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Enhancement of the polycation-mediated DNA uptake and cell transfection with Pluronic P85 block copolymer

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Abstract Polyelectrolyte complexes formed between DNA and poly(*N*-ethyl-4-vinylpyridinium) cations were shown to effectively transfect mammalian cells [7]. This work suggests that the polycation-mediated uptake of the plasmid DNA and cell transfection are significantly enhanced when these complexes are administered simultaneously with a poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide) copolymer, Pluronic P85. The uptake studies were performed using radioactively labeled pRSV CAT plasmid on NIH 3T3, MDCK, and Jurkat cell lines. The transfection was investigated by chloramphenicol acetyltransferase assay using 3T3 cells as a model. The effects reported may be useful for the enhancement of the polycation-mediated cell transfection.

Key words: Transfection; 3T3 cell; Plasmid; Pluronic copolymer; Polycation; Surfactant

1. Introduction

Polyelectrolyte complexes formed between DNA and polycations have recently been used for gene transfer in mammalian and bacteria cells [1–3]. These complexes are spontaneously formed in aqueous media after mixing of DNA and polycation solutions due to the electrostatic binding of oppositely charged polyion chains. Two approaches have been used to deliver these complexes into cells. In the first approach, the polycation chain (e.g. polylysine) is covalently linked to a receptor-recognizing moiety. This provides for specific and selective binding of the complex with a membrane receptor and DNA uptake into the cells [4,5]. The second approach uses synthetic polycations, for example poly(*N*-ethyl-4-vinylpyridinium bromide) (PEVP), which permit uptake of the complex and cell transfection in the absence of binding with the receptor [6,7]. Various cell lines have been successfully transfected using either one of these approaches.

One problem encountered using these techniques is the relatively low efficacy of DNA (or complex) release from endocytic compartments in the cytoplasm and nucleus of cells [1–3]. Further, due to charge neutralization these complexes are often unstable in aqueous solutions and precipitate, thereby hindering their application in gene delivery [3–8]. One approach recently advanced for drug delivery of water insoluble compounds involves the use of micelles of Pluronic block copolymers [9,10]. Recent work on these systems suggests that they enhance the transport of charged molecules across cell membranes [11,12]. This paper reports a significant increase in cell uptake and transfection of mammalian cells using a combination of DNA–PEVP complexes and micelles of Pluronic P85 block copolymer.

2. Materials and methods

2.1. Preparation of DNA–polycation complex

PEVP with a weight-average polymerization degree $P_w = 500$ was synthesized as described in [7]. The pRSV CAT plasmid was obtained from V.A. Engelgard Institute of Molecular Biology, Moscow, Russia. For the uptake study the plasmid was labeled with [^{32}P]CTP using an Amersham nick translation kit (the specific radioactivity of DNA was $3.7 \cdot 10^6$ cpm/μg). To obtain the DNA and polycation complex 64.8 μg of the PEVP in 0.2 ml of HEPES-buffered saline were mixed with 10 μg of the plasmid in 0.2 ml of the same buffer. The mixture was stored 1 h at room temperature and then used in DNA uptake or transfection experiments. This solution was then dissolved in the serum-free culture medium to achieve final DNA concentration of 1 μg/ml. When the effects of copolymer were studied the medium was also supplemented with 1% stock solution of Pluronic P85 (Serva, Germany), to achieve a final copolymer concentration of 0.1%.

2.2. Cell culture conditions

Cells were grown as a monolayers (NIH 3T3, and MDCK cells) or suspension (Jurkat cells) at 37°C under 5% CO_2 atmosphere. The following media were used: (1) Dulbecco's modified Eagle's medium (DMEM) containing 2 mM glutamine and 10% fetal calf serum (FCS) for 3T3 cells; (2) 199 medium containing 10 mM HEPES, 0.075% sodium bicarbonate, 10% FCS and 200 μg/ml gentamycine for MDCK cells; or (3) RPMI-1640 medium containing 2 mM glutamine and 10% FCS for Jurkat cells.

2.3. DNA uptake in cells

Monolayers of 3T3 cells in 90-mm plates were washed with cold (4°C) serum-free medium and then supplemented with 1 ml of the solution of the complex in the serum-free medium containing 0.02–0.4 μg of ^{32}P-labeled plasmid with or without 0.1% Pluronic P85. After 1.5 h incubation with the complex at 4°C or 37°C the cells were transferred on ice and thoroughly washed with cold PBS. The chilled cells were then supplemented with 1 ml of 0.2 mM solution of sodium polyacrylate (PANa) in PBS, pH 7.8, incubated for 1 h, washed, and then treated with DNAse I as previously described [7]. After this treatment the cells were lysed using 0.1% Triton X-100 solution in 1 mM Tris-HCl buffer, pH 7.4, and radioactivity in the lysate was determined in a toluene scincillator. The protein concentration of the lysates was determined using the Bradford method [13]. For comparison the internalization of the same amounts of DNA

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during calcium-phosphate precipitation was investigated using the calcium-phosphate protocol described in detail in [7].

2.4. Cell transfection

Twenty-four hours before the transfection experiment the 3T3 monolayers in 90-mm plates were supplemented with the serum-free medium. During the transfection experiment cell monolayers were supplemented with 5 ml of the solution of the complex in the serum-free medium containing 5 μg of plasmid with or without 0.1% Pluronic P85. The plates were gently agitated for 2 h at 37°C, 5% CO_2. The cells then were thoroughly washed, and supplemented with medium containing 10% FCS, and incubated for 48 h at 37°C, 5% CO_2. (After the first 16 h of incubation the medium was replaced with the fresh medium.) After incubation chloramphenicol acetyltransferase (CAT) activity was determined using the method of Gorman et al. [14]. For comparison the cells were transfected with the same amounts of plasmid using a calcium-phosphate protocol.

3. Results and discussion

Our previous work [7] suggested that the efficacy of transfection with complexes formed between DNA and PEVP strongly depends on the complex composition determined by the base molar ratio [PEVP]/[DNA]. This paper studies the complexes having the optimal composition (= 10) which were shown to be most effective in transfection of 3T3 cells. Binding of the plasmid with the cells was studied at two temperatures: 4°C and 37°C. When the incubation temperature was 4°C, treatment with PANa and DNAse resulted in 85–90% decrease in the recovery of the radioactivity in the cell lysate (data not shown). Since endocytosis is inhibited under these conditions, the residual 10–15% of the radioactivity found in the lysate was probably due to incompletely washed DNA. By contrast, significant recovery of the radioactive DNA was observed when the cells were incubated with the complex at 37°C. In this case 35–40% of the label was found in the cell lysate after PANa and DNAse treatment. This residual fraction apparently corresponds to the plasmid internalized in the cells by endocytosis.

The internalization of the plasmid in 3T3 cells at 37°C is shown in Fig. 1. Practically no recovery of the radioactive label in the lysate was observed when cells were incubated with free DNA. As previously reported, the DNA–PEVP complex is effectively internalized by the cells. In this case the recovery of radioactive label in the lysate exceeds that achieved using calcium-phosphate precipitation by a factor of three. Administration of the complex in the presence of 0.1% Pluronic P85 further increased plasmid uptake, with levels in cells exceeding those observed during calcium-phosphate precipitation by about 15-fold. The substantial enhancement of plasmid binding in cells was also observed with MDCK and human T-lymphoma Jurkat cell lines when the DNA–PEVP complex was administered in the copolymer solution (Table 1). This suggests that the effect of the copolymer on the uptake of the DNA in the complex with PEVP may be a characteristic of a fairly broad range of cell lines.

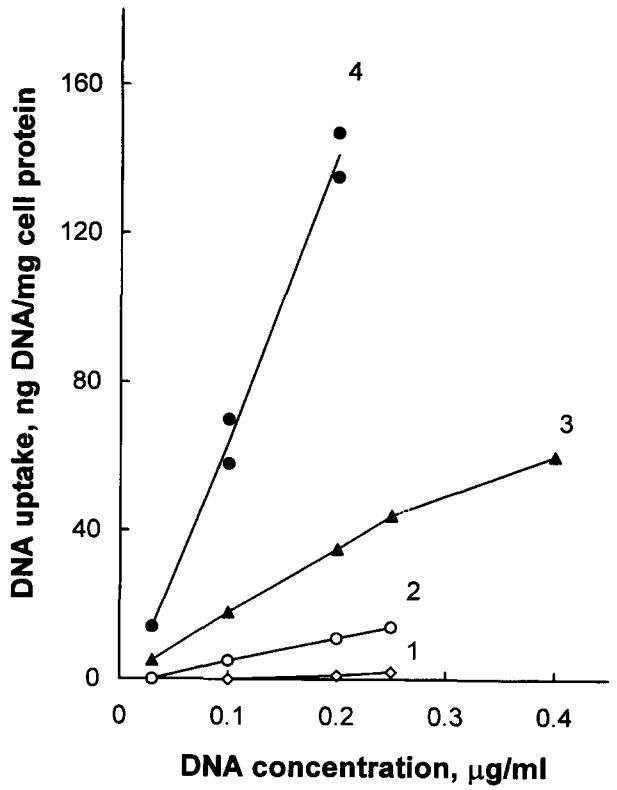

Fig. 1. Internalization of ^{32}P-labeled plasmid in 3T3 cells at 37°C. Cells were incubated with various doses of free DNA (1), DNA precipitated by calcium-phosphate (2), DNA–PEVP complex in the absence (3) and presence (4) of 0.1% Pluronic P85.

3T3 cell transfection with the plasmid is presented in Fig. 2. Enhancement of the transfection was observed when the DNA–PEVP was administered in the copolymer solution. Transfection efficacy was markedly higher than that observed with both calcium-phosphate precipitation and copolymer-free DNA–PEVP complex. Possible reasons for the increased uptake and transfection activity of these complexes were discussed in our previous publication [7]. It was hypothesized that the DNA condensation, and recharging due to the appearance of the cation chains on the surface of the complex, provide for increased binding of the complex to the plasma membrane facilitating its entrapment into endocytic vesicles. Our unpublished data suggest that Pluronic P85 copolymer may intensify endocytic processes in eukariotic cells, and therefore may further enhance the uptake of the complex. Further, these data suggest that this copolymer may enhance the elaboration of the macromolecules from the endocytic compartments in the cytoplasm. In conclusion, the major finding of this paper is that the Pluronic P85 copolymer enhance the internalization and transfection of the DNA–PEVP complexes into cells. This can be used to improve current proto-

Table 1
Internalization of plasmid in MDCK and Jurkat cells at 37°C

DNA form[a]	MDCK	Jurkat
Free DNA	1	6
DNA precipitated with calcium-phosphate	15	30
DNA–PEVP complex	70	135
DNA–PEVP complex + Pluronic P85	150	230

[a]DNA concentration is 0.5 μg/ml (MDCK cells) or 0.2 μg/ml (Jurkat cells).

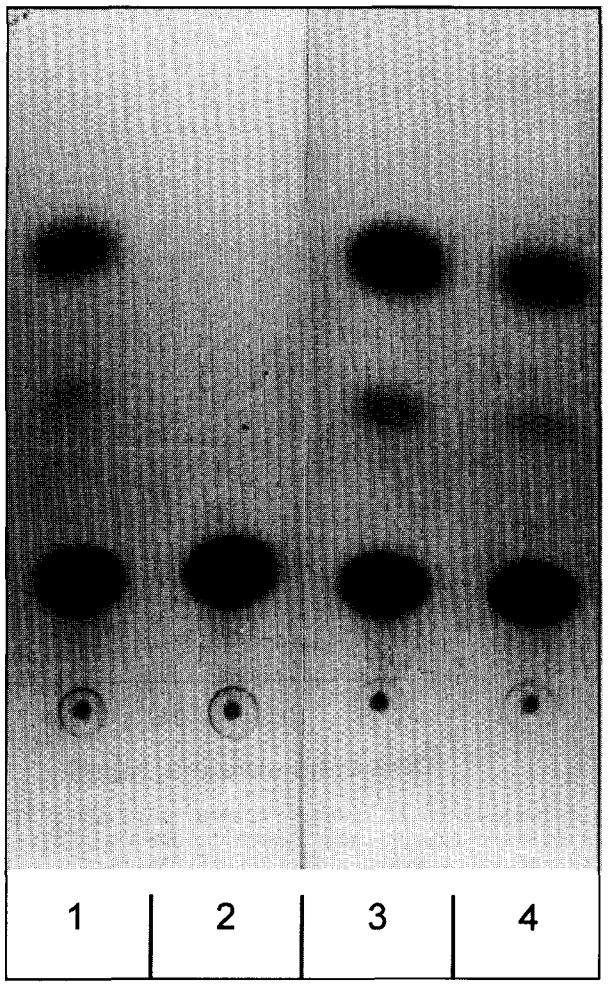

Fig. 2. Transfection of NIT 3T3 cells using calcium-phosphate precipitation (lane 1) DNA–PEVP complex in the presence of 0.1% Pluronic P85 (lane 3), and the copolymer-free DNA–PEVP complex (lane 4). Lane 2, ^{14}C-chloramphenicol sample. The conversion of ^{14}C-chloramphenicol (bottom row) produces two monoacetate forms (upper rows) [14]. The DNA concentration was 1 μg/ml. The base molar ratio [PEVP]/[DNA] equaled 10.

cols for the polycation-mediated cell transfection. These results also warrant further investigation of effects of non-ionic surfactants, specifically, Pluronic copolymers, on the transfection, as more efficient techniques for gene delivery may be developed.

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