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Structural influence of graft and block polycations on the adsorption of BSA

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ABSTRACT

Protein adsorption is considered as an important factor for the low transfection efficiency of polycations *in vivo*. In this study, two typical polycations of equal molecular weight with different structures were chosen to investigate their adsorption on bovine serum albumin (BSA), including the block copolymer named poly (N-vinylpyrrolidone)-b-poly (2-dimethylaminoethyl methacrylate) (PVP-b-PDMAEMA, i.e. PbP) and graft copolymer named PVP-g-PDMAEMA (PgP), respectively. Fluorescence spectroscopy was used to confirm the binding constants and binding sites between polycations and BSA in static state. The binding constants were $4.1 \times 10^4\ M^{-1}$ vs $8.3 \times 10^4\ M^{-1}$ and binding sites were 0.3 vs 1.1 for PbP and PgP, respectively, indicating PgP had stronger binding affinity with BSA. Surface plasmon resonance (SPR) was used to study the dynamical non-specific interaction between BSA and polycations as well as the polyplexes. The numbers of both PbP and PgP adsorbed on BSA increased with concentration of polycations increasing, and the number of PgP adsorbed on BSA is higher compared with PbP when their concentration is low. When their concentration is high, the number of PbP adsorbed on BSA is more than that of PgP. However, PgP/DNA polyplexes showed higher adsorption amount compared with PbP/DNA polyplexes at different N/P ratios.

1. Introduction

Gene therapy is defined as the delivery of exogenous nucleic acids into cell to generate or silence functional protein to treat diseases [1–5]. Exogenous nucleic acids such as naked DNA and siRNA can be hardly delivered into cells due to their inherently negative charges [6,7]. Positively charged materials are suitable to help DNA and siRNA enter the cells because of the favorable electrostatic interaction of cationic particles with the negative exterior of cells [8,9]. Among them, cationic polymers [10–13] (polycations) are widely used due to their advantages such as low immune response, large DNA/siRNA loading capacity, [5,14] simple preparation and adjustable structures [15]. Although polycations show high transfection efficiency *in vitro*, [16,17] the transfection efficiency *in vivo* [18] is still extremely low. It was confirmed that many characters of polycation/DNA polyplexes such as size [13], shape, [19] surface charge, [20] solubility, [21] surface modifications [22], and structures [17,23,24] could influence the transfection efficiency *in vivo*. Notably, the low transfection efficiency is closely related with the adsorption of polyplexes on negatively charged proteins [25–27]. Therefore, it is necessary to illustrate the relationship between the adsorption on protein and the structure of polycations.

When polyplexes contact with biological fluids, approximately fifty proteins [28] could rapidly adsorb to the surface of polyplexes and form protein "corona" [29–31], which mediates the uptake of nanoparticles and controls their pharmacokinetics and biodistribution as well as their eventual fate *in vivo* [31,32]. Among various classes of proteins, albumin (or serum albumin) is the most abundant protein in blood and is responsible for about 80% of the colloidal osmotic pressure, pH buffering and so forth [33]. Bovine serum albumin (BSA) [34] is an ideal model protein to study interaction between protein and polyplexes due to its low cost, wide availability and structural/functional similarity to human serum albumin (HSA) [35,36].

In this study, two typical polycations of equal molecular weight with different structures [37] were used to study the interactions of them and their polyplexes with BSA, including graft copolymer named poly (N-vinylpyrrolidone)-g-poly (2-dimethylaminoethyl

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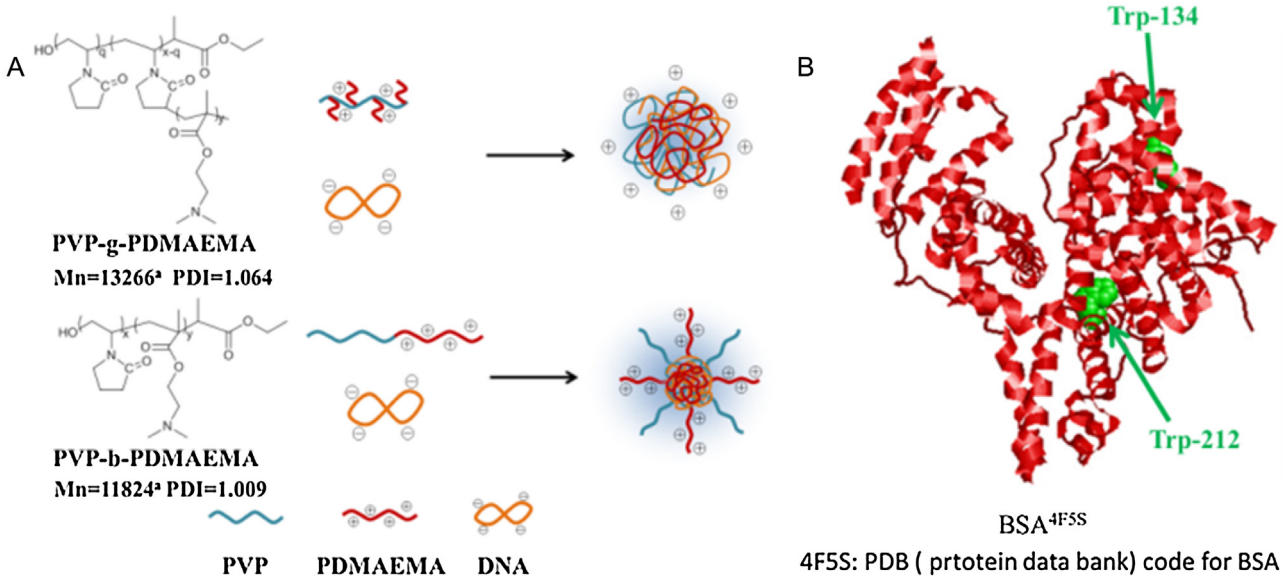

Scheme 1. The chemical structure, molecular weight and polydispersity index of PbP and PgP and the formation of PbP/DNA and PgP/DNA polyplexes (A); Structure of BSA with tryptophan residues shown in green color (B). [a]Determined by GPC. (For interpretation of the references to color in the text, the reader is referred to the web version of this article).

methacrylate) (PVP-g-PDMAEMA, i.e., PgP) and block copolymer named PVP-b-PDMAEMA (PbP). Fluorescence spectrum was used to confirm the binding affinities and binding ratios between polycations and BSA in static state. Surface plasmon resonance sensor (SPR) was chosen to study the dynamical non-specific interaction between BSA and polycations as well as polyplexes.

2. Experimental part

2.1. Chemicals

BSA was purchased from Sigma Chemical Company and used directly. Mercaptoethylamine modified BSA, PVP-b-PDMAEMA and PVP-g-PDMAEMA were prepared according to references [37,38,39]. Other chemicals were of reagent grade and used without further purification. The Au chip was purchased from Biacore (Uppsala, Sweden). Water was double distilled.

2.2. Preparation of polycation/DNA polyplexes

Polycations/DNA polyplexes were prepared at different charge ratios (N/P) (3/1, 5/1, 10/1 and 15/1). N/P refers to the molar ratio of amino groups in polycations to the phosphate groups in DNA. To calculate the amount of polycations in polyplexes at a certain N/P, polycations were firstly diluted to a certain concentration containing appropriate amino group and then blended with DNA of equal volume. The DNA concentration used for polyplexes preparation was 25 ng/μL.

2.3. Fluorescence spectroscopy

Fluorometric experiments were carried out with a FL0903M018 Spectrometer. Stock solution of polycations (200 μM) were prepared at room temperature in PBS buffer (25 ± 1 °C pH = 7.4). Various solutions of polycations (1–100 μM) were prepared. Fluorescence spectra were measured by adding different amounts of polycations with equal volume to a fixed protein solution (25 $\mu g\,mL^{-1}$) to make the final concentration of BSA reach 12.5 $\mu g\,mL^{-1}$. Intrinsic fluorescence spectra of BSA were recorded between 280–500 nm with an excitation of 270 nm. The intensity at 348 nm (from tryptophan residues) was used to calculate the binding constant (K) according to references [30,33,40,41] .

BSA (Scheme 1B) has two tryptophan residues that possess intrinsic fluorescence [40]. when other molecules interact with BSA, tryptophan fluorescence intensity changes due to the change of protein conformation [42,43]. On the assumption that the substantive binding sites for polycation as a quencher [Q] on protein [B] is n, the quenching reaction can be shown as follows:

$$nQ + B \Leftrightarrow Q_nB \quad (1)$$

The binding constant (K_A) can be calculated as:

$$K_A = [Q_nB]/[Q]^n[B] \quad (2)$$

where [Q] and [B] are the quencher and protein concentration, respectively, $[Q_nB]$ is the concentration of nonfluorescent fluorophore-quencher complex, and $[B_0]$ is the total protein concentration:

$$[Q_nB] = [B_0] - [B] \quad (3)$$

$$K_A = \{[B_0] - [B]\}/[Q]^n[B] \quad (4)$$

The fluorescence intensity is proportional to the protein concentration as described:

$$[B]/[B_0] \propto F/F_0 \quad (5)$$

Binding constant of polycation-protein complex can be concluded from Eq. (4):

$$\log[(F_0 - F)/F = \log K_A + n \log[Q] \quad (6)$$

The accessible fluorophore fraction (f) can be calculated by modified Stern–Volmer equation [40]:

$$F_0/(F_0 - F) = 1/fK[Q] + 1/F \quad (7)$$

where F_0 is the initial fluorescence intensity and F is the fluorescence intensities in the presence of quenching agent (or interacting molecule). K is the Stern–Volmer quenching constant, [Q] is the molar concentration of polycations, and f is the fraction of accessible fluorophore to a polar quencher, which indicates the fractional fluorescence contribution of the total emission for an interaction with hydrophobic quencher, The K can be calculated from $F_0/F = K[Q] + 1$.