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Effects of gene carrier polyethyleneimines on the structure and binding capability of bovine serum albumin

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ABSTRACT

Polyethyleneimine (PEI), one of the most effective non-viral gene carriers, is also cytotoxic, however the molecular basis is poorly understood. Little is known about the effects of PEI on the structure and functions of the biomacromolecules. In this work, fluorescence, UV–vis absorption, circular dichroism (CD) spectroscopy and zeta-potential measurement were conducted to reveal the interaction between PEIs (average molecular weight 25, 10 and 1.8 kDa) and bovine serum albumin (BSA), and to evaluate the effects on the conformation of BSA as long as its binding capability to the model compounds, 8-anilino-1-naphthalenesulfonic acid (ANS) and quercetin. PEIs were found to complex with BSA and induced a conformational change of the protein by a major reduction of α-helix at PEI concentration <0.2 mg·mL^{-1} and an increase at higher PEI concentration. The binding efficacy of ANS and quercetin to BSA was greatly reduced by the competitive binding by PEI and influenced by the conformational change of BSA, which was found to display a similar trend to the change of the α-helix content of the protein. The polymer size played an important role in PEI-BSA interaction. PEI of higher molecular weight was more favorable to interact with BSA and more efficient to perturb the conformation and binding capability of the protein.

1. Introduction

Gene therapy is one of the most advanced issues in the field of modern biomedicine [1]. A key factor in the success of gene therapy is the development of delivery systems that are capable of efficient gene transfer, without causing any associated pathogenic effects. Viral vectors have been considered the most efficient vectors by successfully introducing foreign genes into cells. However, the safety concerns and immunological profile of viral vectors set great limits to their clinical applications. The accidents in the gene therapy clinical trials, by using adenovirus as vector, in France and America [2,3], provoke the interesting in non-viral gene delivery system based on cationic lipids and cationic polymers [4,5]. Due to the unique combination of high charge density and enhanced "proton sponge effect" in endolysosome, polyethyleneimine (PEI) is one of the most effective non-viral gene carrier. Branched PEI of molecular weight 25 kDa and linear PEI of molecular weight 22 kDa have been considered as "golden standard" for polymeric gene delivery systems [6–11].

For practical application, the safety of biomaterials is the most important issue and must be strictly evaluated as they will inevitably contact various biomacromolecules, organelles, cells and tissues when used in living system [9–11]. Considerable evidences showed that transfection efficiency and toxicity of PEI was related with the molecular weight. PEI of higher molecular weight is generally more efficient, however more toxic. Many strategies, such as PEGylation, decoration of PEI with functional groups and synthesis of biodegradable PEIs via cross-linking or grafting, were declared to reduce the cytotoxicity and promote the transfection efficiency of PEI [6–8]. However, cytotoxicity tests, such as 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, were based on assessing the in vitro metabolic activity of cells at 4–8 h following treatment with cationic macromolecules. Moghimi et al. [11] suggested that the apoptosis in intact cell was time dependent and that the MTT assay may not provide a true representation of polymer safety in gene therapy trials, as the MTT assay was not indicative of apoptosis which occurred at the later stage long after PEI-treatment. Up to date, little attention has been paid to the effects of PEI on the structure and functions of biomacromolecules. The molecular basis of PEI cytotoxicity is still poorly understood [13–15].

In practical applications, vector/DNA complexes are often introduced into the living system by various routes. In view of the abundant and far-reaching blood vessel network, the vectors or their degradation products would more or less enter the systemic circulation sooner or later, directly or indirectly, regardless of any administration route, and instantaneously become associated with blood cells and plasma proteins, which may alter cell membrane structure and protein conformation, and then perturb blood functions and eventually impair the whole organism [9]. Thus, knowledge of the interaction with blood components is critical to understand the in vivo safety and efficacy of gene vectors. It has been reported that the PEIs have severe impact on

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red blood cell (RBC) membrane structure and blood coagulation process, and induce conformational change of serum albumin [9,12].

Albumin is the most abundant blood plasma protein and of important physiological functions such as maintaining the pH and colloid osmotic pressure of the blood compartment. Albumin also plays a key role in transport, distribution and metabolism of many endogenous and exogenous substances [13–15], which make it the most commonly used protein to test the biocompatibility of many biopolymers [16–21]. However, knowledge about the interaction between PEI and serum albumin and its influence on the conformation and functions of the protein is still limited. In this work, a combinational study of fluorescence, UV–vis absorption, circular dichroism (CD) spectroscopy and zeta-potential measurement were conducted to reveal the interaction between PEI and bovine serum albumin (BSA) and its influence on the conformation of the protein. The binding capability of BSA in the solutions containing PEI was evaluated by using 8-anilino-1-naphthalenesulfonic acid (ANS) and quercetin (Que) as model compounds and related to the interaction of PEI with the model compounds and the conformational change of BSA. PEI of different molecular weight (MW 25, 10 and 1.8 kDa) was chosen in order to clarify the structure-activity relationship, which would facilitate the design of degradable PEIs.

2. Experimental

2.1. Materials

Branched PEI with different molecular weight (average molecular weight 25, 10 and 1.8 kDa, labeled as PEI25k, PEI10k and PEI1.8k, respectively.), BSA (lyophilized powder, ≥98%) and ANS (≥97%, HPLC) were obtained from Sigma-Aldrich (USA). Bioreagent grade quercetin and analytical grade ethanol were from Sinopharm Chemical Reagent Co. Ltd. (China). All of the materials were used as received. All of the stock solutions and the solutions for analysis were prepared in 10 mM phosphate buffered solution (PBS) (pH 7.4).

Stock solutions of PEIs (10 $mg \cdot mL^{-1}$) and BSA (250 μM) were prepared by dissolving the products in PBS buffer and the pH adjusted to 7.4 using HCl and NaOH solution. The stock solutions were stored at 0–5 °C and diluted to the desired concentration when used.

Stock solution of ANS, prepared by dissolving the solid product in PBS buffer, was stored in 0–5 °C and diluted to 5 μM when used. The concentration of ANS in the stock solution was determined by absorbance at 350 nm on a Shimadzu UV-2501PC UV–vis spectrometer, at which the molar absorption efficiency was taken as 6300 $M^{-1} \cdot cm^{-1}$ [22].

Stock solution of quercetin (10.0 mM) was prepared by dissolving the solid product in ethanol and stored in 0–5 °C. The stock solution was diluted in PBS to 400 μM just before used. The final concentration of quercetin for spectra analysis was 5 μM.

It should be noticed that molarity in M was adopted for the concentration of the materials used in this work except PEIs, to which mass volume concentration in $mg \cdot mL^{-1}$ was applied, for the sake of comparison to display the role of polymer size in the PEI-protein interaction.

2.2. Methods

2.2.1. Intrinsic Fluorescence of BSA

Fluorescence measurements were performed for BSA (5 μM) in PBS buffer containing PEI of various concentration (0–1.0 $mg \cdot mL^{-1}$) on a Cary Eclipse spectrofluorimeter (Agilent) using a 1 cm × 1 cm quartz cell thermostated to 25 °C. The fluorescence emission of tryptophan was excited at 295 nm and recorded from 300 to 450 nm. The excitation and emission slit widths were set to 5 nm and scan rate 600 $nm \cdot min^{-1}$.

2.2.2. Zeta-Potential Measurement

Zeta-potential of BSA in PBS buffer containing PEI (0–1.0 $mg \cdot mL^{-1}$) was determined by a Zetasizer Nano-ZS dynamic scattering spectrometer (Malvern) by using disposable folded capillary cell. Zeta-potential was calculated using the Smoluchwski mathematical model. The measurement was performed at least three times and the data in the graph represented the average ± standard deviation.

2.2.3. UV–Vis Absorption

UV–vis absorption spectra of BSA (5 μM) in PBS buffer containing PEI (0–1.0 $mg \cdot mL^{-1}$) were recorded on an UV-2501PC UV–vis spectrophotometer (Shimadzu) equipped with a quartz cell of 1 cm path length at room temperature. PBS buffer containing PEI of the same molecular weight and of the same concentration was used as reference and the absorption spectra were recorded from 200 to 450 nm.

2.2.4. Circular Dichroism (CD) Measurement

CD spectroscopy analysis was carried out on a MOS-450 spectropolarimeter (Bio-Logic, France). The spectra were collected from 190 to 250 nm in a quartz cell of 1 mm path length at 25 °C in nitrogen atmosphere. The final BSA concentration was 5 μM in PBS buffer containing PEI (0–1.0 $mg \cdot mL^{-1}$). All of the CD spectra were obtained by averaging 3 runs with a step interval 0.5 nm and an acquisition duration 0.1 s. A blank for PEIs-containing PBS buffer without BSA, as measured under the same experimental conditions, was subtracted from the data.

2.2.5. Fluorescence Emission of ANS and Quercetin

Fluorometric experiment for ANS and quercetin in PBS buffer containing PEI (0–1.0 $mg \cdot mL^{-1}$), in the absence and presence of 5 μM BSA, were carried out on a Cary Eclipse spectrofluorimeter (Agilent) using a 1 cm × 1 cm quartz cell thermostated to 25 °C. The fluorescence emission for ANS was excited at 388 nm and recorded from 400 to 600 nm. Those for quercetin were excited at 465 nm and recorded from 480 to 650 nm. The excitation and emission slit widths were set to 5 nm and scan rate 600 $nm \cdot min^{-1}$.

2.2.6. Fluorescence Quenching of BSA by Quercetin

BSA-PEI solutions and BSA-quercetin-PEI solutions were prepared by diluting the stock solutions in PBS. The final concentration of both BSA and quercetin was 5 μM. The fluorescence emission of BSA was excited at 270 nm and recorded from 300 to 450 nm on a Cary Eclipse spectrofluorimeter (Agilent) using a 1 cm × 1 cm quartz cell thermostated to 25 °C. The excitation and emission slit widths were set to 5 nm and scan rate 600 $nm \cdot min^{-1}$. The quenching efficiency E of BSA by quercetin was calculated based on the fluorescence intensity at 347 nm

$$E = (F_0 - F)/F_0 \quad (1)$$

in which F_0 and F are the fluorescence emission of BSA in the absence and presence of quercetin.

The absorption of quercetin at the excitation (270 nm) and emission (347 nm) wavelength of BSA was taken into account in the steady-state study of energy transfer. The absorption spectra of BSA, quercetin and total solution (BSA and quercetin) in PEI-containing PBS were obtained following the same method described in subsection 2.2.2. Assuming that the molecular absorption coefficients were the same for free and bound ligand, the effect of quercetin absorption on the fluorescence of BSA at excitation wavelength was corrected according to Eq. (2) [23],

$$F_{corr} = F_{exp} \cdot A_t / A_f \left(1 - 10^{-A_f}\right) / \left(1 - 10^{-A_t}\right) \quad (2)$$

where F_{exp} and F_{corr} are the experimental emission intensity and corrected value, respectively. A_f and A_t are absorbance of BSA and the total solution at excitation wavelength, respectively. The correction for