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Fluorescence studies on PAMAM dendrimers interactions with bovine serum albumin

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Abstract

Polyamidoamine (PAMAM) dendrimers (generation 3.5 and 4) interaction with bovine serum albumin (BSA) was studied. The intensity of intrinsic fluorescence of two tryptophan residues and a shift in wavelength of their emission maxima were chosen as indicators of protein conformational changes. It is shown that the generation 4 has a greater impact on spectral properties of serum albumin than generation 3.5.

Keywords: PAMAM dendrimers; Bovine serum albumin; Fluorescence quenching; Stern–Volmer plot; Tryptophan

1. Introduction

Dendrimers are a relatively new class of polymers with a well-defined, three-dimensional structure. They are synthesised from a polyfunctional core by adding branched monomers that react with the functional groups of the core, in turn leaving end groups that can react again. The number of terminal groups increases after each cycle or ''generation'' of the synthesis. The first synthesised polyamidoamine (PAMAM) dendrimers are based on an ethylenediamine core, possessing amino groups on the surface. The half-generations PAMAM dendrimers have carboxylate groups on the surface.

The structure of dendrimers has a great impact on their utilisation. Dendrimers have found a number of biomedical applications. They have been applied in in vitro diagnostics, as carrier molecules for magnetic resonance imaging (MRI) contrast agents, in the targeted delivery of drugs, and as transfection vectors in gene therapy [1]. Recently, it has been shown that they can also have their own biological activity against viruses. For example, PAMAM dendrimers block herpes simplex virus attachment to cells [2]. However, still little is known about biological properties of dendrimers. More information about dendrimers influence on cells and biomolecules is crucial for further investigations of therapeutic applications of dendrimers. In the present work, we investigated whether PAMAM dendrimers might alter the conformation of bovine serum albumin (BSA).

Serum albumins are the most abundant proteins in plasma. As the major soluble protein constituents of the circulatory system, they have many physiological functions. They contribute to colloid osmotic blood pressure and are chiefly responsible for the maintenance of blood pH [3]. There are evidences of a significant antioxidant activity of serum albumins. These molecules may represent the major plasma components that protect against oxidative stress [4]. The most outstanding property of albumins is their ability to reversibly bind a large variety of endogenous and exogenous ligands. Serum albumins are the principal carriers of fatty acids, which are otherwise insoluble in blood, and have high affinity for hematin, bilirubin, and small, negatively charged, hydrophobic molecules.

2. Experimental

Essentially-fatty-acid-free (fraction V) bovine serum albumin was purchased from Sigma (USA). PAMAM dendrimers (generation 3.5 and 4) were obtained from Aldrich (UK). All other chemicals were of analytical grade.

BSA was dissolved in phosphate-buffered saline (PBS: 150 mmol/l NaCl, 1.9 mmol/l NaH_2PO_4, 8.1 mmol/l Na_2HPO_4, pH 7.4) at a concentration of 5 μmol/l. Dendrimer concentrations ranged from 2.5 to 85 μmol/l. Increas-

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ing concentrations of dendrimers were added from a stock solution in PBS (0.9 mmol/l). Fluorescence spectra were taken with a Perkin-Elmer LS-50B spectrofluorometer using excitation wavelength of 280 nm and the emission range set between 290 and 440 nm. Fluorescence quenching was carried out by measuring the fluorescence intensities at 355 nm as a function of dendrimer concentration. Fluorescence intensities were corrected for dilution. The absorbance of the solutions used was not higher than 0.015.

3. Results and discussion

The present work was aimed to investigate whether PAMAM dendrimers interact with bovine serum albumin and change its conformation. The conformational changes of BSA were evaluated by the measurement of intrinsic fluorescence intensity of protein tryptophan residues before and after addition of dendrimers. BSA, a protein of molecular weight 65 kDa, contains two tryptophan residues (Trp-213 and Trp-134) [4]. Fluorescence measurements give information about the molecular environment in a vicinity of the chromophore molecules. The effect of dendrimers on BSA fluorescence intensity is shown in Fig. 1. Increasing dendrimer concentrations caused a reduction in the fluorescence of the tryptophan residues for both generations. It is also apparent from Fig. 1 that the effect was less pronounced for PAMAM dendrimers generation 3.5.

The fluorescence quenching data are usually analysed by the Stern–Volmer equation [5]:

$$\frac{F_0}{F} - 1 = K_{SV}[Q] \quad (1)$$

where F_0 and F are, respectively, the fluorescence intensity in the absence of a quencher and in its presence at [Q] concentration, K_{SV} is the Stern–Volmer dynamic quenching constant.

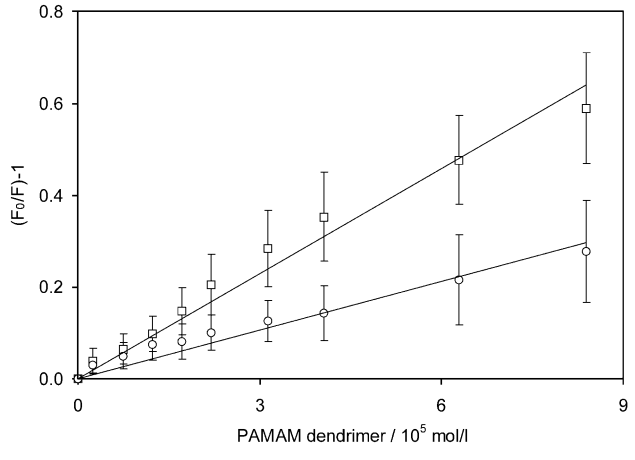

Fig. 1. Stern–Volmer plots of the quenching of BSA tryptophan residues fluorescence by PAMAM dendrimers: generation 4 (□) $A_{max} = 0.015$ and 3.5 (○) $A_{max} = 0.013$.

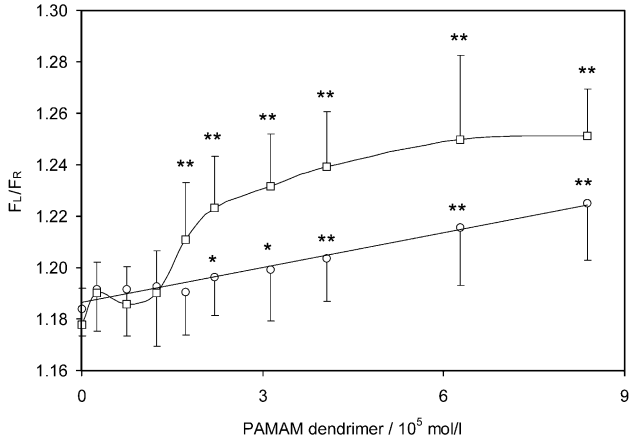

Fig. 2. A shift in the position of the emission maximum after the addition PAMAM dendrimers: generation 4 (□) and 3.5 (○). The significance of the results expressed as mean ± standard deviation was determined by comparison with control values using one-way analysis of variance ANOVA; $*P < 0.01$, $**P < 0.001$.

The Stern–Volmer constants express tryptophan accessibility to the quencher. In case of generation 4 PAMAM dendrimers, K_{SV} was (7.6 ± 1.7) mM^{-1}, whereas for generation 3.5, K_{SV} was (3.5 ± 1.4) mM^{-1}. These results indicate that the changes of the environment of tryptophan residues depend on the applied dendrimer. Both types of dendrimers possess the same core molecule and are built from the same monomers. Generation 3.5 and generation 4 PAMAM dendrimers have similar molecular mass of 12419 and 14215 Da, respectively. The only difference between them is their functional end groups. Fluorescence quenching proceeds mainly via physical contact of the quencher with chromophores, and hence, is dependent on the extent of the approach to the chromophores in the protein. This in turn makes the process extremely dependent on the nature of quenchers themselves. Due to the comparable dimensions of dendrimer and BSA molecules, it is not expected that the dendrimers can penetrate into the protein matrix. The probable reason for the decrease in the fluorescence intensity is the electrostatic dendrimer–protein interactions. Thus, generation 4 PAMAM dendrimers, terminated with $-NH_2$ groups stronger interact with BSA than PAMAM dendrimers of generation 3.5 ended with –COOH groups.

Another useful method to study the environment of tryptophans is by measuring the possible shift in wavelength emission maximum. The shift in the position of emission maximum corresponds to the changes of the polarity around the chromophore molecule. The position of emission maximum was registered as a ratio of fluorescence intensities at two wavelengths: on the left (F_L) and on the right (F_R) slopes of the spectrum [6]. Fig. 2 shows the effect of generation 4 and generation 3.5 dendrimers on the position

of emission maximum. A slight blue-shift of tryptophan fluorescence upon addition of these dendrimers was observed. Dendrimers of generation 4 alter the position of emission maximum a little stronger than dendrimers of generation 3.5 over the same concentration range. This shift indicates that tryptophan residues were placed in a more hydrophobic environment and less exposed to the solvent [7]. This may be due to the aggregation of protein molecules caused by the presence of dendrimers. It is also possible that dendrimers stick to BSA molecules and consequently rearrange the tryptophan microenvironment.

4. Conclusions

The main purpose of this work was to check the effect of dendrimers possessing amino or carboxylate groups on BSA conformational changes. The results support the concept that full-generation dendrimers have stronger effect on biomolecules than the half-generation ones. There is a relationship between the structure and the effects of dendrimers on biological molecules, as exemplified by the study of the PAMAM dendrimers on BSA. The biological behaviour of dendrimers depends to a large extent on their surface groups.

These conclusions are consistent with the previous studies of Malik et al. [8] who showed that dendrimers bearing carboxylate groups are less cytotoxic and haemolytic than dendrimers possessing amino surface.

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