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Surface Behaviors of Nucleic Acid Base-Containing Lipids in Monolayer and Bilayer Systems

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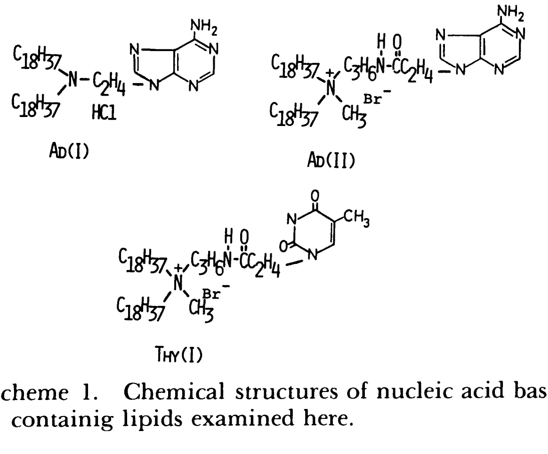
Three kinds of nucleic acid base-containing lipids were synthesized and the surface behaviors of these compounds in monolayer and bilayer systems were examined. In the monolayer systems, the addition of a complementary nucleoside to a subphase solution extended the monolayer much more efficiently than that of other nucleosides because of hydrogen bonding between a pair of complementary bases. In the monolayer and bilayer systems, the photodimerization reaction of a thymine ring was confirmed by the change in the surface pressure of the monolayer and the decrease in absorbance and the broadening of the phase-transition peak in the DSC curve of the liposome solution.

Mutual recognition of complementary nucleic acid bases is a fairly essential factor for the reproduction and generation of cells. To mimic these phenomena, many researchers have introduced nucleic acid bases into soluble polymers, polymer gels and micelles.[1–3] Considering the fact that replication of DNA occurs in cells, it is worthwhile to examine the mutual recognition of nucleic acid bases in model cell systems. In this project we studied the mutual recognition of nucleic acid bases in monolayer and bilayer systems which resembled membrane systems. Nucleic acid bases with lipophilic groups are also very interesting as both drug delivery systems and drugs themselves. Researchers have observed various remarkable effects of lipophilic nucleotides as anti-tumor reagents,[4a–c] and it is worthwhile to obtain basic information about the properties of nucleic acid base derivatives with long alkyl chains.

Experimantal

Materials. Three kinds of amphiphiles with nucleic acid bases (Ad(I), Ad(II), and Thy(I)) were synthesized (Scheme 1).

a) Ad(I). 9-(2-Chloroethyl)adenine was synthesized by the method of Ueda *et al.*[5,6] Dioctadecylamine (Fluka, three times recrystallized from acetone before use) (0.625 g, 1.2 mmol) and 0.12 g (0.607 mmol) of 9-(2-chloroethyl)adenine were dissolved in 1-butanol (50 cm^3) and the solution was refluxed for 5 d. After evaporating the solvent, an oily mixture was purified by silica-gel column chromatography using ethanol as an eluent. The eluate was evaporated and the oily product was precipitated in cold acetonitrile (Ad(I) 67 mg, 14.2% yield). Found; C, 69.45; H, 11.72; N, 10.40. Calcd for $C_{43}H_{83}N_6Cl$; C, 71.82; H, 11.55; N, 11.69.



Scheme 1. Chemical structures of nucleic acid base-containig lipids examined here.

b) Ad(II). A *p*-Nitrophenyl ester of 9-(2-carboxyethyl)adenine was synthesized by the same method of Overberger and Inaki.[7] (3-Aminopropyl)methyldioctadecylammonium bromide hydrochloride[8] (1.22 g, 1.72 mmol) and 0.548 g (1.5 mmol) of 9-(2-carboxyethyl)adenine *p*-nitrophenyl ester were dissolved in 50 cm^3 of a dichloromethane-*N,N*-dimethylformamide mixture (4:1) and 0.5 cm^3 (3.59 mmol) of triethylamine was added. The mixture was continuously stirred for 2 d at room temperature. After evaporating the solvent, an oily yellow mixture was purified using silica-gel column chromatography with ethanol as an eluent. Just after the yellow colored *p*-nitrophenol flowed out of the column, the eluent was changed to $CHCl_3$–methanol (2:1). After the addition of a few drops of concd HBr, the collected eluate was evaporated and the oily product was precipitated in cold acetonitrile (HBr salt of Ad(II) 0.573 g, 40.5% yield). Found; C, 60.38; H, 9.83; N, 10.24. Calcd for $C_{48}H_{93}N_7OBr_2$; C, 61.06; H, 9.93; N, 10.38.

c) Thy(I). One g (1.41 mmol) of (3-aminopropyl)methyldioctadecylammonium bromide hydrochloride[8] and 0.5 g (1.57 mmol) of 1-(2-carboxyethyl)thymine *p*-nitrophenyl ester[7] were reacted using a similar method for Ad(II) (Thy(I) 0.412 g, 34.2% yield). Found; C, 66.85; H, 11.02; N, 6.50. Calcd for $C_{48}H_{93}N_4O_3Br$; C, 67.49; H, 10.97; N, 6.56.

Monolayer Measurements. Surface-pressure measurements were carried out using a Wilhelmy system connected to a Commodore PET 4032 microprocessor with a floppy-disc memory system. The volume of the subphase solution was about 200 cm^3. Millipore water was used for the preparation of a subphase solution. The temperature of the subphase was maintained by circulating water using a Lauda K4R water bath.

Bilayer Structure Formation. The necessary amount of nucleic acid base-containing amphiphile was suspended in distilled water using a vortex mixer. The solution was ultrasonicated using a Branson sonifier (Model B15P, 200 W 20 kHz) at 60 °C for 5 min. The liposome that was obtained was negatively stained using a 2(w/v)% ammonium molybdate solution on a carbon-evaporated copper mesh (10 nm thickness on G200 mesh, Polaron Equipment Ltd., Watford, England)

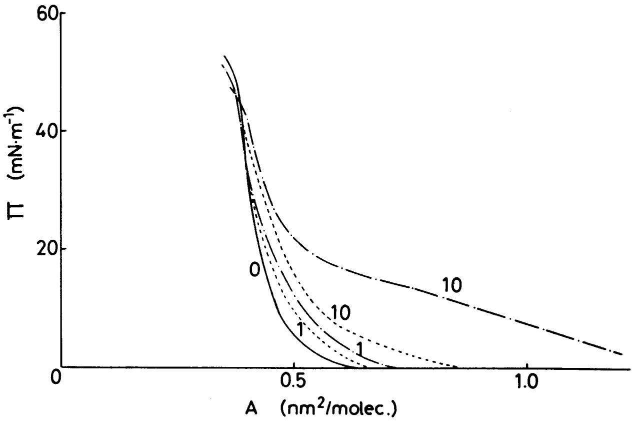


Fig. 1. Effects of nucleosides on the monolayer of Ad(I) at 10 °C in mmol dm^{-3}.
—·—·—, in the presence of thymidine, ----, in the presence of adenosine.

for measurements using a JEM-100U electron microscope (Nihon Denshi, Tokyo, Japan).

UV Measurements. To observe any decrease in the absorption of the Thy(I) liposome solution due to photodimerization, a Beckman spectrophotometer (Model DU-6) was used.

UV Irradiation. To examine the photodimerization reaction of a Thy(I) liposome solution, a 200-V mercury lamp (Oriel) was used at 100 mA. The sample solution (10 mg cm^{-3}) was poured into a quarz cuvette (1×1×4 cm) and was placed at the distance of 20 cm from the light source at room temperature. The surface behavior of the Thy(I) monolayer was also observed before and after UV-irradiation. During a photo-reaction involving the monolayer of Thy(I) for 45 min (at 14 °C), the surface tension of the monolayer was automatically maintained at 30 mN m^{-1}.

DSC Measurements. Phase transition behaviors of the Ad(II)- and Thy(I)-liposomes were observed using a Perkin Elmer DSC-2C. The volumes and concentration of samples in DSC alminium cells were 70×10^{-6} dm^3 and 10 mg cm^{-3}, respectively. The temperature was increased at a rate of 2.5 °C min^{-1}.

Results and Discussion

A. Monolayer System. At first, we observed the surface behavior of the nucleic acid-base-containing amphiphiles in the monolayer system. Figure 1 shows the effects of adenosine and thymidine on the Ad(I) monolayer. Upon an addition of thymidine to the subphase, the monolayer expanded extraordinarily. However, an addition of adenosine induced a relatively smaller expansion. The smaller increase in the surface area in the Ad(I)–adenosine system can be attributed to a hydrophobic base-stacking effect since purine rings of adenosine and adenine are more hydrophobic than a pyrimidine ring of thymine. On the contrary, the larger expansion effects observed for an Ad(I)–thymidine system is due to the hydrogen-bonding-induced complementary base pairs between thymine and adenine rings. By the addition of 0.01 M (1 M=1 mol dm^{-3}) or 0.1 M of HCl to the subphase solution, the expanding effect of thymidine became much smaller because of a protonation of the amino group of Ad(I). This is consistent with the hydrogen-bonding effect regarding the thymidine–adenine ring interaction. Similar tendencies were also observed in the Ad(II)- and Thy(I)-monolayer systems. The surface area of the Thy(I) monolayer was, for example, 0.78 nm^2 molecule^{-1} at 15 mN m^{-1}. In the presence of 1 mM of adenosine, the surface area of the Thy(I) monolayer was increased by 0.24 nm^2 molecule^{-1}, whereas a smaller increase (0.10 nm^2 molecule^{-1}) was observed in the presence of 1 mM of thymidine.

The UV irradiation of the Thy(I) monolayer at 30 mN m^{-1} (densely packed conditions) for 45 min increased the surface area (surface area at 40 mN m^{-1}; from 0.54 nm^2 molecule^{-1} to 0.62 nm^2 molecule^{-1}). Though the amount of the reaction product was too small for a confirmation, the photodimerization of the thymine moiety of Thy(I) probably occured. In the case of the photodimerization of an amphiphilic cinnamic acid derivative in the monolayer system, a slight decrease in the surface area was observed.[9] In this case, the cinnamic acid moiety existed in the lipophilic region. On the contrary, the thymine moiety of Thy(I) existed near the ammonium head group. These differences might be the reason for the different tendencies regarding the photodimerization.

Bilayer System. Upon ultrasonication, suspended solutions of both Ad(II) and Thy(I) turned opaque and the diameters of the liposomes formed were estimated to be 60—300 nm (widely distributed) from electron micrographs. The bilayer structures of these solutions were confirmed by a small-angle X-ray scattering method (SAXS) using a Kratky U-slit system with a position-sensitive-proportional counter (Rigaku Denki, Tokyo, Japan).[10] In plots of the relative intensities *vs.* the scattering vectors of the Thy(I) solution, for example, a sharp peak at 4.3—4.2 nm, which reflects the thickness of the bilayer of Thy(I), was clearly observed (using a CPK model, the length of the octadecyl chain of a stretched Thy(I) molecule was estimated to be about 2.1 nm). The phase-transition temperatures of these liposome solutions were quite similar (Ad(II), 42.1 °C, Thy(I), 42.5 °C) with each other. This was probably due to a similar structure regarding the lipophilic parts.

Upon UV irradiation of the Thy(I)-liposome solution, a decrease in absorbance at 270 nm could be observed and attributed to the photodimerization of the thymine moiety (Fig. 2). This was because upon dimerization the thymine ring loses its aromaticity. There was no recovery of absorption at 270 nm after heating at 60 °C for 10 min. This excludes the probability of a hydration of the thymine moiety during the UV irradiation. From electron micrographs and SAXS measurements, the liposome structure was observed to be maintained both before and after UV

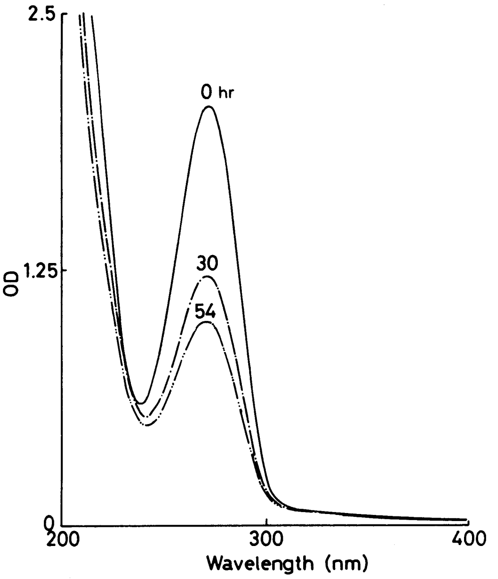


Fig. 2. Effect of uv irradiation on the absorbance of Thy(I)-liposome solution. [Thy(I)]=0.2 mmol dm^{-3}.

irradiation. Dimerization of Thy(I) was also confirmed by gel-permeation chromatography. After UV irradiation, a Thy(I) liposome solution was lyophilized and the obtained powder was dissolved in dichloromethane and carried on a Pharmacia Sephadex LH-20 column (2×20 cm). It was then eluted using dichloromethane. Elution of dimeric compounds from a column was observed using a Shodex SE-11 refractometer and the conversion of the dimerization reaction was estimated to be 50% after UV irradiation for 54 h. We tried to evaluate the structure of the dimerization product using ^{13}C NMR. However, the conversion of the reaction was so low and the amount of product was so small that it was very difficult to resolve the ^{13}C NMR spectra. At present we consider that a head-to-head and cis form dimerization is most probable from the molecular structure of Thy(I). The rate of the observed dimerization was much smaller than the polymer solution system.[11] This was probably because the long distance between thymine moieties was unfavorable for realizing dimerization (the distance between thymine moieties must be shorter than 0.4—0.5 nm for photodimerization).[12] Due to the bulkiness of the head group of the liposome-forming molecule, such a tight packing might be difficult. The unfavorable turbidity of the liposome solution and the lack of a cooperative action of the neighboring thymine ring might also be possible reasons for the slow dimerization reaction observed. After dimerization, the peak of a Thy(I)-liposome solution in DSC measurements was observed to be broader than that of the starting Thy(I)-liposome solution (peak width at half height; 3.7 °C→6.8 °C). This is probably because of the heterogenicity of the partially dimerized membrane surface. Upon mixing Ad(II) and Thy(I) liposome solutions, no large change in the DSC profiles was observed compared to those of Ad(II)- and Thy(I)-liposome solutions. This is probably because of the electrostatic repulsive forces between the cationic liposome particles. After incubating the mixed liposome solution at 350 K for 8 h, the peak was observed to be much broader than the starting solution (peak width at half height; 3.7 °C→7.1 °C) and the transition temperature shifted to a lower region by 2.3 °C during the slow fusion of these Ad(II)- and Thy(I)-liposomes and the following base-base interaction in the same liposome particle.

In conclusion, a mutual recognition of complementary nucleic acid bases could be observed in a monolayer system. A dimerization reaction involving thymine derivatives could also be observed in these systems. Especially in the liposome system, a clear decrease in the absorption of the thymine ring was observed. Further systematic studies of various kinds of nucleic acid base-containing lipids are presently in progress.

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