

# Effect of zeta potential of cationic liposomes containing cationic cholesterol derivatives on gene transfection

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Received 17 September 1996

**Abstract** Cationic liposomes are known to be useful tools for gene transfection. However, the relation between transfection efficiency and physicochemical properties of liposomes has not been well understood. Here, we synthesized eight cationic derivatives of cholesterol which contain a tertiary amino head group with a different spacer arm. Transfection of plasmid pSV2CAT DNA into cells was done by cationic liposomes made of a mixture of dioleoylphosphatidylethanolamine (DOPE) and each cationic cholesterol derivative. At the same time we measured zeta potential of cationic liposomes by laser Doppler spectroscopy. The present results indicated that zeta potentials of cationic liposomes were well related to transfection activity of pSV2CAT DNA. This suggested that zeta potential of cationic liposomes is one of important factors which control gene transfection.

**Key words:** Cationic liposome; Gene transfection; pSV2CAT DNA; CAT assay; Cationic cholesterol; Zeta potential

## 1. Introduction

Cationic liposomes have proven to be useful tools for delivery of plasmid DNA and RNA into cells [1–8]. Improved delivery of antisense oligonucleotides using cationic liposomes has also been demonstrated [9,10]. For the experiments of such cationic liposomes, cationic cholesterol derivatives were justified by their high transfection efficiency and low toxicity [5–8]. However, little is known for the relation between transfection activity and physicochemical property of cationic liposomes. In the present experiments we have synthesized eight cationic derivatives of cholesterol which contain a tertiary amino head group with a different spacer arm. Cationic liposomes were made of a mixture of dioleoylphosphatidylethanolamine (DOPE) and each cationic derivative of cholesterol. By them we have studied transfection of plasmid pSV2CAT DNA into three kinds of mammalian cultured cells. Transfection efficiency of pSV2CAT was elucidated from chloramphenicol acetyltransferase (CAT) activity. At the same time we measured zeta potential of the cationic liposomes by laser Doppler spectroscopy [11,12]. Values of zeta potential of the cationic liposomes were well consistent with those of transfection efficiency of pSV2CAT in the cells. This indicated that zeta potential of cationic liposomes was one of the important factors which control gene transfection.

## 2. Materials and methods

### 2.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) was purchased from Sigma (St. Louis, MO). The syntheses of cholesteryl-3 $\beta$ -oxysuccinamidomethylamine (I), cholesteryl-3 $\beta$ -oxycarboxyamidoethylenedimethylamine (II), cholesteryl-3 $\beta$ -oxycarboxamido-propanedimethylamine (III), cholesteryl-3 $\beta$ -carboxyamidoethylenedimethylamine (IV), cholesteryl-3 $\beta$ -carboxyamido-propanedimethylamine (V) were done following the previous procedures by Huang et al. [5,6]. Cholesteryl-3 $\beta$ -oxycarboxyamidoethylenediethylamine (II(ethyl)), cholesteryl-3 $\beta$ -oxycarboxyamidoethylenediisopropylamine (II(isopropyl)) and cholesteryl-3 $\beta$ -carboxamidoethylenediethylamine (IV(ethyl)) were synthesized by employing *N,N*-diethylethylenediamine or *N,N*-diisopropylethylenediamine, respectively. Plasmid pSV2CAT was given by Prof. Kikuo Onozaki (Nagoya City University).

### 2.2. Liposome preparation

DOPE was combined with cationic derivatives of cholesterol in chloroform and a mixture was dried under reduced pressure to remove chloroform solvent. A mol ratio of 3:2 was used DOPE/cholesterol liposomes [5]. The dried lipid film was vacuum desiccated for at least half an hour and suspended by vortexing and the samples were sonicated in a bath type sonicator (Branson model B1200) to generate small unilamellar vesicles [5,6,13]. The diameter of the cationic liposomes was measured using a multiangle light scattering instrument (Otsuka Electronics). The average diameter of liposomes was 200–350 nm.

### 2.3. Cell culture and transfection

NIH3T3 cells were cultured in RPMI-1640 medium from Gibco (Grand Island, NY) supplemented with 10% FCS (HyClone, Logan UT). HeLa cells were cultured in Eagle's MEM medium from Nissui (Tokyo) supplemented with 10% FCS and COS-7 cells were cultured in D-MEM medium from Gibco supplemented with 10% FCS. RPMI1640 and D-MEM media contained antibiotics: 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin.

Plasmid pSV2CAT DNA was complexed to cationic liposomes in SFM101 (Nissui) at room temperature for 15 min and then the complex was incubated with target cells for 4 h at 37°C. Then the cells were washed and cultured for another 40 h in growth medium at 37°C before the CAT assay.

### 2.4. Transfection activity

The CAT assay was done by modification of the previous paper [12]. The cells were washed once with PBS and lysed in 0.25 M Tris-HCl buffer by a sonicator (Tomy, UR-20P) at room temperature. The samples were centrifuged in a microfuge at high speed at 4°C for 10 min and the supernatants were heat-inactivated at 70°C for 5 min. The CAT reaction was performed at 37°C for 1 h using 60  $\mu$ g protein of cell lysates [14].

### 2.5. Laser Doppler spectroscopy

Measurements of electrophoretic mobilities by laser Doppler spectroscopy were done by an electrophoretic light scattering spectrometer from Otsuka Electronics (ELS-800). The electrophoresis cell was made of quartz. Platinum electrodes were fixed to the outer buffer compartments to apply constant voltages following the procedure of the previous method [11,12]. All experiments were done at 25°C.

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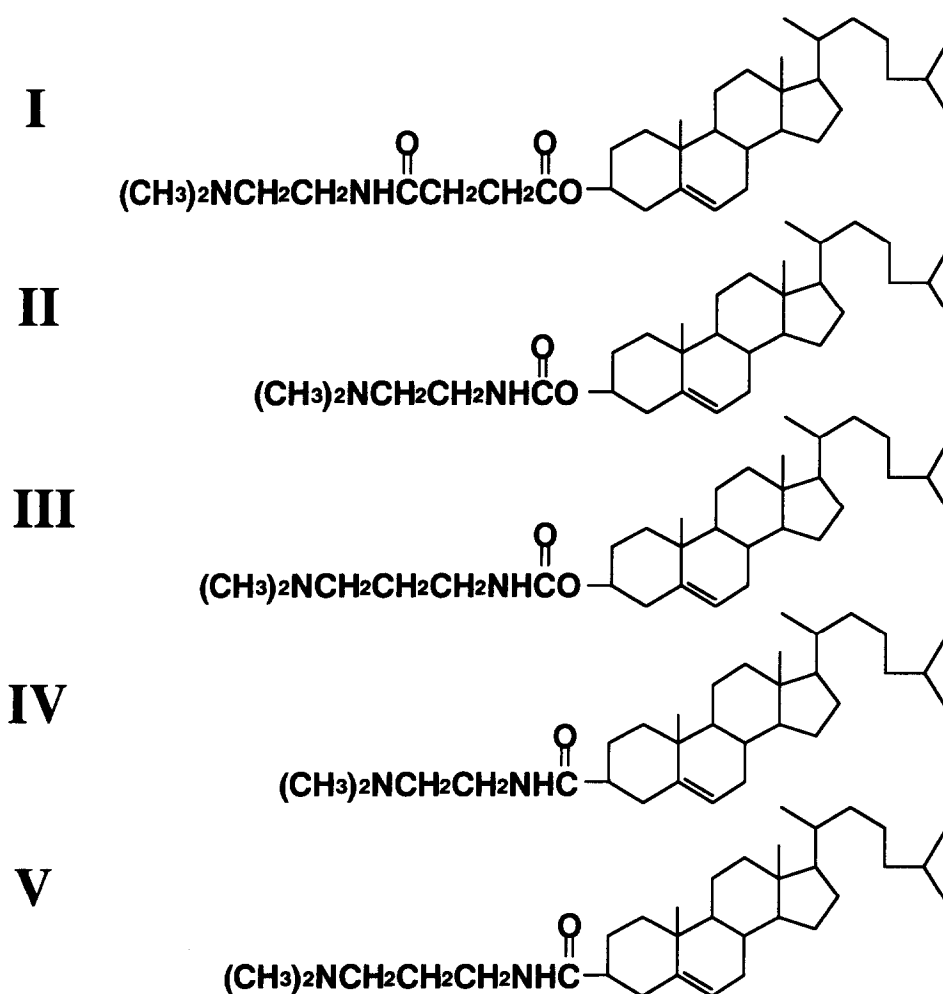


Fig. 1. The structure of five cationic derivatives of cholesterol with a tertiary amino head group. The derivatives II(ethyl), IV(ethyl) and II(isopropyl) were substituted a dimethylamino head group of the derivatives II and IV with a diethylamino or a diisopropylamino head group.

### 3. Results

#### 3.1. Transfection activity of pSV2CAT plasmid DNA

We have synthesized eight derivatives of cholesterol which contain a tertiary amino head group with a different spacer arm. Five derivatives (I, II, III, IV and V) of cholesterol had a dimethylamino head group as shown in Fig. 1. Others had a diethylamino (II(ethyl) and IV(ethyl)) or a diisopropylamino head group (II(isopropyl)) instead of a dimethylamino head group of the derivative II and IV. Cationic liposomes which contained a mixture of DOPE (30 nmol) and a cationic cholesterol derivative (20 nmol) were tested for the transfection activity of pSV2CAT in three kinds of cell lines (NIH3T3, HeLa and COS-7) following the previous procedure [5,6]. pSV2CAT plasmid DNA was mixed with the cationic liposomes and then chloramphenicol acetyltransferase (CAT) activity of the cell extracts were determined by a standard protocol. As shown in Fig. 2, cationic liposomes with the derivative IV showed the highest transfection efficiency, although CAT activity itself was very different among three cell lines (NIH3T3, HeLa and COS-7) (Fig. 2). Cationic liposomes with the derivative I were much less effective in transfection efficiency. Transfection efficiency of the derivative I was less than 20% of that of the derivative IV. Liposomes made from the derivatives II, III or V gave moderate transfection

efficiency. Substitution of a dimethylamino head group of the derivative II with a bulky head group such as a diethylamino (II(ethyl)) or a diisopropylamino head group (II(isopropyl)) showed significant decreases in transfection efficiency. Substitution of dimethylamino head group of the derivative IV with a diethylamino head group (IV(ethyl)) decreased also transfection efficiency, however, the transfection efficiency of the derivative IV(ethyl) was much higher than those of the derivatives II(ethyl) and II(isopropyl) shown in Fig. 2.

Table 1

Values of zeta potential of cationic liposomes with a different cationic derivative of cholesterol

Derivative <sup>a</sup>	Zeta potential (mV)
I	12.6 ± 1.0
II	26.5 ± 4.0
II (ethyl)	9.0 ± 1.4
II (isopropyl)	11.1 ± 1.3
III	27.5 ± 2.5
IV	33.6 ± 1.3
IV (ethyl)	22.2 ± 2.4
V	27.5 ± 1.1

<sup>a</sup>The derivatives without parentheses have a dimethylamino head group.

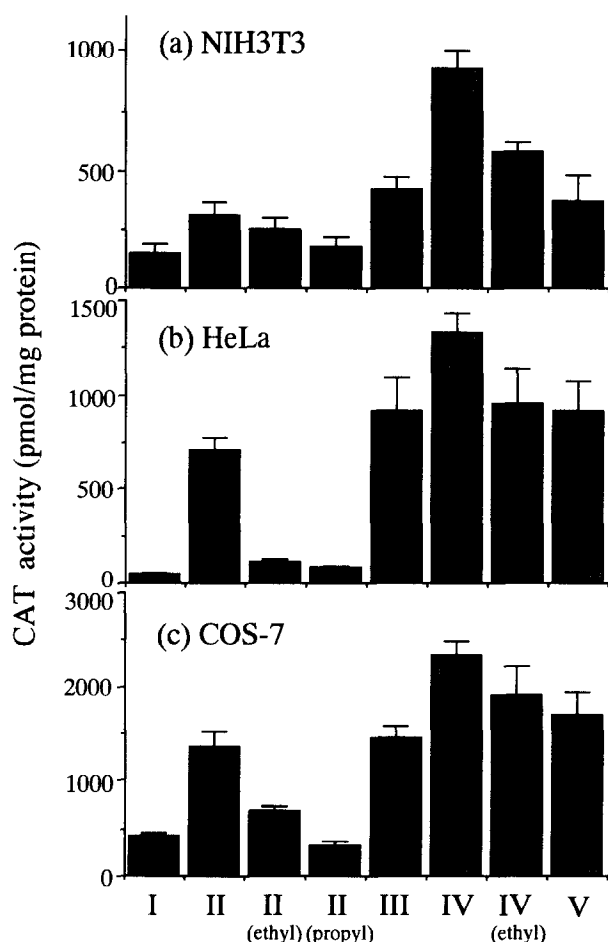


Fig. 2. Effects of cationic liposomes with a different cholesterol derivative on transfection efficiency. Vertical axes show the CAT activity which was measured following the standard procedures described in Section 2 [14]. Bars are standard deviations. (a) NIH3T3, (b) HeLa and (c) COS-7 cells.

### 3.2. Zeta potential of cationic liposomes measured by laser Doppler spectroscopy

Next, we measure zeta potential of cationic liposomes by laser Doppler spectroscopy. In laser Doppler spectroscopy particle mobility was measured by Doppler shifts of laser light [11,12]. The laser Doppler electrophoresis has been successfully applied for the characterization of the surface groups of cells and vesicles [15–17]. Values of zeta potential of cationic liposomes made from different cholesterol derivatives are shown in Table 1. The derivative IV gave the highest zeta potential ( $33.6 \pm 1.3$  mV) and the derivative I gave the lowest zeta potential ( $12.6 \pm 1.0$  mV) in the liposomes containing a cationic cholesterol with a dimethylamino head group. Substitution of a dimethylamino head group with a diethylamino or a diisopropylamino head group decreased zeta potential of cationic liposomes (Table 1). Zeta potential of cationic liposomes seemed to be well correlated to transfection efficiency of pSV2CAT DNA in the cells.

### 4. Discussion

DNA-mediated transfection has become an important tool in modern biology [1–9]. Among the conventional reagents

such as calcium phosphate, DEAE-dextran and other particulate reagents, cationic liposomes have become increasingly acceptable as a convenient and reproducible reagent for DNA-mediated transfection. However, little information has been obtained about the relation between efficiency of gene transfection and physicochemical property of cationic liposomes.

Then, in this paper we synthesized eight cationic derivatives of cholesterol which contain a tertiary amino head group with a different spacer arm. Using cationic liposomes made of a mixture of DOPE and cationic derivatives of cholesterol we studied transfection of plasmid pSV2CAT DNA in three kinds of cultured cells. Transfection efficiency of pSV2CAT DNA was well consistent with the values of zeta potential (positive charge) of cationic liposomes which contain a tertiary amino head group. Substitution of a dimethylamino head group with a diethylamino or a diisopropylamino head group decreased significantly the values of zeta potential of cationic liposomes. This may be due to the shielding effects of positive charge of a tertiary amino head group by a bulky dialkyl (diethyl or diisopropyl) residue. The values of zeta potential were well consistent with the values of transfection activity of pSV2CAT DNA as shown in Fig. 2. The results suggested that zeta potential of cationic liposomes was one of important factor which effected on gene transfection by cationic liposomes.

**Acknowledgements:** We thank Prof. Leaf Huang (University of Pittsburgh) for his good advice.

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