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Size-dependent binding of pristine fullerene (nC_{60}) nanoparticles to bovine/human serum albumin

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ABSTRACT

The size of nC_{60} nanoparticles may exert profound influence on their binding to serum albumins and their ability to alter protein structure and conformation. To verify this speculation, in this work, five fractions of nC_{60} dispersions with different particle size distributions were prepared by centrifuge method and characterized using UV–Visible absorption spectroscopy and transmission electronic microscopy. In this work, the binding of five fractions of nC_{60} nanoparticles with bovine serum albumin (BSA) was demonstrated using fluorescence and synchronous fluorescence spectroscopy. Through the analysis and comparison of binding interactions, it was found that the diameter of nC_{60} nanoparticles plays a key role in the binding patterns of nC_{60} nanoparticles to BSA. The smaller nC_{60} nanoparticles had stronger binding to BSA and exhibited greater influence on the conformation change of BSA. Further study showed that human serum albumin (HSA) has similar binding interaction to nC_{60} nanoparticles, but the conformation changes of human serum albumin caused by five fractions of nC_{60} nanoparticles were different from bovine serum albumin. Despite the different particle sizes, the binding sites of nC_{60} nanoparticles to BSA/HSA were mainly in the vicinity of tryptophan residues. The possible interaction mechanism and the prospects were also discussed.

1. Introduction

Protein is an extremely important biological macromolecules and regarded as the physical basis of life. Entry of nanomaterials into organisms might cause the interaction between nanoparticles and various proteins, thereby affecting the protein structure and function and resulting in unknown physiological effects, therefore, research on the interaction between nanomaterials and proteins plays important roles in nanotoxicology and nanomedicine areas.

C_{60} fullerene is an important carbon nanomaterial which has potential applications in chemistry, biology, medicine, materials, etc. [1–5]. Since pristine C_{60} is insoluble in water, study on its interaction with proteins was subject to certain restrictions and thus water-soluble fullerene derivatives were often chosen instead [6–10]. To obtain pristine nC_{60} nanoparticles aqueous dispersions, it has been reported that several methods such as direct ultrasonic method, solvent displacement methods [11], and addition of surfactant [12] or polymer [13] to water were usually utilized, and research on the binding of pristine C_{60} to protein was also based on these methods. However, due to the difference of preparation methods, the obtained nC_{60} nanoparticles often have different properties such as different shapes and size distributions, and this might further lead to the different binding patterns of nC_{60} nanoparticles to the same protein, which has been observed in our previous work [14,15]. However, in many studies, the factors resulted from the different preparation methods of nC_{60} nanoparticles were rarely involved, which made the lack of comparability between the results of different research groups.

In our previous work, two pristine nC_{60} nanoparticles aqueous dispersions were obtained by ultrasonic method and solvent (toluene) replacement method, and spectroscopic methods were applied for the investigation of the binding of nC_{60} nanoparticles to serum proteins (HSA and BSA) [14,15]. Results showed that nC_{60} nanoparticles prepared by different methods exhibited different effects on serum proteins (HSA and BSA) conformation and secondary structure. The main difference between the two experiments was the preparation methods of nC_{60} nanoparticles, and main differences between the nC_{60} nanoparticles obtained by the two methods are the particle size distributions and the residual

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toluene which could be absorbed on the nC_{60} surface in solvent replacement method. Since in our previous research, it was shown that the solvent (toluene) residue was too low to cause protein fluorescence quenching, here we speculate that particle size might be the main factor which causes the difference of binding of nC_{60} nanoparticles to serum proteins.

Spectroscopic methods are widely used in the investigation of interactions between protein and other substances such as drugs [16–19] and nanoparticles [20,21]. In this work, the fluorescence and synchronous fluorescence spectroscopy were utilized to verify our speculation and to further comprehend the effect of particle size on the binding of nC_{60} nanoparticles to serum albumins. The nC_{60} nanoparticles aqueous dispersions with different particle size distributions were obtained by centrifuge method and characterized using UV–Visible spectroscopy and transmission electronic microscopy, and BSA/HSA served as subject, the binding of nC_{60} nanoparticles to serum albumins were investigated by fluorimetric method.

2. Experimental

2.1. Materials

BSA, HSA (free fatty acid fraction V) and PBS Premixed Powder (pH = 7.2–7.4) were purchased from Sigma-Aldrich(USA). C_{60} powder (purity: 99.9%) was obtained from Henan Puyang Yongxin Fullerene Technology Co. Ltd. (China). Distilled ultra-pure water (18.3 MΩ) (Millipore, USA) was used to prepare all the solutions. During the experiments the samples should be kept away from light. All the experiments were performed at room temperature.

2.2. Instruments

UV-2450 spectrophotometer (Shimadzu, Japan); HT-7700 transmission electron microscopy (TEM) (Hitachi, Japan); FL-4500 spectrofluorimeter (Hitachi, Japan).

2.3. Preparation of stock aqueous dispersion of nC_{60} nanoparticles

The nC_{60} stock aqueous dispersion was prepared according to our previously published work [14].

2.4. Preparation of nC_{60} dispersions with different particle size distributions

Appropriate amounts of C_{60} stock aqueous dispersion were divided into three fractions, two of which were centrifuged at 8000 r/m (rcf = 6797 g) and 12000 r/m (rcf = 15294 g)for 10 min, respectively. The supernatants were removed for further experiments and named as S_8 and S_{12}. The third fraction was centrifuged at 4000 r/m(rcf = 1699 g) for 10 min, and then the supernatant was removed and named it as S_4, while the left bottom part was diluted by appropriate amount of water and then named as S_{4b}. The C_{60} stock aqueous dispersion was named as S_0.

2.5. UV–Vis spectra measurements of BSA/HSA

The UV–Vis spectra of BSA/HSA were performed on UV-2450 spectrophotometer equipped with a 1.0 cm quartz cell.

2.6. TEM observation of nC_{60} nanoparticles

Drop some nC_{60} aqueous dispersion on the copper girds (150-mesh) and dry them in the air. The nC_{60} nanoparticles were characterized by H-7000 transmission electron microscopy.

2.7. Fluorescence and synchronous fluorescence spectra measurements of protein

The BSA/HSA fluorescence and synchronous fluorescence spectra measurements were performed on FL-4500 spectrofluorimeter equipped with a 1.0 cm quartz cell (excitation and emission slit width: 5 nm; scan speed: 1200 nm/min).

3. Results and discussion

3.1. Characterization of five nC_{60} dispersions with different particle size distributions

Nanoparticles with different particle sizes can be separated by centrifugal method. As well known, the larger centrifugal velocity is, the faster particles settling velocities are, and the larger particle size is, the faster particles settling velocities are. In this work, to obtain nC_{60} nanoparticles with different size distributions, according to the description of reported work, five fractions of nC_{60} dispersions with different particle size distributions were prepared by centrifuge method (see Methods section) and characterized using UV–Visible spectroscopy and TEM images. The concentrations of the five prepared nC_{60} dispersions were quantitated by UV–Vis spectrophotometric method [22].

3.2. UV–Visible spectra of five nC_{60} dispersions

Nanoparticles with different size distributions show different spectral properties. In this work, UV–Vis spectrophotometry was used to characterize the nC_{60} nanoparticles in five fractions of nC_{60} dispersions, and the results were shown in Fig. 1.

According to the centrifugal principle, the size distributions of nC_{60} nanoparticles in five dispersions should be in the order of $S_{4b} > S_0 > S_4 > S_8 > S_{12}$. From Fig. 1, all the five dispersions had absorption at about 320–420 nm, which results from the absorption of nC_{60} nanoparticles. However, the maximum absorption wavelengths of their spectra were different from each other with an order of $S_{4b} > S_0 > S_4 > S_8 > S_{12}$, which indicates the five dispersions had different size distributions of nC_{60} nanoparticles. With the decrease in size of nC_{60} nanoparticles, their maximum absorption peaks exhibited red-shift phenomenon, which were in consistent with the findings of previous work.

The five nC_{60} dispersions are very stable, and no precipitation

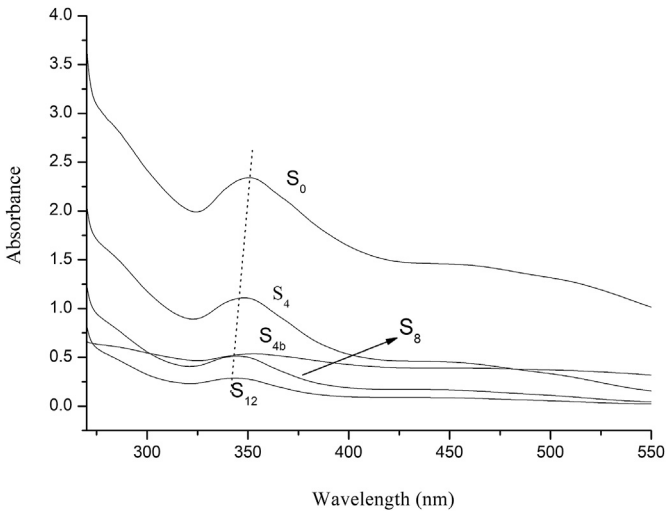

Fig. 1. UV–Visible spectra of five nC_{60} dispersions. (Concentrations of nC_{60}: S_0, S_4, S_{4b}, S_8 and S_{12} were 43.4, 18.6, 10.6, 8.7 and 4.5($\times 10^{-6}$ mol/L), respectively.).