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Spectroscopic and electrochemical studies on the interaction of an inclusion complex of β-cyclodextrin/fullerene with bovine serum albumin in aqueous solution

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ABSTRACT

The potential effect of human exposure to carbonaceous nanomaterials (e.g., fullerenes or their derivatives) in the environment has become a concern. In the current study, we report the interaction of one water-soluble fullerene with bovine serum albumin using spectroscopic and electrochemical methods under aqueous solutions. The novel supramolecular inclusion complex of the water-soluble fullerene $(\beta\text{-CD})_2/C_{60}$ was synthesized and characterized. In the mechanism discussed, the spectroscopic methods such as fluorescence quenching and ultraviolet–visible absorption, proved that the fluorescence quenching of BSA by $(\beta\text{-CD})_2/C_{60}$ was the result of the formation of $(\beta\text{-CD})_2/C_{60}$–BSA complex and that the mechanism of quenching might be a static quenching procedure. The binding constants K_a, the number of binding sites n, and the corresponding thermodynamic parameters ΔG, ΔH, and ΔS at different temperatures were calculated through fluorescence spectroscopy, then as an auxiliary method, the electrochemical impedance spectroscopy (EIS) experiments confirmed this conclusion. The results indicated that the electrostatic interactions play a major role in $(\beta\text{-CD})_2/C_{60}$–BSA association. The circular dichroism spectra show the conformation change of the effect of $(\beta\text{-CD})_2/C_{60}$ on the conformation of BSA, which was confirmed by the results of the three-dimensional fluorescence spectra. Site marker competitive experiments indicate that the binding of $(\beta\text{-CD})_2/C_{60}$ to BSA primarily took place in site I. The distance r between donor (BSA) and acceptor ($(\beta\text{-CD})_2/C_{60}$) was obtained according to fluorescence resonance energy transfer (FRET). This work aims to demonstrate the mechanisms of the formation of the complex between water-soluble fullerene and protein under physiological conditions, as well as the remediation for the possible unwarranted biological effects of water-soluble fullerene.

1. Introduction

There is growing concern about the use of nanomaterials in the field of life science and biological applications, medicines and cosmetic ingredients, notably human exposure to carbonaceous nanomaterials (e.g., fullerenes) in the environment [1]. Nanomaterials are a very important basis for the future social development, and many new breakthroughs in the areas of science and technology urgently need the support of the nanomaterials and nanotechnology. C_{60}, one of the most promising nanomaterials, has been called a "radical sponge" [2] because of its extremely highly reactivity to radical species applicable in enzyme inhibition, antiviral activity, DNA cleavage, photodynamic therapy, electron transfer, etc. [3,4]. Scientists who engaged in fullerene research decades ago have made considerable progress. Generally, these works can be summarized as follows: (1) research on chemical properties of fullerenes, development of new chemical reactions and summary of the reaction rules; (2) modification of fullerene, in which by using fullerene as a platform and connecting it to groups with special functions, scientists have changed the electrical, optical, and magnetic properties of fullerene; and (3) increasing the water solubility of fullerenes to use their properties of anti-cancer and sterilization. In recent years, more and more research have focused on the performance of fullerene in biomedical applications, such as Friedman et al. [5], Nakamura and Isobe [6], Chiang et al. [7], among others.

However, their poor water solubility of fullerene has limited these biological applications. Recently, the water-soluble fullerenes have been obtained either by the non-covalent encapsulation of fullerene molecules in soluble polymeric or host molecules, or the reliance on covalent functionalization with hydrophilic groups by chemical modification [8]. As a common host molecule in the

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inclusion reaction, cyclodextrin (CD) is the amylase produced by bacillus cyclodextrin glycosyltransferase produced under the general term for a series of cyclic oligosaccharides. In the role of the cyclodextrin glycosyltransferase, CD constitutes a number of α-1,4-glycosidic bonds linked end to end generated by starch (mainly amylopectin) and usually contains 6–12 D-pyranose glucose units [9]. As the outer edge of the CD (rim) is hydrophilic and the cavity is hydrophobic, it has well-defined chemical structures with many sites for chemical modification or conjugation, which can provided a hydrophobic binding site as a main host for all appropriate guest. In this work, we successfully synthesized an excellent water-soluble fullerene derivative using CD.

As one of the most common and abundant carrier proteins, bovine serum albumin (BSA) has been widely applied in biology, physics, chemistry, medicinal chemistry, and computational chemistry studies, among others. As reported in [10,11], the distribution and metabolism of many biologically active compounds in the body are correlated with their affinity to serum albumin, and serum albumins are also found in tissues and body secretions throughout the body. Moreover, the extravascular protein comprises 60% of the total albumin [12]. Resent research reveals that various drugs can be significantly affected as a result of their binding to serum albumins in the circulatory system and that their interactions usually affect the secondary and tertiary structure of albumin. However, these drugs are often selected as small organic molecules, or low molecular weight dyes, and their ligands or derivatives, but nanomaterials are rarely selected, including carbonaceous materials. To this end, studying the interaction of nanomaterials with serum albumin is important. As the interactions between natural molecules and native proteins remain largely unexplored, as the great homologous protein of human serum albumins [10–12], BSA has been used widely as a model protein and has no exception in the current work.

The growing interest in fullerenes provides a compelling reason to investigate their interaction with proteins. Resent research have proved their interaction, such as the cholesterol modified fullerene/γ-cyclodextrin inclusion complex with BSA (HSA and DNA) [13], the polyhydroxylated fullerene derivative fullerol with BSA, etc. [14], the organophosphate-containing water-soluble derivative of fullerene with HSA [4], and the formation of a soluble stable complex between pristine fullerene and a native blood protein [15], among others. Although there is great interest in the biological effect of different drugs on protein especially in our group [11], the specific mechanism and conformational behavior of BSA changed by $(\beta\text{-CD})_2/C_{60}$ are poorly understood at present. Moreover, little work has been conducted to determine the poisonous and pharmacology adverse effect of fullerene. In this work, we first employ various experimental methods to detect comprehensively the influence of $(\beta\text{-CD})_2/C_{60}$ on the function and conformation change of BSA at the molecular level. We use several spectral methods to obtain information on the binding mechanisms of to BSA, such as quenching rate constant, binding modes, binding constants, binding number, binding sites, etc. We hope this study can provide valuable information to address the growing concern on the safety and toxicology of water-soluble fullerene derivatives, which have special molecular structures under physiological conditions and have become fundamental elements of biomedicine in future research.

2. Experimental details

2.1. Chemicals

BSA (defatted BSA, approx. 99%) and warfarin were obtained from Sigma–Aldrich (St. Louis, MO, USA); Ibuprofen was obtained from Hubei Biocause Pharmaceutical Co., Ltd. (Hubei, China; the purity no less than 99.7%); crystalline fullerene (C_{60}) powder of 99.9 wt.% purity was purchased from Yongxin Chemical Reagent Company. BSA was dissolved in Tris–HCl buffer solution with a purity of no less than 99.5% (0.05 mol L^{-1} of Tris, 0.10 mol L^{-1} of NaCl, and pH 7.4 ± 0.1). β-Cyclodextrin, N,N-dimethylformamide (DMF), sodium, naphthalene, iodine, and all other reagents and solvents used in synthesis and analysis were of analytical reagent grade and purchased from Sinopharm Group Chemical Reagent Company Ltd., Shanghai, P.R. China. Water used in all procedures was prepared using a Millipore water purification system. Sample masses were accurately weighted on a microbalance (Sartorius, ME215S) with a resolution of 0.1 mg.

2.2. Apparatus

The LS-55 spectrofluorophotometer from Perkin-Elmer equipped with a thermostat bath was used to measure all of the fluorescence spectras. The absorption spectra of the synthesized Inclusion Complex and BSA were recorded by a UNICO 4802 UV–vis double-beam spectrophotometer. The CD spectra were recorded on Cirsular Dichroism Photomultiplier from Applied Photophysics Limited, UK, using a cylindrical with 0.1 cm path length. The electrochemical properties studied were based on a CHI660C electrochemical workstation from Shanghai Chenhua Instrument Company. For characterizations, element analysis was measured on the 1112SERIES elemental analysis system produced by FLASH Company from Italy. The morphology and microstructure were observed by a scanning electron microscopy (SEM) with a Hitachi X-650. Infrared spectra were recorded on an Avatar 360 FT-IR spectrophotometer as KBr pellets. TGA analyses were performed in nitrogen with a temperature scanning rate of 10 K/min in a thermal analyzer of the NETZSCH STA 449C system. The crystal structures of the powers were characterized by X-ray diffraction analysis using Shimadzu XRD-6000 diffractometer with Cu Kα radiation (λ = 1.54056 Å). ^{13}C NMR spectra were recorded on mercury 600 MHz NMR spectrometer (Varian, Inc.) using the DMSO-d_6 as the solvent.

2.3. Preparation of water soluble fullerene $(\beta\text{-CD})_2/C_{60}$

The water-soluble derivative of C_{60}, two equivalents of β-cyclodextrin-bicapped C_{60}–fullerene inclusion complex ($(\beta\text{-CD})_2/C_{60}$) was synthesized by an improved method from Refs. [16,17] via the primary rim of the β-CD. Briefly, by using N,N-dimethylformamide (DMF) as the solvent, a certain amount of naphthalene (56 mg, 0.44 mmol) and elemental sodium (12 mg, 0.52 mmol) was added into the DMF solvent by a dry flask under the nitrogen atmosphere. After stirred for 6 h, sodium cannot be dissolved completely, and then a darker solution was achieved by soaking fullerene (30 mg, 0.04 mmol) into the flask with continuous stirring. β-Cyclodextrin (94.6 mg) was added into the solution to package fullerene after 12 h reaction. At last, iodine (17 mg, 0.07 mmol) was used to change the anion fullerene into neutral.

2.4. Fluorescence spectral measurements

All fluorescence spectra were measured on an LS55 fluorophotometer (Perkin-Elmer Co., USA) equipped with a 1.0 cm quartz cell and a thermostat bath. The widths of the excitation slit and the emission slit were set to 15 nm and 5.5 nm separately. An excitation wavelength of 295 nm was used throughout to minimize the contribution of the tyrosine residues to the emission. BSA solution was prepared on the basis of its molecular weight of 67,000 and kept in a refrigerator at 4 °C which was purchased from Sigma Aldrich and dissolved in PBS (pH 7.4) at the concentration of 2×10^{-6} mol L^{-1}.