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Cationized bovine serum albumin as gene carrier: Influence of specific secondary structure on DNA complexibility and gene transfection

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ABSTRACT

In this research, BSA, one of the natural rigid globular proteins with ca. 51% of α-helix secondary structure, was utilized to prepare cationized BSA (cBSA) as gene carrier. Tetraethylenepentamine (TEPA) or polyethylenimine (PEI_{1800}) was grafted to BSA with different grafting levels. Based on the circular dichoism (CD) spectra, all cBSA remained α-helical structure to some degree. This was exciting to endow cBSA with quite different DNA complexibility and cellular biology behavior from the random coiled and flexible polycations such as PEI and poly-L-lysine (PLL). Strangely, the DNA condensability decreased with the increment of TEPA or PEI_{1800} grafting level. Also, the cBSA could condense DNA effectively to form irregular nanoparticles around 50–200 nm above N/P ratio of 10. On account of the excellent hydration of BSA, the cBSA/DNA complexes revealed good colloidal stability under physiological salt condition. Cell culture experiments indicated this BSA-based gene carrier possessed good cellular compatibility. Surprisingly, cBSA/DNA complexes could be uptaken excellently by up to 90% cells. This might be owing to the agitation effect of α-helical structure and the positive potential of these complexes. BSA-PEI_{1800}/DNA complexes with quick endosome escape even had transfection efficiency as high as PEI_{25k}/DNA complexes. Overall, this paper provided us the potential of cBSA as gene carrier and might have some instructions in the design of protein-based gene delivery system.

1. Introduction

Recent years have witnessed a large number of researches on non-viral gene carriers [1–5]. Among them, some synthesis and natural polycations have drawn much attention because of the safety and easy way to control in gene therapy. Traditional polycations, such as PEI and PLL, are usually random-coided and flexible in dilute solution, which facilitate them to collapse and fold gene to form tight particles via electrostatic interaction [6,7]. The structure of polycation and assembly condition demonstrated great influence on gene delivery properties [8,9]. Take the "gold tranfection agent" PEI and PEI-based derivates for instance, as the molecular weight and charge density increased, their DNA complexibility would increase and then enhance the gene transfection efficiency. Furthermore, with the increment of N/P ratio (mole ratio between nitrogen atoms of PEI segment and the phosphate groups on DNA), the polycations would condense DNA to form tighter nanoparticles [10–13].

However, compared with viral gene carriers, the in-vivo transfection of polycation-based gene carriers should be further improved. Virus is a kind of natural supramolecular assembly with core-shell structure, enclosed infective nucleic acid in the capsid of proteins. And this structure might give us some inspiration. Thus, natural or synthesized polypeptides have been widely researched in gene delivery for low immunogenicity and potential to be applied clinically [14,15]. It was interesting to find that the secondary structure of polypeptide played an important role in gene transfection [14,16]. Yin et al. synthesized a series of cationic polypeptides for gene delivery. They found that α-helical structure of polypeptide could effectively agitate cell membrane and promote cellular uptake [16]. This inspired us to think of the secondary structure of polypeptide gene carriers.

Bovine Serum Albumin (BSA) is a kind of natural protein with rigid globular structure. For example, its secondary structure is constituted of ca. 51% of α-helix [17]. BSA had a high number of reactive residues (e.g. glutamic acid, aspartic acid and lysine), including ca. sixty amino groups and one hundred carboxylic groups, which made it easy to be modified [18–20]. And many researchers reported that BSA had good hydration property [21–23]. This hydration property could endow protein-coated particles with

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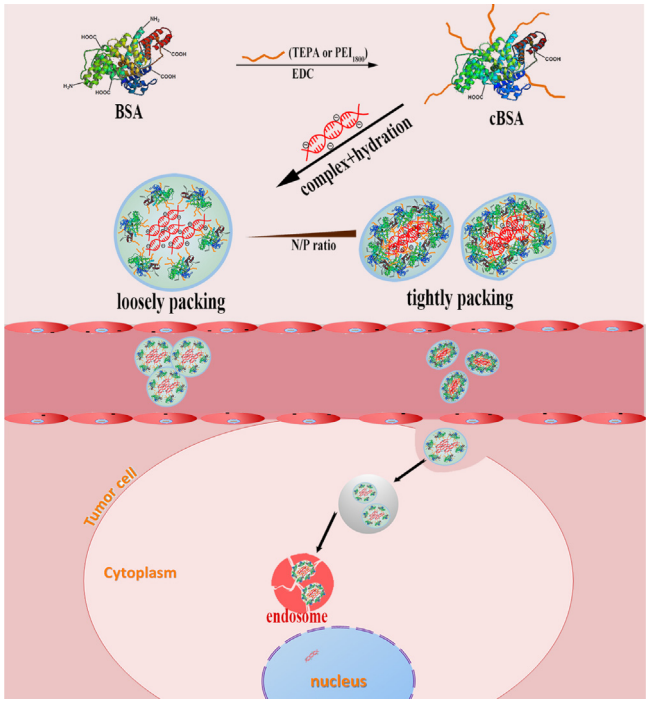

Fig. 1. Schematic showing DNA complex and gene delivery of cBSA.

good colloidal stability in salt solution [22]. Based on these properties, BSA had been widely applied in drug/gene delivery. For instance, Zhang et al. synthesized modified BSA as charge-reversal platform to prepare pDNA–PEI–mBSA ternary complexes, which could simultaneously improve biocompatibility and transfection efficiency of polyplexes [24]. And Zophel et al. prepared cBSA by introducing ethylenediamine onto BSA as gene carrier with good cyto-compatibility and transfection efficiency [19,20]. However, never had they focused on the different gene delivery properties caused by the specific secondary structure.

In this research, the cBSA was synthesized by the grafting of TEPA and PEI_{1800} onto BSA, respectively (as depicted in Fig. 1). TEPA and PEI_{1800} are composed of different number of ethylenediamine units with low cytotoxicity. Based on CD spectra, all cBSA remained α-helical structure to some degree. Comparing with random-coided and flexible polycations, cBSA with specific secondary structure might show different DNA complexibility and cellular biology behavior. This paper thus provides us the potential of cBSA as gene carrier and shows the way to design protein-based gene delivery system.

2. Materials and methods

2.1. Materials

Bovine Serum Albumin (BSA) was purchased from Sigma-Aldrich Co. LLC. Tetraethylenepentamine (TEPA), branched polyethylenimine with molecular weight of 1800 Da (PEI_{1800}) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was obtained from Aladdin Industrial Corporation. 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) was purchased from Bio Basic Inc. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPEs) was purchased from Sangon Biotech (Shanghai, China). 6-Diamidino-2-phenylindole (DAPI) was obtained from J&K Chemical Ltd.

Deoxyribonucleic acid (DNA, fish sperm, sodium salt) were obtained from AMRESCO for physiological measurements such as DLS, zeta potential and transmission electron microscope (TEM). Cy3-labeled DNA was purchased from Sangon Biotech (Shanghai, China) for cellular uptake determination and intracellular trafficking of complexes. Plasmid DNA pGL3 was obtained from Promega (USA) for gel retardation assay and gene transfection.

2.2. Synthesis of cBSA

The synthesis of BSA-TEPA was proceeded as reported [19]. Briefly, 7.64 mL of TEPA was dissolved in 20 mL of three distilled water and the pH was adjusted to 4.75 using 6 N hydrochloric acid. Then, 200 mg of BSA was dissolved in this solution and stirring for 15 min. This resulting solution was separated into 2 equal aliquots. Aliquot A was mixed with 35 mg of EDC and reacted for 2 h. Aliquot B was mixed with 40 mg of EDC and reacted for 3 h. After that, 1 mL of acetate buffer (4 M, pH 4.75) was added to stop this reaction. The solution was ultrafiltrated four times to remove non-reacted EDC and TEPA through 30k Da ultrafiltrated membrane. The condensed solution was then lyophilized for further analysis.

BSA-PEI_{1800} was synthesized similar with the synthesis of BSA-TEPA. Briefly, 5 g of PEI_{1800} was dissolved in 20 mL of water and adjusted pH to 4.75. Then, 200 mg of BSA was added. After 15 min of stirring, the solution was divided into two equal aliquots. Aliquot C was mixed with 15 mg of EDC and reacted for 30 min while aliquot D was added by 35 mg of EDC and reacted for 2 h. The reaction was stopped by the addition of acetate buffer and the product was ultrafiltrated for purification. The condensed solution was lyophilized. The final products were characterized by MALDI-TOF, CD and FTIR.

2.3. Characterization of cBSA

MALDI-TOF mass spectra was recorded on a Bruker UltrafleXtreme MALDI TOF/TOF instrument using gentisic acid (2,5 dihydroxy benzoic acid) as the matrix. The CD spectra of cBSA solution were scanned on a MOS-450 spectropolarimeter (Bio-Logic, France) with a sample cuvette pathlength of 1.0 mm in the range of 190–250 nm at room temperature under constant nitrogen flush. All cBSA samples maintained the same mole concentration with BSA solution. IR spectra were recorded on a Fourier-transform infrared (FTIR) spectrometer. KBr pellets of BSA and cBSA were used in the IR instrument. FTIR spectra were acquired after 31 scans between 4000 and 400 cm^{-1}.

2.4. cBSA/DNA complexes formation protocol

cBSA/DNA complexes were prepared in HEPEs buffer as reported [25]. Briefly, cBSA/DNA complexes at different N/P ratios were prepared by votexing equal volume of cBSA solution with DNA solution (100 μg mL^{-1} dissolve in 20 mM HEPEs buffer solution). The amount of DNA was fixed for each experiment and N/P ratio was adjusted by changing the concentration of cBSA. For all the complexes, N/P ratio referred to the mole ratio between protonatable nitrogen atoms of grafted TEPA (or PEI_{1800}) and the phosphate groups of the DNA. Take BSA-TEPA-39/DNA complexes for example, N/P ratio referred to the mole ratio between nitrogen atoms of all grafted TEPA chains with the phosphate groups of the DNA. Taking DNA condensability, cellular viability and gene transfection into consideration, we chose N/P ratios of 10, 20 and 50 in the following study if there were no special instructions.

2.5. Chemo-physical characterization of different cBSA/DNA complexes

The DNA condensation capability at pH of 7.4 and 5.0 was examined by gel retardation assay. The cBSA/DNA complexes at different pH containing 450 ng pDNA were prepared, mixed with loading buffer (5:1 by volume) and electrophoresed at 100 V for