Formation of a DNA Complex with Lipoglutamide Having Tetraethyleneglycol Tails and Its Interaction with a Tumor Cell

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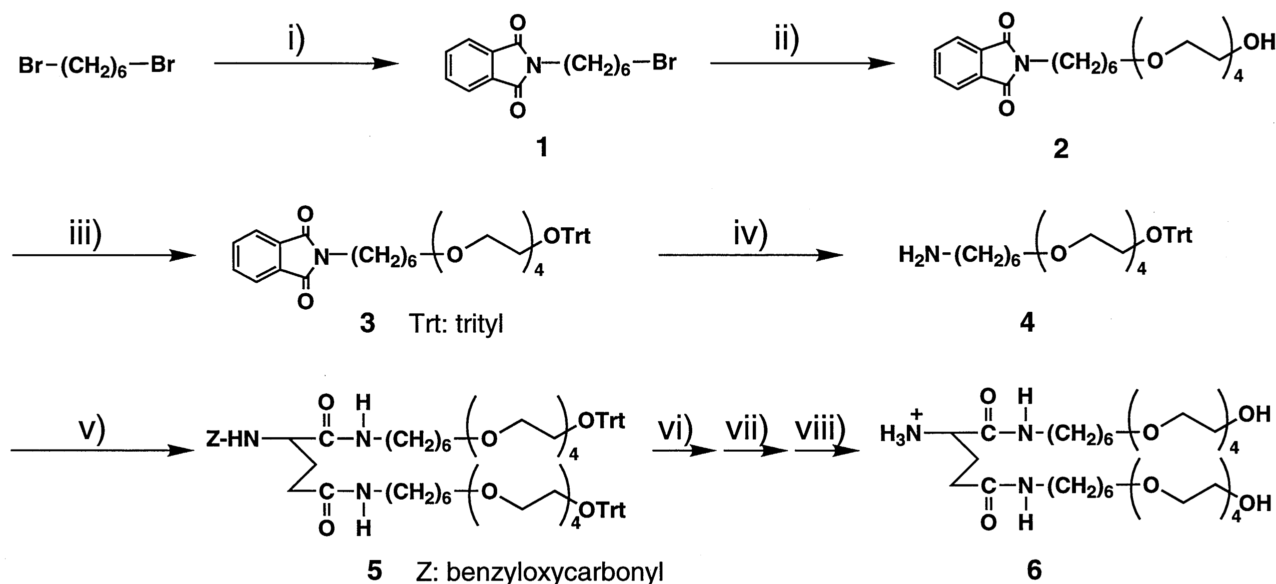
A cationic lipoglutamide having tetraethyleneglycol tails was synthesized. A DNA complex with the cationic lipoglutamide was formed in water, and showed efficient uptake into Hela cells.

Recently, a number of new techniques have been devised to introduce foreign DNA into cells. Basic requirement for the therapeutic use of nucleotides is efficient cell uptake. Encapsulations of nucleic acid into a liposome resulted in the increase of the macrophage activation.[1] Bindings of nucleic acid to polycation such as a cationic liposome,[2] lipopolyamine,[3] poly(L-lysine),[4] and DEAE-dextran[5] through ionic interaction have been developed for the efficient cell uptake. Though the DNA complexes showed the high cell uptake, problems of those DNA ion complexes are poor solubility in water. Especially DNA ion complexes with cationic lipids and cation polymers become precipitates. Production of precipitates restricts the dose of the DNA complex. If a water soluble DNA complex could be prepared, aggregation of DNA complex is expected to be avoidable. Exploration of a DNA complex that does not produce precipitate and shows strong interaction with cells is necessary for the progress of DNA delivery system.

In this paper, we synthesized a cationic lipoglutamide having tetraethyleneglycol tails, N,N'-di(18-hydroxy-7,10,13,16-tetraoxaoctadecyl)-L-glutamide hydrochloride (abbreviated to $2EO_4C_6N^+$). The chemical structure was given as compound **6** in Scheme 1. The $2EO_4C_6N^+$ has two functional terminals; one is a cationic primary amino group to bind with DNA through ionic interactions, and the another is hydrophilic ethyleneglycol group.

Synthetic procedures of $2EO_4C_6N^+$ were shown in Scheme 1. Dibromohexane and potassium phthalimide was allowed to react in DMF at 70 - 80 °C for 2 days to give **1** in yield of 41%. Reaction of tetraethyleneglycol with **1** in THF by refluxing for 15 h gave **2** in yield of 31%. The compound **2** was reacted with trityl chloride in pyridine at r. t. for 15 h. Hydroxy group of **2** was protected with trityl group in 55%. The compound **3** was transformed to **4** in 90% yield by refluxing for 15 h in the presence of hydradine monohydrate. Conjugation of **4** with benzyloxycarbonyl-L-glutamic acid was carried out in the presence of diethyl dicyanophosphate at r. t. for 15 h. The product was purified with silica gel chromatography (ethyl acetate:methanol = 95:5). Yield of **5** was 32%. Removal of carbobenzoxy group from **5** was carried out by reflux in the



Reagent, condition and yield: i) Potassium Phthalimide (0.2 eq) DMF, 70-80 °C, 2 days, 41 %; ii) tetraethyleneglycol (10 eq), THF, reflux, 15 h, 31 %; iii) tritylchoride (1.1 eq), pyridine, r.t., 15 h, 55 %; iv) hydradine monohydrate (11 eq), ethanol, reflux, 15 h, 90 %; v) benzyloxycarbonyl-L-glutamic acid (0.4 eq), dicyanophosphatidylether, THF, r.t., 15 h, 32 %; vi) ammonium formate (12 eq)-PdC (35 eq), methanol, reflux, 5 h; vii) formic acid, r.t., 15 h, 63 %; viii) hydrochloride (20 vol%), ethanol, r.t., 4 h, 100 %

Scheme 1.

presence of ammonium formate and Pd-C for 15 h. Furthermore removal of trityl group was carried out in the presence of formic acid overnight. Overall yield of deprotection process was 63%. The product was mixed with 20 vol% HCl in ethanol to give product **6** (yield, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$), δ=8.0 (3H, NH_3^+), 4.1 (1H, α-CH of glu), 3.5-3.8 (38H, 6, 8, 9, 11, 12, 14, 15, 17, 18-CH_2), 3.3 (4H, 1-CH_2), 2.1-2.5 (4H, β-, γ-CH_2 of glu), 1.5-1.7 (4H, 2-CH_2), 1.3-1.4 (8H, 3, 4-CH_2). From the ^{1}H-NMR, removal of Z- and trityl-groups was confirmed by the disappearance of peak at 7.2-7.5 ppm due to phenyl group.

Salmon sperm DNA (Sigma) was used after ultrasonic irradiation with a probe-type sonifier at 10 W and 4 °C for 40 min. 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. Elution solvent was 100 mM NaCl aqueous solution. Weight average molecular weight of the sonicated DNA was 236 kD.

The sonicated DNA solution (1 mg ml^{-1}) was mixed with $2EO_4C_6N^+$ solution (2.2 mg ml^{-1}). The molar ratio of phosphate anion of DNA to $2EO_4C_6N^+$ was 1:1. The mixing of aqueous solutions of DNA and $2EO_4C_6N^+$ did not show any turbidity. Molecular weight of the DNA-$2EO_4C_6N^+$ complex was determined to be 441 kD. The number of $2EO_4C_6N^+$ per hundred phosphate groups of DNA was calculated to be 36 from the molecular weight of the DNA complex. Complex formation was also confirmed from gel chromatography and elemental analysis. Previously, we have prepared the DNA complex with α, γ-dihexyl glutamate ($2C_6N^+$) without ethyleneglycol group.[6] When an aqueous solutions of DNA (1 mg ml^{-1}) was mixed with $2C_6N^+$(4.8 mg ml^{-1}), the mixed solution became precipitate. This indicated that this DNA-$2C_6N^+$ complex aggregated in aqueous solution because of hydrophobicity of the DNA complex. Compared with the solution state of the DNA-$2C_6N^+$ and the DNA-$2EO_4C_6N^+$ complexes, we could understand that introduction of hydrophilic ethyleneglycol tails resulted in the depression of the aggregation of DNA complex.

The interaction between the DNA-$2EO_4C_6N^+$ complex and tumor cell was investigated. Human adenocarcinoma Hela cells (2 × 10^5 cells) were co-incubated with FITC-labeled DNA complex ([DNA] = 40 μg ml^{-1}) for 24 h at 37 °C in 1 ml of serum-free culture medium (ASF104, Ajinomoto Co. Inc., Japan). No cell damage was observed at the given concentration. Thereafter, cells were collected by treating with the aqueous solution of 0.05% trypsin-0.02% ethylendiamine tetraacetic acid. The cells loading FITC-DNA were detected by flow cytofluorometer (EPICS-XL, Coulter, USA). Figure 1 shows fluorescence cytograms of Hela cells incubated with DNA and DNA-$2EO_4C_6N^+$ complexes. Native DNA showed small shift of fluorescence intensity of Hela cells compared with spontaneous fluorescence of cells. While in the case of the DNA-$2EO_4C_6N^+$ complex, almost 100% of Hela cells shifted to the higher fluorescence intensity.

We are investigating the mechanism of cell uptake, intracellular localization and transfection efficiency, will report them soon.

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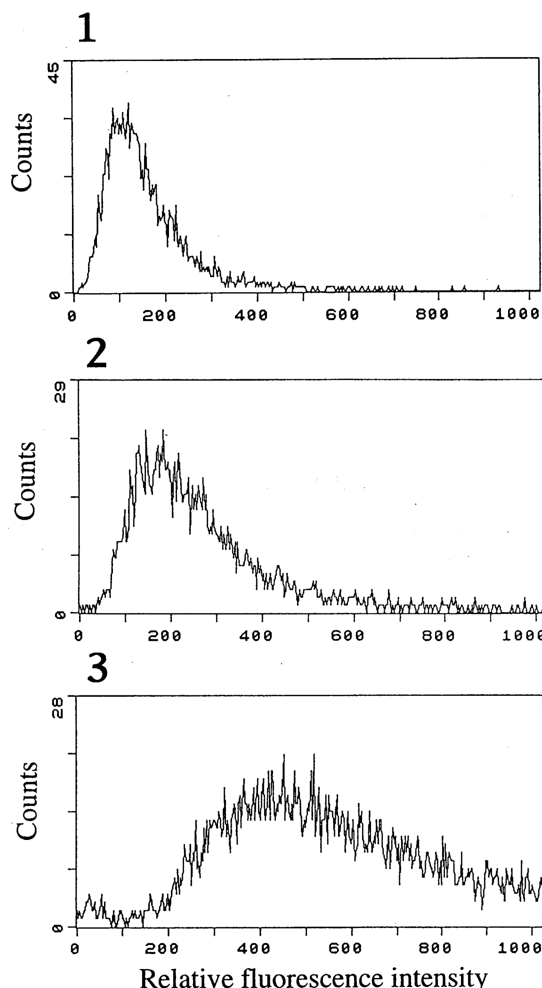

Figure 1. Fluorescence histograms of (1) control Hela cells, (2) Hela cells incubated with conventional DNA, and (3) incubated with the DNA-$2EO_4C_6N^+$ at 37 °C for 24 h. [Hela cells] = 2 × 10^5 cells, [DNA] = 40 μg ml^{-1}, [$2EO_4C_6N^+$] = 88 μg ml^{-1}.

References and Notes

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