Preparation and Characterization of DNA–Lipoglutamate Complexes

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We newly prepared DNA–lipoglutamate polyion complexes by mixing aqueous solutions of DNA and cationic lipoglutamates such as α,γ-dibutyl glutamate ($2C_4N^+$), α,γ-dihexyl glutamate ($2C_6N^+$), and α,γ-dioctyl glutamate ($2C_8N^+$). Formation of the DNA–lipoglutamate complex was confirmed by gel chromatography, elemental analysis, CD spectra, and light scattering measurement. Compaction of DNA by binding with the cationic lipoglutamate was revealed by multi-angle light scattering. The DNA–lipoglutamate complexes showed the high stability for enzymatic hydrolysis by DNase I, which was confirmed by a quartz-crystal microbalance (QCM) technique. The DNA–lipoglutamate complexes showed the strong interactions with the lipid membranes and tumor cells compared with native DNA.

Recently, development of delivery system for oligonucleotides and genes into mammalian cells has been remarkably improved. A number of new techniques have been devised to introduce foreign DNA into cells. Basic requirements for the therapeutic use of nucleotides are both of increase in their stability and efficient cell uptake. Structural analogs such as phosphorothioate,[1] methylphosphonate,[1] and zwitterionic oligonucleotides[2] have been synthesized to increase the resistance to enzymatic hydrolysis. Several modifications have been proposed to increase the cell uptake of nucleic acids. Substitutions of nucleic acids with lipophilic group[3—6] or cell receptor ligand[7,8] have been explored as a method of efficient transport into cells. An alternative approach would be to use molecular assemblies without any chemical modification of nucleic acids itself. It seems convenient and easy for us to employ molecular assemblies of chemically unmodified nucleic acids. Because in many cases chemical modifications of nucleic acid could not escape from the complication for the separation of isomers and the decrease of activity. Encapsulations of nucleic acid into a neutral[9] or a polysaccharide-coated liposome[10] resulted in the increase of the activity. Bindings of nucleic acid to polycation such as cationic lipid liposome,[11] lipopolyamine,[12] poly(L-lysine),[13] and DEAE-dextran[14] through ionic interaction have been developed for the efficient cell uptake.

In this paper, we newly developed a DNA complex with cationic dialkyl glutamate (lipoglutamate) through ionic interactions as shown in Fig. 1. Synthesis of lipoglutamate and preparation procedure of the DNA–lipoglutamate complexes are simple and convenient. The lipoglutamates employed in this study would be expected to be biodegradable and biocompatible. We investigated complex formation, structure, enzymatic hydrolysis, interaction with lipid membrane, and cell uptake of the DNA–lipoglutamate complexes.

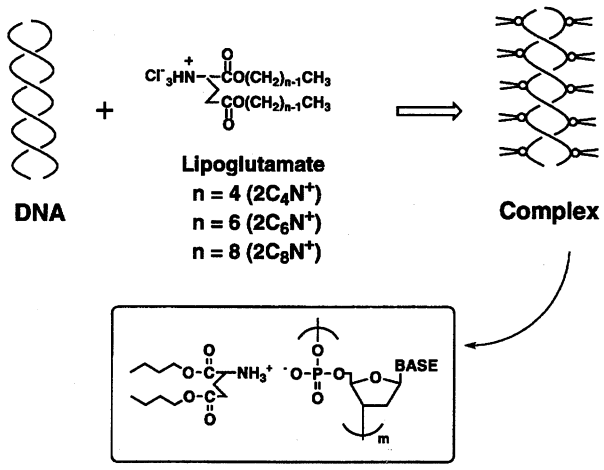

Fig. 1. A schematic illustration of the DNA–lipoglutamate complex.

Experimental

Syntheses of Lipoglutamates; α,γ-Dibutyl Glutamate ($2C_4N^+$), α,γ-Dihexyl Glutamate ($2C_6N^+$), and α,γ-Dioctyl Glutamate ($2C_8N^+$): Dibutyl glutamate ($2C_4N^+$) was synthesized as follows. The mixture of L-glutamic acid (7 g, 0.048 mol) and 1-butanol (10 g, 0.13 mol) was bubbled with HCl gas under stirring at 80 °C for 5 h, thereafter was stirred for 12 h. The mixture was extracted with dichloromethane–water. Dibutyl glutamate hydrochloride salt was obtained by the evaporation of dichloromethane under reduced pressure (Yield 2.1 g, 24%) and the purity was inspected on thin-layer chromatogra-

phy (TLC, Iatroscan MK-5, Iatron, Tokyo); retention fraction (R_f)=0.65 (with chloroform : methanol=8 : 2 (v/v)). IR (neat) 1200 (C–O–C), 1730 (C=O), and 2870 cm^{-1} (C–H); ^1H NMR ($CDCl_3$) δ=0.9 (6H, CH_3), 1.5 (8H, C–$(CH_2)_2$–C of alkyl chain), 2.5 (4H, C–$(CH_2)_2$–C of glutamate), and 4.2 (5H, C–CH_2–O and N–CH).

Dihexyl glutamate ($2C_6N^+$) was synthesized as follows. The mixture of L-glutamic acid (13 g, 0.088 mol), 1-hexanol (21 g, 0.20 mol), and *p*-toluenesulfonic acid (21 g, 0.11 mol) suspended in toluene (250 ml) was refluxed for 12 h. Thereafter toluene was removed by evaporation. The residue was dissolved in dichloromethan and washed with water in the presence of triethylamine. Organic phase was evaporated, and the resulting liquid was dissolved in anhydrous benzene. To the solution HCl gas was bubbled, and the HCl salt of $2C_6N^+$ could be obtained as a solid after the evaporation. Yield 20% (6 g). TLC (chloroform : methanol=8 : 2 (v/v)) R_f=0.65; IR (neat) 1200 (C–O–C), 1730 (C=O), and 2870 cm^{-1} (C–H); ^1H NMR ($CDCl_3$) δ=0.9 (6H, CH_3), 1.5 (16H, C–$(CH_2)_4$–C of alkyl chain), 2.5 (4H, C–$(CH_2)_2$–C of glutamate), and 4.2 (5H, C–CH_2–O and N–CH).

Dioctyl glutamate ($2C_8N^+$) was synthesized by the same procedure with $2C_6N^+$. Yield 50% (2 g). TLC (chloroform : methanol=8 : 2 (v/v)) R_f=0.75; IR (NaCl) 1200 (C–O–C), 1740 (C=O), and 2870 cm^{-1} (C–H); ^1H NMR ($CDCl_3$) δ=0.9 (6H, CH_3), 1.5 (24H, C–$(CH_2)_6$–C of alkyl chain), 2.5 (4H, C–$(CH_2)_2$–C of glutamate), and 4.2 (5H, C–CH_2–O and N–CH).

Preparation of a DNA–Lipoglutamate Complex: Salmon sperm DNA (Na salt) was purchased from Sigma. The DNA was irradiated with a probe-type sonifier (UR-200P, Tomy Co., Tokyo) at 10 W and 4 °C for 40 min. Molecular weight of the sonicated DNA was determined to be 430 kD (weight-average molecular weight, M_w) by multi-angle light scattering method. The length of the sonicated DNA was estimated from the weight-average molecular weight to be ca. 500 base-pair.

The sonicated DNA solution (10 mg DNA in 10 ml pure water) was mixed with 10 ml of pure water containing 8 mg of $2C_4N^+$, 9.6 mg of $2C_6N^+$ or 11 mg of $2C_8N^+$. The molar ratio of phosphate anion of DNA to lipoglutamate was 1 : 1. The mixture was stirred at 20 °C (room temperature) for a couple of hours. Collection and purification of the DNA–lipoglutamate complexes were carried out by gel chromatography (Sephacryl S-1000 super fine, ϕ 20×400 mm) for the DNA–$2C_4N^+$ and the DNA–$2C_6N^+$ or centrifugation (4000 rpm, 10 min) for the DNA–$2C_8N^+$.

Spectroscopic Observation: Absorption spectra were recorded on a UV-240 spectrophotometer (Shimadzu Co., Tokyo) with a 10 mm path-length cuvette. Circular dichroism (CD) spectra were recorded on a J-20A spectropolarimeter (JASCO Co., Tokyo) with a 10 mm path-length cuvette. The concentration of DNA was 0.2 mg ml^{-1}.

Absolute molecular weight of DNA was determined by multi-angle light scattering photometers (Dawn® Model F, Wyatt Technology, California) connected with high-performance size exclusion chromatography (pump; JASCO Model PU-980, injector; Rheodyne Model 7125, column; Shodex KW-804). The quantity of DNA injected was 0.1 mg/20 μl. Elution solvent was 100 mM NaCl aqueous solution. Elution rate was 1 ml min^{-1}. Elution was monitored by both of refractive index detector (Showa Denko K.K. Model SE-61) and light scattering (Dawn® Model F). Analysis such as molecular weight and conformation parameter at elution peak was carried out according to the Wyatt Technology Software Program, ASTRA®GPC.

Assay for Hydrolysis of DNA by Deoxyribonuclease: Quantitative and time-dependent measurements of hydrolysis of DNA by deoxyribonuclease (DNase I) were carried out by using a quartz-crystal microbalance (QCM) technique. A QCM is known to provide a very sensitive mass measuring device because the resonance frequency decreases upon the increase of a given mass on the QCM in a nanogram level.[15—19] The QCM employed is a commercially available 9-MHz, AT-cut quartz (9 mm diameter) on both sides of which gold electrodes were deposited. The one side of the QCM plate was covered with a rubber to avoid the contact with an aqueous phase. The QCM was connected to a handmade oscillator designed to drive the quartz at its resonance frequency in water. The frequency changes were followed by universal frequency counter Model SC7201 (Iwatsu Co., Tokyo) attached to a microcomputer system Model PC9801 (NEC Co., Tokyo). Calibration showed that a frequency decrease of 1 Hz corresponded to a mass increase of 1.05 ng on the QCM electrode (16 mm^2) both in aqueous solution and in air.[15—19]

Assay procedures for enzymatic hydrolysis of DNA by a QCM were shown in Fig. 2. Sonicated salmon sperm DNA (ca. 500 bp, 50 mg) was reacted with bis(2-hydroxyethyl)-disulfide (150 mg, Tokyo Kasei, Tokyo) in the presence of 1-cyclohexyl-3-(2-morpholinylethyl)carbodiimide metho-*p*-toluenesulfonate (1 g, Nacalai tesque, Kyoto) in 25 ml of 10 mM MES buffer (pH 6.0) for 1 d at 25 °C under stirring to introduce disulfide groups to the terminal ends of the DNA chains.[20] The DNA bearing disulfide group was precipitated by the addition of 20 ml acetone into the reaction mixture. The precipitate was collected by centrifugation and lyophilized.

The DNA chains were immobilized on the gold electrode on one side of the QCM plate by immersing it in the aqueous solution of 0.3 M NaCl (1 M=1 mol dm^{-3}) and the DNA bearing disulfide group (5 mg/10 ml) at 37 °C for 5 h. The amount of the probe nucleotide immobilized was calculated to be ca. 50 ng on the electrode from the frequency decrease of 45±5 Hz.[16] The QCM immobilized with DNA–lipoglutamate complex was prepared by immersing the DNA–immobilized QCM in 10 mM NaCl aqueous solution containing lipoglutamate at 17 °C for 12 h. The amount of the lipoglutamate bound to DNA was ca. 50 ng from the frequency decrease. This value means that 90—100% of phosphate anions of DNA was bound with lipoglutamates.

The QCM was immersed in 10 mM acetate buffer (pH 5.0) containing 10 mM NaCl and 4.2 mM $MgCl_2$, and frequency changes upon the addition of DNase I (60 μg) were monitored at 25 °C for 2 h. When DNAs immobilized on the QCM were cleaved by DNase I, frequencies of the QCM increased due to the mass decrease. Percentages of DNA cleavage were calculated from the frequency increase of the QCM.

Detection of Interaction between a Phospholipid Monolayer and DNA–Lipoglutamate Complexes: A monolayer of distearoylphosphatidylcholine (DSPC, Nippon Oil and Fats Co., Ltd., Tokyo) was spread on Milli-Q water containing 150 mM NaCl (pH 5.8) in a Teflon®-coated

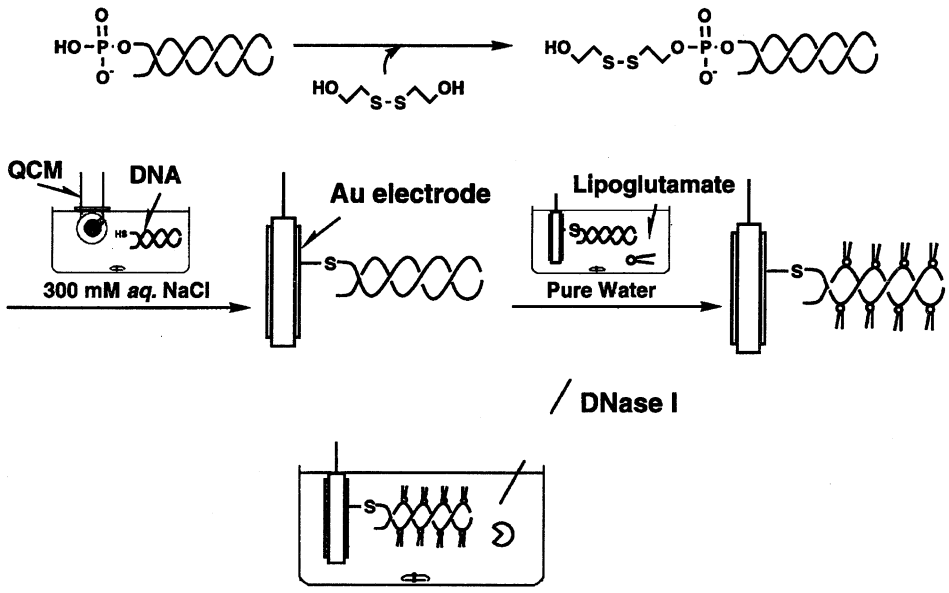

Fig. 2. Assay procedure for enzymatic hydrolysis of DNA–lipoglutamate complexes by using a quartz-crystal microbalance, on which a DNA–lipoglutamate was immobilized. The immobilization methods were described in the text. The hydrolysis of DNA immobilized on a QCM was followed by frequency increases (mass decrease) of the QCM, responding to the addition of DNase I (60 μg) in the aqueous solution (25 °C, 10 mM acetate buffer, pH 5.0, 10 mM NaCl, and 4.2 mM $MgCl_2$).

trough with a microcomputer-controlled Teflon® barrier (USI Co., Fukuoka, Japan). The stable monolayer formation was confirmed from surface pressure (π)-area (A) isotherms at 20 °C. Surface pressure changes ($\Delta\pi$) at the constant area of the monolayer (25 cm^2) were monitored responding to the addition of a native DNA and the DNA–lipoglutamate complexes (2 ppm) into the subphase.[17]

Assay for Cellular Interaction of DNA Complexes: FITC-labeled DNA was prepared as follows: A 50-mg DNA solution in 50 ml of 100 mM MES buffer (pH 6.0) was reacted with ethylenediamine (75 mg) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (96 mg), and allowed to react under stirring at room temperature for 24 h. The product was precipitated by the addition of sodium chloride (1.45 g) and cold ethanol. The precipitates were collected by centrifugation (35000 rpm, 10 min), washed twice with cold ethanol/water (7:3 by vol), and lyophilized. To the ethylenediamine-conjugated DNA (50 mg) solubilized with 10 ml of carbonate buffer (pH 9.0)/*N,N*-dimethylformamide (4:1 by vol), fluoresceinisothiocyanate (97 mg, FITC, Dojindo Laboratories, Japan) was added, and allowed to react in the dark under stirring at room temperature for 48 h. The product was precipitated by the addition of sodium chloride (1.0 g) and cold ethanol. The precipitates were collected by centrifugation twice (35000 rpm, 10 min). FITC-labeled DNA was purified by gel chromatography (Sephacryl S-1000 superfine). The substitution degree of FITC per DNA was 0.22.

Human adenocarcinoma Hela cells (2×10^5 cells) in 1 ml of serum-free culture medium (ASF104, Ajinomoto Co., Inc., Japan) was seeded into 24-well multiplate, and was incubated in 5% CO_2–95% air at 37 °C. Then the cells were co-incubated with FITC–DNA and FITC–DNA– lipoglutamate complexes ([DNA]=40 $\mu g\,ml^{-1}$) for 6 h at 37 °C. Thereafter, cells were collected by treating with the aqueous solution of 0.05% trypsin-0.02% ethylendiaminetetraacetic acid. The cells loading FITC-DNA was detected by flowcytometer (EPICS-XL, Coulter, USA).

Results and Discussion

Formation of a DNA–Lipoglutamate Complex: The mixing of aqueous solutions of DNA with $2C_4N^+$, $2C_6N^+$, and $2C_8N^+$ at a given concentration in experimental section resulted in transparent solution, turbid solution, and precipitate, respectively. Isolations of the DNA–$2C_4N^+$ and the DNA–$2C_6N^+$ complexes were carried out by gel filtration (Sephacryl S-1000 superfine, $\phi20\times400$ mm). Elution peak of the DNA–$2C_4N^+$ appeared near the same volume (115 ml) with the native DNA. On the other hand, the DNA–$2C_6N^+$ was eluted at the void volume (50 ml). The DNA–$2C_8N^+$ was isolated by centrifugation (4000 rpm, 10 min), because the complex was not eluted through gel filtration.

The numbers of lipoglutamate per hundred phosphate groups of DNA were calculated to be 46 for the DNA–$2C_4N^+$, 55 for the DNA–$2C_6N^+$ and 100 for the DNA–$2C_8N^+$ complexes from C/N ratio of elemental analyses. The weight-average molecular weights (M_w) of the native DNA and the DNA–$2C_4N^+$ complex were observed from multi-angle light scattering measurements to be 430 kD and 640 kD, respectively. The number of $2C_4N^+$ per hundred phosphate groups of DNA was calculated to be 63 from the molecular weight of the DNA–$2C_4N^+$ complex. This value was near to the results of elemental analysis. We could not obtain the molecular weight of the DNA–$2C_6N^+$ and the DNA–$2C_8N^+$ complexes by light scattering method

because of the turbidity of those samples.

Conformational Changes of DNA upon the Binding of Lipoglutamate: Figure 3 shows CD spectra of DNA–lipoglutamate complexes in aqueous solutions. The maximum absorption of DNA is red-shifted from 277 to 284 nm by mixing with lipoglutamates, and the peak intensities at 277 nm are different for each of DNA–lipoglutamate complexes. These results suggested that the conformation of DNA double strand of the DNA–lipoglutamate complexes was somewhat different from the native DNA in aqueous solution.

Information of DNA structures in aqueous solution was also obtained by multi-angle light scattering measurements. It is known that the slope of log–log plots of (root mean square radius, $\langle S^2 \rangle^{1/2}$) vs. (molecular weight, M) gives a conformation parameter.[21] This conformation parameter is correspondence to the "α" value of the following equation.

$$\log \langle S_0^2 \rangle^{1/2} = ((\alpha + 1)/3) \log M + A. \quad (1)$$

For example, the conformation parameters (α) for rod-like polymers, random coil polymers, and spherical polymers are obtained as to be 2, 0.5, and 0, respectively. The log–log plots for DNA and DNA–$2C_4N^+$ complex gave straight lines as shown in Fig. 4. The α values were calculated from the slopes of solid lines according to Eq. 1. The conformation parameter of the native DNA used in this study was found to be about 0.40, which expected that the conformation of the native DNA in water is near to random coil. In contrast, the structure parameter of the DNA–$2C_4N^+$ complex was about 0.05, which expected that DNA duplex was condensed by binding with lipoglutamate. It has been reported that similar condensation of DNA into small sphere was also induced by spermine,[22] lipospermine,[23] and other counter cations.[22] Wilson and Bloomfield demonstrated that DNA collapsed occurred at 89—90% neutralization of phosphate anion.[22] In the present study, DNA collapse was observed though the neutralization of DNA by $2C_4N^+$ was 46—63%. It is considered from these results that DNA collapsed was induced by hydrophobicity of lipoglutamates bound to DNA and neutralization of phosphate anions of DNA.

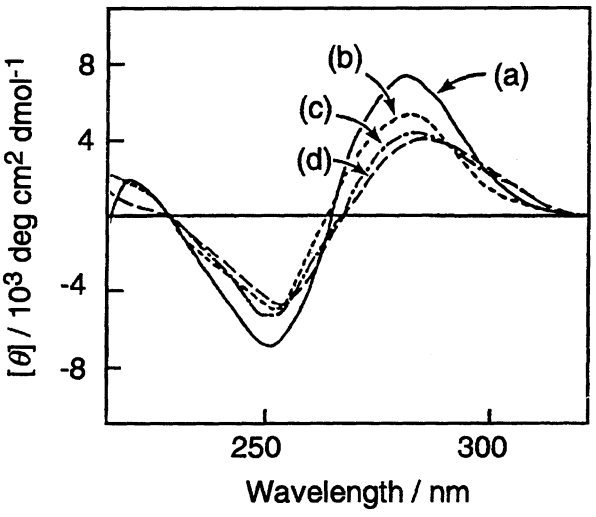

Fig. 3. CD Spectra of (a) native DNA, (b) DNA–$2C_4N^+$, (c) DNA–$2C_6N^+$, and (d) DNA–$2C_8N^+$ complexes in aqueous solution (20 °C, [DNA]=0.2 mg ml^{-1}).

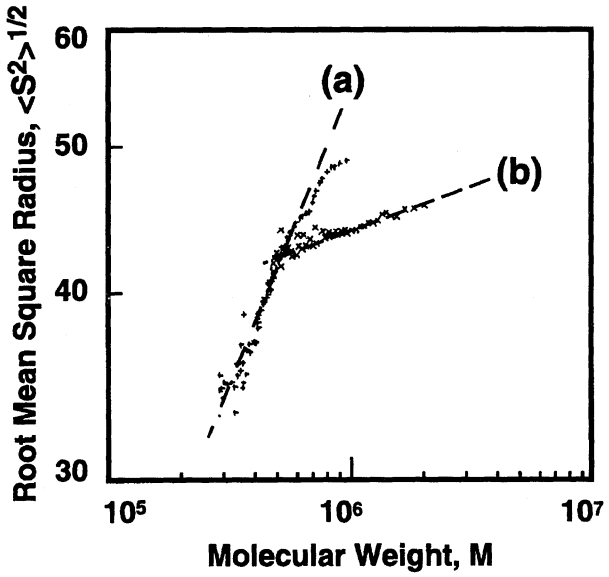

Fig. 4. Log–log plots of (root mean square radius) vs. (molecular weight) for (a) native DNA and (b) DNA–$2C_4N^+$ complex. According to Eq. 1 (see text), conformation parameters 'α' were calculated from the slopes of the solid lines.

T_m of DNA–Lipoglutamate Complexes: The melting temperatures (T_m) of DNA–lipoglutamate complexes were obtained from hyperchromicity (increase in the absorbance at 260 nm) at elevated temperatures. The temperature dependence of hyperchromicity of the native DNA and the DNA–lipoglutamate complexes was shown in Fig. 5. Thermal profiles of the DNA–$2C_4N^+$ and the DNA–$2C_6N^+$ are almost identical with that of the native DNA. In contrast, the DNA–$2C_8N^+$ complex did not the show significant change of hyperchromicity near T_m. This is explained that the aggregated DNA–$2C_8N^+$ complex hardly melted to a single strand even at the higher temperature.

Hydrolysis of DNA–Lipoglutamate Complexes Catalyzed by DNase I: Instability of DNA against nuclease is one of the serious problems when nucleic acids are injected into body. So, stability of DNA–lipoglutamate complexes against DNase I was quantitatively investigated by using a QCM method. Fig. 6 shows the percentage of the DNA cleavage, which was calculated from the frequency increase (mass decrease) of the DNA–immobilized QCM responding to the addition of DNase I. In the case of native DNA,

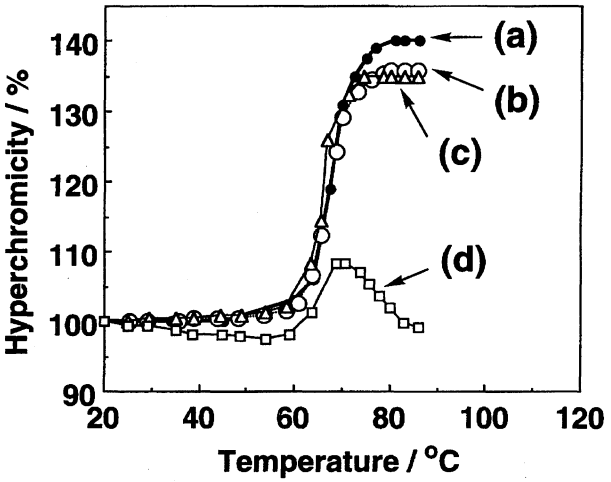

Fig. 5. Hyperchromicity changes of (a) native DNA, (b) DNA–$2C_4N^+$, (c) DNA–$2C_6N^+$, and (d) DNA–$2C_8N^+$ complexes in aqueous solution at elevated temperatures. Hyperchromicity was estimated from the absorbance at 260 nm ([DNA]=0.2 mg ml^{-1}, 20 mM NaCl).

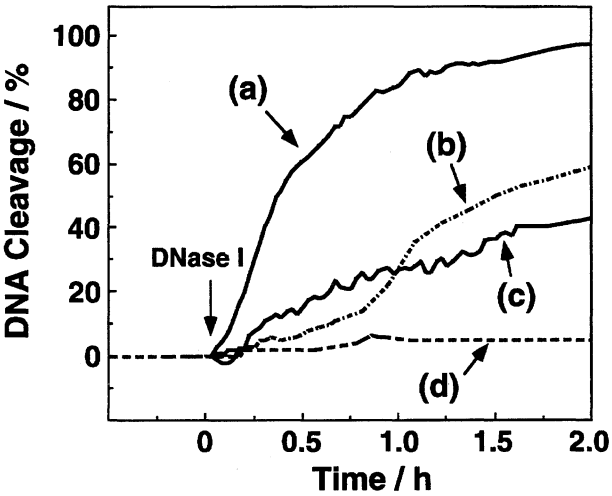

Fig. 6. Time courses of DNA cleavage of (a) native DNA, (b) DNA–$2C_4N^+$, (c) DNA–$2C_6N^+$, and (d) DNA–$2C_8N^+$ complexes upon the addition of DNase I in 10 mM acetate buffer (pH 5.0) containing 10 mM NaCl and 4.2 mM $MgCl_2$. Experimental set up is shown in Fig. 2. Frequency changes upon the addition of DNase I were monitored for 2 h at 25 °C. Percentages of DNA cleavage were calculated from the frequency increase (mass decrease) of the DNA–immobilized QCM ([DNA]=50 ng) responding to the addition of DNase I (60 μg) at the arrow in the solution.

the frequency was gradually increased with time and reached $\Delta F=50$ Hz (mass decrease of ca. 50 ng) in 2 h. Thus, the native DNA was completely hydrolyzed within 2 h. Hydrolysis of the DNA–$2C_4N^+$ and the DNA–$2C_6N^+$ complexes was apparently depressed compared with the native DNA. Furthermore, no hydrolysis of the DNA–$2C_8N^+$ complex was observed within the measurement time. The obstruction for enzymatic hydrolysis increased with increasing the hydrophobicity of the lipoglutamate complex. This is explained due to the hydrophobic counter cations of lipoglutamates or condensed structures of the DNA–lipoglutamates complexes in aqueous solutions. The DNA–immobilized QCM was useful as a new method for measuring the quantitative hydrolysis of DNA, because of high sensitivity and good reproducibility.

Interaction between DNA–Lipoglutamate Complexes and a Phospholipid Monolayer: Interaction between DNA complexes and lipid membranes was investigated to know the hydrophobicity of DNA–lipoglutamate complexes. Figure 7 shows the time courses of surface pressure changes ($\Delta\pi$) of the DSPC monolayer at the constant area, responding to the addition of native DNA and the DNA–lipoglutamate complexes into the aqueous subphase.

When the native DNA was injected into the subphase, the surface pressure of the monolayer hardly changed. On the contrary, the surface pressure was increased largely responding to the injection of the DNA–lipoglutamate complexes and the extent was increased with increasing the hydrophobicity of the complex. This indicates that the native DNA hardly interacts with the DSPC monolayer, but the DNA–lipoglutamate complexes can penetrate into the lipid monolayer by hydrophobic interaction and expand the lipid monolayer.

Interaction of DNA–Lipoglutamate Complexes with Hela Cells: Figure 8 shows fluorescence cytograms of Hela cells incubated with native

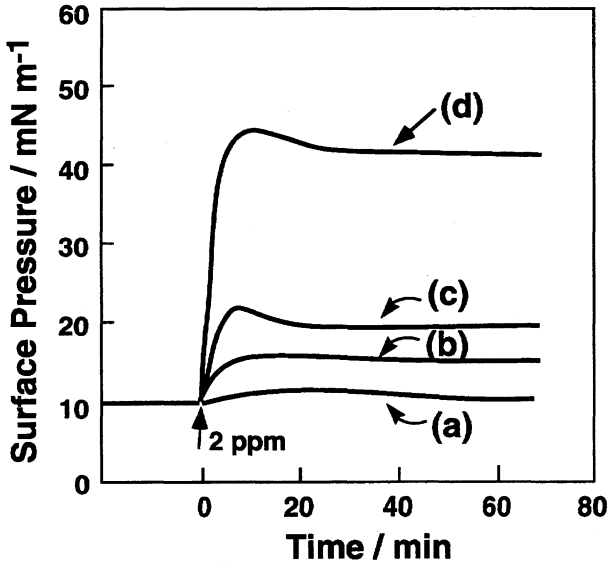

Fig. 7. Time courses of surface pressure changes ($\Delta\pi$) on the distearoylphosphatidylcholine (DSPC) monolayer at the surface pressure of 10 mM m^{-1}, responding to the addition of (a) native DNA, (b) DNA–$2C_4N^+$, (c) DNA–$2C_6N^+$, and (d) DNA–$2C_8N^+$ complexes.

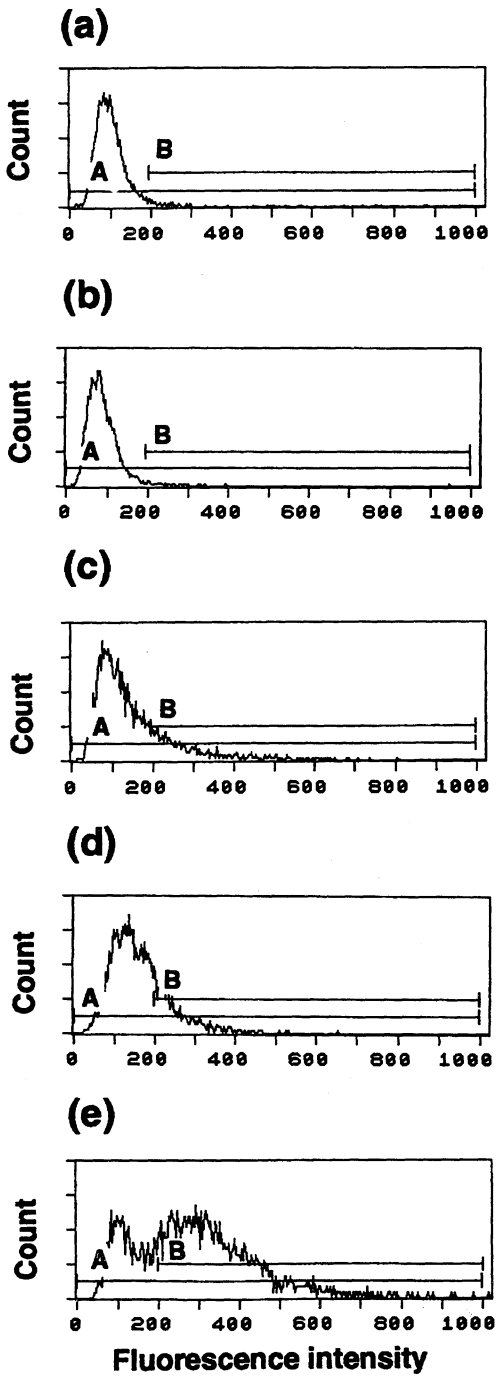

Fig. 8. Fluorescence diagrams of Hela cells incubated with DNA and DNA–lipoglutamate complexes; (a) Hela cell (control), (b) native DNA, (c) DNA–$2C_4N^+$, (d) DNA–$2C_6N^+$, and (e) DNA–$2C_8N^+$ complexes. DNA was partly labeled with fluorescent probe (FITC). Incubation was carried out at 37 °C for 6 h. Cell number was 2×10^5 cells ml^{-1} and DNA concentration was 40 $\mu g\,ml^{-1}$. Area A and B in histograms means all cells and FITC–DNA bearing cells, respectively.

DNA or DNA–lipoglutamate complexes at 37 °C for 6 h. FITC-labeled DNA was employed here. Native DNA did not show the shift of fluorescence intensity of Hela cells compared with spontaneous fluorescence of cells. While DNA–$2C_4N^+$, DNA–$2C_6N^+$, and DNA–$2C_8N^+$ complexes showed significant increase of fluorescence intensity. To calculate the interaction index of DNA with cells, gates for the fluorescence intensities of histograms were created as shown in Fig. 8 (area A and B in histograms). The ratios of cell number in area B against area A were determined as interaction index. The calculated ratios were 3%, 6%, 24%, 26%, and 72% for Hela cells (control), DNA, DNA–$2C_4N^+$, DNA–$2C_6N^+$, and DNA–$2C_8N^+$ complexes, respectively. Most hydrophobic DNA–$2C_8N^+$ complex showed the highest interaction index. Tabata et al. demonstrated that the appropriate hydrophobicity of the surface of polymer particle enhanced the internalization into macrophages.[24] Considered that the DNA–lipoglutamate complexes are more hydrophobic than DNA, we concluded that higher interaction of DNA–lipoglutamate complexes with Hela cells can be explained in terms of the hydrophobic interaction.

Conclusion

We newly prepared and studied the fundamental properties of the DNA–lipoglutamate complex. The preparation method is simple as just mixing aqueous solutions of native DNAs and lipoglutamates. The DNA–lipoglutamates form collapsed particle in the aqueous solutions, and show the high stability for catalytic hydrolysis by deoxyribonuclease. Furthermore, the DNA–lipoglutamate complexes showed the high penetration behavior into the lipid membrane, and interaction with tumor cells. We expect that the DNA–lipoglutamate complexes would be one of the vehicle for the delivery of DNA into cells.

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