CHEMISTRY LETTERS, pp. 1899-1902, 1985.

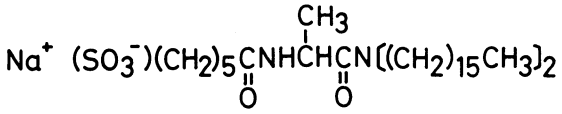
PARTICIPATION OF NONLAMELLAR PHASE IN FUSION OF BILAYER MEMBRANES FORMED WITH SYNTHETIC PEPTIDE LIPIDS

Yukito MURAKAMI,* Jun-ichi KIKUCHI, and Toshihiko TAKAKI
Department of Organic Synthesis, Faculty of Engineering,
Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812

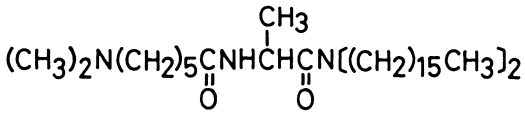
Participation of the nonlamellar phase in the fusion process of bilayer membranes was examined with an equimolar mixture of sodium N,N-dihexadecyl-N^{α}-(6-sulfohexanoyl)-L-alaninamide and N,N-dihexadecyl-N^{α}-[6-(dimethylamino)hexanoyl]-L-alaninamide in aqueous media by means of electron microscopy, differential scanning calorimetry, and turbidity measurements.

The membrane fusion is an extremely important morphological process in biological systems. It has been postulated recently that nonbilayer structures having molecular packing modes of inverted micelles or lipidic particles are directly involved in the fusion process.[1] Although nonbilayer structures such as inverted hexagonal and cubic ones often appear as stable phases in natural lipid systems,[2] such morphological states are difficult to be trapped during fusion even by adopting quick-freezing techniques.[3] In this communication, we show the first example of stable nonbilayer aggregates appeared in an intermediate state of the membrane fusion by employing a combination of two kinds of synthetic peptide lipids; sodium N,N-dihexadecyl-N^{α}-(6-sulfohexanoyl)-L-alaninamide [$(SO_3^-)C_5Ala2C_{16}$][4] and N,N-dihexadecyl-N^{α}-[6-(dimethylamino)hexanoyl]-L-alaninamide [NC_5Ala2C_{16}].[5]

We have clarified previously that globular aggregates having highly ordered and three-dimensional arrangement of small internal aqueous compartments are formed with an equimolar mixture of cationic and anionic peptide lipids in the aqueous dispersion state.[6] A similar nonlamellar assembly has also been observed in the aqueous dispersion of a nonionic peptide lipid having the quinoyl moiety as its head group.[7] Strong associative interactions, such as electrostatic and hydrogen bonding ones, among head moieties of such lipids seem to control the dynamic packing mode of lipid molecules and to be responsible for stabilization of the nonlamellar phase. This aggregate morphology is structurally related to lipidic particles observed for natural lipid systems. Since NC_5Ala2C_{16} employed here acts



$(SO_3^-)C_5Ala2C_{16}$



NC_5Ala2C_{16}

as either a cationic lipid or a nonionic one as controlled by pH, the mixed lipid system composed of $(SO_3^-)C_5Ala2C_{16}$ and NC_5Ala2C_{16} may provide bilayer and non-bilayer aggregates alternatively by changing the medium pH.

When an equimolar mixture of $(SO_3^-)C_5Ala2C_{16}$ and NC_5Ala2C_{16} was dispersed in water, multiwalled bilayer aggregates were observed exclusively at pH 12.0 where the NC_5Ala2C_{16} molecule is present in the nonionic form. Turbidity of an aqueous dispersion of the mixed lipids is shown in Fig. 1 (point A). The phase transition of the aggregate between gel and liquid-crystalline states occurred at 22.9 °C (T_m) with an enthalpy change (ΔH) of 29 kJ mol^{-1} as confirmed by differential scanning calorimetry (DSC) (line A in Fig. 2). These parameters are in good agreement with those evaluated for multiwalled bilayer membranes composed of the individual ionic peptide lipids: T_m = 25.0 °C, ΔH = 30 kJ mol^{-1} for $(SO_3^-)C_5Ala2C_{16}$;[4] T_m = 24.8 °C, ΔH = 28 kJ mol^{-1} for the cationic form of NC_5Ala2C_{16}.[8] Upon sonication of this aqueous dispersion with a probe-type sonicator for 10 min at 50 W, the turbidity decreased to an extent that gives a clear solution (point B in Fig. 1). The corresponding DSC thermogram (line B in Fig. 2) shows a broader phase transi-

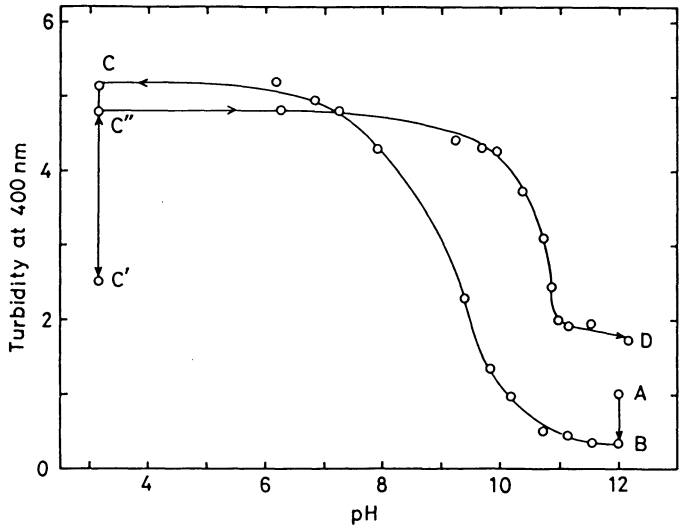


Fig. 1. Correlations of turbidity with pH for an equimolar mixture (0.5 mmol dm^{-3} each) of $(SO_3^-)C_5Ala2C_{16}$ and NC_5Ala2C_{16}: A, dispersed in water at pH 12.0 and 40 °C; B, after sonication of A; C, pH of B being adjusted to 3.0; C', temperature of C being lowered to 20 °C; C", temperature of C' being raised to 40 °C; D, pH of C" being adjusted to 12; temperature for measurements, 40 °C except for C'.

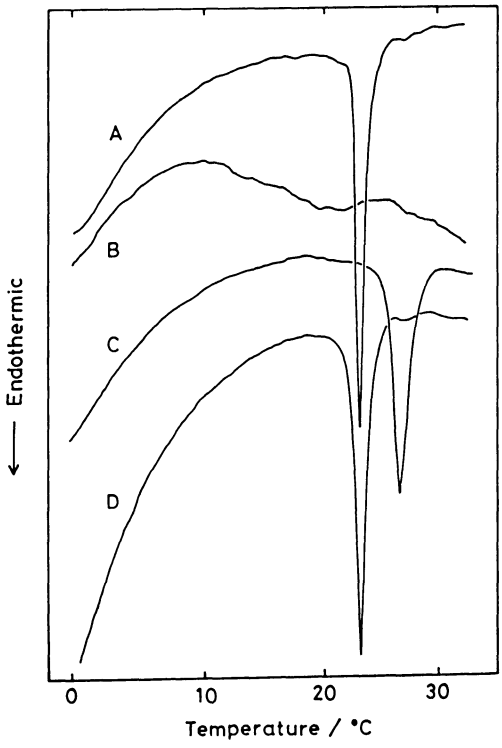


Fig. 2. DSC thermograms for an equimolar mixture (2.5 mmol dm^{-3} each) of $(SO_3^-)C_5Ala2C_{16}$ and NC_5Ala2C_{16}: refer to the respective symbols (A, B, C, and D) in Fig. 1 for preparative conditions of samples.

tion peak along with a shift to the lower temperature range (20 ± 5 °C). This thermodynamic behavior is identical with that observed for N,N-dihexadecyl-N^{α}-[6-(trimethylammonio)hexanoyl]-L-alaninamide bromide [$N^{+}C_5Ala2C_{16}$],[5] indicating the formation of single-walled bilayer vesicles having large vesicular curvatures. This aggregate morphology remained the same for several days at the same pH as confirmed by DSC and turbidity measurements.

Turbidity of the aqueous solution containing single-walled vesicles gradually increased as the pH of medium was lowered at 40.0 °C (above T_m), reflecting a morphological change induced by protonation of NC_5Ala2C_{16} at the tertiary amino group (Fig. 1, B ⟶ C). When the protonation of NC_5Ala2C_{16} was completed at pH 3.0, formation of the nonlamellar phase, having internal aqueous compartments with a repeating distance of 120 Å, was confirmed by the negative staining electron microscopy for a sample prepared at 50 °C (Fig. 3). We have already clarified that such a nonlamellar phase formed with a mixture of cationic and anionic peptide lipids

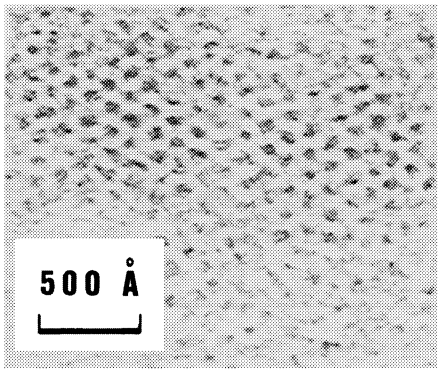


Fig. 3. Electron micrograph for an equimolar mixture (2.5 mmol dm^{-3} each) of $(SO_3^{-})C_5Ala2C_{16}$ and NC_5Ala2C_{16} in the dispersion state at pH 3.0; negatively stained with uranyl acetate at 50 °C, taken on a JEOL JEM-200B electron microscope.

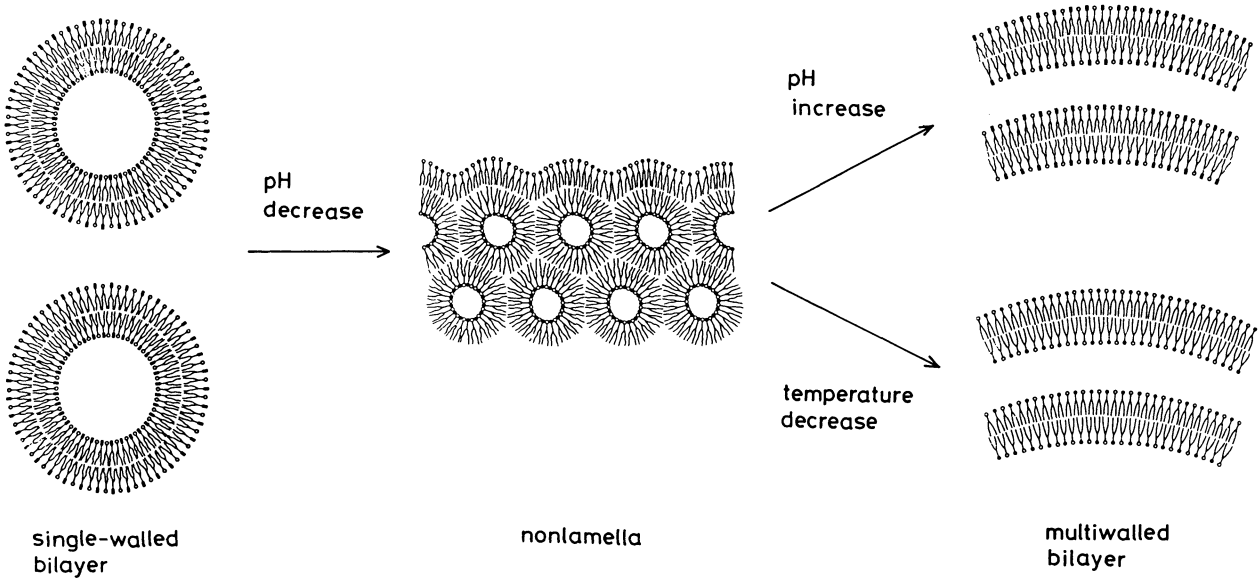


Fig. 4. Schematic representation of morphological changes for an equimolar mixture of $(SO_3^{-})C_5Ala2C_{16}$ (○⊂) and NC_5Ala2C_{16} (■⊂ and ●⊂ for nonionic and cationic forms, respectively); fusion of single-walled bilayer vesicles to afford multiwalled ones via an intermediate nonlamellar phase.

appears under the conditions that an overall charge on the aggregate surface is completely neutralized in a temperature range above T_m.[6,8] The nonlamellar phase was spontaneously converted into the multiwalled bilayer below T_m (point C' in Fig. 1).[8] The T_m value for the aggregate at pH 3.0 (27.0 °C) is somewhat higher than that observed at pH 12. This effect presumably originates from the compact molecular packing of the head moieties in the former aggregate through the formation of tight ion-pairs (line C in Fig. 2). Transformation of the nonlamellar assembly into the multiwalled bilayer also took place as the pH of medium was raised in the temperature range above T_m; reflected on a turbidity change (Fig. 1, C'' → D) and a DSC thermogram (T_m = 23.2 °C, ΔH = 30 kJ mol^{-1}; line D in Fig. 2).

In conclusion, it became apparent that the fusion of bilayer vesicles takes place via formation of the intermediate nonlamellar phase as induced by changing medium conditions, pH and/or temperature (Fig. 4). At first, single-walled bilayer vesicles with the negative surface charge are formed with an equimolar mixture of $(SO_3^-)C_5Ala2C_{16}$ and NC_5Ala2C_{16} and converted into the nonlamellar aggregate upon neutralization of the surface charge performed by protonation of the latter nonionic lipid at the tertiary amino group in the temperature range above T_m. Secondly, the nonlamellar phase is transformed into the multiwalled bilayer either by deprotonation of the cationic NC_5Ala2C_{16} or by lowering temperature below the phase transition. The morphological changes observed for the present mixed lipid system suggest possible participation of the nonlamellar phase during the fusion process of biomembranes. In contrast, single-walled vesicles composed of a single species of cationic peptide lipids, such as $N^+C_5Ala2C_{16}$, do not undergo fusion to any detectable extent.[5] Accordingly, any physicochemical perturbations on the vesicular surface may induce vesicular fusion via formation of the intermediate nonlamellar phase. Our further studies are in progress along this line.

References

1) A. J. Verkleij, Biochim. Biophys. Acta, 779, 43 (1984).
2) V. Luzzati, T. Gulik-Krzywicki, and A. Tardieu, Nature (London), 218, 1031 (1968); S. W. Hui and L. T. Boni, ibid., 296, 175 (1982); P. R. Cullis and B. De Kruijff, Biochim. Biophys. Acta, 559, 399 (1979).
3) E. L. Bearer, N. Düzgüneş, D. S. Friend, and D. Papahadjopoulos, Biochim. Biophys. Acta, 693, 93 (1982).
4) Y. Murakami, J. Kikuchi, T. Takaki, K. Uchimura, and A. Nakano, J. Am. Chem. Soc., 107, 2161 (1985).
5) Y. Murakami, A. Nakano, A. Yoshimatsu, K. Uchitomi, and Y. Matsuda, J. Am. Chem. Soc., 106, 3613 (1984).
6) Y. Murakami, J. Kikuchi, T. Takaki, and K. Uchimura, J. Am. Chem. Soc., 107, 3373 (1985).
7) Y. Murakami, A. Nakano, J. Kikuchi, T. Takaki, and K. Uchimura, Chem. Lett., 1983, 1891.
8) Y. Murakami, J. Kikuchi, T. Takaki, and K. Uchimura, Bull. Chem. Soc. Jpn., submitted for publication.

(Received September 26, 1985)