Conformational change in DNA induced by cationic bilayer membranes

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Abstract The effect of synthetic cationic lipids on the structure of DNA was studied. The fluorescence enhancement of ethidium bromide on intercalation into DNA was suppressed by the addition of bilayer-forming lipids, but not by micellar ones. Results on the fluorescence depolarization index suggest that ethidium bromide is not released from DNA by lipids intercalated into DNA. CD spectra of the DNA-lipid complexes revealed that the structure of DNA was changed only by bilayer-forming lipids at temperatures lower than their T_c values. Thus, the conformation of DNA is forced to change by cationic lipids forming the rigid bilayer membrane so that ethidium bromide fluorescence might be reduced, and the conformation can be controlled by selection of the appropriate lipid and temperature.

Key words: Bilayer membrane; Cationic lipid; DNA conformation; Ethidium bromide

1. Introduction

DNA in aqueous solution exhibits various conformations, including the A-, B-, C- and Z-forms [1,2]. Although the conformations are dependent on the GC content of the DNA [3], interconversion between these forms can be readily induced by a temperature change and interaction with various substances, such as metal ions [4], peptides [5] and proteins [6]. A change in the conformation of the DNA could regulate biological processes such as replication, transcription, and transfection.

Binding of metal ions to the phosphate groups of DNA lowers the electrostatic repulsion between these groups and then stabilizes the DNA structure [2]. At higher concentration of metal ions, however, the ions bind to the bases in addition to the phosphate groups and lead to the destabilization of double-stranded DNA structures by weakening the hydrogen bonds between the base pairs. The cationic proteins, histones, associate with particular phosphate anions of the DNA to neutralize the repulsive interaction between them [7]. The elimination of ionic repulsion at one helical face of the DNA makes it possible to bend the DNA to form a highly folded compact structure of chromatin. This process is quite important for storing long DNA molecules in the nucleus. Spontaneous bending of the DNA molecule by neutralization of the ionic repulsion at one helical face was demonstrated by incorporation of a neutral analog of the phosphate group into DNA [8].

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Abbreviations: CTAB, cetyltrimethylammonium bromide; TMAB, trimethyammonium bromide.

Bilayer membranes formed with cationic lipids have been successfully used as DNA carriers into cultured eukaryotic cells [9–13]. Since these membranes have fluid two-dimensional surface cationic groups neutralizing the anionic phosphate groups of the DNA, DNA interacting with the membranes would exhibit a different conformational change from that interacting with metal ions and proteins. In this communication, we studied the effects on the conformation of DNA of synthetic cationic lipids with various properties of membrane fluidity and aggregate morphology, and show that cationic lipids forming the rigid bilayer membrane can induce a structural change in DNA and that the structure of DNA can be controlled by selection of the lipids and temperature.

2. Materials and methods

Double-chain lipids **1** and **2** (Fig. 1) were prepared according to the procedure reported by Kunitake et al. [14,15]. Lipid **3** was purchased from Sogo Pharmaceutical Co. (Tokyo, Japan). Cetyltrimethylammonium bromide (CTAB), a micellar lipid, and trimethyammonium bromide (TMAB), a cationic monomeric compound, were from Wako Pure Chemical (Osaka, Japan). Fluorescence spectra were measured using a Hitachi spectrofluorophotometer. Intercalation to plasmid DNA using ethidium bromide [16] was performed as follows: plasmid DNA (pCH110, 10 mM Tris-HCl, 1.0 mM EDTA, pH 8.0) was prepared to 6.28×10^{-6} unit M in 1.0 ml of water. Ethidium bromide (1.6 equimolar per base pair of DNA) was added to the DNA solution. The degree of polarization (P) was used as a measure of the rotational motion of the excited state [17]. Circular dichroism (CD) spectra were recorded using a JASCO J-700 spectropolarimeter at various temperatures.

3. Results and discussion

The synthetic cationic bilayer-forming lipids listed in Fig. 1 were selected as cationic compounds in order to examine the interaction with DNA. The first series of lipids, **1**(12,2) and **1**(14,2), are composed of two long-chain alkyl tails (length of alkyl chain: m, 12 or 14), trifunctional glutamate and phenylene groups as the connector, a methylene spacer (number of methylene carbon: n, 2) and an ammonium group as the cationic head. The lipids of the second series, **2**(12,6) and **2**(14,6), have the same basic structure as **1**, except there is no phenylene group in the connector. Compound **3** consists of two long-chain alkyl tails and an ammonium group. These compounds form bilayer liposomes in aqueous solution at room temperature, although they do have various phase-transition temperatures (T_c); **1**(12,2), 24.8°C; **1**(14,2), 41.1°C; **2**(12,6), 9.0°C; **2**(14,6), 25.8°C; **3**, 28.0°C [18,19]. Other cationic compounds, CTAB and TMAB, which are unable to form the bilayer structure, were used for purposes of comparison.

When ethidium bromide was mixed with DNA, an about 10-fold enhancement in fluorescence took place on intercala-

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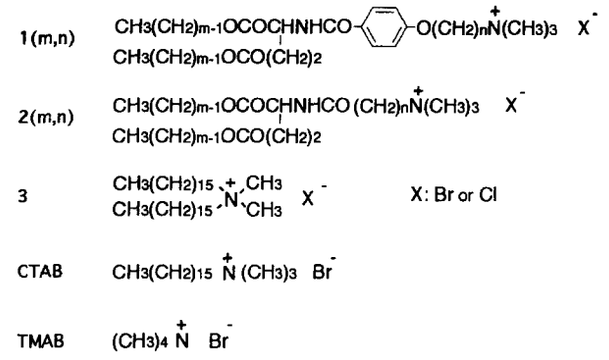

Fig. 1. Structures and nomenclatures of the synthetic cationic lipids examined.

tion of the compound into DNA and the spectrum was maximized at 590 nm [16]. The enhanced fluorescence was reduced to nearly the basal level on the addition of the lipid **1**(12,2). Further addition of Triton X-100 to disrupt the bilayer structure, however, led to recovery of the quenched fluorescence to the original level. When various concentrations of lipid **1**(12,2) were added to the DNA-ethidium bromide solution, maximum quenching was observed at a concentration below an equimolar amount of the phosphate groups of DNA (Fig. 2). Similar quenching behavior was observed with various lipids, including **1**(14,2), **2**(12,6), **2**(14,6) and **3**. A micellar compound (CTAB) also quenched the fluorescence, but 6-fold equimolar amounts of the lipid were required to quench up to 30% of the original fluorescence. In addition, a non-micellar compound (TMAB) led to no change in the fluorescence. Thus, only the bilayer-forming lipids seem to quench effectively the fluorescence of ethidium bromide intercalated into DNA. Triton X-100 destroyed the bilayer structure of the lipids and consequently ethidium bromide fluorescence could be recovered.

To examine the possibility that the quenching of fluorescence was caused by the removal of ethidium bromide from the DNA to the lipid liposomes, the microviscosity of the environment surrounding ethidium bromide was examined by measuring in the fluorescence depolarization index, *P*, of the excited state of the intercalator. As shown in Table 1, ethidium bromide alone had a *P* value of 0.001, while the value was much higher (0.21) in the presence of DNA. Thus, the rotational mobility of ethidium bromide was markedly suppressed by intercalation into DNA. Although the addition of lipid **1**(12,2) to the DNA-ethidium bromide complex lowered the *P* value, a substantial level of the value, 0.07, was still observed. The addition of CTAB had no effect on the value. Neither **1**(12,2) nor CTAB affected the polarization of ethidium bromide in the absence of DNA, indicating that the intercalator does not interact directly with these lipids. These results suggest that ethidium bromide still interacts with DNA even after the addition of **1**(12,2) and that the fluorescence quenching is not due to the release of ethidium bromide molecules from DNA by the lipids. Thus, since the effect was only observed with the bilayer-forming lipid and not with other lipids, interactions between DNA and ethidium bromide seem to be weaker depending on the bilayer structure of the lipids.

Table 1
Fluorescence polarization (*P*) of ethidium bromide (EB, 2.54×10^{-8} M) in the complex with plasmid DNA and/or cationic amphiphiles at 25°C

Complex	*P*
EB	0.001
EB+DNA (3.14×10^{-5} unit M)	0.210
EB+DNA+**1**(12,2) (6.5×10^{-6} M)	0.073
EB+DNA+**1**(12,2) (1.95×10^{-5} M)	0.074
EB+DNA+CTAB (6.5×10^{-6} M)	0.208
EB+DNA+CTAB (1.95×10^{-5} M)	0.223
EB+**1**(12,2) (6.5×10^{-6} M)	0.002
EB+**1**(12,2) (1.95×10^{-5} M)	0.001
EB+CTAB (6.5×10^{-6} M)	0.000
EB+CTAB (1.95×10^{-5} M)	0.000

The interaction between DNA and **2**(14,6) was studied using CD spectroscopy, an approach which provides information on the helical structure of DNA. Fig. 3 shows the CD spectra of the DNA-lipid complex in an aqueous solution at 25°C. The DNA exhibited a spectrum with a peak at 273 nm, which is derived from the B-form DNA [1]. The dichroic intensity of the DNA was suppressed with increasing concentration of **2**(14,6). A decrease in peak height reaching a plateau at concentrations below an equimolar amount of the lipid to the phosphate groups of DNA was found, and a marked decrease was observed over an equimolar concentration of the lipid. The second decrease was accompanied by a red shift

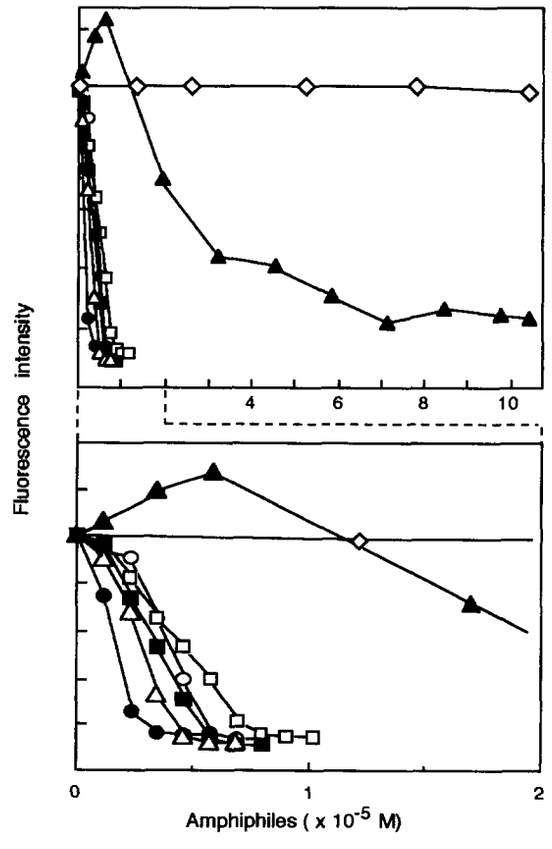

Fig. 2. Effect of various lipids on the fluorescence intensity of ethidium bromide-DNA complex. Fluorescence spectra of ethidium bromide (5×10^{-6} M) intercalated into pCH110 (6.28×10^{-6} unit M) in the presence of various lipids at 25°C. ○, **1**(12,2); ●, **1**(14,2); □, **2**(12,6); ■, **2**(14,6), △, **3**; ▲, CTAB; ◇, TMAB.

in the peak to 280 nm. A similar red shift and reduction of the $[\theta]_{273}$ value in the CD spectrum of DNA were also observed with other lipids, including **1**(12,2), **1**(14,2), **2**(12,6), and **3**, whereas CTAB and TMAB had practically no effect on the spectrum (Fig. 4A). In Fig. 4, the shoulder peaks, which appear around 240 nm in lines 6 and 7, are due to a chiral phenylene group contained in **1**(12,2) and **1**(14,2), respectively. Thus, only the bilayer-forming lipids were able to change the structure of DNA. The results show that the extent of the effect of the lipids on the spectral change was closely related to the T_c values of the lipids. The higher the T_c of an lipid, the greater the effect it has on the CD spectrum of DNA. The change in the CD spectrum due to various lipids at constant concentration (1.74×10^{-4} M) was very similar to that observed on increasing the concentration of **2**(14,6) as shown in Fig. 3. The phenomena are more clearly shown in Fig. 4B, in which the effects of these lipids on the [θ] value of the CD spectrum at 273 nm are plotted for various concentrations of the lipids. The values at 1.74×10^{-4} M of the lipids in Fig. 4B correspond to those at 273 nm of the spectra in Fig. 4A. The findings suggest that the fluidity of the lipids in the bilayer membrane determines the extent of change in the spectrum and that the rigid membrane induces a larger structural change in DNA. To test this possibility, the effect of temperature on the CD spectrum was examined using **1**(12,2), which has a T_c of 24.8°C (Fig. 5). Although no change was observed in the spectra derived from the phenylene group of the lipid over the temperature range from 15 to 30°C, the CD spectrum around 270 nm, which is derived from DNA, was lowered and shifted even at 30°C and the extent of the change was more marked at 15°C than at 30°C. These results indicate that the rigid bilayer membrane induces more drastically the conformational change in DNA than do the fluid membranes.

The CD spectrum of DNA in the absence of lipid is typical of the B form DNA, which exhibits a longwave positive and a shortwave negative band of nearly equal magnitude with an

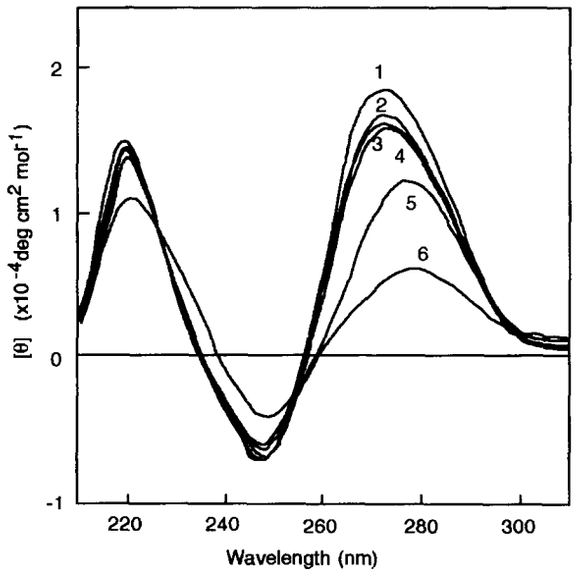

Fig. 3. Effect of lipid **1**(12,2) on CD spectra of plasmid DNA. CD spectra of pCH110 (2.09×10^{-4} unit M) were measured using various concentrations of **2**(14,6) at 25°C. Curves: 1, 0 M; 2, 0.174×10^{-4} M; 3, 0.348×10^{-4} M; 4, 0.871×10^{-4} M; 5, 1.74×10^{-4} M; 6, 4.18×10^{-4} M.

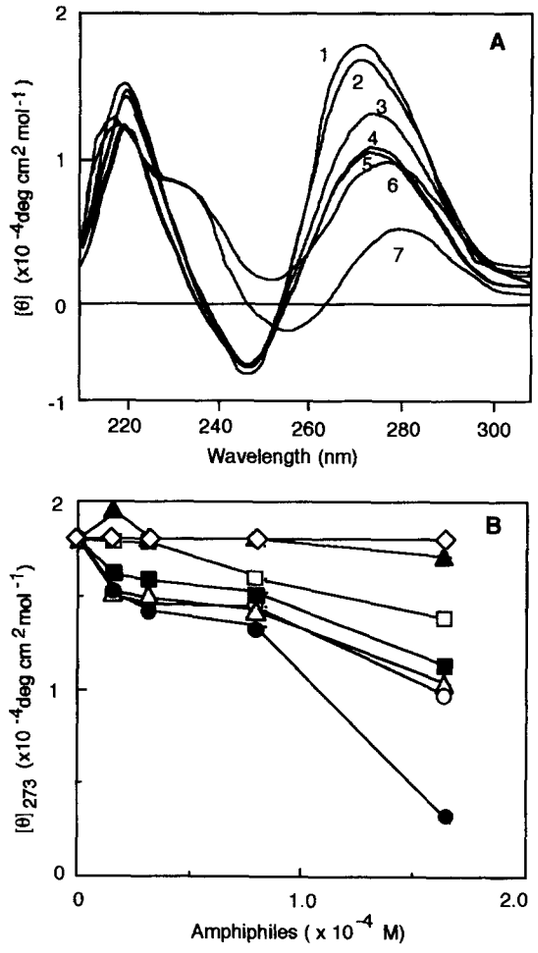

Fig. 4. Effect of various lipids on CD spectra of plasmid DNA. (A) CD spectra of pCH110 (2.09×10^{-4} unit M) were measured using various lipids (1.74×10^{-4} M) at 25°C. 1, TMAB; 2, CTAB; 3, **2**(12,6); 4, **2**(14,6); 5, **3**; 6, **1**(12,2); 7, **1**(14,2). (B) Concentration dependence of CD spectra of pCH110 (2.09×10^{-4} unit M) measured at 25°C. ○, **1**(12,2); ●, **1**(14,2); □, **2**(12,6); ■, **2**(14,6); △, **3**; ▲, CTAB; ◇, TMAB.

intersection point at about the absorption maximum. On the other hand, the spectrum in the presence of lipids is very similar to that of C form DNA, which has a negative band similar to that of the B form and a small positive band at slightly longer wavelength [1,20]. Thus, the addition of the bilayer-forming lipids to DNA probably induces the transformation of the DNA conformation from the B form to the C form, especially at temperatures below the T_c. Mou et al. [21] recently showed, using atomic-force microscopy, that DNA adsorbed to the bilayer surface of the cationic lipid, dipalmitoyltrimethylammonium propane, has a periodicity of 3.4 nm, which is expected from the B-DNA, at elevated temperatures (50–55°C). The major structural differences between the B and C forms are the rotational angle between adjacent base pairs, which are 36 and 38.6° in the B and C forms, respectively, and the inclinations of the base to the helix axis, which are −2 and −6° in the B and C forms, respectively [1]. The C form has a larger winding angle and smaller narrow groove than the B-form. At temperatures lower than the T_c of the lipid, the interaction between the lipids in the bilayer membrane might be sufficiently tight to change the distance between the phosphates and induce further winding of the helix. Thus, an in-

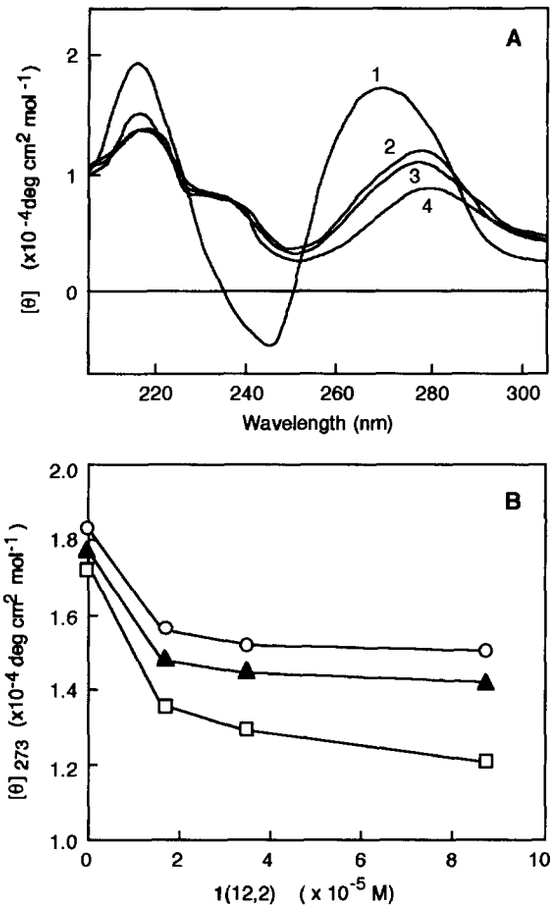

Fig. 5. Effect of temperature on CD spectra of plasmid DNA-lipid complex. (A) CD spectra of complex consisting of pCH110 (2.09×10^{-4} unit M) and **1**(12,2) (1.74×10^{-4} M) was measured at various temperatures. 1. pCH110 alone at 25°C; 2, pCH110 plus **1**(12,2) at 30°C; 3, pCH110 plus **1**(12,2) at 25°C; 4, pCH110 plus **1**(12,2) at 15°C. (B) Concentration dependence of CD spectra of complex consisting of pCH110 (2.09×10^{-4} unit M) and **1**(12,2) at various temperatures. □, 15°C; ▲, 25°C; ○, 30°C.

crease in the rotational angle and inclination probably make intercalation of the ethidium bromide molecule to DNA weaker and consequently, the efficiency of energy transfer from DNA to the ethidium bromide molecules decreases and the fluorescence would be reduced. In conclusion, the present findings indicate that cationic lipids forming a rigid bilayer membrane can induce a structural change in DNA and that the structure of DNA can be controlled by selection of the lipids and temperature.

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