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MOLECULAR DESIGN OF SYNTHETIC LIPIDS FORMING INVERTED HEXAGONAL PHASE

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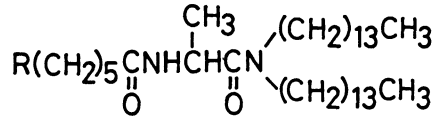
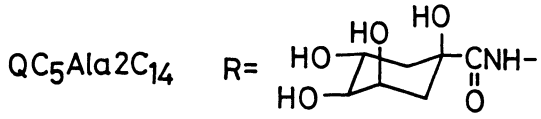
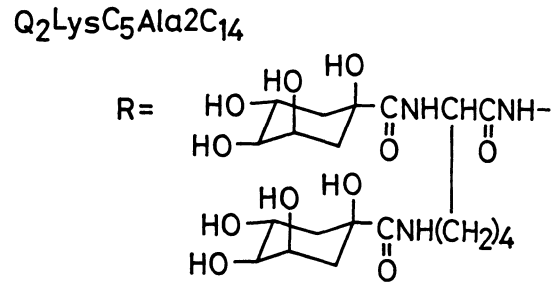
The inverted hexagonal phase was formed with mixed lipid systems composed of two species of synthetic lipids, the one having two quinoyl moieties and the other holding a single hydroxyl moiety as their head groups, as confirmed by negative staining electron microscopy and selected area electron diffraction measurements.

We have recently reported that nonbilayer aggregates were readily formed with synthetic peptide lipids by enhancing intramembrane packing of polar head moieties of the lipids through hydrogen-bonding[1] or electrostatic interactions.[2] In the light of low-angle X-ray diffraction measurements carried out above the phase transition temperatures, the nonbilayer phase was constituted with highly developed globular domains with the face-centered cubic lattice.[3] Much attention has been focused on nonbilayer structures in relation to their postulated dynamic functions in biomembranes,[4] and some plausible theories have been proposed to explain self-assembling behavior of lipid molecules to afford micelles, bilayers, and nonbilayers.[5] Although we have successfully developed synthetic lipid systems that produce inverted cubic (C_{II}) aggregates, molecular design of synthetic lipids, which selectively constitute the inverted hexagonal (H_{II}) phase, remains to be explored. In this regard, we report here that appropriate combinations of nonionic synthetic lipids can afford the H_{II} phase through a homogeneous self-assembling process at room temperature and discuss the molecular basis for designing nonbilayer-forming lipids.

While a nonionic peptide lipid having a head moiety with four hydroxyl groups, QC_5Ala2C_{14}, affords a stable C_{II} phase composed of three-dimensional network of







globular aggregates with small internal aqueous compartments (diameter, ca. 30 Å),[1] another nonionic lipid having eight hydroxyl groups, $Q_2LysC_5Ala2C_{14}$,[6] forms normal bilayer aggregates in the aqueous dispersion state as confirmed by negative staining electron microscopy (NSEM).[7] This marked difference in aggregate morphology led us to explore the formation of another nonbilayer phase, i.e. the H_{II} phase, by controlling the intramembrane packing mode of polar head moieties of synthetic lipids.

We examined the aggregate morphology of mixed lipid systems composed of two different nonionic peptide lipids having hydroxyl groups, $Q_2LysC_5Ala2C_{14}$ and $(HO)C_5Ala2C_{14}$,[8] on the following grounds. (i) In order to adopt the nonbilayer phase (H_{II} or C_{II}), critical packing parameter v/a_0l_c must be greater than 1, where v, a_0, and l_c are the hydrocarbon volume, optimal surface area, and critical chain length of a lipid molecule, respectively.[5a,b] The hydrogen-bonding interaction among polar head moieties of lipids may provide a suitable effect in controlling the packing geometry of lipid molecules. (ii) Since both lipids have the common molecular structure except for their head moieties, the structural difference is mainly reflected on the a_0 value but not on the v and l_c values. Thus, a lipid with a smaller a_0 value relative to that for QC_5Ala2C_{14} tends to form nonbilayer aggregates with smaller inner aqueous compartments in dilute aqueous solutions and presumably results in molecular packing to afford the H_{II} phase rather than the C_{II} one. (iii) Bulkiness of the head moieties of these nonionic lipids can be roughly estimated on the basis of the number of hydroxyl groups per lipid molecule (n), and a lipid having the number smaller than 4 would tend to constitute the H_{II} phase. As for a mixed lipid system, an average number of the hydroxyl groups is used for estimation of the bulkiness and subjected to change by the mixing ratio.

$Q_2LysC_5Ala2C_{14}$ and $(HO)C_5Ala2C_{14}$ were dispersed in water at the 1:3 molar ratio (n = 2.75), and the aggregate morphology was observed by NSEM. As shown in Fig. 1, two types of striped patterns with different layer thickness, 50 and 30 Å of repeating distances of the layers (ratio, ca. $\sqrt{3}$:1), were observed in different areas of the specimen, suggesting formation of the H_{II} phase. Similar electron micrographs have been obtained for the H_{II} phase formed with hydrated phosphatidylethanolamine.[9] In an aqueous dispersion of $Q_2LysC_5Ala2C_{14}$ and $(HO)C_5Ala2C_{14}$ at the 1:2 molar ratio (n = 3.33), the H_{II} phase was also observed by NSEM, together with the bilayer phase. A fraction of the H_{II} phase in the aggregates, f, was evaluated by differential scanning calorimetry on the basis of phase transitions at 4.6 and 1.8 °C for the aggregates forming H_{II} and bilayer phases, respectively. The f values calculated from the phase transition enthalpies were 0.7 and 0.9 for the $Q_2LysC_5Ala2C_{14}$—$(HO)C_5Ala2C_{14}$ systems at molar ratics of 1:2 and 1:3, respectively.

To confirm the H_{II} phase formation, we measured electron diffractions for selected areas of the electron micrograph[10] by tilting the specimen in the electron microscope. As for the specimen prepared from an aqueous dispersion of $Q_2LysC_5Ala2C_{14}$ and $(HO)C_5Ala2C_{14}$ at the 1:2 molar ratio, an electron diffraction pattern with a spacing of 31 Å (Fig. 2B) was obtained for the selected area shown in Fig. 2A. When this specimen was tilted by 30° rotation around the y-axis in Fig. 2A, the image was converted into Fig. 2D and the spacing was expanded to 52 Å, $\sqrt{3}$ of

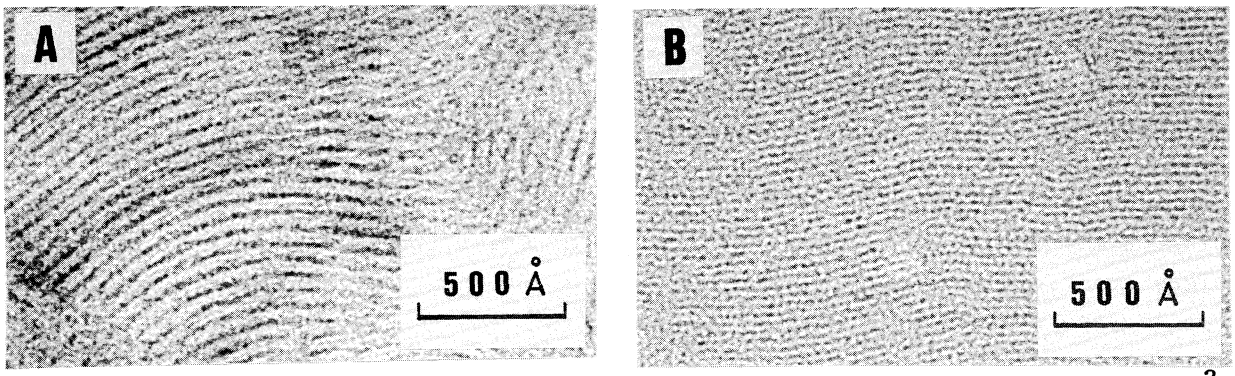


Fig. 1. Electron micrographs for a mixture of $Q_2LysC_5Ala2C_{14}$ (1.25 mmol dm^{-3}) and $(HO)C_5Ala2C_{14}$ (3.75 mmol dm^{-3}) in the aqueous dispersion state at room temperature; negatively stained with uranyl acetate. The images shown in A and B were observed in different visual areas of the specimen.

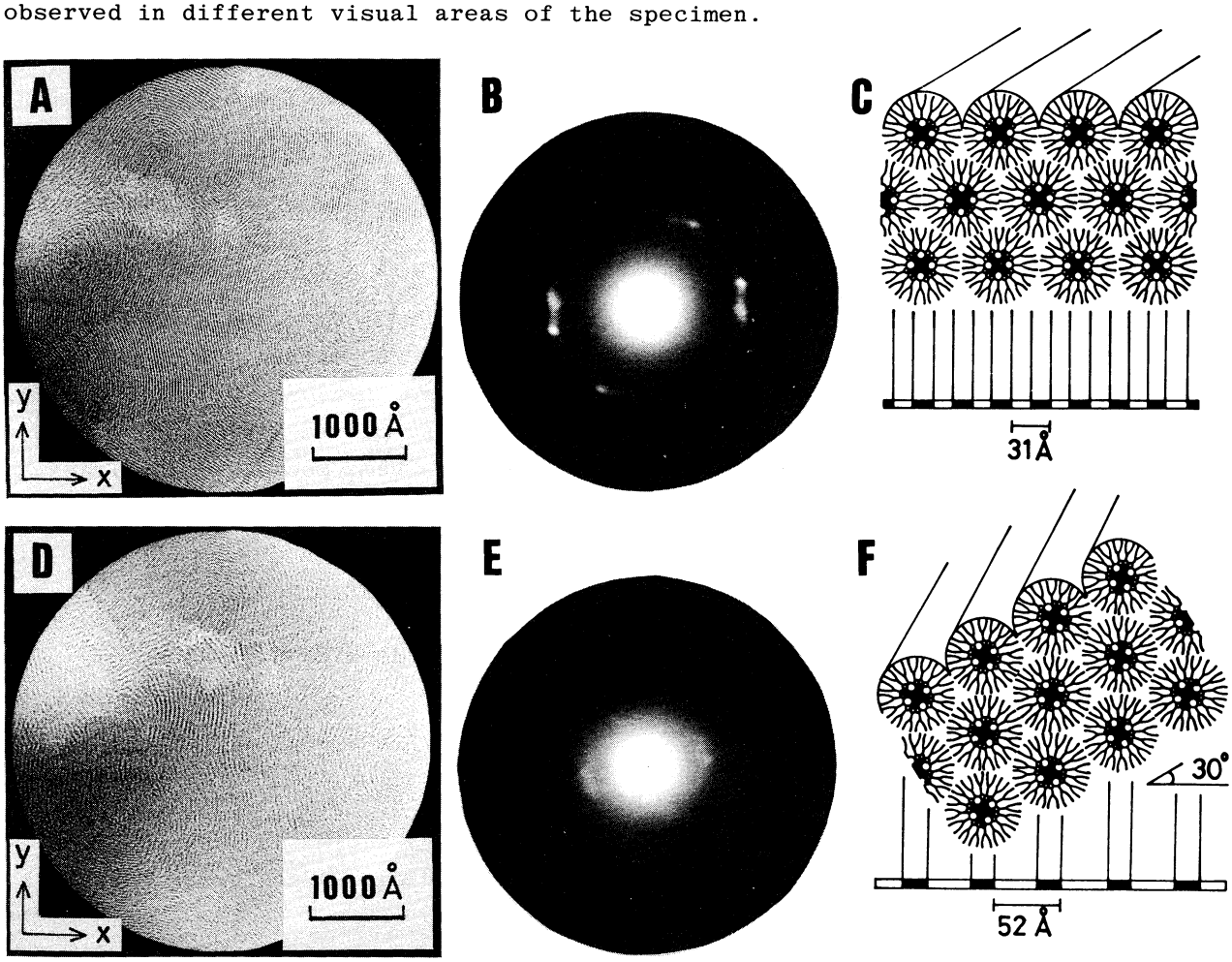


Fig. 2. Electron micrographs for a mixture of $Q_2LysC_5Ala2C_{14}$ (1.67 mmol dm^{-3}) and $(HO)C_5Ala2C_{14}$ (3.33 mmol dm^{-3}) in the aqueous dispersion state at room temperature, as negatively stained with uranyl acetate (A and D); their selected area electron diffraction patterns (B for A and E for D, respectively); schematic representations to show relations between their images and aggregate structures (C for A and F for D, respectively). Image A and diffraction pattern B were converted into D and E, respectively, by 30° rotation of the specimen around the y-axis.

the original spacing (Fig. 2E). These changes are consistent with the aggregate arrangements schematically shown in Figs. 2C and 2F.

In conclusion, it became apparent that the nonbilayer phases, H_{II} and C_{II}, and the bilayer one are readily constituted with the synthetic nonionic peptide lipids having hydroxyl groups by controlling the molecular packing mode in the surface domain of aggregates. Synthetic peptide lipids are superior to natural lipids in the following aspects; morphological stability,[11] incorporation of various functional groups by molecular design,[12] and ready control of aggregate morphology as demonstrated here.

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6) Found: C, 62.93; H, 10.32; N, 6.45%. Calcd for $C_{57}H_{107}N_5O_{13}\cdot H_2O$: C, 62.89; H, 10.09; N, 6.43%.
7) A JEOL JEM-2000FX electron microscope installed at the HVEM Laboratory of Kyushu University was used for the measurements.
8) Found: C, 74.34; H, 12.39; N, 4.75%. Calcd for $C_{37}H_{74}N_2O_3$: C, 74.69; H, 12.54; N, 4.71%.
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