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DNA-lipid complexes carrying azobenzene moieties: Preparation, characterization, and photoisomerization

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ABSTRACT

Novel DNA-lipid complexes carrying azobenzene moieties were prepared by substituting sodium counter cations with cationic amphiphilic lipids, namely lipid(AZO) and lipid(diAZO), in which the actual molar ratios of phosphate to lipid were 1:1.05 and 1:1.02, respectively. DNA-lipid(AZO) and DNA-lipid(diAZO) complexes were soluble in common organic solvents including $CHCl_3$, CH_2Cl_2, methanol, and ethanol, while insoluble in THF, toluene, and water. CD spectroscopy revealed that DNA-lipid(AZO) and DNA-lipid(diAZO) complexes took a predominantly double helical structure in methanol and $CHCl_3$ and that the helical structure was fairly stable against heating. The *trans*-azobenzene of the DNA-lipid complexes in the side chain isomerized into *cis* upon UV irradiation, while the helical conformation of DNA backbone hardly changed. The *cis*-azobenzene moiety reisomerized into *trans* upon visible-light irradiation, but they did not recover completely the original geometry of azobenzene moieties in the side chain. Both DNA-lipid(AZO) and DNA-lipid(diAZO) exhibited lyotropic LC properties. The onset temperatures of weight loss of DNA-lipid(AZO) and DNA-lipid(diAZO) were both 226 °C according to TGA in air.

1. Introduction

Azobenzene is a well-known photoresponsive chromophore that undergoes reversible photoisomerization between *trans* and *cis* forms [1]. Polymers carrying azobenzene moieties in main or side chain have attracted much attention due to their unique properties, which allow various photonic applications such as holographic and digital storage of information [2,3]. Many studies have been reported concerning polymers carrying azobenzene moieties that display photoresponsive, photoswitchable, optical memories and liquid crystalline properties [4–7]. Thus far, most of the studies have been carried out with synthetic polymers. However, there is a growing interest in natural polymers and biomacromolecules for practical applications as functional materials especially from the viewpoints of bio- and nanotechnologies and sustainable materials science. Among various biomacromolecules, DNA is one of the most abundant substances in the biosphere and quite interesting as a candidate of source material for these applications.

Since DNA is an important source of biological information depending on the base sequence [8], it has been gathering much attention as a powerful protocol for gene therapy, vaccination in biotechnology and medical applications. Okahata developed a facile method of synthesizing DNA-cationic lipid complexes and succeeded in the fabrication of membranes from them by casting their organic solutions [9]. DNA-cationic lipid complexes appear as promising gene delivery vehicles, and the structural and morphological studies have been reported. Specifically, cryo-TEM [10], freeze-fracture electron microscopy [11], synchrotron X-ray scattering [12], optical and fluorescence microscopies [13], and small-angle X-ray scattering (SAXS) [14] have given a fairly good picture of the structure of these complexes as a function of the lipid content and charge ratio between the cationic lipid and DNA. Properties and electronic functions of DNA and its organosoluble derivatives were studied considerably either in the pure form or in the form complexed with the double helix [15–19]. Recently, we synthesized DNA-cationic lipid complexes carrying 2,2,6,6-tetramethyl-1-piperidine-1-oxy (TEMPO), applied them as positive electrode materials of organic radical battery (ORB), and found that the complexes displayed two-stage discharge process [20]. The total capacity of one TEMPO-containing DNA-cationic lipid complex reached 192% of the theoretical value for one electron redox

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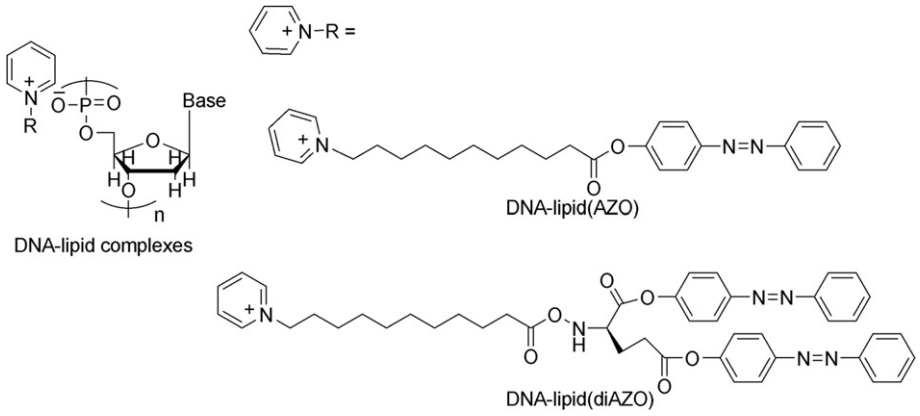


Scheme 1. The structures of DNA-lipid complexes.

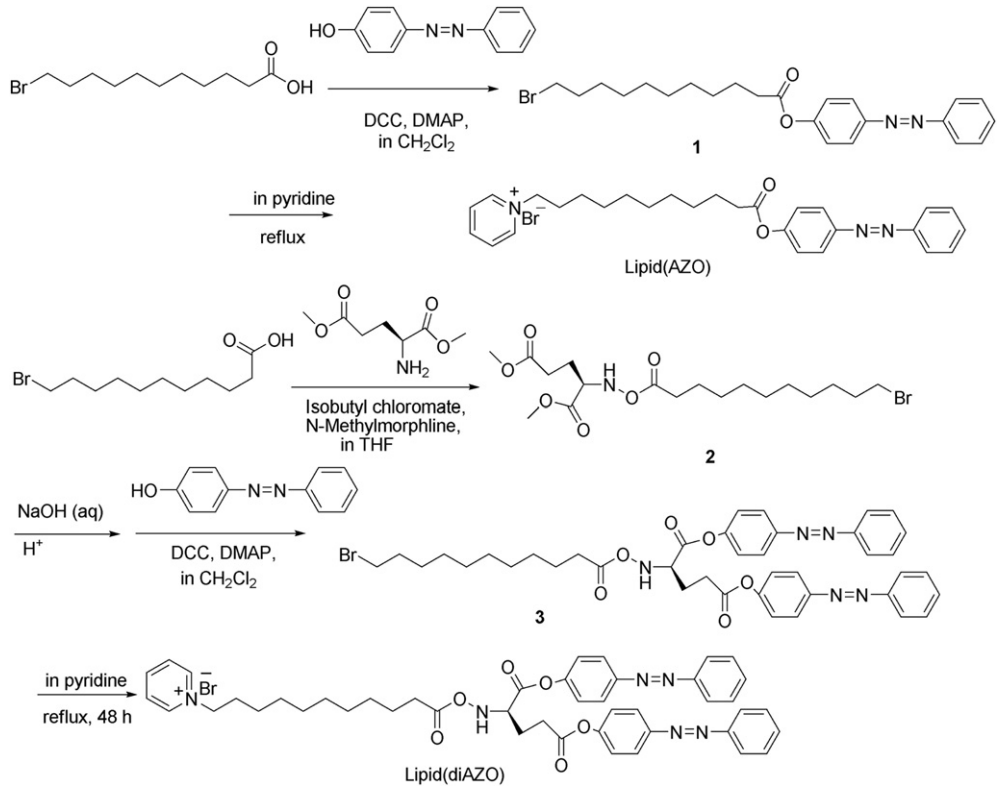
reaction, suggesting two-electron redox reactions between the cation and the anion. We have also reported the DNA-lipid complexes carrying carbazole and triphenylamine, and found that their solution emitted fluorescence in 5.7 and 76.4% quantum yields, and displayed electrochemical properties [21].

Although no research has been performed about DNA complexes carrying azobenzene, incorporation of azobenzene into DNA will possibly lead to the development of novel functional materials based on synergistic actions of azobenzene and DNA main chain. Such polymeric materials may form helical azobenzene strands based on the helical DNA main chain, which may endow efficient photoisomerization properties for potential applications such as nonlinear optics, field-effect transistors, photovoltaics, and so on. In the present paper, we would like to report for the first time the preparation and properties of azobenzene-carrying DNA-lipids, namely DNA-lipid(AZO) and DNA-lipid(diAZO) (Scheme 1), aiming at the future development of advanced polymeric functional materials.

2. Experimental section

2.1. Materials

Sodium salts of DNA from salmon testes (>95%) were donated from Japan Chemical Feeding Company, and used without farther purification. According to the data of Japan Chemical Feeding Company, the weight-average molecular weight of the DNA sample is 6.6×10^6 (ca. 30 000 bp) (tested by electrophoresis). *N,N′*-Dicyclohexylcarbodiimide (DCC, Aldrich), 4-dimethylaminopyridine (DMAP; Wako), 11-bromoundecanoic acid (Aldrich), *p*-



Scheme 2. Synthetic routes of lipid(AZO) and lipid(diAZO).

Table 1
Preparation of DNA-lipid complexes.

lipid	Yield (%)[a]	Ratio of replaced Na^+ (%)[b]	Molar ratio of phosphate to lipid (N)[c]
Lipid(AZO)	94	98	1:1.05
Lipid(diAZO)	90	97	1:1.02

[a] DNA-lipid complex is the insoluble part in water. The yield was determined according to equation (1).
[b] Determined by ICP.
[c] Calculated by P elemental analysis according to equation (3).

phenylazophenol (Wako), and H–Glu(OMe)–OMe.HCl (Aldrich) were purchased and used without further purification.

2.2. Measurements

^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JEOL EX-400 spectrometer using tetramethylsilane as an internal standard. IR and UV-vis spectra were measured on JASCO FT/IR-4100 and V-550 spectrophotometers, respectively. Circular dichroism (CD) spectra were recorded on a JASCO J-820 spectropolarimeter. Melting points (m.p.) were measured on a Yanaco micro melting point apparatus. Elemental analysis was carried out on an Elementar Vario EL-III instrument. Thermogravimetric analysis (TGA) was carried out on a Shimadzu TGA-50 thermal analyzer in air. The content of Na ion was determined by inductively coupled plasma (ICP) emission spectrometry using a Shimadzu ICP-1000 IV spectrometer; DNA-lipid samples were dissolved in 2 N HCl. Polarized optical microscope images were observed with a Nikon ECLIPSE LV100POL.

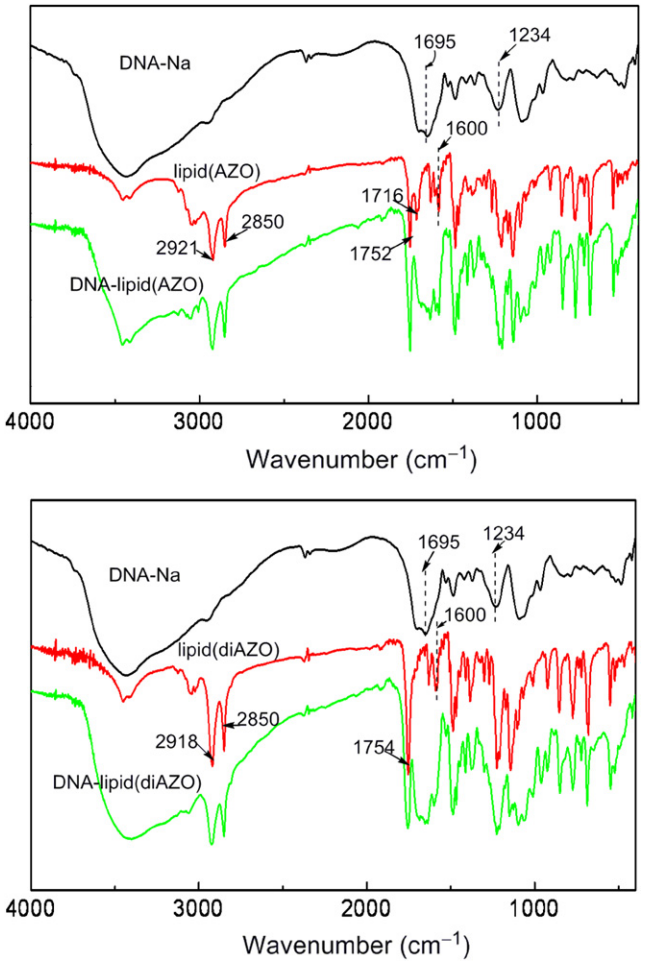


Fig. 1. IR spectra of DNA-Na, lipid(AZO), lipid(diAZO), DNA-lipid(AZO), and DNA-lipid(diAZO) (KBr pellet).

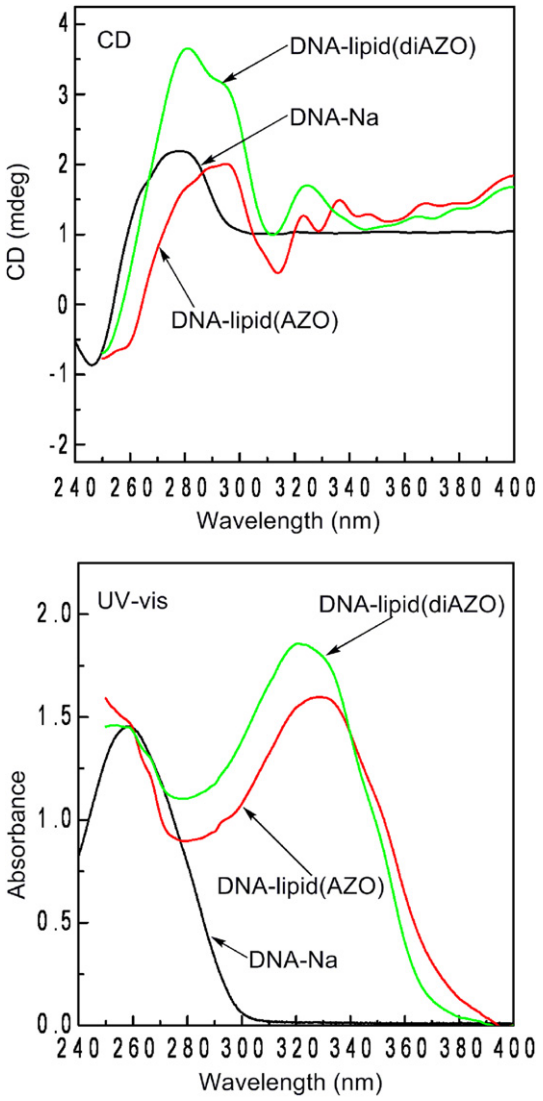


Fig. 2. CD and UV-vis spectra of DNA-lipid(AZO) and DNA-lipid(diAZO) in methanol ($c = 0.05$ mg/mL) and of DNA-Na in water ($c = 0.04$ mg/mL) at 22 °C.

2.3. Synthesis of lipids

Scheme 2 illustrates the synthetic procedures of 11-oxo-11-[4-(phenyldiazenyl)phenoxy]undecylpyridinium bromide [lipid(AZO)] and a pyridinium bromide containing two azobenzene moieties [lipid(diAZO); see the Scheme].

Lipid(AZO) was prepared as follows [21]: 11-Bromoundecanoic acid (1.33 g, 5.0 mmol) was added to a solution of DCC (1.0 g, 5.2 mmol) and DMAP (60 mg, 0.50 mmol) in CH_2Cl2 (45 mL) at room temperature. *p*-Phenylazophenol (1.13 g, 5.7 mmol) was added to the solution and the resulting mixture was stirred at room temperature overnight. The reaction mixture was washed with water (50 mL) three times, and the organic layer was dried over anhydrous MgSO4. It was filtered, and the filtrate was concentrated on a rotary evaporator. The residual mass was purified by silica gel column chromatography eluted with *n*-hexane/ethyl acetate = 19/1 (volume ratio) to give 4-(phenyldiazenyl)phenyl 11-bromoundecanoate (**1**) as a red solid. Yield 1.90 g (85%). ^1H NMR (400 MHz, $CDCl_3$): $\delta = 1.30$ (s, 12H,

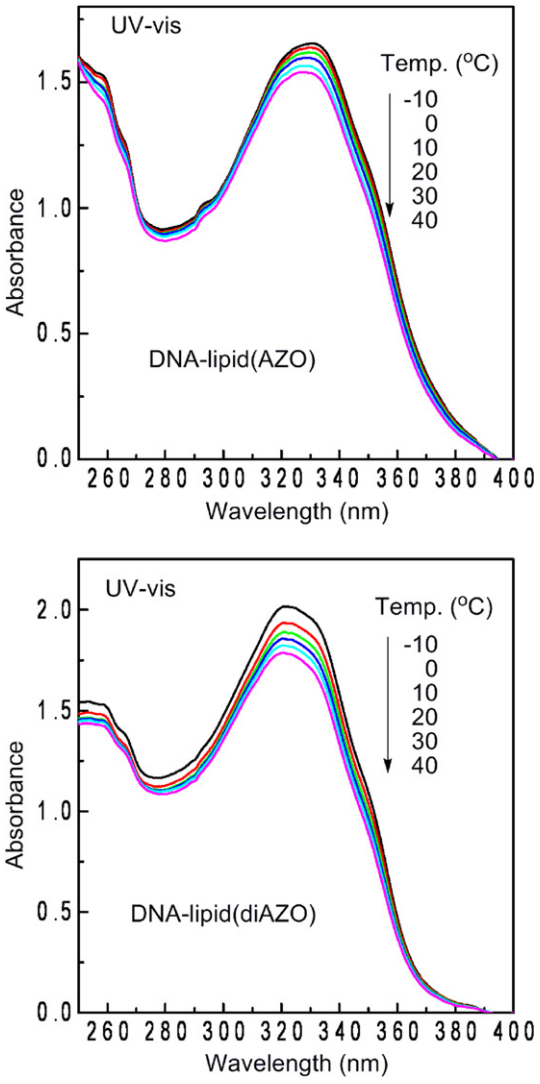


Fig. 3. Temperature-variable UV-vis spectra of DNA-lipid(AZO) and DNA-lipid(diAZO) measured in a range of −10 to 40 °C in methanol ($c = 0.05$ mg/mL).

$6CH_2$), 1.42 (m, 2H, $-OCOCH_2CH_2$), 1.80–1.87 (m, 2H, $-CH_2CH_2Br$), 2.55–2.59 (m, 2H, $-OCOCH_2$), 3.38–3.41 (m, 2H, $-CH_2Br$), 7.21–7.97 (m, 9H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.8, 28.1, 28.7, 29.0, 29.1, 29.2, 29.3, 32.7, 34.0, 34.3, 122.2, 122.7, 129.0, 131.0, 150.1, 152.5, 152.7, 171.9 ($-CO_2-$). Anal. Calcd for $C_{23}H_{29}BrN_2O_2$: C, 62.02; H, 6.56; N, 6.29. Found: C, 62.26; H, 7.04; N, 6.10.

The above-stated product **1** (1.50 g, 3.4 mmol) was dissolved in 50 mL of pyridine and stirred at reflux temperature for 2 days. After cooling to room temperature, the reaction mixture was concentrated on a rotary evaporator, and then poured into a large amount of diethyl ether to precipitate lipid(AZO). The red crystals in this step were dried in vacuum: yield 1.3 g (73%, 2.5 mmol); mp 134–136 °C; IR (KBr): 3450, 3413, 3043, 2918, 2850, 1753, 1631, 1590, 1487, 1468, 1386, 1226, 1144, 854, 774, 680 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24–1.28 (m, 12H, $6CH_2$), 1.57–1.62 (m, 2H, $COCH_2CH_2$), 1.75 (m, 2H, pyridine-CH_2CH_2), 2.51–2.54 (m, 2H, $-OCOCH_2$), 4.60–4.64 (m, 2H, pyridine-CH_2), 7.17–7.21 (m, 1H, Ar), 7.48–8.39 (m, 9H, Ar), 7.35–7.37 (m, 2H, pyridine$^+$), 8.60–8.68 (m, 2H, pyridine$^+$), 9.13–9.14 (m, 1H, pyridine$^+$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.2, 25.3, 28.3, 28.7, 30.7, 33.4, 60.7, 115.9, 122.0, 122.9, 123.8, 124.8, 128.0, 129.3, 129.5, 131.6, 144.7, 145.4, 151.8, 152.7, 171.6. Anal. Calcd for $C_{28}H_{34}N_3O_2Br$: C, 64.12; H, 6.53; N, 8.01. Found: C, 64.16; H, 6.64; N, 8.10.

Lipid(diAZO) was synthesized in a manner similar to that of Lipid(AZO).

Compound **2** was synthesized according to the literature [20]: Yield 66%, white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.29 (m, 12H, $6CH_2$), 1.42 (s, 2H, $-OCCH_2CH_2CH_2$), 1.61–1.63 (m, 2H, $-COOCH_2CH_2$), 1.81–1.86 (m, 2H, $Br-CH_2CH_2$) 2.33–2.36 (m, 4H, $COOCH_2CH_2$), 3.39–3.42 (m, 1H, NHCH), 3.40–3.50 (m, 2H, $BrCH_2$), 3.60–3.70 (m, 6H, $2CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.6, 28.1, 28.7, 29.0, 29.1, 29.2, 29.3, 30.2, 32.8, 34.0, 50.2, 61.7, 171.5, 173.3, 180.2.

Compound **3** was synthesized according to the literature [21] Yield 88%, red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.31 (s, 12H, $6CH_2$), 1.43 (s, 2H, $-OCCH_2CH_2CH_2$), 1.73–1.78 (m, 2H, $-COOCH_2CH_2$), 1.81–1.87 (q, 2H, $Br-CH_2CH_2$), 2.32 (m, 2H, CH_2COO), 2.56–2.60 (m, 4H, $COOCH_2CH_2$), 3.32–3.38 (m, 1H, NHCHCOO), 3.40–3.52 (m, 2H, $BrCH_2$), 6.22–7.97 (m, 18H, Ar). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.8, 28.1, 28.7, 29.0, 29.1, 29.2, 29.3, 30.2, 32.8, 34.4, 61.7, 122.2, 122.8, 124.0, 129.1, 131.0, 150.1, 152.5, 152.7, 171.9, 173.3, 181.2.. Calcd for $C_{40}H_{44}BrN_5O_6$: C, 62.34; H, 5.75; N, 9.09. Found: C, 62.01; H, 5.72; N, 9.04.

Lipid(diAZO): Yield 67%, pale grey solid, m.p. 170–172 °C. IR (KBr): 3450, 3409, 3043, 2919, 2850, 1753 ($\nu_{C=O}$), 1631, 1591, 1487, 1467, 1386, 1226, 1144, 1104, 854, 774, 680 cm^{-1}. ^{1}H NMR (400 MHz,$CDCl_3$): δ = 1.28 (s, 12H, $6CH_2$), 1.47 (s, 2H,–$OCCH_2CH_2CH_2$), 1.62–1.67 (m, 2H, pyridine ring–CH_2CH_2), 1.92 (m, 2H, $CHCH_2CH_2$), 2.51(m, 2H, $CHCH_2CH_2$), 2.60–2.64 (m, 2H, CH_2COO), 3.26–3.29 (m, 1H, $NHCHCH_2$), 4.41–4.64 (m, 2H, pyridine $^+CH_2$), 7.17–7.21 (m, 2H, Ar), 7.58–8.19 (m, 18H, Ar), 7.35–7.39 (m, 2H, pyridine$^+$), 8.60–8.64 (m, 2H, pyridine$^+$), 9.14–9.15 (m, 1H, pyridine$^+$). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.2, 25.3, 25.6, 28.3, 28.6, 28.7, 28.8, 28.9, 30.7, 32.8, 33.4, 51.3, 60.7, 122.5, 122.8, 123.8, 128.0, 129.5, 131.6,.2, 144.7, 145.4, 151.8, 152.7, 171.5, 173.1, 181.3 ($-CO_2-$). Calcd for $C_{45}H_{49}BrN_6O_6$: C, 63.60; H, 5.81; N, 9.89. Found: C, 63.51; H, 5.52; N, 9.84.

2.4. Preparation of DNA-lipid complexes

A small amount of lipid (2.0 mmol) in THF was added slowly into double-distilled water to form a uniform solution. An aqueous solution (200 mL) of DNA (expressed as DNA-Na below) from salmon testes (0.50 g, 0.68 mmol bp^{-1}) was added dropwise into the aqueous lipid solution (the feed mole ratio of phosphate to lipid was 1.50). The formed DNA-lipid complex immediately precipitated

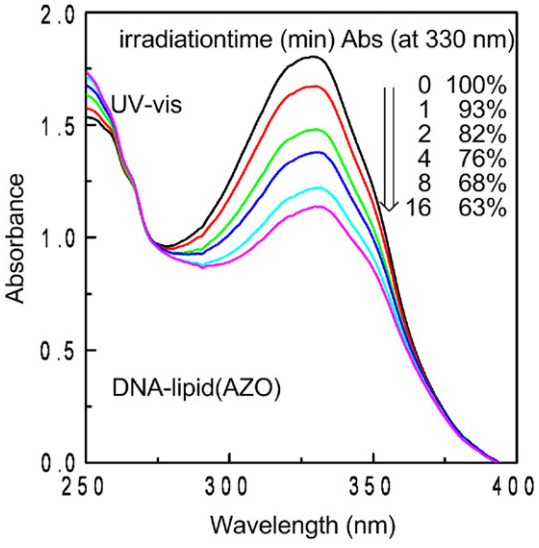


Fig. 4. Variations of the UV-vis spectra of DNA-lipid(AZO) with UV irradiation at 300 nm < λ < 400 nm in methanol at room temperature ($c = 0.05$ mg/mL).

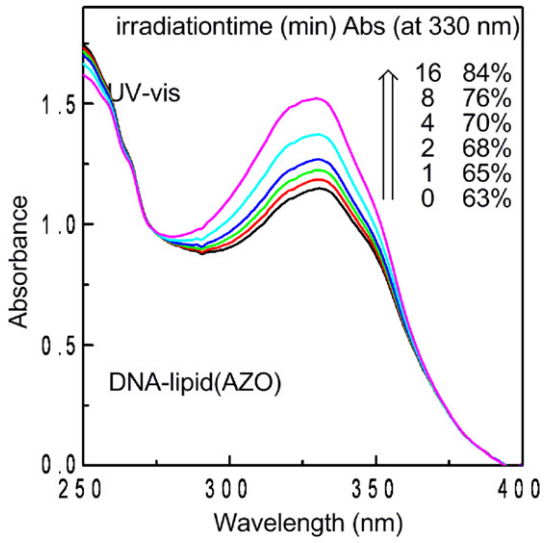


Fig. 5. Variations of the UV-vis spectra of DNA-lipid(AZO) with visible-light irradiation at 400 nm < λ in methanol, after UV irradiation at 300 nm < λ < 400 nm at room temperature for 16 min ($c = 0.05$ mg/mL).

out from the aqueous solution. After mixing for 24 h, the precipitate was collected by filtration, washed with water to remove free DNA, and then dried in a vacuum oven at 50 °C for 24 h. The white DNA-lipid complex was dissolved in chloroform and reprecipitated in THF two times. The obtained DNA-lipid complex was examined by elemental analysis to decide the actual composition of phosphate anion and the cationic lipid in the DNA-lipid complex.

DNA-lipid(AZO): IR (KBr): 3455, 3058, 2922, 2852, 1753, 1654, 1637, 1486, 1466, 1208, 1141, 1099, 849.5, 775, 688, 548.7, cm^{-1}. Anal. Calcd for DNA-lipid(AZO) complex with 1:1 ratio of phosphate anion to cationic lipid(AZO): C, 58.92; H, 6.06; N, 12.29; P, 4.02. Found: C, 60.28; H, 6.15; N, 10.85; P, 1.58.

DNA-lipid(diAZO): IR (KBr): 3403, 2923, 2851, 1755, 1654, 1599, 1486, 1466, 1377, 1226, 1150, 1097, 1010, 962, 849, 686 cm^{-1}. Anal. Calcd for DNA-lipid(diAZO) complex with 1:1 ratio of phosphate anion to cationic lipid(diAZO): C, 60.06; H, 5.64; N, 12.47; P, 2.83. Found: C, 60.25; H, 6.52; N, 10.62; P, 2.86.

2.5. Calculation of the yield of DNA-lipid complexes

We define the yield of DNA-lipid complex as the ratio of the actual weight of DNA-lipid complex to the theoretical weight of DNA-lipid complex based on DNA-Na. The yield of DNA-lipid complex was calculated using the following equation.

$$\text{yield}(\%) = \frac{W_{\text{DNA-lipid}} \times 100}{W_{\text{DNA-Na}}\left(1 + \frac{M_{\text{lipid}} - M_{\text{NaBr}}}{M_{\text{base}}}\right)} \quad (1)$$

where $W_{\text{DNA-lipid}}$ is the actual weight of DNA-lipid complex, $W_{\text{DNA-Na}}$ is the feed weight of DNA-Na, M_{lipid} and M_{NaBr} are the molecular weights of lipid and NaBr, respectively, and M_{base} is the average molecular weight of base groups in the repeating unit of DNA-Na [the value is 347.91 which is calculated from the structures of base couple (according to the fragment sequence of salmon DNA with an AT/GC ratio of approximately 56:44)] [15a].

2.6. Determination of the actual mole ratios of phosphate to lipid in the DNA-lipid complexes

The actual mole ratio of phosphate to lipid in the DNA-lipid complexes was estimated from the amounts of phosphorus before and after complexation. Phosphorus was determined by elemental analysis. The actual percent content of phosphorus in a DNA-lipid (P_a) was calculated from the following equation:

$$P_a = \frac{P_0}{1 + \frac{M_{\text{lipid}} - M_{\text{NaBr}}}{N \times M_{\text{base}}}} \quad (2)$$

where P_0 is the percent content of phosphorus in DNA-Na, which was determined by elemental analysis to be 8.90%, M_{lipid} and M_{NaBr} are the molecular weights of lipid and NaBr, respectively, M_{base} is the average molecular weight of base groups in the repeating unit of DNA-Na [the value is 347.91, which was calculated from the structures of base couple (according to the fragment sequence of salmon DNA with an AT/GC ratio of approximately 56:44)] [22], and N is the actual mole ratio of phosphate to lipid in the DNA-lipid complexes. When solved for N, the equation is

$$N = \frac{P_a \times \left(M_{\text{lipid}} - M_{\text{NaBr}}\right)}{(P_0 - P_a) \times M_{\text{base}}} \quad (3)$$

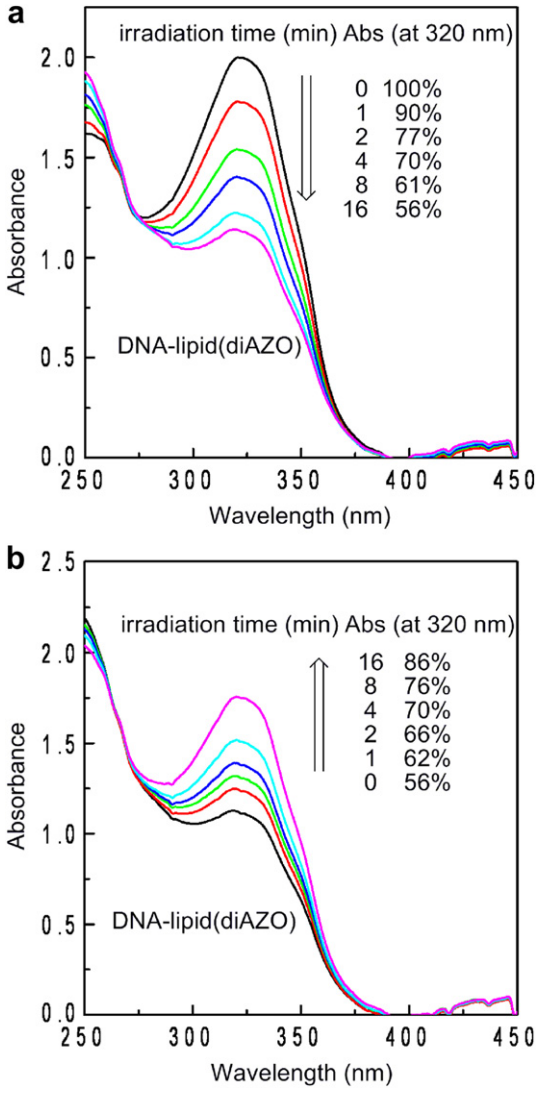


Fig. 6. Variations of the UV-vis spectra of DNA-lipid(diAZO) in methanol at 20 °C with (a) irradiation at 300 nm < λ_1 < 400 nm and (b) visible-light irradiation at 400 nm < λ after UV irradiation at 300 nm < λ < 400 nm at room temperature for 16 min ($c = 0.05$ mg/mL).

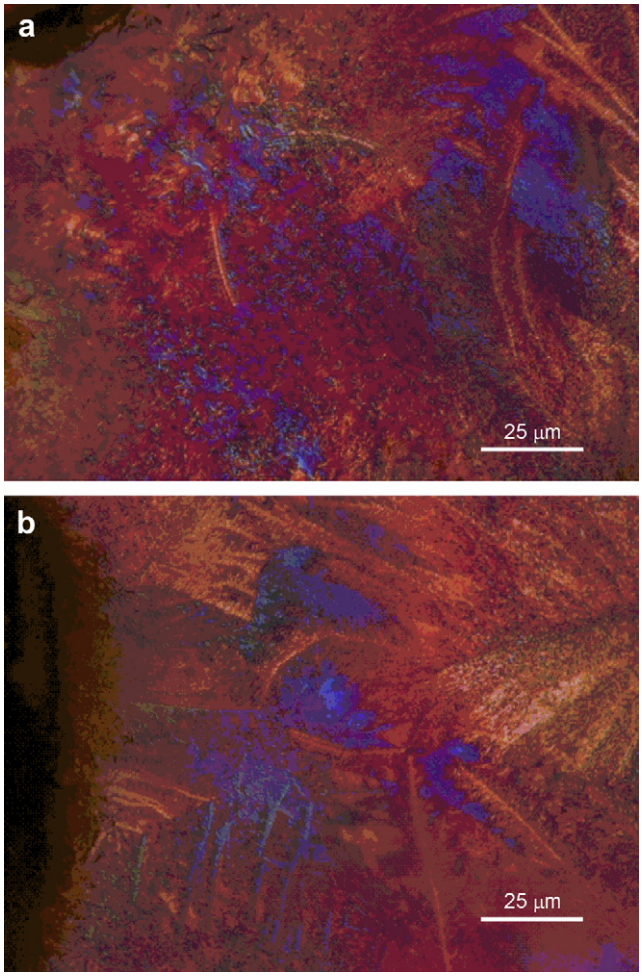


Fig. 7. Polarized optical microscope images of solutions of (a) DNA-lipid(AZO) and (b) DNA-lipid(diAZO) in $CHCl_3$ (20 wt%) observed at room temperature.

2.7. Photoisomerization

A sample solution was irradiated in a quartz glass tube with a 400 W high-pressure mercury lamp (Fuji Glass Work HB-400). To irradiate at 300 nm $< \lambda <$ 400 nm, Pyrex glass and a Toshiba D33S color glass filter were used. To irradiate at 400 nm $< \lambda <$ 500 nm, Pyrex glass and a Toshiba L42 color glass filter were used. The distance between the sample and lamp was set at 20 cm.

3. Results and discussion

3.1. Synthesis of lipids

Scheme 2 illustrates the synthetic routes for the azobenzene-containing lipid(AZO) and lipid(diAZO). Lipid(AZO) was synthesized by the reaction of 11-bromoundecanoic acid with *p*-phenylazophenol using DCC as a condensation agent, DMAP as a catalyst, followed by the reaction of product **1** with pyridine. Lipid(diAZO) was prepared similarly by the condensation of the *p*-phenylazophenol with compound **2** and the subsequent reaction of product **3** with pyridine. The lipids were identified by ^{1}H, ^{13}C NMR, and IR spectroscopies besides elemental analysis.

3.2. Preparation of DNA-lipid complexes

Table 1 summarizes the conditions and results of preparation of DNA-lipid(AZO) and DNA-lipid(diAZO) complexes. When the aqueous solution of DNA-Na was added into the aqueous lipid solutions, the DNA-lipid complexes immediately precipitated from the aqueous solution. After stirring for 24 h, the white flocculent products were easily isolated by filtration to afford DNA-lipids in good yields (94 and 90%). The ICP data revealed that the Na^+ ion of DNA was almost completely replaced by the cationic lipids. The formed DNA-lipids were completely soluble in $CHCl_3$, CH_2Cl_2, methanol, and ethanol, while insoluble in water, THF, toluene, diethyl ether, and *n*-hexane. The elemental analysis showed that the obtained DNA-lipid complexes possessed 1:1.05 and 1:1.02 compositions of a phosphate anion to the cationic amphiphile (Table 1).

3.3. Properties of DNA-lipid complexes

Fig. 1 shows the FTIR spectra of DNA-Na, lipid(AZO), and DNA-lipid(diAZO) complexes in the range of 4000–400 cm^{-1}. Judging from the absorption band at 1234 cm^{-1} (asymmetric stretching vibration of PO_2^-) in the DNA-Na FTIR spectrum, DNA adopts the B-form conformation [19]. The absorption peak at 1695 cm^{-1} is attributed to the hydrogen-bonded C=O stretching in the base pairs [thymine (T), guanine (G), and cytosine (C)]. The asymmetric shape in this absorption band is due to the C=N and C=C stretching in the aromatic bases of DNA around 1640 cm^{-1}. In the spectrum of lipid(AZO) in Fig. 1, the absorptions at 2921 and 2850 cm^{-1} are assignable to $-CH_2-$ stretching vibration (see Scheme 1), and the absorption at 1752 cm^{-1} to the non-hydrogen-bonded ester C=O stretching vibration. The absorption band observed at 1600 cm^{-1} is due to the stretching of C=C in aromatic azobenzene rings. The spectrum of the DNA-lipid(AZO) complex displays almost all the absorption bands of both lipid(AZO) and DNA-Na, suggesting the presence of both lipid(AZO) and double-strand DNA in the complex. The spectrum of the DNA-lipid(diAZO) also exhibits absorptions characteristic of both lipid(diAZO) and DNA-Na.

The top part of Fig. 2 shows the CD spectra of DNA-lipid(AZO) and DNA-lipid(diAZO) measured in methanol, along with the one of DNA-Na in water for comparison. Pristine DNA in an aqueous solution exhibited a positive Cotton effect at 270 nm and a negative Cotton effect at 245 nm, while lipid(AZO) and lipid(diAZO) were CD inactive (their CD spectra are not shown). On the other hand, both DNA-lipid(AZO) and DNA-lipid(diAZO) displayed a large plus CD signal at 290 nm and a minus one at 260 nm in methanol. This was also the case in $CHCl_3$ solution. Thus it is evident that DNA-lipid(AZO) and DNA-lipid(diAZO) adopt a double helical C-form conformation different from that of virgin DNA [9b]. In the UV-vis spectra, DNA-lipid(AZO) and DNA-lipid(diAZO) displayed similar absorption peaksat 330 and 320 nm, respectively, which are attributable to azobenzene. Further, these complexes showed absorption shoulders attributable to DNA around 260 nm. Thus the absorptions of the DNA complexes in the range of 250–400 nm confirm that lipid(AZO) and lipid(diAZO) moieties have been incorporated into DNA.

When the measuring temperature was raised from −10 to 40 °C in methanol, the magnitude of Cotton effect decreased only slightly (CD spectra are not shown). This phenomenon resembles the aqueous DNA-Na solution which exhibited slightly changes of Cotton effect in a temperature range of 10–80 °C in aqueous solution (the Figure is not shown). Thus it can be said that the helical structure of the DNA-lipids is thermally very stable in the temperature range. The UV-vis spectrum of DNA-Na in water hardly changed in a range of 10–80 °C. The temperature dependence of UV-vis spectra of DNA-lipid(AZO) and DNA-lipid(diAZO) was examined (Fig. 3). The UV-vis spectral peaks of DNA-lipid(AZO) and DNA-lipid(diAZO) slightly decreased when the measuring

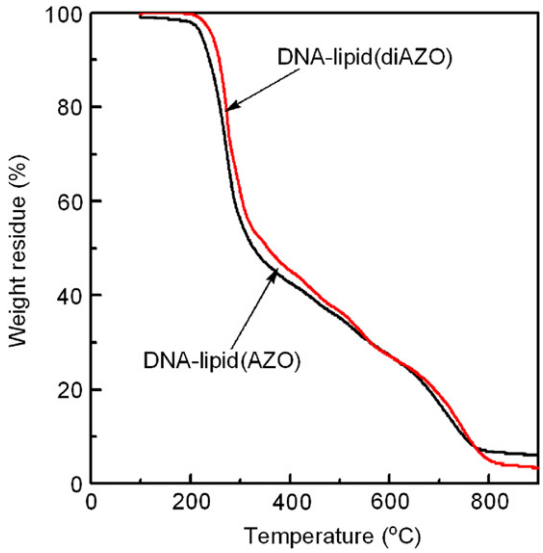


Fig. 8. TGA curves of DNA-lipid(AZO) and DNA-lipid(diAZO) measured at a heating rate of 10 °C/min in air.

temperature was raised from −10 to 40 °C. This suggests that the azobenzene moieties in the side chain of the present polymers slightly suffered the *trans-cis* isomerization with changing the temperature [23].

3.4. Photoinduced conformational change

Fig. 4 depicts the change of UV-vis spectra of DNA-lipid(AZO) during the photoisomerization process. When the complex solutions in methanol were irradiated in a quartz glass cell with a 400 W high-pressure mercury lamp through Pyrex glass and a color glass filter to exclude the light of the wavelength below 300 nm and above 400 nm, the UV-vis absorption at 330 nm decreased with the irradiation time. The reduction of the absorption leveled off after 16 min. This data indicates that the *trans*-azobenzene moiety isomerized into the *cis* form upon irradiation of UV light with the wavelength of 300–400 nm. The CD signal at 290 nm based on the helical DNA backbone hardly changed upon UV irradiation (CD spectra are not shown).

We next examined visible-light irradiation ($420\ \text{nm} < \lambda$) to achieve isomerization of azobenzene from cis form to *trans* one (Fig. 5). As a result, the absorption at $\lambda_{max} = 330$ nm recovered 84% of the original magnitude upon photoirradiation for 16 min. The CD signal at 260 nm of the DNA-lipid(AZO) hardly changed upon visible-light irradiation. We continued visible-light irradiation for 1 h to find no obvious change of the CD spectrum after 16 min. As seen from Fig. 6, the photoisomerization of azobenzene moieties of DNA-lipid(diAZO) was similar to that of DNA-lipid(AZO).

3.5. Liquid crystalline (LC) properties

Fig. 7 shows the polarized optical microscope (POM) images of solutions of DNA-lipid(AZO) and DNA-lipid(diAZO) in $CHCl_3$. They exhibited birefringence patterns at room temperature, which hardly changed upon raising temperature to 200 °C, presumably due to regulated aggregation. We examined LC properties of DNA-Na in water along with lipid(diAZO) with POM, and found that they did not show LC properties. It is likely that the pendent azobenzene groups are effective for the complexes to be arranged in a regulated manner, leading to the formation of lyotropic liquid crystals. We measured the DSC of DNA-lipid(diAZO) and DNA-lipid(AZO), but could not observe melting and phase-transition temperatures in the range of 30–220 °C, indicating that the polarized optical microscope images were not crystals but liquid crystals.

3.6. Thermal stability

DNA-Na gradually started weight loss around 150 °C, which seems due to water loss, and probably the real degradation appeared to occur at about 250 °C in TGA. Fig. 8 depicts the TGA traces of DNA-lipid(AZO) and DNA-lipid(diAZO). The onset temperatures of weight loss of DNA-lipid(AZO) and DNA-lipid(diAZO) were both 226 °C under air, and exhibited similar thermal stability to each other. They did not completely lose weight even at 900 °C, which is attributable to phosphorus pentoxide.

4. Conclusions

Novel DNA-lipid complexes carrying azobenzene were prepared by substituting sodium counter cations with cationic amphiphilic lipid(AZO) and lipid(diAZO); the actual molar ratios of phosphate to lipid were 1:1.05 and 1:1.02, respectively. The DNA-lipid(AZO) and DNA-lipid(diAZO) were soluble in common organic solvents including $CHCl_3$, CH_2Cl_2, methanol, and ethanol, but insoluble in THF, toluene, and water. CD spectroscopic studies revealed that DNA-lipid(AZO) and DNA-lipid(diAZO) took predominantly double helical structure in $CHCl_3$ and methanol, and the helical structure was very stable against heating. The *trans*-azobenzene of the DNA-lipid complexes in the side chain isomerized into *cis* upon UV irradiation, while the helical conformation of DNA backbone hardly changed. The *cis*-azobenzene moiety reisomerized into *trans* upon visible-light irradiation, but they did not recover completely the original geometry of azobenzene moieties in the side chain. Both DNA-lipid(AZO) and DNA-lipid(diAZO) displayed lyotropic liquid crystalline properties. The onset temperatures of weight loss of DNA-lipid(AZO) and DNA-lipid(diAZO) were both 226 °C under air.

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