wrobel, I., Collins, D. (1995) Fusion of cationic liposomes with mammalian cells occurs after endocytosis. *Biochim. Biophys. Acta* **1235**: 296–304