  Bull. Chem. Soc. Jpn., 59, 3145—3151 (1986)

Fusogenic Behavior of Single-Walled Vesicles Composed of Synthetic Peptide Lipids†

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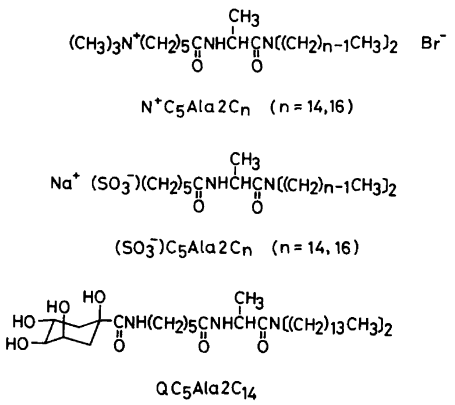
(Received April 9, 1986)

Fusogenic behavior of the single-walled vesicles formed with mixed or individual synthetic peptide lipids, $N^+C_5Ala2C_n$ and $(SO_3^-)C_5Ala2C_n$ (n=14, 16), was investigated in the presence of various water-soluble polymers or a nonbilayer-forming lipid by means of electron microscopy, differential scanning calorimetry, and turbidity measurements. The following ionic polymers having opposite charges to the net surface charges of the vesicles immediately induced fusion via vesicle aggregation; heparin, chondroitin sulfate C, chondroitin, and potassium salt of poly(vinyl sulfate) for the cationic vesicles, and polybrene for the anionic ones. On the other hand, ionic polymers having electrostatic charges identical with the vesicular surface charges and nonionic ones, such as amylose, poly(vinyl alcohol), polyethylene glycol 20000, and polyethylene glycol 6000, induced neither fusion nor aggregation. A nonbilayer-forming lipid, QC_5Ala2C_{14}, incorporated into the $N^+C_5Ala2C_n$ vesicles induced fusion at kinetic rates much slower than those triggered by the ionic polymers. The observations were discussed with attention to the dehydration effect and the participation of nonbilayer structures in the fusion process.

In the field of membrane mimetic chemistry,[1] synthetic lipid membranes have been widely used in place of liposomes formed with natural phospholipids, because the former aggregates attain greater morphological stability relative to the latter and provide specified functions through chemical modifications. In the light of the Brockerhoff's concept[2] which claims that the intramembrane hydrogen-belt domain is much important for morphological stabilization of biomembranes, we have prepared various peptide lipids; each composed of a polar head moiety, a hydrophobic double-chain segment, and an amino acid residue interposed between them as a hydrogen-bonding component.[3-5] We have previously clarified the characteristic morphological features of aggregates composed of the peptide lipids. In general, ionic peptide lipids form multiwalled bilayer membranes in the aqueous dispersion state and the corresponding single-walled vesicles are formed upon sonication of the aqueous dispersions,[3-5] in analogy with morphological behavior observed for liposomal membranes. Physicochemical stability of the single-walled vesicles formed with the cationic lipids, $N^+C_5Ala2C_n$, is much larger than that of the liposomal membrane formed with dimyristoylphosphatidylcholine under comparable conditions.[5] Meanwhile, nonbilayer structures such as inverted hexagonal (H_{II}) and inverted cubic (C_{II}) phases can be created with the peptide lipids by controlling intramembrane interaction among polar head moieties of the lipids.[6-10] Such nonbilayer structures have been often observed in biological systems and claimed to play crucial roles in the membrane fusion process as key intermediates.[11] Thus, the peptide lipid system can be utilized as a suitable model for investigation of the plausible mechanism of nonbilayer-mediated fusion. In the present work, we studied on morphological changes of the single-walled vesicles as induced by fusogens such as water-soluble polymers and nonbilayer-forming lipids.



†Contribution No. 831 from this Department.

Experimental

Materials. The following compounds were obtained from commercial sources as the purest grade available and used without further purification: heparin, chondroitin 6-(sodium sulfate) (chondroitin sulfate C), amylose (molecular weight, ca. 2900), potassium salt of poly(vinyl sulfate) (PVSK: degree of polymerization (DP), 162.2; degree of esterification, 94.5%) (all four species from Nakarai Chemicals, Kyoto); 2,6-pyridinedicarboxylic acid, poly(vinyl alcohol) (PVA; DP, 1500—1800), polyethylene glycol 6000 (PEG 6000; DP, ca. 7500), polyethylene glycol 20000 (PEG 20000; DP, 20000 ±5000) (all four compounds from Wako Pure Chemical Industries, Osaka); chondroitin, 2-amino-2-deoxy-3-*O*-(β-D-glucopyranuronosyl)-α-D-galactopyranose (chondrosin) (both from Seikagaku Kogyo Co., Tokyo); poly[(dimethyliminio)-1,3-propanediyl(dimethyliminio)-1,6-hexanediyl dibromide] (polybrene; from Sigma Chemical Co., Missouri, U.S.A.). Preparation of the following lipids has been reported previously: *N,N*-ditetradecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{14}$),[5] *N,N*-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide ($N^+C_5Ala2C_{16}$),[5] sodium *N,N*-ditetradecyl-N^{α}-(6-sulfonatohexanoyl)-L-alaninamide [(SO_3^-)-C_5Ala2C_{14}],[7] sodium *N,N*-dihexadecyl-N^{α}-(6-sulfonatohexanoyl)-L-alaninamide [(SO_3^-)C_5Ala2C_{16}],[7] and *N,N*-ditetradecyl-N^{α}-[6-(quinoylamino)hexanoyl]-L-alaninamide (QC_5Ala2C_{14}); quinoyl being the conventional name for 1,3,4,5-tetrahydroxy-[1*R*-(1α,3α,4α,5β)]cyclohexylcarbonyl.[6]

Measurements. Aqueous dispersions of lipids were prepared according to the previous method.[7] Whenever necessary, a dispersion sample was subsequently sonicated with a Heat Systems-Ultrasonics W-220F probe-type sonicator and allowed to stand for 20 min at room temperature. The sonicated solution was adjusted to an appropriate pH with aqueous hydrochloric acid or aqueous potassium hydroxide. A Beckman Φ71 pH meter equipped with a Beckman 39505 combined electrode was used for the pH measurements after calibration with a combination of appropriate standard aqueous buffers. Turbidity measurements were run at 400 nm on a Union Giken SM-401 high sensitivity spectrophotometer equipped with a cell of 10.0- or 1.0-mm path length. A Daini Seikosha SSC-560U calorimeter was used for differential scanning calorimetry (DSC). The phase transition parameters (T_m, temperature at a peak maximum of the DSC thermogram; ΔH, enthalpy change for phase transition) were determined in a manner similar to that reported previously.[7] Electron micrographs were taken on a JEOL JEM-2000FX electron microscope installed at the Research Laboratory for High Voltage Electron Microscopy of Kyushu University. Negatively stained samples for the measurements were prepared in a manner as described previously.[7]

Results

First, we examined aggregation behavior of the single-walled vesicles by means of turbidity measurements as typically shown in Fig. 1. Addition of anionic polymers immediately caused aggregation of the cationic single-walled vesicles composed of $N^+C_5Ala2C_{16}$ with the diameter range 250—800 Å. When heparin or PVSK was added to the vesicular solution,

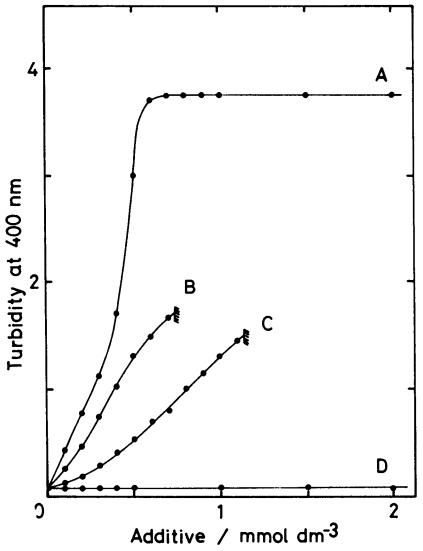


Fig. 1. Effects of additive-concentrations (in monosaccharide or monomer unit) on turbidity of the single-walled vesicles formed with $N^+C_5Ala2C_{16}$ (1.0 mmol dm^{-3}) at pH 7 and 40 °C.
A, heparin; B, chondroitin sulfate C; C, chondroitin; D, polybrene, PVA, PEG 20000, PEG 6000, amylose, or chondrosin.

turbidity increased and reached a maximum level under conditions that the whole cationic charges on the vesicular surfaces were completely neutralized with anionic charges involved in the polymer species at the 1 : 1 ratio of positive to negative charge, and further addition of the polymer gave no additional turbidity change (Fig. 1, line A). Addition of chondroitin sulfate C or chondroitin also resulted in an turbidity increase. However, precipitates were formed before neutralization of the cationic charges of the lipid vesicles with these anionic polymers (Fig. 1, lines B and C). On the other hand, a cationic polymer, polybrene, and nonionic ones, amylose, PVA, PEG 20000, and PEG 6000, did not trigger aggregation of the cationic vesicles (Fig. 1, line D) under the conditions employed here: [$N^+C_5Ala2C_{16}$], 1.0 mmol dm^{-3}; [polymer], 0—5 mmol dm^{-3} in a monosaccharide or a monomer unit. Anionic compounds with low molecular weights, chondrosin (measured at pH 5—11) and 2,6-pyridinedicarboxylic acid (measured at pH 6), also exhibited no capacity to induce aggregation of the $N^+C_5Ala2C_{16}$ vesicle.

The growth of single-walled vesicles to multiwalled lamellae was observed by negative staining electron microscopy (NSEM) for the $N^+C_5Ala2C_{16}$ membrane upon addition of one of the following anionic polymers; heparin (Fig. 2A), chondroitin sulfate C, chondroitin, and PVSK (Fig. 2B). On the other hand, the single-walled $N^+C_5Ala2C_{16}$ vesicle remained morphologically unchanged in the presence of any one of the following additives; polybrene (Fig. 2C), amylose, PVA, PEG 20000 (Fig. 2D), PEG 6000, chondrosin, and 2,6-pyridinedicarboxylic acid.

The vesicle growth observed by NSEM was also

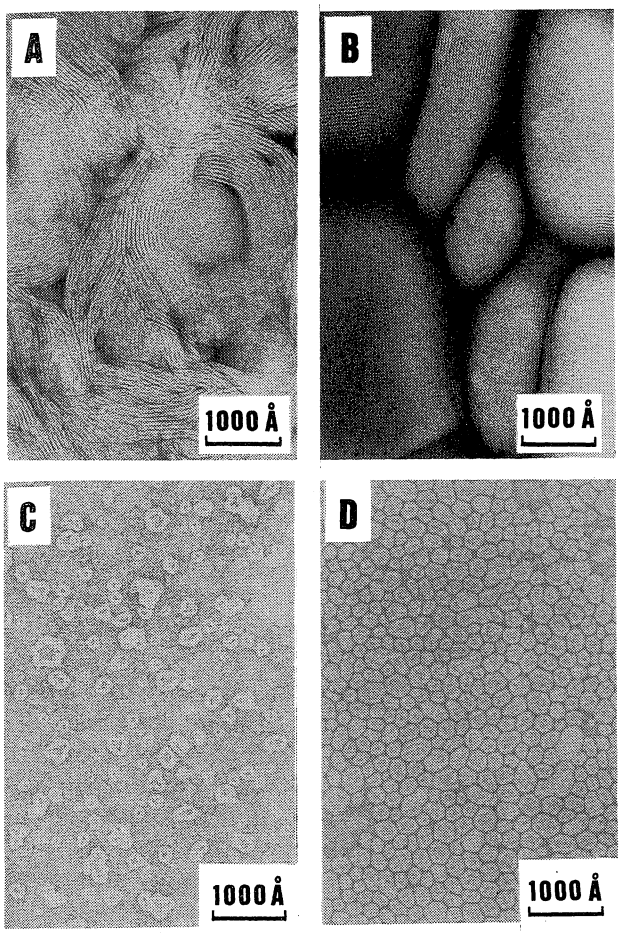


Fig. 2. Electron micrographs negatively stained with uranyl acetate.
Each specimen was prepared immediately after addition of the following additives to the single-walled vesicles formed with $N^+C_5Ala2C_{16}$ at pH 7 and 40 °C: A, heparin (2.5 mmol dm^{-3} in the anion unit); B, PVSK (0.8 mmol dm^{-3} in the monomer unit); C, polybrene (1.0 mmol dm^{-3} in the cation unit); D, PEG 20000 (5.0 mmol dm^{-3} in the monomer unit). Lipid concentrations: 5.0 mmol dm^{-3} for A and D; 1.0 mmol dm^{-3} for B and C.

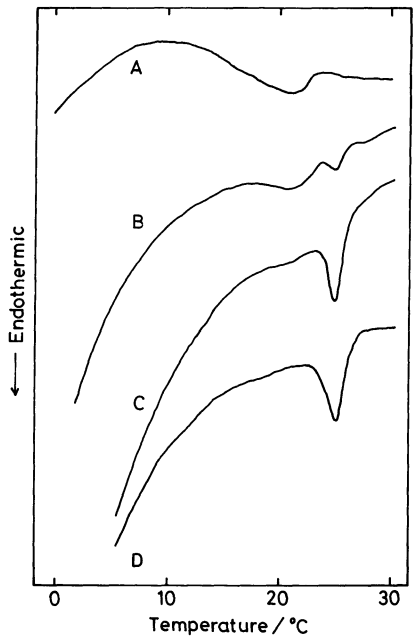


Fig. 3. Effect of added polymer on DSC behavior of the $N^+C_5Ala2C_{16}$ (5.0 mmol dm^{-3}) vesicle at pH 7.
A, aqueous solution sonicated for 3 min at 40 W without additives and incubated for 2 months at 20 °C; immediately after addition of heparin (B, 0.25 mmol dm^{-3}; C, 1.25 mmol dm^{-3}; D, 2.5 mmol dm^{-3}) at 20 °C to the aqueous solution sonicated for 3 min at 40 W. Concentrations of heparin are given in the anion unit.

confirmed by differential scanning calorimetry (DSC). The single-walled $N^+C_5Ala2C_{16}$ vesicle showed a broad phase transition at 20±5 °C (Fig. 3, curve A). The DSC thermogram remained unchanged after incubation without additives for 60 d at 20 °C or with PEG 20000 for 6 d at 20 °C. On the other hand, a phase transition peak, reflecting formation of the multi-walled bilayer membrane,[5] appeared immediately at 25.0 °C upon incubation of the vesicular solution in the presence of heparin. This DSC peak was intensified along with an increase in an amount of heparin added, and the peak originated from the single-walled vesicles nearly disappeared when a half portion of the positive charges of the lipid aggregates was neutralized with the negative ones of heparin (Fig. 3, curves B, C, and D). The results obtained from DSC measurements for the $N^+C_5Ala2C_{16}$ vesicle containing other additives, chondroitin sulfate C, chondroitin, PVSK, amylose, PVA, PEG 6000, chondrosin, and 2,6-pyridinedicarboxylic acid, were consistent with those from NSEM. Similar morphological behavior induced by anionic polymers was observed for the single-walled $N^+C_5Ala2C_{14}$ vesicle.

The aggregation-mediated vesicle growth was observed for the anionic vesicles formed with (SO_3^-)-C_5Ala2C_{16} upon addition of a cationic polymer, polybrene, as confirmed by NSEM and turbidity measurements. On the other hand, addition of heparin to the $(SO_3^-)C_5Ala2C_{16}$ vesicle did not cause any morphological changes.

In the mixed system composed of cationic and anionic peptide lipids, bilayer aggregates are formed under conditions that the total electrostatic charge on the membrane surface is not completely neutralized.[9] Multiwalled bilayers were observed in an aqueous dispersion of $N^+C_5Ala2C_{16}$ and $(SO_3^-)C_5Ala2C_{14}$ mixed at the 3:1 molar ratio (Fig. 4A), showing a relatively sharp phase transition peak (T_m) at 17.8 °C with a transition enthaply change (ΔH) of 24 kJ mol^{-1} (Fig. 5, curve A). Even though these individual lipids show phase transitions at 25.5 and 2.1 °C for $N^+C_5Ala2C_{16}$ and $(SO_3^-)C_5Ala2C_{14}$, respectively, in the aqueous dispersion state,[5,7] any phase separation between the cationic and anionic lipids was not detected by DSC when they were mixed. Upon sonication of the dispersion of the mixed lipid system for 2 min at 40 W, the

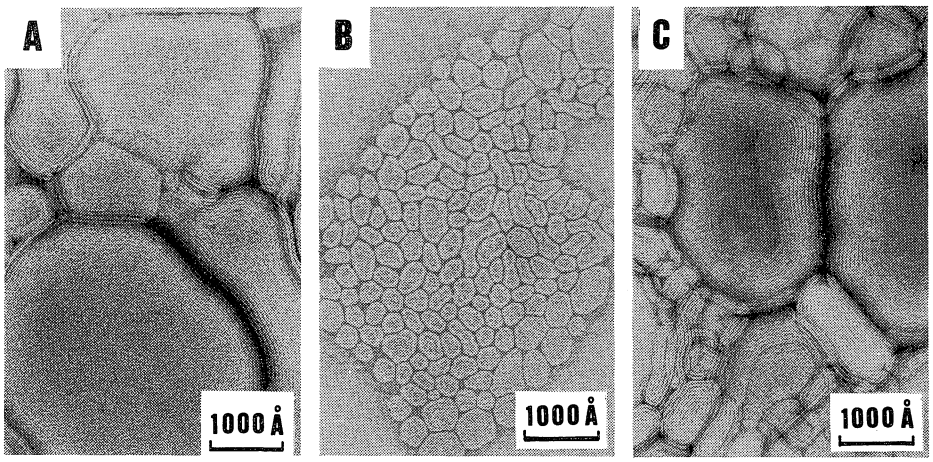


Fig. 4. Electron micrographs for a mixture of $N^+C_5Ala2C_{16}$ (1.88 mmol dm^{-3}) and $(SO_3^-)C_5Ala2C_{14}$ (0.63 mmol dm^{-3}), as negatively stained with uranyl acetate.
A, aqueous dispersion; B, aqueous solution sonicated for 3 min at 40 W; C, immediately after addition of heparin (2.0 mmol dm^{-3} in the anion unit) to B at pH 7 and 20 °C.

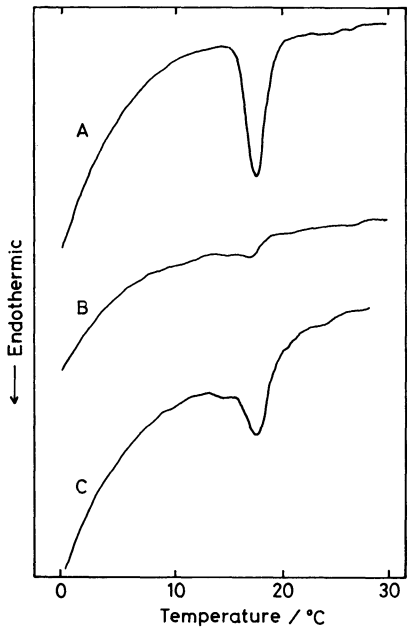


Fig. 5. DSC thermograms for a mixture of $N^+C_5Ala2C_{16}$ (1.88 mmol dm^{-3}) and $(SO_3^-)C_5Ala2C_{14}$ (0.63 mmol dm^{-3}).
Refer to the respective symbols (A, B, and C) in Fig. 4 for preparative conditions of samples.

aggregates were converted into the single-walled vesicles in the diameter range 250—800 Å (Fig. 4B), and a broad phase transition peak was observed at 17±5 °C (Fig. 5, curve B). Addition of heparin to the single-walled vesicles resulted in both aggregation and vesicle growth (Fig. 4C). The DSC thermogram showed a recovery of the sharp phase transition peak at 17.6 °C, without indicating any phase separation after such morphological changes (Fig. 5, curve C).

A mixed lipid system composed of the cationic $N^+C_5Ala2C_n$ and the nonionic QC_5Ala2C_{14} showed a unique vesicle-growing behavior in the absence of additives. The single-walled vesicles with the diameter range 200—500 Å were formed upon sonication of a homogeneous aqueous dispersion of $N^+C_5Ala2C_{14}$ and QC_5Ala2C_{14} at the 3:1 molar ratio (Fig. 6A). Although the vesicle aggregation was not detected by turbidity measurements, gradual growth of the aggregates was observed by NSEM. As shown in Fig. 6B, formation of elongated vesicles was observed in the specimen prepared after incubation for 0.5 h in addition to the original small single-walled vesicles. These aggregates were converted into larger vesicles with the diameter range 1000—4000 Å after incubation for 2 h (Fig. 6C). Such morphological changes were further examined with a combination of the cationic and nonionic lipids having different double-chain lengths. A homogeneous dispersion of $N^+C_5Ala2C_{16}$ and QC_5Ala2C_{14} at the 3:1 molar ratio showed a phase transition as detected by DSC: T_m, 19.9 °C; ΔH, 27 kJ mol^{-1}. A half-width of the endothermic peak is 3 °C and larger than that (2 °C) observed for the mixed lipid system composed of $N^+C_5Ala2C_{16}$ and (SO_3^-)-C_5Ala2C_{14} at the 3:1 molar ratio. This seems to indicate that the intramembrane domains formed by microscopic phase separation between the cationic and nonionic lipids are relatively larger than those in the cationic-anionic lipid system, although the nonlamellar phase formed with QC_5Ala2C_{14} alone was not detected by NSEM (Fig. 7A). Upon sonication of the dispersion, the multiwalled vesicles were converted into the single-walled ones with the diameter range 200—800 Å along with appearance of a broadened phase transition peak (T_m, 16±5 °C). The aggregate structure did not undergo an immediate change after sonication was performed (Fig. 7B). After the sonicated solution was allowed to stand for 3—6 d at 20 °C, very large vesicles in a diameter range of 1000—4000 Å were observed by NSEM (Fig. 7C). On the other hand,

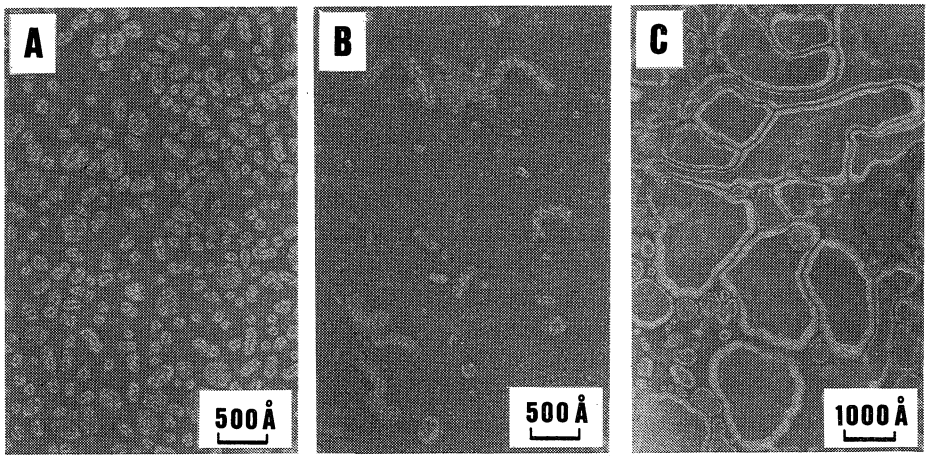


Fig. 6. Electron micrographs for a mixture of $N^+C_5Ala2C_{14}$ (3.75 mmol dm^{-3}) and QC_5Ala2C_{14} (1.25 mmol dm^{-3}), as negatively stained with uranyl acetate.
The aqueous solution sonicated for 3 min at 40 W was incubated at pH 7 and 20 °C: A, immediately after sonication; B, for 0.5 h; C, for 2 h.

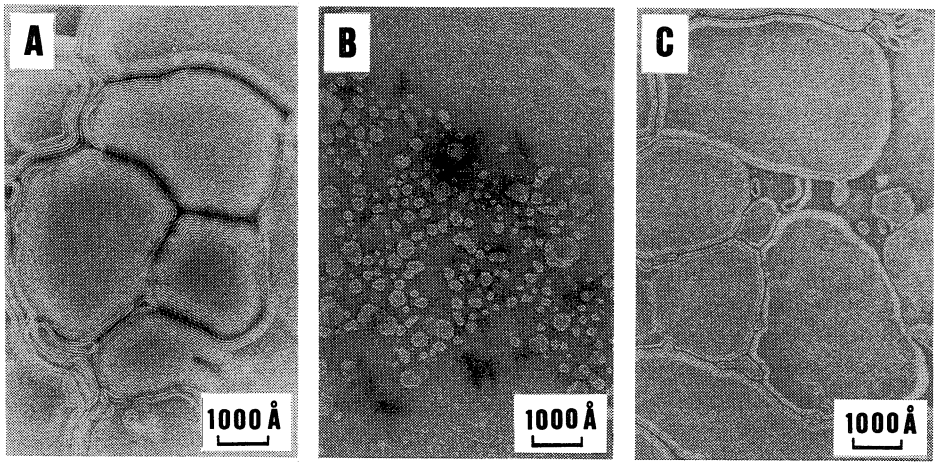


Fig. 7. Electron micrographs for a mixture of $N^+C_5Ala2C_{16}$ (3.75 mmol dm^{-3}) and QC_5Ala2C_{14} (1.25 mmol dm^{-3}), as negatively stained with uranyl acetate.
A, aqueous dispersion at pH 7 and 20 °C; B, A being sonicated at pH 7 and 20 °C for 3 min (40 W); C, B being left at 20 °C for 6 d.

the DSC thermogram characteristic of single-walled vesicles remained practically unchanged even after incubation for 6 d.

Discussion

The membrane fusion is one of the most important phenomena for a wide variety of cellular activities, and molecular mechanisms involved in the fusion process have been extensively studied with the use of liposomal membranes.[12] The fusion of bilayer membranes is generally considered to proceed through the following sequential steps; (i) aggregation of vesicles, (ii) contact of two lipid bilayers at a limited area (adhesion), (iii) intermixing of lipid molecules by transient destabilization of bilayers at the contact point (joining), and (iv) restabilization of the bilayer structure by intermixing of separated aqueous compartments of vesicles (fission).[11a,13] Medium conditions such as temperature[13a,14] and pH,[14a,15] and many effectors such as divalent cations,[12b,16] polymers,[15,17] and lipophilic agents[18] have been reported to induce the fusion of liposomal membranes. Importance of local dehydration triggered by these fusogens has been emphasized for the adhesion step.[11b,19] Furthermore, deformation of the bilayer structure must take place in the joining step, and formation of nonbilayer structures (inverted micelles or small inverted hexagonal tubes) in the bilayer-connecting domain has been suggested.[11] In this article, we are to discuss the fusion behavior of the peptide lipid vesicles with attention to the dehydration effect and the participation of nonbilayer structures.

We have previously clarified fusogenic tendencies of the single-walled vesicles of $N^+C_5Ala2C_n$ by means of NMR, ESR, DSC, NSEM, and turbidity measurements.[3,5] The single-walled vesicles prepared upon sonication are so stable as to inhibit fusion of them under ordinary conditions and stay in solution over at

least a month without meaningful morphological change. Such structural stability of the peptide lipid vesicles is superior to that of liposomes and seems to originate partly from the formation of effective hydrogen-belt domains through intravesicular hydrogen-bonding interaction among the amino acid residues. On the other hand, nonlamellar assemblies composed of three-dimensional network of globular aggregates with small internal aqueous compartments (C_{II} phase) are formed with $N^+C_5Ala2C_n$ and $(SO_3^-)C_5Ala2C_n$ mixed at the 1 : 1 molar ratio in the aqueous dispersion state.[8,9] Since these cationic and anionic lipids form bilayer membranes individually, the polar head moieties of these lipids must be considerably dehydrated through an effective electrostatic interaction to afford such a nonlamellar phase.

It has been pointed out that the interbilayer hydration force is predominant at bilayer separations less than 20 to 30 Å and that the interaction between bilayers is dominated by electrostatic and van der Waals forces beyond about 30 Å separation.[11b,19] Thus, an essential part of fusion event must arise from the ability of membrane surfaces to overcome or circumvent the hydration barrier in order that bilayer surfaces get into contact with each other.[19] In our preliminary communication,[20] we have shown that fusion of the single-walled vesicles formed with peptide lipids takes place via formation of the intermediate nonlamellar phase as triggered by medium conditions, pH and/or temperature. Apparently, repulsion between the hydrated vesicular surfaces is circumvented by formation of the nonbilayer structure in which the lipid molecules are largely dehydrated due to an electrostatic intramembrane attraction. Dehydration of the peptide lipid bilayer may also be achieved through an electrostatic interaction on the membrane surface between lipid and additive molecules having opposite charges to each other. Indeed, fusion of the single-walled vesicles took place upon addition of polymers having the opposite charges (Figs. 2 and 3) via aggregation of the vesicles (Fig. 1). Thus, the electrostatic interaction between the vesicle and the polymer to give charge neutralization on the membrane surface becomes a potent driving force which allows not only the vesicles to aggregate but also the two membrane surfaces to come into contact. An amount of heparin, which neutralizes a half portion of the whole cationic charges of the lipid molecules present, is required for complete transformation of the cationic single-walled vesicle into the multiwalled bilayer, as confirmed by DSC (Fig. 3). This implies that the electrostatic interaction between the cationic vesicular surface and the anionic polymer is so large as to inhibit migration of the polymer molecule to the other vesicles. Since neither aggregation nor fusion was observed for the cationic vesicles in the presence of chondrosin or 2,6-pyridinedicarboxylic acid, the extended charge neutralization on the vesicular surface with polymer species seems to be essential to induction of the fusion. Accordingly, deformation of the bilayer structure can be achieved through strong electrostatic interactions between lipid and polymer molecules on the membrane surface and among charged head moieties of cationic and anionic peptide lipids.[8,9] Such changes in the packing mode of lipid molecules are accompanied undoubtedly with dehydration of lipid molecules so as to reduce the hydration barrier.

As for the mixed lipid systems composed of cationic and anionic peptide lipids, another characteristic feature was found to be present in the polymer-lipid interaction. The single-walled vesicles formed with $N^+C_5Ala2C_{16}$ and $(SO_3^-)C_5Ala2C_{14}$ at the 3 : 1 molar ratio underwent the heparin-induced fusion (Figs. 4 and 5), although the apparent surface charge density of the mixed-lipid vesicle is a half of that provided with the $N^+C_5Ala2C_{16}$ vesicle. Since homogeneous mixing of these ionic lipids was retained even after completion of the fusion as confirmed by DSC, a polymer-triggered phase separation does not seem to occur during the fusion. Thus, deformation of the bilayer structure, which results in the fusion, presumably comes from the cooperative assistance of polymer-lipid and lipid-lipid electrostatic interactions in the system.

QC_5Ala2C_{14} forms the inverted cubic phase through strong hydrogen-bonding interaction among the polar head moieties in the aqueous dispersion state.[6] Addition of this nonbilayer-forming lipid to the cationic peptide lipid, $N^+C_5Ala2C_n$, resulted in slow vesicle growth relative to the vesicle growth induced by ionic polymers (Figs. 6 and 7). In spite of such a disadvantage in the fusion rate, the membrane adhesion followed by deformation of the bilayer structure occurred in this system, QC_5Ala2C_{14} undoubtedly acting as a fusogen toward the vesicles. Formation of the elongated vesicles observed for the $N^+C_5Ala2C_{14}$-QC_5Ala2C_{14} (3 : 1) system (Fig. 6B) at the initial stage of morphological changes needs to be subjected to mechanistic consideration. Such vesicle growth to the transverse direction must be originated from a preferential fusogenic tendency of the vesicles at the surface domain having larger curvature. In fact, smaller liposomes formed with phosphatidylserine or phosphatidylcholine have an intrinsically greater capacity to fuse than larger vesicles.[14d,21] Relaxation of the packing strain imposed on the highly curved portion of the bilayers becomes a trigger of such vesicle growth. In the QC_5Ala2C_{14}-rich domains formed by microscopic phase separation as detected by DSC, such a strain effect must be much enhanced.

In conclusion, the fusion of single-walled vesicles formed with the ionic peptide lipids proceeds under the following conditions: (i) as for the vesicular systems of $N^+C_5Ala2C_n$ and $(SO_3^-)C_5Ala2C_n$ individually or mixed together to an extent that the surface charges are not completely neutralized, ionic polymers having the opposite charges to the net vesicular surface

charges are added; (ii) as for the $N^+C_5Ala2C_n$ vesicular system containing QC_5Ala2C_{14}, the latter lipid acts as a fusogen without polymer additives. Since vesicle aggregation is not always followed by fusion of bilayers,[21b,22] the present ionic polymers induce aggregation of the vesicles prior to the membrane adhesion and subsequently give out perturbation on the vesicular surface so that the bilayer structure is effectively deformed. Even in the absence of fusion-inducing polymers, incorporation of a nonbilayer-forming lipid into the vesicular system results in induction of the fusion process. Although the nonbilayer structure was not detected during the membrane fusion process under the present conditions, formation of such an intermediate phase in a microscopic portion of the bilayer-joining domain is much plausible as a consequence of dehydration of the polar head moieties of lipids.

References

1) J. H. Fendler, "Membrane Mimetic Chemistry," John Wiley, New York (1982), Chap. 6.

2) H. Brockerhoff, "Bioorganic Chemistry," ed by E. E. van Tamelen, Academic Press, New York (1977), Vol. 3, Chap. 1.

3) Y. Murakami, A. Nakano, and K. Fukuya, *J. Am. Chem. Soc.*, **102**, 4253 (1980).

4) Y. Murakami, A. Nakano, and H. Ikeda, *J. Org. Chem.*, **47**, 2137 (1982).

5) Y. Murakami, A. Nakano, A. Yoshimatsu, K. Uchitomi, and Y. Matsuda, *J. Am. Chem. Soc.*, **106**, 3613 (1984).

6) Y. Murakami, A. Nakano, J. Kikuchi, T. Takaki, and K. Uchimura, *Chem. Lett.*, **1983**, 1891.

7) Y. Murakami, J. Kikuchi, T. Takaki, K. Uchimura, and A. Nakano, *J. Am. Chem. Soc.*, **107**, 2161 (1985).

8) Y. Murakami, J. Kikuchi, T. Takaki, and K. Uchimura, *J. Am. Chem. Soc.*, **107**, 3373 (1985).

9) Y. Murakami, J. Kikuchi, T. Takaki, and K. Uchimura, *Bull. Chem. Soc. Jpn.*, **59**, 515 (1986).

10) Y. Murakami, J. Kikuchi, T. Takaki, and K. Uchimura, *Chem. Lett.*, **1986**, 325.

11) a) A. J. Verkleij, *Biochim. Biophys. Acta*, **779**, 43 (1984); b) R. P. Rand, *Ann. Rev. Biophys. Bioeng.*, **10**, 277 (1981); c) A. J. Verkleij, J. Leunissen-Bijvelt, B. De Kruijff, M. Hope, and P. R. Cullis, "Cell Fusion," Ciba Foundation Symposium 103, Pitman Books, London (1984), pp. 45—59.

12) a) M. Gratzel, C. Schudt, R. Ekerdt, and G. Dahl, "Membrane Structure and Function," ed by E. E. Bittar, John Wiley, New York (1980), Vol. 3, pp. 59—92; b) D. Papahadjopoulos, G. Poste, and W. J. Vail, *Methods Membr. Biol.*, **10**, 1 (1979).

13) a) S. W. Hui, T. P. Stewart, L. T. Boni, and P. L. Yeagle, *Science*, **212**, 921 (1981); b) P. R. Cullis and M. J. Hope, *Nature (London)*, **271**, 672 (1978).

14) a) A. M. Haywood and B. P. Boyer, *Biochemistry*, **21**, 6041 (1982); b) M. Wong, F. H. Anthony, T. W. Tillack, and T. E. Thompson, *ibid.*, **21**, 4126 (1982).

15) P. S. Uster and D. W. Deamer, *Biochemistry*, **24**, 1 (1985).

16) a) D. Papahadjopoulos, G. Poste, B. E. Schaefer, and W. J. Vail, *Biochim. Biophys. Acta*, **352**, 10 (1974); b) S. Nir, J. Bentz, J. Wilschut, and N. Düzgünes, *Prog. Surf. Sci.*, **13**, 1 (1983).

17) C.-Y. Wang and L. Huang, *Biochemistry*, **23**, 4409 (1984); A. E. Gad, B. L. Silver, and G. D. Eytan, *Biochim. Biophys. Acta*, **690**, 124 (1982); R. I. MacDonald, *Biochemistry*, **24**, 4058 (1985).

18) D. Papahadjopoulos, S. Hui, W. J. Vail, and G. Poste, *Biochim. Biophys. Acta*, **448**, 245 (1976); J. Sunamoto, T. Hamada, and H. Murase, *Bull. Chem. Soc. Jpn.*, **53**, 2773 (1980).

19) V. A. Parsegian, R. P. Rand, and D. Gingell, "Cell Fusion," Ciba Foundation Symposium 103, Pitman Books, London (1984), pp. 9—27.

20) Y. Murakami, J. Kikuchi, and T. Takaki, *Chem. Lett.*, **1985**, 1899.

21) a) J. Wilschut, N. Düzgünes, R. Fraley, and D. Papahadjopoulos, *Biochemistry*, **19**, 6011 (1980); b) J. Wilschut, N. Düzgünes, and D. Papahadjopoulos, *ibid.*, **20**, 3126 (1981); c) S. Nir, J. Wilschut, and J. Bentz, *Biochim. Biophys. Acta*, **688**, 275 (1982); d) J. Bentz and N. Düzgünes, *Biochemistry*, **24**, 5436 (1985); e) D. Lichtenberg, E. Freire, C. F. Schmidt, Y. Barenholz, P. L. Felgner, and T. E. Thompson, *ibid.*, **20**, 3462 (1981).

22) F. Schuber, K. Hong, N. Düzgünes, and D. Papahadjopoulos, *Biochemistry*, **22**, 6134 (1983); A. E. Gad, M. Bental, G. Elyashiv, and H. Weinberg, *ibid.*, **24**, 6277 (1985); L. A. M. Rupert, D. Hoekstra, and J. B. F. N. Engberts, *J. Am. Chem. Soc.*, **107**, 2628 (1985).