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A DNA−Lipid Complex in Organic Media and Formation of an Aligned Cast Film[1]

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Abstract: A novel DNA−lipid complex is prepared by replacing sodium counter cations by cationic amphiphilic lipids (**1**). The DNA−lipid complex is insoluble in aqueous solutions, but is soluble in most organic media such as benzene, ethanol, and chloroform. The DNA−lipid complex was confirmed to form double helical strands even in organic media, and the internal conformation of DNA strands could be reversibly changed from B-form to C-form in the organic solution ($CHCl_3$/EtOH = 4:1) by changing the water content. A self-standing, water-insoluble DNA−lipid (**1**) film was prepared by casting from the organic solution and could be stretched to produce oriented DNA strands with their axes aligned along the stretching direction. The film can be impregnated with the drug ethidium bromide in the aqueous solution. The linear dichroism characteristics of the drug−DNA film show that the DNA strands are well oriented and the ethidium molecules are intercalated, as they are in normal aqueous solutions.

Introduction

DNA is important as a source of biological information depending on base sequences. DNA is also interesting as a molecular material that shows a rod-like, stereochemical double helical structure with π-electron-rich base-pair stacking: the base separation is 3.4 Å while the diameter of the helix is about 20 Å.[2] DNAs are soluble only in aqueous solutions, and their fibrous crystals can be prepared by slow evaporation from the aqueous solution. Duplex structures in the fibers have been studied by X-ray diffraction[3,4] and solid state NMR.[5−7] Orientation of DNA strands by using hydrodynamic flow gradients in the dilute aqueous solution[8] and lyotropic liquid crystal properties of rod-like DNA in the concentrated aqueous solution[9−13] have been investigated. Water molecules have been shown to play an important role of the internal conformation of DNA strands by interacting with minor grooves of DNA strands.[14] However, it is difficult to discuss effects of water molecules in aqueous media and fibers. If DNA strands can be solubilized in non-aqueous organic media, it is useful to study effects of water molecules on the conformation of DNA strands. If a self-standing film in which rod-like DNA strands are aligned in one direction could be prepared by a simple method, it is interesting as molecular materials for supramolecular aggregates, electron transfer, rod-like liquid crystals, and so on.

We propose a simple preparation of a DNA−lipid complex

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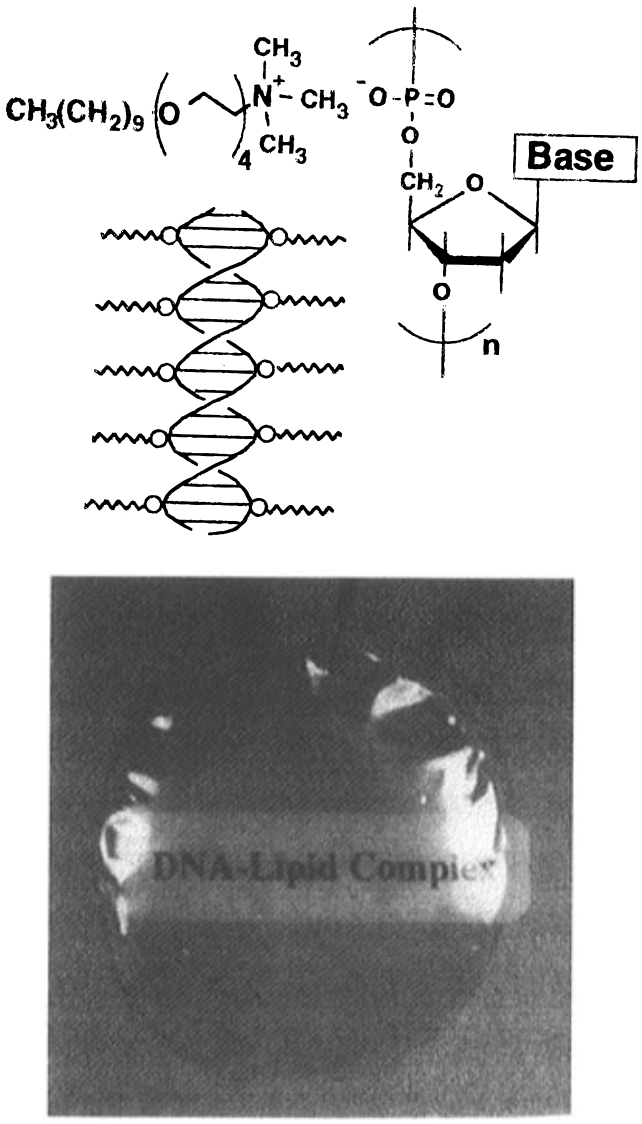


Figure 1. Schematic illustration and a photograph of a DNA−lipid (**1**) complex cast film.

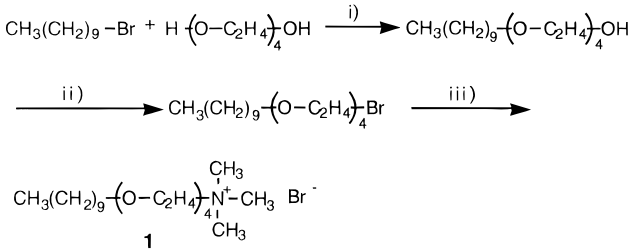
that is soluble in organic media and a simple formation of a DNA-aligned, self-standing, water-insoluble film. The DNA−lipid complex in which counter cations of phosphate anions are changed to cationic lipophilic amphiphiles (**1**) was prepared by simply mixing aqueous solutions of DNA and cationic amphiphiles (see Figure 1). The DNA−lipid complex is soluble and forms a double-strand structure in organic solutions. A self-standing, water-insoluble DNA−lipid film could be prepared by casting from the organic solution, and DNA strands were easily aligned in the film by stretching it in one direction. The film structure was confirmed by X-ray diffraction, CD spectra of DNA strands, and polarized absorption spectra of intercalated dye molecules.

Experimental Section

Materials. Sodium salts of DNA from Salmon testes (average M_w: 1.3×10^6, ca. 2000 bp) were purchased from Sigma Chemical Co. (St. Louis, MO) and used without farther purification.

Synthetic procedures of a cationic amphiphile (**1**), *N*,*N*,*N*-trimethyl-*N*-(3,6,9,12-tetraoxadocosyl)ammonium bromide are shown in Scheme 1. Decylbromide was allowed to react with an excess amount of tetraethylene glycol in dry benzene in the presence of NaH to give 3,6,9,12-tetraoxadocosanol in a yield of 51% (bp. 152 °C/0.1 mmHg). Reaction of the alcohol with thionyl bromide in dry benzene gave the bromo compound in a yield of 76% (140 °C/0.2 mmHg). The bromo

Scheme 1[a]



[a] Reagent condition, and yield: (i) tetraethylene glycol (10 equiv) and Na (13 equiv), dry benzene under reflux for 1 day, 51% (ii) thionyl bromide (2 equiv), pyiridine (2 equiv), dry benzene under reflux for 1 day, 76%; (iii) trimethylamine (20 equiv), ethanol under pressure at 50 °C, 3 days, 87%.

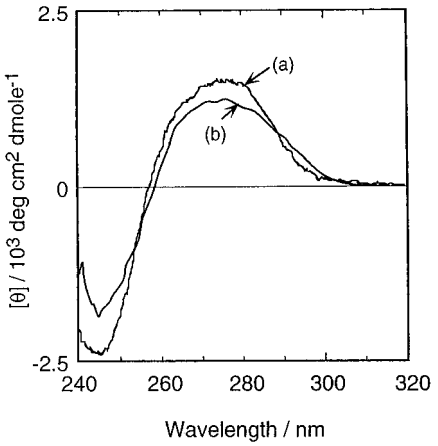


Figure 2. CD spectra of (a) DNA−lipid (**1**) complex in $CHCl_3$/EtOH/H_2O = 4:1:0.07, and (b) native DNA in aqueous buffer solution (20 mM NaCl, pH 7.8, 10 mM Tris, [DNA] = 50 μM bp^{-1}, 23 °C).

compound was converted to the quaternary ammonium salts in a yield of 87%. ^{1}H-NMR (300 MHz, $CDCl_3$), δ 0.8−0.9 (*t*, 3H, CH_3), 1.1−1.4 (m, 32H, CH_2), 3.3−3.5 (s and t, 11H, N^+CH_3 and N^+CH_2, respectively), 3.5−3.7 (m, 16H, OCH_2). Elemental analysis of C, N, and Br was consistent with the calculated values within ±0.1%.

Preparation of a DNA−Lipid Complex and a Cast Film. An aqueous solution (100 mL) of DNA^- Na^+ from salmon testes (0.50 g, 0.68 mmol bp^{-1}, ca. 2000 bp) and an aqueous solution (100 mL) of 1.1 equiv mol of cationic amphiphiles (**1**, 1.5 mmol) were mixed at room temperature, and polyion complex precipitates were gathered by centrifugation and freeze-dried. The white powder was solubilized in chloroform and reprecipitated to diethyl ether two times. The obtained DNA−lipid complex was confirmed by elemental analysis to form 1:1 complexes of a phosphate anion and the cationic amphiphile (**1**). The DNA−lipid complex was soluble only in organic solvents such as chloroform, benzene, and ethanol, but not in aqueous buffer solution. CD spectra were taken in $CHCl_3$/EtOH solution by changing an amount of water content.

The $CHCl_3$/EtOH (4:1) solution (10 mL) of the DNA−lipid complex (0.25 g) was cast on a Teflon plate, and the solvent was evaporated slowly under the saturated vapor at room temperature. The obtained self-standing film was transparent (ca. 60 μm thick), water-insoluble, and physically stable (see Figure 1). When the cast film was soaked in water, the wet film could be stretched ca. 3 times in length (ca. 20 μm thick) at room temperature. The stretched film was still transparent and physically strong.

Measurements. CD spectra of a native DNA in aqueous solution or a DNA−lipid complex in organic solution were taken by J-600 spectropolarimeter (Nippon Bunkou Co., Tokyo). The X-ray diffraction pattern of the DNA−lipid complex film was recorded on an imaging plate by using Model R-AXIS (Rigaku-Denki, Co., Tokyo). The film was cut and piled up into a fine strip with a size of 8 × 0.5 × 0.3 mm for X-ray measurements. Polarized absorption spectra of the DNA−lipid complex film intercalated with dyes were measured on a quartz

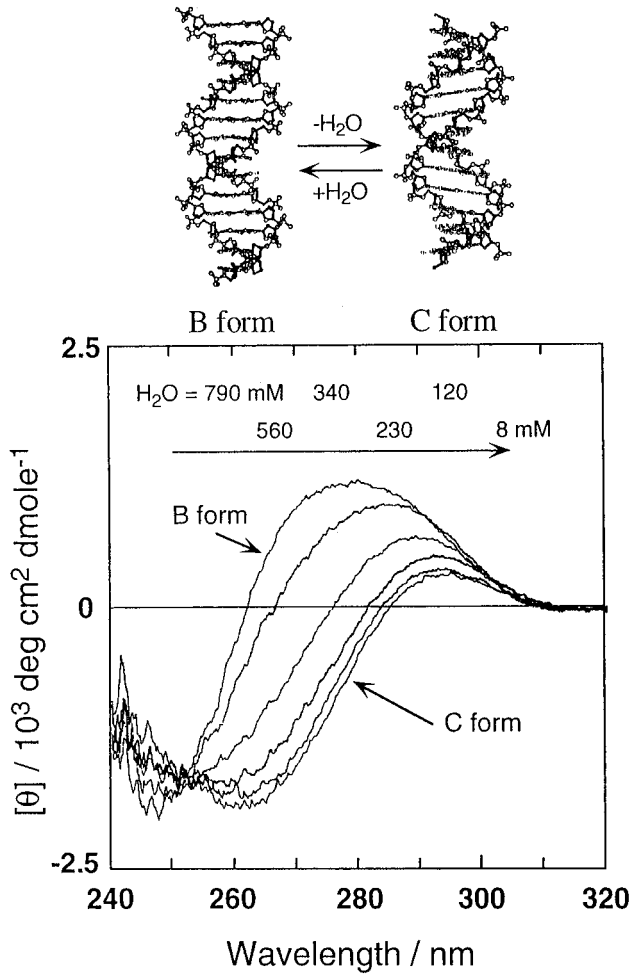


Figure 3. Effect of water content on CD spectra of the DNA−lipid (**1**) complex in $CHCl_3$/EtOH (4:1) solution ([DNA] = 50 μM bp^{-1}, 23 °C).

plate by a Model HP8452A diode array (Hewlett Packard Co., Tokyo). The dye-intercalated film was prepared by soaking the film into aqueous solution (25 μM) of ethidium bromide for 1 day at room temperature.

Results and Discussion

A DNA−Lipid Complex in Organic Solution. A DNA−lipid (**1**) complex obtained simply by mixing aqueous solutions was soluble in most organic solvents such as chloroform, benzene, and ethanol. Figure 2 shows CD spectra of (a) the DNA−lipid complex in organic solution containing a small amount of water ($CHCl_3$/EtOH/H_2O = 4:1:0.07, 790 mM H_2O) and (b) native DNA in aqueous buffer solution (20 mM NaCl, 10 mM Tris, pH 7.8). The DNA−lipid complex shows a positive Cotton effect at 270 nm and a negative Cotton effect at 245 nm similar to native DNA in aqueous solution, which indicates B-form structure of DNA strands.[15] Thus, the DNA−lipid complex forms a double helical B-form structure even in organic solvent similar to the native DNA in an aqueous solution.

Figure 3 shows the effect of water content in organic solvent ($CHCl_3$/EtOH = 4:1, water content: 8−790 mM) on CD spectra. The positive Cotton effect at 270 nm was shifted to long wavelength, and the θ value was decreased gradually with decreasing water content from 790 to 8 mM in $CHCl_3$/EtOH solution. The CD spectrum of the DNA−lipid complex in the dry solution (8 mM water) was similar to that of the C-form of native DNA strands in aqueous solution in the presence of high salts[16] or high ethanol concentration.[17] Dickerson and coworkers reported that water molecules are important to form B-structures of DNAs, in which water molecules interact with oxygen molecules of ribose and phosphate and minor or major groove of DNA strands.[14] Thus, the conformation of the DNA−lipid complex can be reversibly changed (B-form and C-form) by controlling water contents in organic solution as well as native DNA depending on salt and ethanol concentrations.

X-ray Diffraction of Cast Film. The $CHCl_3$/EtOH/H_2O (4:1:0.07) solution of the DNA−lipid (**1**) complex was cast on a Teflon plate, and the solvent was evaporated slowly under the saturated vapor at room temperature. The obtained self-standing film was transparent (ca. 60 μm thick), water-insoluble, and physically stable (see Figure 1). Figure 4a shows X-ray diffraction patterns of an as-cast film of the DNA−lipid (**1**) complex. When the incident beam was irradiated perpendicular to the film plane, the circular reflection with 41 Å was observed that corresponds to a diameter of a DNA−lipid complex. When the beam was exposed parallel to the film plane (side-edge view), hexagonal spots with 41-Å spacing were observed. They indicate the DNA−lipid strands whose diameter is 41 Å aggregate into hexagonal close-packed structures in the film plane. Thus, aggregates of hexagonal-packed DNA−lipid strands aligned on the film plane in *random* directions, as schematically shown in Figure 4a.

The DNA−lipid cast film was stretched ca. 3 times in length (ca. 20 μm thick) in wet state, and X-ray diffraction patterns are shown in Figure 4b. When the incident beam was irradiated parallel to the top edge of the stretched film, the circular reflection with 41 Å was observed. When the beam was exposed parallel to the side edge and perpendicular to the film plane, diffraction on the equator appeared as two spots of 41 Å, indicating the distance between DNA−lipid strands, and the diffraction on the meridian of 3.4 Å, indicating the distance between parallelly stacked base pairs was clearly observed.[2−4] These findings clearly show that DNA strands aligned parallel to the stretched direction in the film and that base pairs stacked perpendicular to the direction of DNA strands as shown in the illustration. Neither the diameter nor the distance between strands (41 Å) was affected by stretching.

When the stretched film was dried in air, only diffraction on the equator as two spots of 41 Å were observed but not diffraction on the meridian for base pairs (Figure 4c). This suggests that the orientation of base pairs is not perpendicular to the stretched direction, although DNA strands are aligned parallel to the stretched direction. Figure 5 shows CD spectra of the cast film of the DNA−lipid complex in (a) wet and (b) dry states. The CD spectrum of the wet film was consistent with B-form structure of native DNA in fibers obtained from aqueous solution.[2−4] On the contrary, the CD spectrum in the dry state resembles the A-form of DNA in which base pairs slant to the axis of strands.[3,4] These results are consistent with CD spectra of the DNA−lipid complex in organic solution: DNA exists in B-form in the presence of water and in C-form in the absence of water. The conformational changes between B-form and A-form of DNA strands in the film depending on water moisture were reversible.

Polarized Spectra of Intercalated Cast Film. When the stretched DNA−lipid (**1**) film was soaked in an aqueous solution of ethidium bromide (λ_{max} = 480 nm) for 1 day at room temperature, the transparent film turned red (λ_{max} = 520 nm), and the aqueous solution became clear. Thus, the ethidium intercalated completely between base pairs of the DNA film.

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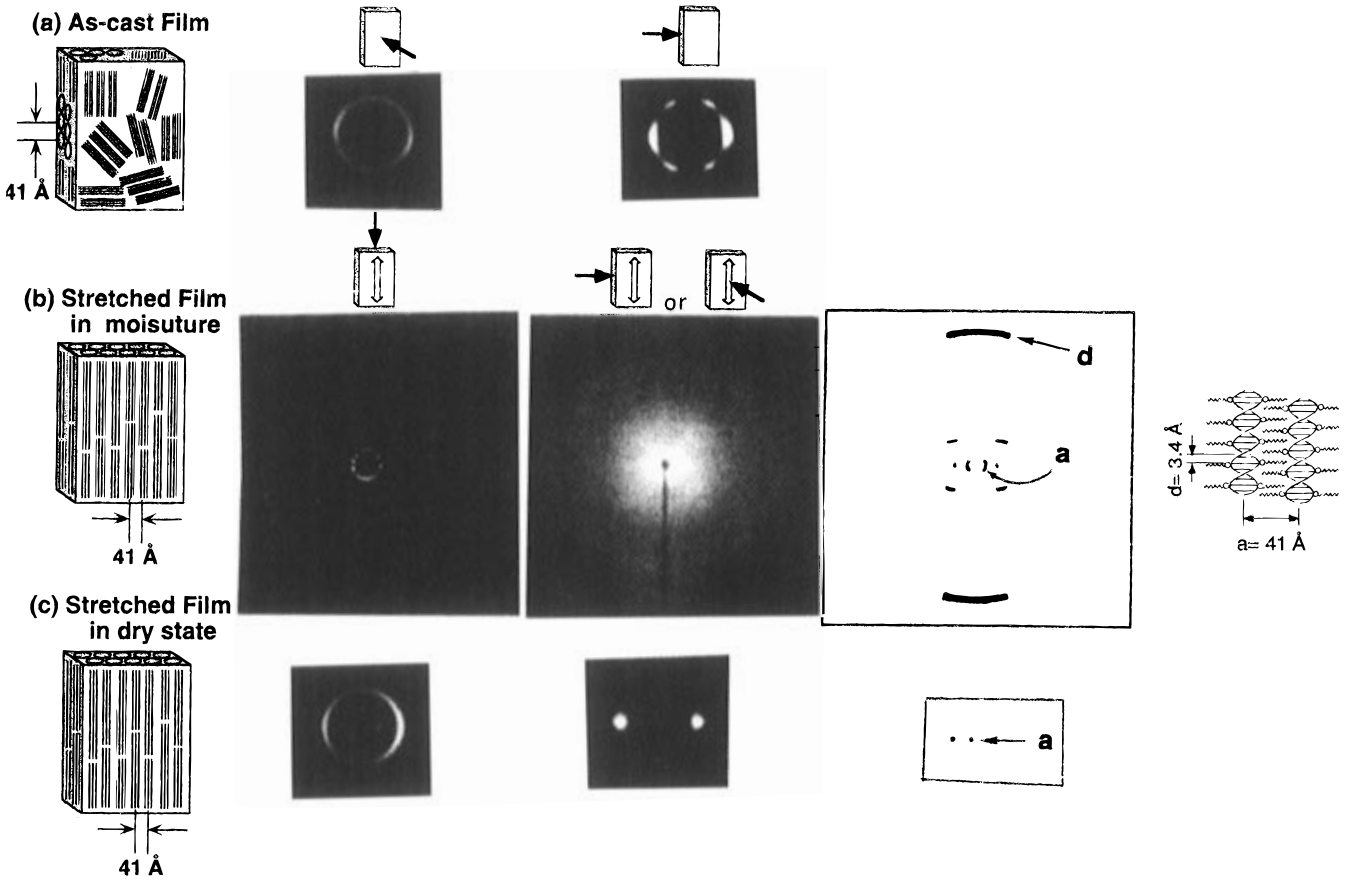

Figure 4. X-ray diffraction patterns of (a) the as-cast film, (b) the stretched film of the DNA−lipid complex in water moisture, and (c) the stretched DNA−lipid film in dry state. Open, two-headed arrows show the stretching direction of the film. Closed arrow shows the incident beam of X-ray. X-ray photographs of panels a and panels c were enlarged to clearly see the diffraction.

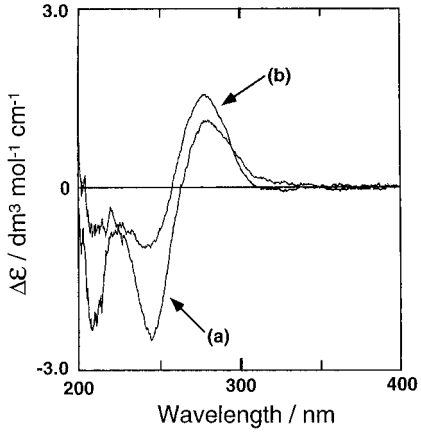

Figure 5. CD spectra of the cast film of DNA−lipid complex (a) in water and (b) in dry state (23 °C, film thickness: 3 μM).

When the film was moved into the new aqueous buffer solution, the intercalated dye molecules were hardly removed from the film at least for 1 day. Similar intercalation behavior into the film was observed for other dyes such as proflavine, acridine orange, and safranin T.[18,19] Figure 6a shows polarized absorption spectra of ethidium intercalated in the stretched DNA−lipid film in water. The absorption at 520 nm of the light polarized perpendicularly to the stretched direction of the film was 3.3 times larger than that the light polarized parallel to the stretched direction. In the case of the as-cast DNA−lipid film, there were no differences in absorption of the parallel and perpendicular light. The large dichroic ratio of $A_{\perp}/A_{//} = 3.3$ indicates that the intercalated dyes are aligned perpendicular to the stretched direction of the film: the DNA strands are aligned parallel to the stretched direction. The secondary order parameter (orientation moment)[20] of DNA strands in the film was calculated to be ca. 0.7 from the $A_{\perp}/A_{//} = 3.3$.[21] This value is close to the orientation of synthetic rod-like polymers such as poly(arylenes) prepared by a uniaxicial stretching method.[22,23]

When a polarized spectrum of the film was taken in the dry state, a small dichroic ratio was observed ($A_{\perp}/A_{//} = 1.3$, see Figure 6b). This is due to the slant structures of base pairs and intercalated dyes to the axis of strands, since DNA strands were confirmed to be aligned along the stretching direction even in the dry film (see Figure 4c). These changes of polarized absorption spectra depending on water moisture were reversible at least 10 times.

Effects of Cationic Amphiphiles on DNA Structures. Several single-chain and double-chain amphiphiles as shown in Scheme 2 were used to prepare DNA−lipid complexes. DNA−lipid complexes could be prepared from all cationic lipids (**2**−**8**) shown in Scheme 2, and they were soluble in most

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(20) Stein, R. S. *J. Polym. Sci.* **1958**, *31*, 327; **1959**, *34*, 709.

(21) Secondary order parameter (f_{20}) is calculated according to the following equation, which was defined for orientation of dye molecules in a polymer film (see ref 20).

$$f_{20} = \frac{(A_{\perp}/A_{\parallel}) - 1}{(A_{\perp}/A_{\parallel}) + 2}$$

(22) Skotheim, T. A., Ed. *Handbook of Conducting Polymers, Vols. I and II*; Marcel Dekker: New York, 1986.

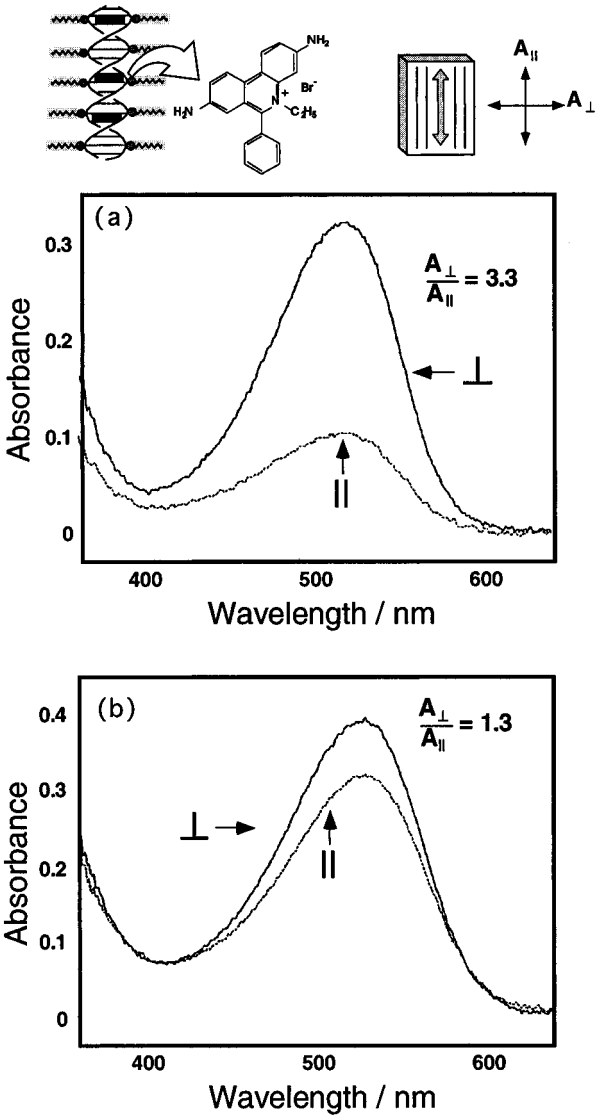
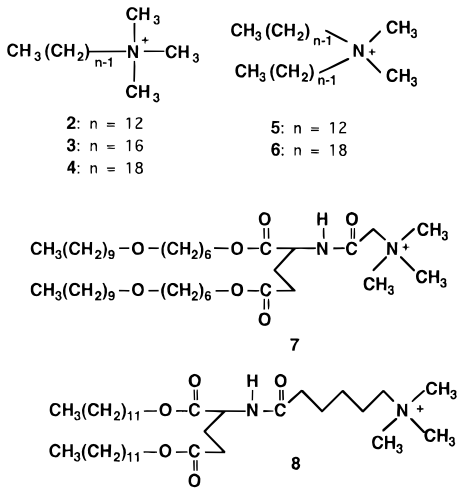


Figure 6. Polarized absorption spectra of ethidium bromide intercalated into the stretched DNA−lipid film (a) in water and (b) in the dry state. Ethidium bromide was intercalated one molecule per 10 base pairs at 25 °C. Open, two-headed arrows show the stretching direction of the film.

organic solutions. All of these complexes, as well as the complex from the amphiphile **1**, were also confirmed from CD spectra to exist as double-strand structures in the organic solution of $CHCl_3/EtOH/H_2O$ = 4:1:0.1. When cast films were prepared from the complexes of **2**−**8**, they were rigid or fragile compared to the film from the lipid **1**. DNA−lipid complexes from **2** to **8** showed the typical lamellar structures of amphiphiles whose spacing is 30−45 Å depending on alkyl chain length, but not the distance of DNA strands and stacked base pairs. These cast films did not show significant Cotton effects in CD spectra. Ethidium bromide hardly intercalated into these films in the aqueous solution, and no anisotropy was observed in polarized absorption spectra. These data clearly indicate that these DNA−lipid complexes can form double-strand structures in organic solution independent of chemical structures of cationic lipophilic amphiphiles. In cast films, however, double strands are broken and lipid lamellar structures are observed due to the strong aggregation of solid alkyl chains. This is the reason we chose the amphiphile **1** having the flexible oligoethyleneglycol unit in the single alkyl chain as counter cations of DNA. The flexible amphiphile seems not to disturb the double stranded structure, even in the cast film.

Scheme 2



Conclusion

The DNA−lipid (**1**) complex was soluble in organic solution and formed double helical structures whose conformation could be changed reversibly depending on water content. In the self-standing cast film of DNA−lipid complex, DNA strands could be aligned in one direction simply by stretching the cast film. The conformation of DNA strands could be changed reversibly even in the film state depending on moisture content. Water molecules are shown to be important to form B-structure of DNAs even in organic solvents and in the film.

The DNA−lipid film is useful as an adsorption membrane of carcinogens such as ethidium bromide in the aqueous solution. The DNA-aligned film is expected as molecular materials for chiral supramolecules and for one-dimensional electron transfer and conduction along the DNA strands. The flexible film, in which DNA strands having hairy alkyl side chains aligned in one direction, is also expected to show thermotropic liquid crystal properties. These applications are currently under investigation in our laboratory.

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