

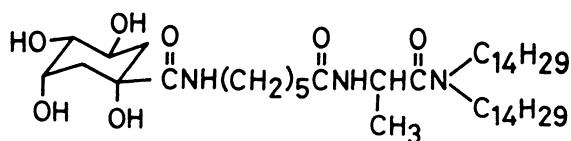
MORPHOLOGICAL CHANGE INDUCED BY INTERMEMBRANE INTERACTION OF SYNTHETIC PEPTIDE LIPIDS BEARING CATIONIC AND NONIONIC HEAD GROUPS

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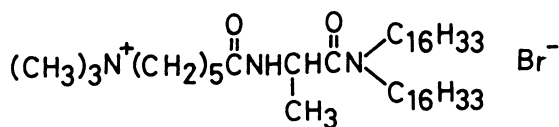
The intermembrane lipid transfer between two different aggregates individually composed of *N,N*-ditetradecyl-*N*^α-(6-quinoylamino)hexanoyl-L-alaninamide and *N,N*-dihexadecyl-*N*^α-(6-trimethylammonio)hexanoyl-L-alaninamide bromide induced the morphological change of individual aggregates and ultimately resulted in the formation of small vesicles as confirmed by electron microscopy and differential scanning calorimetry.

Extensive studies on organizations and functions of biomembranes gave impetus to rapid growth of "synthetic bilayer membrane" chemistry.¹⁾ We reported previously that the cationic and zwitterionic peptide-amphiphiles, having two long alkyl chains, form stable bilayer assemblies in aqueous media.²⁾ In order to gain further understanding on the physicochemical characteristics of peptide-amphiphile aggregates, we prepared nonionic peptide-amphiphile 1, having the quinic acid moiety as a head group.³⁾ We report here the intermembrane interaction between two different kinds of membranes formed with amphiphiles having a nonionic head group on one hand and a cationic one on the other.

While cationic peptide-amphiphile 2 forms multilayered bilayer aggregates (vesicle and lamella) in dispersion state,⁴⁾ the aggregate morphology for nonionic amphiphile 1 in aqueous media is quite different from that for the cationic one (2). As shown in Fig. 1a, the electron micrograph for an aqueous dispersion of 1 shows some highly ordered network structure with a repeating distance of 70 Å, which corresponds to the magnitude of the thickness of two lipid monolayers (ca. 40 Å) plus the diameter of an inner aqueous compartment (ca. 30 Å) as shown schematically in Fig. 2. The formation of relatively small aqueous compartments (ca. 35 Å diameter), as compared with the inner water pool of single-walled liposomes (150-200 Å diameter), has been observed for a sonicated aqueous dispersion of mono- and digalactosyldiacylglycerols mixed at molar ratio 2:1.⁵⁾ Since both head groups, galactosyl and quinoyl, bear a large number of the hydroxyl group, lipid molecules involving such head groups readily undergo an effective intermolecular hydrogen-bonding interaction within each vesicle. Thus, the hydrogen-bonding effect must be the primary important factor controlling the aggregate morphology observed for 1.



1



2

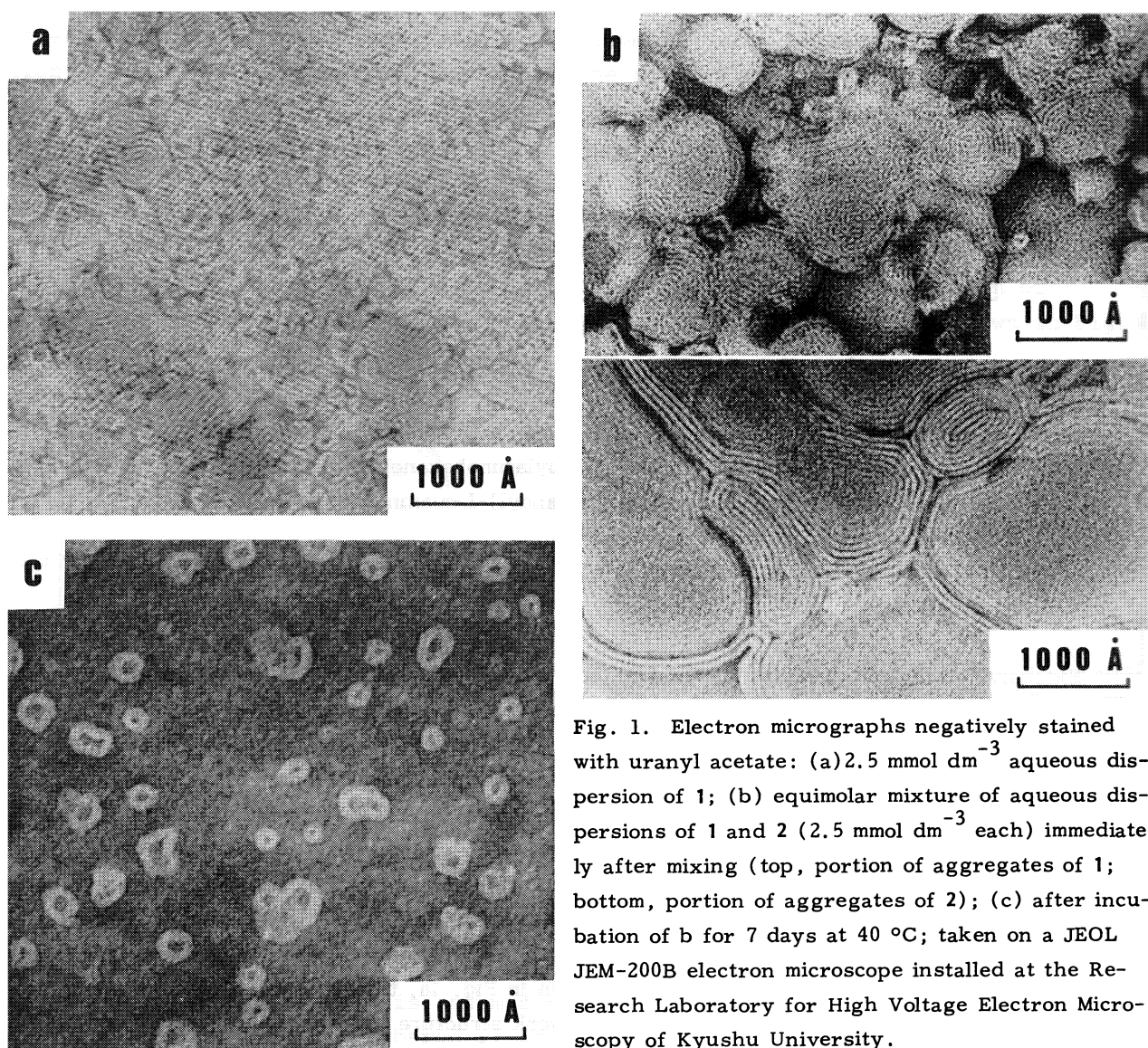


Fig. 1. Electron micrographs negatively stained with uranyl acetate: (a) 2.5 mmol dm^{-3} aqueous dispersion of 1; (b) equimolar mixture of aqueous dispersions of 1 and 2 (2.5 mmol dm^{-3} each) immediately after mixing (top, portion of aggregates of 1; bottom, portion of aggregates of 2); (c) after incubation of b for 7 days at $40 \text{ }^\circ\text{C}$; taken on a JEOL JEM-200B electron microscope installed at the Research Laboratory for High Voltage Electron Microscopy of Kyushu University.

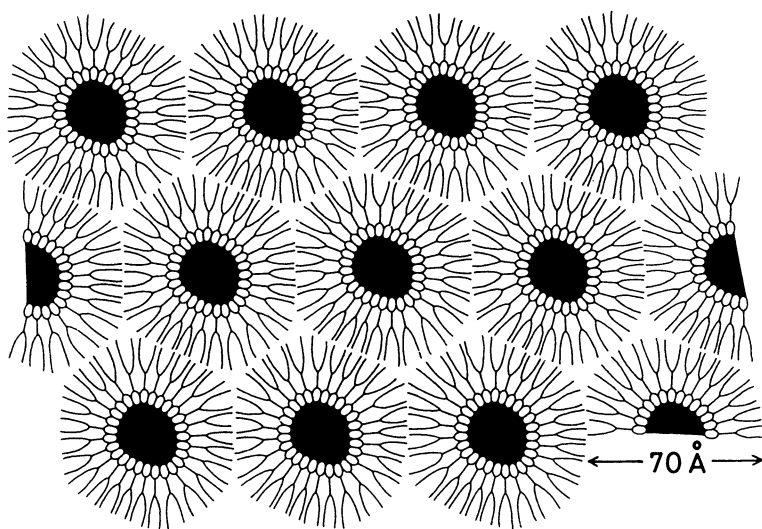


Fig. 2. Schematic representation of the aggregate structure of nonionic amphiphile 1 in aqueous media (refer to Fig. 1a); each black portion indicates an aqueous compartment, and each small ellipse is the head group.

When an equimolar mixture of 1 and 2 (2.5 mmol dm^{-3} each), prepared by mixing individual aqueous dispersions, was incubated at $40 \text{ }^\circ\text{C}$, the aggregate morphology for each species underwent gradual change. The turbidity observed for the mixture at 400 nm decreased in a reverse sigmoidal manner showing a half-life of about one day. This implies that the size of the molecular aggregates was reduced with time. In the light of an electron micrograph observed for the mixture immediately after the incubation was initiated, aggregate structures of individual amphiphile species were retained independent of each other (Fig. 1b). However, double-layered small vesicles with $250\text{--}500 \text{ \AA}$ diameter appeared after incubation for 7 days (Fig. 1c), even though larger multilayered aggregates still remained.

The change in aggregate morphology was further confirmed by the differential scanning calorimetry (DSC). The phase transition temperatures (T_m , temperature at a peak maximum of DSC thermogram) for amphiphiles 1 and 2 were observed to be 2.5 and $25.5 \text{ }^\circ\text{C}$, respectively (Fig. 3, a and b). The DSC thermogram for the mixture immediately after initiation of the incubation gave two endothermic peaks which exactly correspond to those for aqueous dispersions of 1 and 2 as measured separately (Fig. 3c). These endothermic peaks were, however, broadened with time and new peaks appeared in the region lying between both peaks (Fig. 3, d-g) after prolonged incubation time. Undoubtedly, these changes reflect the intermembrane lipid transfer. An equimolar mixture of 1 and 2 dissolved in chloroform was evaporated to dryness and then dispersed in water. The sample showed a broad DSC peak at $15 \text{ }^\circ\text{C}$ (Fig. 3h). Since the sample involves amphiphiles 1 and 2 homogeneously distributed within each aggregate, the DSC thermogram for this sample must correspond to that for the mixture of individual dispersions incubated for an infinitely prolonged period of time. In addition, the phase transition temperature for a homogeneous dispersion of 1 and 2 was found to be

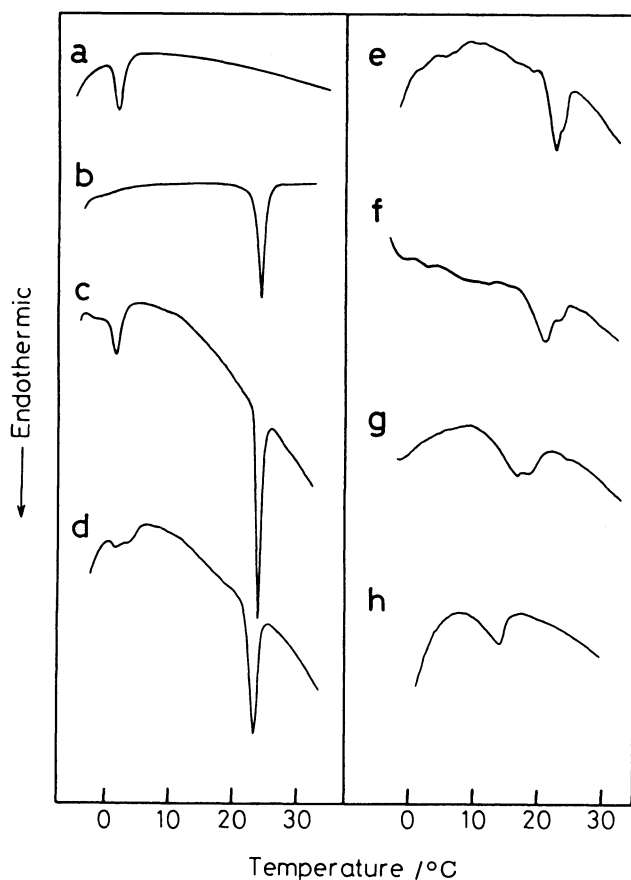


Fig. 3. DSC thermograms for aqueous dispersions of peptide lipids: (a) 1 (2.5 mmol dm^{-3}), (b) 2 (2.5 mmol dm^{-3}). An equimolar mixture of aqueous dispersions of 1 and 2 (2.5 mmol dm^{-3} each) was incubated at $40 \text{ }^\circ\text{C}$ for the following periods of time: (c) immediately after mixing, (d) 8 h, (e) 20 h, (f) 50 h, and (g) 240 h; (h) a homogeneous mixture of 1 and 2 (2.5 mmol dm^{-3} each), being equivalent to the sample incubated for an infinitely prolonged time.

dependent on the molar ratio of the two amphiphiles. Analogous DSC behavior due to the intermembrane lipid transfer has been observed for the dimyristoyl and dipalmitoyl phosphatidylglycerol systems.⁶⁾

It became apparent in this study that the intermembrane lipid transfer between aggregates composed of a cationic peptide lipid and those of a nonionic one induces the morphological change of individual aggregates and leads to the formation of smaller vesicles. Several factors controlling formation and stabilization of small vesicles are pointed out as follows: molecular shapes of amphiphiles,⁷⁾ strong hydration of hydroxyl groups placed in the head portion of nonionic amphiphile molecules,⁸⁾ hydrogen-bonding interaction effective in the so-called hydrogen belt domain,^{2,8)} unequal distribution of two different kinds of amphiphiles between outer and inner layers in each vesicle, etc. While extensive studies have been carried out on the vesicle fusion,^{6,9)} the reversed morphological change of bilayer aggregates, fission, has been little studied. Further investigations on the lipid-lipid interactions between molecular aggregates formed separately with peptide lipids bearing various head groups are expected to shed light on mechanisms of the vesicle fission.

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- 3) Prepared from the reaction of tetraacetylquinoyl chloride with N,N-ditetradecyl-N^α-(6-aminohexanoyl)-L-alaninamide and purified by repeated gel-filtration chromatography: liquid crystal with final mp 85 °C, $[\alpha]_D^{25} -44.3^\circ$ (c 1.00, EtOH); ¹H-NMR (CDCl₃, Me₄Si) δ 0.88 [6H, t, (CH₂)₁₃CH₃], 1.17 [51H, m, CH₂(CH₂)₁₂CH₃ and CH(CH₃)], ≈ 1.5 [6H, m, NHCH₂(CH₂)₃CH₂CO], 2.9-3.3 [6H, m, CONHCH₂ and NCH₂(CH₂)₁₂CH₃], 3.45 [1H, dd, CH(OH)CH(OH)CH(OH)], 3.8-4.5 [2H, m, CH(OH)CH₂], and 4.75 [1H, m, CH(CH₃)]. Found: C, 68.45; H, 11.32; N, 5.29%. Calcd for C₄₄H₈₅N₃O₇: C, 68.79; H, 11.15; N, 5.48%.
- 4) Cationic peptide-amphiphiles involving an L-alanine residue, [(CH₃)₃N⁺(CH₂)_mCONHCH(CH₃)CO-N(C_nH_{2n+1})₂]Br⁻ (m = 5, n = 8, 10, 12, 14, 16, 18; n = 16, m = 2, 7, 10), have been prepared and their aggregate structures in dispersion and sonicated states have been identified in our laboratory: Y. Murakami, A. Nakano, K. Uchitomi, and A. Yoshimatsu, unpublished results.
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