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Rapid report

Delipidated serum abolishes the inhibitory effect of serum on in vitro liposome-mediated transfection

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Abstract

All the standard in vitro lipofection has been routinely performed in serum-free medium as the transfection activity of liposome/DNA complexes is sensitive to the presence of serum. In this study, we have demonstrated that lipid-rich serum lipoprotein included in the transfection medium strongly inhibited the transfection activity of DC-chol liposome/DNA complexes in five different cell types (CHO, 293, A2780CP, A431 and SKBR3). The levels of inhibition by serum lipoprotein were rather greater than those by serum and varied with cell types. However, this inhibition was completely abolished by delipidation of serum. Thus, delipidated serum can be included in the transfection medium. The complexes formed in the presence of serum ($\zeta = -18.2 \pm 1.07$ mV), delipidated serum ($\zeta = -19.6 \pm 0.54$ mV), IgG ($\zeta = -21.6 \pm 1.92$ mV) or serum lipoprotein ($\zeta = -10.5 \pm 2.33$ mV) were as much negatively charged as those in serum-free medium ($\zeta = -21.3 \pm 1.60$ mV). The results suggest that the inhibition of liposome-mediated transfection by serum was not associated with charges of serum proteins but with lipids or lipid-associated proteins present in serum.

Keywords: Lipofection; DNA/liposome complex; Surface charge; Lipoprotein; Delipidated serum

Delivery of genetic material into cells is mediated by a variety of methods, DEAE-dextran, calcium phosphate, electroporation, cationic liposomes, virus, biolistic particles, microinjection, etc. Among them, cationic liposomes have been demonstrated as an efficient and safe transfection reagent in experimental animals and human gene therapy clinical trials [1,2]. However, one drawback of liposome-mediated transfection (lipofection) is that the presence of serum in medium during transfection dramatically lowers transfection efficiency [3–7]. In this reason, the optimal condition for in vitro transfection with a liposome reagent omits serum in the lipofection medium. This drawback also hampers the application of cationic liposomes for in vivo delivery of genes. The liposomes currently in use typically contain a mixture of cationic and neutral lipids. Transfection complex formation is based on the interaction of the positively charged liposomes with the negatively charged phosphate groups of the nucleic acid. Thus, finding factor(s) in serum inhibiting the transfection efficiency of cationic liposome/DNA complexes (lipoplexes) can lead a rational design of transfection

Abbreviations: CAT, chloramphenicol acetyltransferase; DC-chol, 3β[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl]cholesterol; FBS, fetal bovine serum; IgG, immunoglobulin G; DMEM, Dulbecco's modification of Eagle's medium; HDL, high density lipoprotein; LDL, low density lipoprotein; ζ, zeta potential

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Table 1
Concentrations of serum protein used in the lipofection medium for transfecting five cell lines shown in Fig. 1

Serum protein	Concentration (%)				
	0	2.5	5.0	10	100[a]
IgG (μg/ml)	0	7.5	15	30	50–300
Lipoprotein (μg/ml)	0	12.5	25	50	290–500

[a]Normal untreated fetal bovine serum.

complex formulation for in vitro transfection and in vivo gene delivery.

To find the inhibitory factor(s) present in serum, we examined the effect of serum proteins on the transfection efficiency in five different cell types. Those cell lines were CHO Chinese hamster ovary (a gift from Dr. M.M. Gottesman, National Cancer Institute, Bethesda, MD, USA), 293 human embryonal kidney (ATCC), A2780CP cisplatin-resistant variant of human ovarian carcinoma (a gift from Dr. K.J. Scanlon, City of Hope National Medical Center, Duarte, CA, USA), A431 human epidermoid carcinoma (ATCC), and SKBR3 human breast carcinoma (ATCC). Cells were grown in RPMI 1640 or Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) containing penicillin (100 U/ml)/streptomycin (100 μg/ml), and glutamine (2 mM). Albumin, the most abundant protein in serum, was tested and confirmed to enhance in vitro lipofection efficiency as reported [8]. The other two major serum proteins are immunoglobulins and lipoproteins. Table 1 shows the concentrations of immunoglobulin G (IgG) (Sigma I5506) and lipoprotein (Sigma L9906) equivalent to 2.5, 5.0 and 10% of serum, which were used for transfecting five cell lines shown in Fig. 1. Serum lipoprotein used was commercially available lipoprotein concentrate that contained a very high amount of lipid (9.8 mg/ml of cholesterol) and relatively low amount of protein (14.5 mg/ml) (no other information available from Sigma). Normal FBS (Gibco/BRL) used contained 1.9–2.3 mg/ml of total lipids (32–40 mg/dl of cholesterol, 11–13 mg/dl of HDL, 16–21 mg/dl of LDL, 50–65 mg of phospholipid and 82 mg/dl of triglycerides) and 35 mg/ml of total protein. Thus, lipid content was 4.3–5.2-fold higher but protein content was 2.4-fold less in serum lipoprotein concentrate than in serum.

Inclusion of IgG in the transfection medium did not significantly affect the transfection activity in all five cell lines even at 30 μg/ml, while inclusion of serum lipoprotein did in a dose-dependent manner with the degree of inhibition varying with cell type (Fig. 1). In vitro transfection was performed by mixing 1 μg of pUCCMVCAT DNA with 10 nmol of 3β[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl]cholesterol (DC-chol) in an appropriate medium and adding to the cells at 70% confluency as described [9]. Chloramphenicol acetyltransferase (CAT) gene expression in the transfected cells was determined as described [10]. CAT is the bacterial CAT, a reporter gene, that was used to measure its expression [11]. pUCCMVCAT (5.1 kb) is a pUC18-based plasmid containing the full-length CAT cDNA and was purified by Qiagen Plasmid Giga kit (Valencia, CA, USA) according to the manufacturer's instructions and dissolved in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0). DC-chol liposomes were composed of DC-chol and dioleoyl phosphatidylethanolamine (3:2 mol ratio) which were combined in chloroform, dried thoroughly, and hydrated with deionized water (pH 7.50 ± 0.25) as described [12]. The transfection activity in CHO and A431 cells was completely inhibited by the presence of 50 and 25 μg/ml of serum lipoprotein, respectively, in the transfection medium (Fig. 1A,D). This inhibition was somewhat less pronounced in 293 (maximum 61%), A2780CP (maximum 65%) and SKBR3 (maximum 53%) cells trans-

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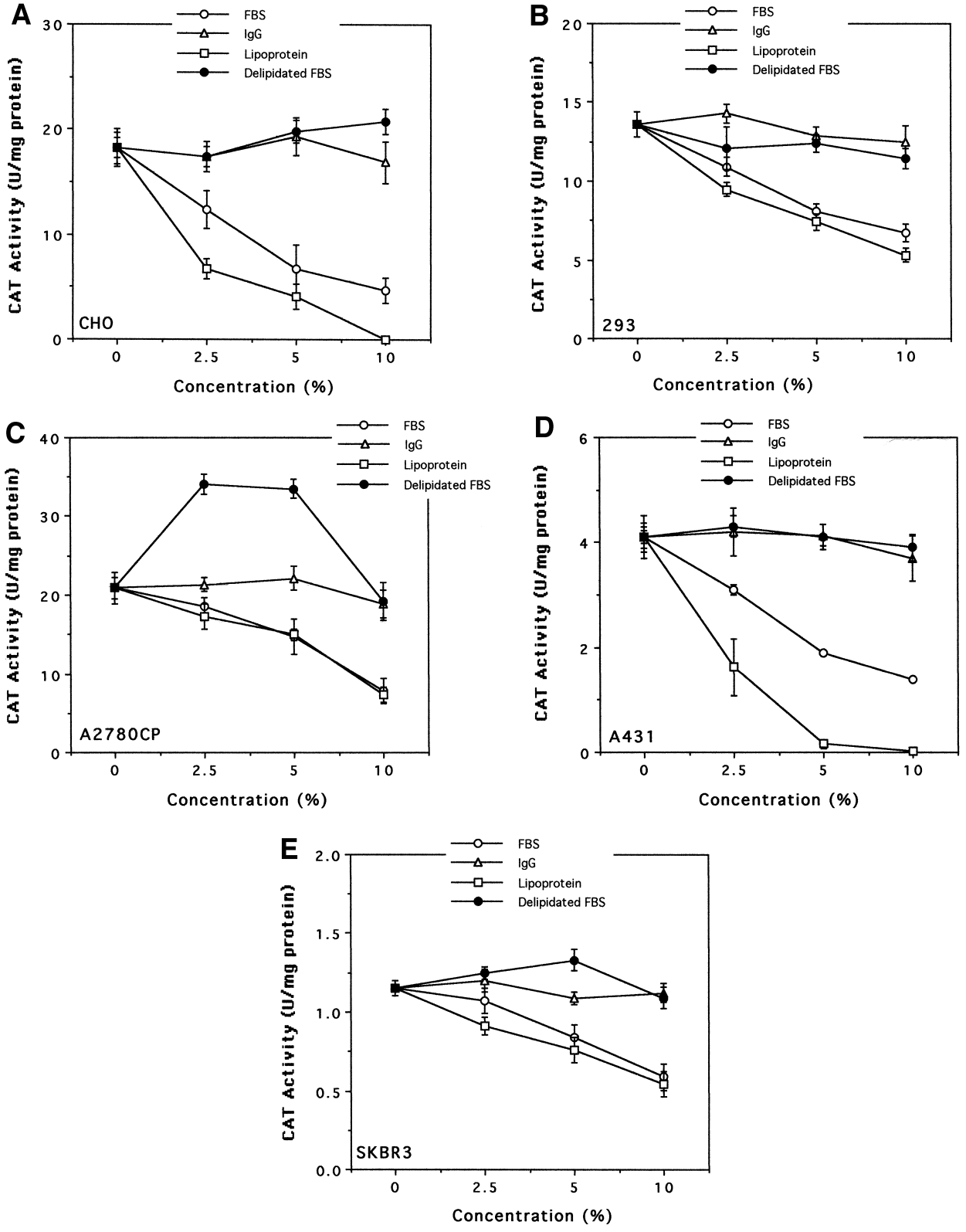
Fig. 1. CAT gene expression in five cell lines transfected with liposome/DNA complexes in the presence of serum, serum protein or delipidated serum. (A) Chinese hamster ovary CHO, (B) human embryonal kidney 293, (C) cisplatin-resistant variant of human ovarian carcinoma A2780CP, (D) human epidermoid carcinoma A431 and (E) human breast carcinoma SKBR3. Cells were grown in complete medium. The cells at 70% confluency were seeded into 24 well plates overnight before transfection. One μg of pUCCMVCAT DNA (1 μg/μl) and 10 nmol of DC-chol (2 nmol/μl) were mixed in medium (RPMI 1640 or DMEM) containing three different concentrations of serum, IgG, lipoprotein, or delipidated serum, added to the washed cells and incubated for 5 h. The transfected cells were further incubated in complete medium for 48 h followed by lysis and CAT activity assay as described [9]. Each data point is mean ± S.D. of three replicate cultures. FBS, fetal bovine serum; IgG, immunoglobulin G.


A
FBS
IgG
Lipoprotein
Delipidated FBS
CAT Activity (U/mg protein)
CHO
Concentration (%)
B
293
C
A2780CP
D
A431
E
SKBR3

fected in the presence of 50 μg/ml of serum lipoprotein (Fig. 1B,C,E). Unlike lipoprotein, there was no complete inhibition of lipofection in any five cell lines in the presence of serum (Fig. 1). In fact, the strong inhibitory effects of serum lipoprotein on lipofection were even more dramatic than those of serum in CHO and A431 cell lines (Fig. 1A,D). The degree of inhibition by serum lipoprotein in 293, A2780CP and SKBR3 cells was similar to that by serum as the concentration increased. We also observed that expression of a target gene inducible nitric oxide synthase, routinely used for the treatment of drug-resistant ovarian and breast tumor in our laboratory, in A2780CP cells was also inhibited by the presence of serum or serum lipoprotein in the transfection medium to a similar extent to CAT expression (data not shown). The results suggest that lipoprotein is a major inhibitory factor present in serum on lipofection.

To test if the inhibition was associated with lipids, we used the medium containing delipidated serum during transfection. Delipidated FBS used contained 6 mg/dl of cholesterol, 3 mg/dl of HDL, 1 mg/dl of LDL, 12 mg/dl of phospholipid (no information on triglycerides content) and 3.5 g/dl of total protein (Cocalico Biologicals). Thus, delipidated serum had 85–95% less lipid than normal serum. Surprisingly enough, inclusion of delipidated serum in the transfection medium not only abolished the strong inhibitory effect of lipoprotein on the lipofection activity in four cell lines, CHO, 293, A431 and SKBR3, but also significantly enhanced transfection of A2780CP cells (maximum 1.6-fold) (Fig. 1). These results support that the inhibitory effect of lipoprotein on lipofection is due to lipids associated with it. Thus, delipidated serum can be included in the transfection medium and cells can be incubated longer than 5 h, a time used in serum-free medium, to achieve higher transfection efficiency. It is evident that in vivo gene delivery is much less efficient than in vitro transfection as normal human plasma contains a large amount of lipids (a total lipid content of 3.6–6.8 g/l). A better transfection complex formulation to block the inhibitory effect of lipids present in serum, blood or tissue will greatly enhance in vitro transfection as well as in vivo gene delivery.

To test if the inhibition of lipofection by serum was also associated with large amounts of negatively charged serum proteins as previously reported [5], we measured zeta potential (surface charge density) of liposome/DNA complexes formed in the absence and presence of serum, serum protein or delipidated serum (Fig. 2). The parameters used to measure ζ were as follows [13]: ζ model = Smoluchowski, field frequency = 2.00 Hz, voltage = 3.00 V, temperature = 25°C, viscosity = 0.0890 cP, reflex index = 1.330, angle = 90°, and wavelength = 676 nm. We used DMEM (pH = 7.27 ± 0.02, the ionic strength calculated from the formulation = 0.17) which was filtered through a 0.2 μm membrane. Charge-associated transfection efficiency can be predicted by zeta potential [13]. Cationic liposomes were slightly positively charged in serum-free medium ($\zeta = 4.16 \pm 1.75$ mV) but negatively charged in the presence of serum ($\zeta = -7.50 \pm 1.70$ mV). This indicates that liposomes did interact with negatively charged serum proteins. However, as we previously demonstrated [9], when DNA was present, the complexes were highly negatively charged not only in serum-free medium ($\zeta = -21.3 \pm 1.60$ mV) but also in the presence of serum ($\zeta = -18.2 \pm 1.07$ mV) or delipidated serum

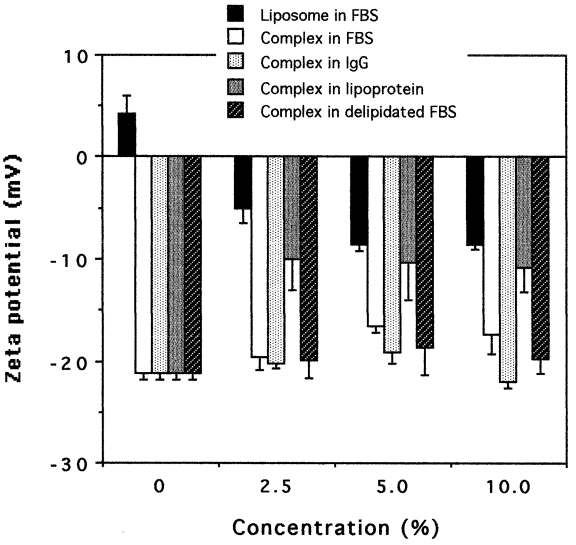


Fig. 2. Zeta potential of liposome/DNA complexes formed in the presence of serum, serum protein or delipidated serum. Appropriate concentration of serum or serum protein in DMEM was mixed with 1 μg of pUCCMVCAT DNA and then added to 10 nmol of DC-chol liposomes (the total volume of 250 μl). These mixtures were subjected to the measurement of zeta potential. All measurements were made in triplicate and expressed as the mean ± S.D. FBS, fetal bovine serum; IgG, immunoglobulin G.

($\zeta = -19.6 \pm 0.54$ mV). Furthermore, the complexes were still negatively charged in the presence of IgG ($\zeta = -21.1 \pm 1.92$ mV) or lipoprotein ($\zeta = -10.5 \pm 2.33$ mV), although those with lipoprotein were slightly less negative (Fig. 2). Despite a dramatic difference in the lipofection efficiency of the complexes formed between normal and delipidated serum in the transfection medium, zeta potential of the complexes remained little changed. These results indicate that the inhibition effect of serum was not due to negative charges introduced from serum proteins. This finding supports, in part, our previous report that negatively charged complexes were not only much more efficient to deliver gene to solid tumors than positively charged ones but also as efficient as positively charged ones to deliver gene to ascitic-like tumors [14]. Furthermore, we did not observe significant changes in the size of the complexes in the presence of serum or serum proteins (the hydrodynamic diameter = 300–370 nm) (data not shown). Therefore, less efficient in vivo gene delivery may not be associated with neither charges or size of the complexes. It is lipids or lipid-associated proteins present in serum, blood or tissue that liposome/DNA complexes interact with, reducing in vitro and in vivo gene delivery.

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