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Rapid report

Zeta potential of transfection complexes formed in serum-free medium can predict in vitro gene transfer efficiency of transfection reagent

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Abstract

We have tested the zeta potential (ζ, the surface charge density) of transfection complexes formed in serum-free medium as a rapid and reliable technique for screening transfection efficiency of a new reagent or formulation. The complexes of CAT plasmid DNA (1 μg) and DC-chol/DOPE liposomes (3–20 nmol) were largely negatively charged ($\zeta = -15$ to -21 mV), which became neutral or positive as 0.5 μg or a higher amount of poly-L-lysine (PLL, MW 29 300 or MW 204 000) was added (-3.16 ± 3.47 to $+6.04 \pm 2.23$ mV). However, the complexes of CAT plasmid DNA (1 μg) and PLL MW 29 300 (0.5 μg or higher) were neutral or positively charged (-3.22 ± 2.3 to $+6.55 \pm 0.64$ mV), which remained the same as 6.6 nmol of the liposomes was added. The complexes formed between two positively charged compounds, PLL MW 29 300 (0.5 μg) and the liposomes (3–20 nmol), were as closely positively charged as DNA/PLL or DNA/liposomes/PLL complexes ($+3.31 \pm 0.41$ to 7.16 ± 1.0 mV). These results indicate that PLL determined the overall charge of the DNA/liposome/PLL ternary complexes. The complexes formed with histone (0.75 μg or higher) were also positively charged, whose transfection activity was as high as PLL MW 29 300. However, the complexes formed with protamine or PLL MW 2400 remained negatively charged. These observations are in good agreement with the transfection activity of the formulation containing each polycationic polymer. The presence of PLL MW 29 300 did not change the hydrodynamic diameter of DNA/liposome/PLL complexes ($d_H = 275$–312 nm). The complexes made of different sizes of PLL (MW 2400 and 204 000) also did not significantly change their size. This suggests that DNA condensation may not be critical. Therefore, ζ of the transfection complex can predict the transfection efficiency of a new formulation or reagent.

Keywords: Zeta potential; Liposome-mediated gene transfer; Polycation; Particle size

Delivery of foreign molecules such as DNA into eukaryotic cells (transfection) 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 to be an efficient and safe transfection reagent in experimental animals and human gene therapy clinical trials [1,2]. 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. It has been known that cationic liposome-mediated transfection (lipofection) may be enhanced by (i) the overall positive charges of transfection complexes and/or (ii) condensation of DNA into liposome [3]. This means that the

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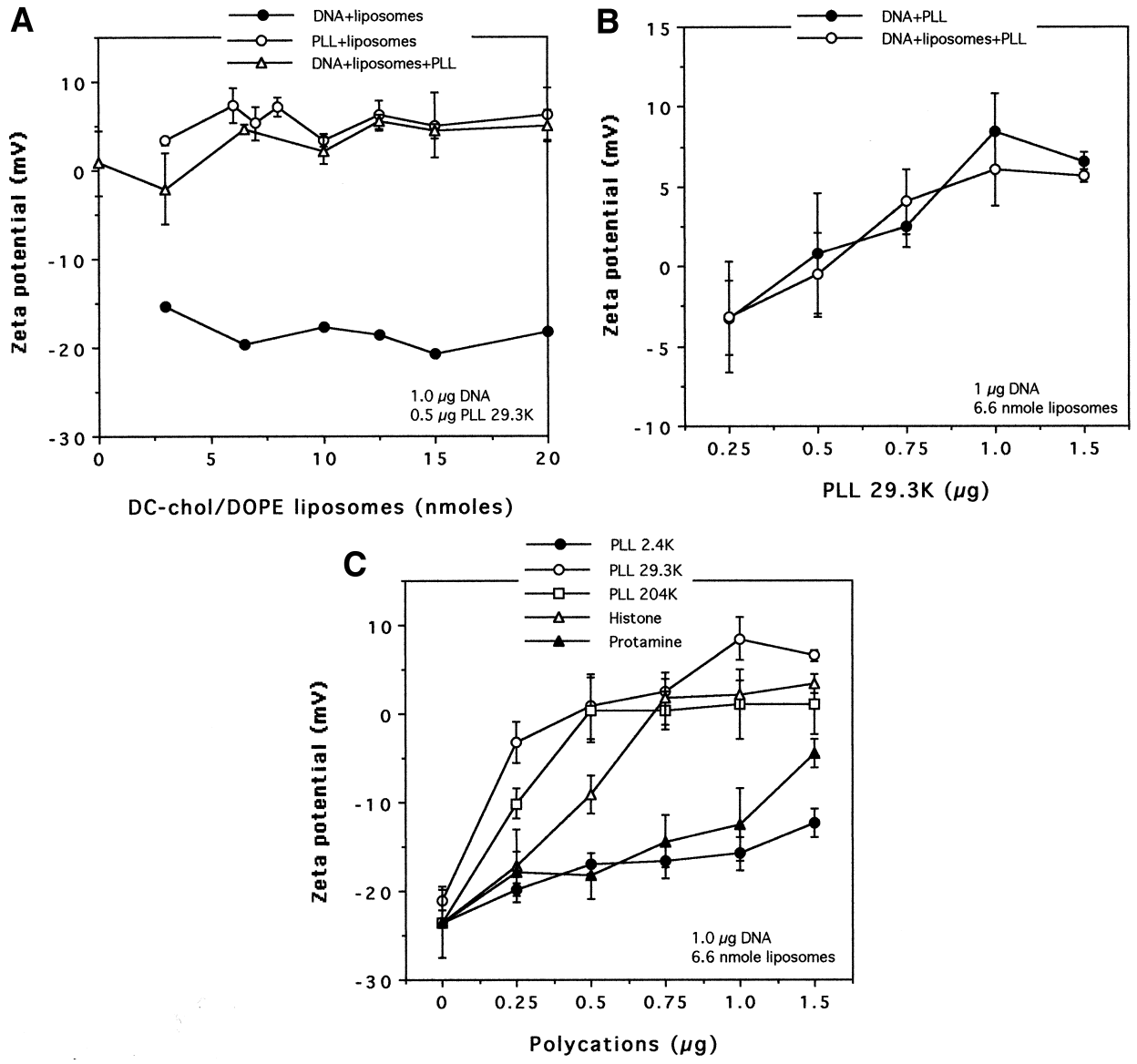

Fig. 1. The charge of DNA/liposome/polycation complexes formed in serum-free DMEM medium (500 μl). (A) ζ of the complexes formed at different liposome concentration. DNA (1 μg) was mixed with different amounts of DC-chol liposomes with or without 0.5 μg of PLL MW 29 300 simultaneously and ζ was measured. (B) ζ of the complexes formed at different PLL MW 29 300 concentrations. DNA (1 μg) and PLL (0.25–1.5 μg) were mixed with or without liposomes (6.6 nmol) simultaneously and ζ was measured. (C) ζ of the complexes formed with different polycations at different concentrations. DNA (1 μg) and liposomes (6.6 nmol) were mixed with different amounts of various polycations simultaneously and ζ was determined.

size and surface charge density of transfection complexes can be related to the transfection efficiency of a reagent. Although currently available formulations are efficient, the search for improvement continues by experimentally testing the transfection activity of potential transfection reagents using an in vitro standard transfection protocol [4]. This trial and error approach is labor-oriented and time-consuming.

Here we present a simple, rapid and reliable approach, zeta potential (ζ, surface charge density) and size (the hydrodynamic diameter, d_H) of transfection complexes using the laser light scattering technique (Brookhaven ZetaPals, New York), for screening the transfection efficiency of a new reagent or formulation. The parameters used to measure ζ were: zeta potential model = Smoluchowski, field frequency =

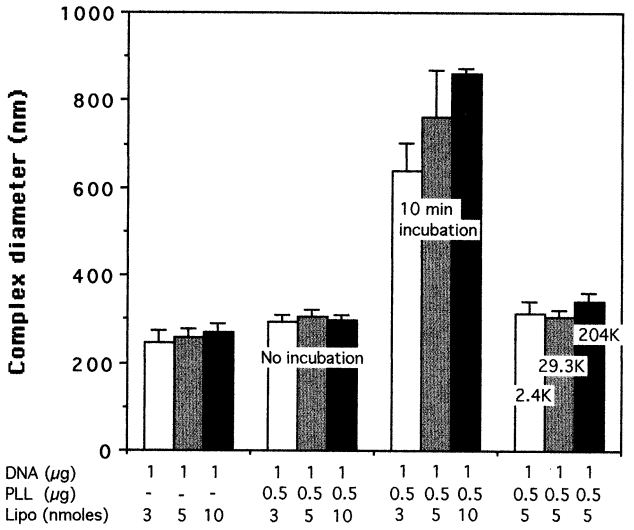

Fig. 2. The size (d_H) of DNA/liposome/polycation complexes formed in serum-free DMEM medium (500 μl). DNA (1 μg) and liposomes (3.3–6.6 nmol) were mixed with or without PLL MW 29 300 (0.5 μg) for the size measurement with or without incubation. The complexes made of two other PLLs (MW 2400 and 204 000) were also measured for their diameters.

2.00 Hz, voltage = 3.00 V. The other parameters, temperature = 25°C unless specified, viscosity = 0.0890 cP, reflex index = 1.330, angle = 90°, and wavelength = 676 nm, were used for measuring both ζ and d_H as described previously [5]. The medium contains a variety of ions and charged molecules, and thus behaves differently from low salt buffer, in which a number of structural studies of DNA/liposome complexes have been done [6–8]. We used Dulbecco's modification of Eagle's medium (DMEM) (pH 7.27 ± 0.02, ionic strength calculated from the formulation = 0.17) which was filtered through a 0.2 μm membrane. All measurements were made in triplicate and are expressed as the mean ± S.D.

The formulations we tested in serum-free medium were those containing a synthetic polycation such as poly-L-lysine (PLL) or a biological polycation such as histone and protamine which was shown to potentiate in vitro lipofection activity [4]. The complexes of CAT plasmid DNA (1 μg) and DC-chol/DOPE liposomes (3–20 nmol) were largely negatively charged (ζ = −15 to minus;21 mV), which became neutral or positive as 0.5 μg or a higher amount of poly-L-lysine (PLL MW 29 300 or MW 204 000, Sigma) was added (−3.16 ± 3.47 to +6.04 ± 2.23 mV) (Fig. 1A). However, the complexes of CAT plasmid DNA (1 μg) and PLL MW 29 300 (0.5 μg or higher) were neutral or positively charged (−3.22 ± 2.3 to +6.55 ± 0.64 mV), which remained the same as 6.6 nmol of the liposomes was added (Fig. 1B). The complexes formed between two positively charged compounds, PLL MW 29 300 (0.5 μg) and DC-chol liposomes (3–20 nmol), were as closely positively charged as DNA/PLL or DNA/liposomes/PLL complexes (+3.31 ± 0.41 to 7.16 ± 1.0 mV) (Fig. 1A). These results indicate that PLL determined the overall charge of the DNA/liposome/PLL ternary complexes. CAT is the bacterial chloramphenicol acetyltransferase, a reporter gene, that was used to measure its expression [9]. pUCCMVCAT (5.1 kb) is a pUC18-based plasmid containing the full-length CAT cDNA and was purified with the Qiagen Plasmid Giga Kit (Valencia, CA) 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 3β[*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl] cholesterol (DC-chol) and dioleoyl phosphatidylethanolamine (DOPE) (3:2 molar ratio) which were combined in chloroform, dried

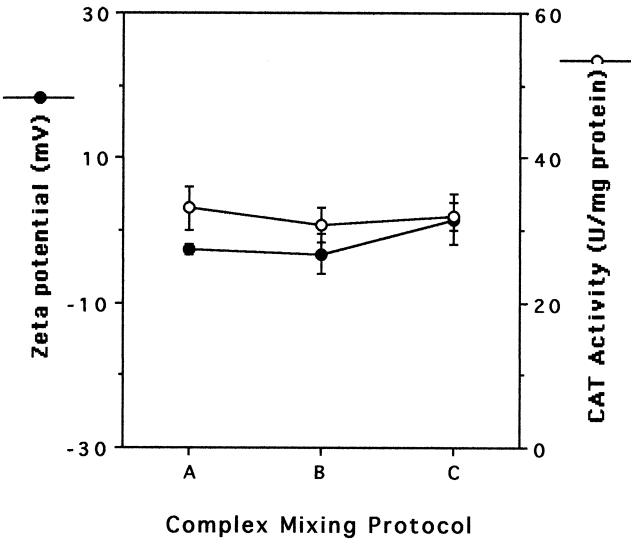

Fig. 3. ζ and transfection activity of DNA/liposome/PLL ternary complexes. CHO cells were treated with 1 μg of pUCCMVCAT complexed with 6 nmol of liposomes for 5 min, (A) followed by incubation with 0.5 μg of PLL MW 29 300 for another 5 min, (B) preceded by complexation with 0.5 μg of PLL MW 29 300, or (C) complexed simultaneously with 0.5 μg PLL MW 29 300. CAT activity was measured after 36 h. Ternary complexes prepared by three different orders, A, B and C, were measured for ζ.

thoroughly, and hydrated with deionized water (pH 7.50 ± 0.25) as previously described [10]. Three other commercial transfection reagents we tested, Lipofectin, LipofectAMINE 2000, and SuperFect, were also largely negatively charged when complexed with DNA in medium [11]. PLL MW 29 300 may be a better polycation than PLL MW 204 000 as a high amount or a high molecular weight of cationic reagents is toxic to cells and thus decreases transfection efficiency. The complexes formed with histone (0.75 μg or higher) (Sigma) were also positively charged, whose transfection activity was as high as PLL MW 29 300, while the complexes formed with protamine (Sigma) or PLL MW 2400 (Sigma) remained negatively charged (Fig. 1C). These observations are in good agreement with the transfection activity of transfection complexes containing a polycationic polymer as reported previously [4].

The presence of PLL MW 29 300 did not change the diameter of DNA/liposome/PLL complexes (d_H = 275–312 nm) (Fig. 2). However, upon incubation for 10 min, PLL lowered ζ by 5–10 mV (data not shown) and increased the size to 640–875 nm. The complexes made of different sizes of PLL (MW 2400 and 204 000) also did not significantly change their size. This suggests that DNA condensation may not be critical.

Since DNA/liposome complexes were negatively charged, we did not expect that the protocol of complex preparation affected the structure and function of the complex. To test whether this is the case, DNA/polycation/liposome complexes were prepared exactly as previously described [4]. Three complex mixing protocols were: DNA was mixed with either cationic polymer or liposomes first, and then, after 10 min incubation, the second component was added (A and B of Fig. 3). Alternatively, all three components were mixed simultaneously (C of Fig. 3). After another 10 min incubation, the mixture was used to measure ζ or transfect cells. The results indicated that there was no difference in either ζ or the CAT activity of the complexes prepared by these three different orders of mixing, indicating that the order of addition in complex formation was not important (Fig. 3). This observation was not consistent with the previous report [4].

The DNA/liposome complexes formed in serum-free medium were largely negatively charged but when cationic polymers were introduced into the liposomes and DNA at appropriate ratios they were slightly positively charged. This significant enhancement of the overall charge of the ternary complexes was attributed to the polycation. However, a low molecular weight of polycations such as PLL MW 3400 or protamine (approximately MW 4100) did not significantly contribute to the negatively charged transfection complexes, being poor gene delivery potentiators. This charge information was consistent with the transfection activity data published previously [4]. Therefore, ζ of transfection complexes can predict in vitro transfection efficiency of new formulations and can be used for screening transfection reagents. Research in improving the transfection activity of currently available cationic liposome formulations or in synthesizing new cationic lipids has been slowly progressing by trial and error experiments. The information on the overall charge of transfection complexes by ζ can speed up the development of better non-viral DNA delivery vectors for gene therapy.

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