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Spectroscopic studies on the interaction of Phacolysin and bovine serum albumin

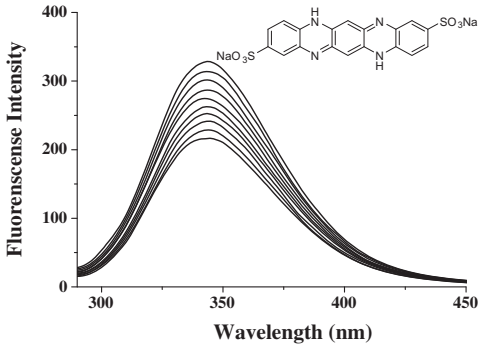
Xianyong Yu [a,b,c,*], Zhixi Liao [a], Qing Yao [a], Heting Liu [a], Wenlin Xie [a,*]

[a] Key Laboratory of Theoretical Chemistry and Molecular Simulation of Ministry of Education, Hunan Province College Key Laboratory of QSAR/QSPR, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan 411201, China
[b] State Key Laboratory of Physical Chemistry of Solid Surfaces, Xiamen University, Xiamen 361005, China
[c] Key Laboratory of Computational Physical Sciences, Fudan University, Ministry of Education, Shanghai, China

HIGHLIGHTS

- We explored the interaction of BSA and PCL by spectroscopic methods.
- The fluorescence quenching mechanism is static quenching.
- The binding constants and binding sites were calculated.
- Hydrogen bonds and van der Waals forces were the main force in stabilizing the complex.
- The conformation of BSA was changed affected by PCL.

GRAPHICAL ABSTRACT



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ABSTRACT

The interaction between Phacolysin (PCL) and bovine serum albumin (BSA) under imitated physiological conditions was investigated by spectroscopic (fluorescence, UV–Vis absorption and Circular dichroism) techniques. The experiments were conducted at different temperatures (294 K, 302 K, 306 K and 310 K) and the results showed that the PCL caused the fluorescence quenching of BSA through a static quenching procedure. The binding constant (K_a), binding sites (n) were obtained. The corresponding thermodynamic parameters (ΔH, ΔS and ΔG) of the interaction system were calculated at different temperatures. The results revealed that the binding process was spontaneous and the acting force between PCL and BSA were mainly hydrogen bonding and van der Waals forces. According to Förster non-radiation energy transfer theory, the binding distance between PCL and BSA was calculated to be 2.41 nm. What is more, both synchronous fluorescence and Circular dichroism spectra confirmed the interaction, which indicated the conformational changes of BSA.

Introduction

As we all know, serum albumins (SA) contribute to colloid osmotic blood pressure and the maintenance of blood pH. They also play a dominant role in drug disposition and efficacy. Most drugs are transported as a complex with SA, which makes SA an important part of drug metabolism [1–3]. The serum protein used

* Corresponding authors. Address: Key Laboratory of Theoretical Chemistry and Molecular Simulation of Ministry of Education, Hunan Province College Key Laboratory of QSAR/QSPR, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan 411201, China (X. Yu). Tel.: +86 731 58290187; fax: +86 731 58290509.
E-mail addresses: yu_xianyong@163.com (X. Yu), xwl2000zsu@163.com (W. Xie).

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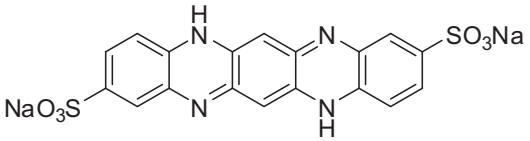

Scheme 1. Molecular structure of Phacolysin.

in these studies is bovine serum albumin (BSA) which has an inherent fluorescing property attributed to the presence of aromatic amino acids [4–6]. BSA is a natural globular protein with a relatively high molecular mass (68,000 Da) and a single polypeptide chain containing 583 amino acid residues. It consists of three linearly arranged and structurally distinct homologous domains (I–III), which were divided into nine loops (L1–L9). Each domain contains two sub-domains (*A* and *B*). What's more, tertiary structures of BSA and human serum albumin (HSA) are similar in 76% and all the study results are consistent with the fact that HSA and BSA are homologous protein [7,8].

Phacolysin (PCL) (Scheme 1) also known as Phacolin, which is a proteolytic enzyme activator. As eye drops, it can prevent cataracts [9,10]. When instill it into the denatured protein and once it is absorbed, it can promote the degradation of protein and penetrate into the lens. Thus it can maintain the transparency of the lens, improve eye tissues metabolism and prevent from the progression of cataract [11,12].

Protein–drug interaction is the hot point in the fields of medicine, chemistry and biology [13]. Many drugs and other bioactive small molecules bind reversibly to albumin, which then function as carriers [14]. Consequently, it is important to study the interactions of drugs with this protein. In this paper, the binding of Phacolysin to BSA was studied under imitated physiological conditions by fluorescence, ultraviolet spectroscopy and Circular dichroism spectrum. The binding constants were calculated and binding mechanism was investigated. In addition, the effect of PCL on the conformational change of BSA was also studied. We hope this work will not only provide useful information for understanding of the PCL, but also illustrate its binding mechanisms at a molecular level.

Materials and methods

Reagents

BSA (⩾99%) was obtained from Huamei Bioengineering Co. (Shanghai, China) and was dissolved in a Tris–HCl (0.05 mol L^{-1}, pH = 7.43) buffer to form the BSA solution with a concentration of 1.00×10^{-5} mol L^{-1}. A Tris–HCl buffer (0.05 mol L^{-1}, pH = 7.43) containing 0.10 mol L^{-1} NaCl was selected to keep the pH value constant and to maintain the ionic strength of the solution. Phacolysin was obtained from Liye Pharmaceutical Co. (Nanjing, China). The Phacolysin (6.14×10^{-5} mol L^{-1}) solution was prepared in double–distilled water. All other reagents were of analytical grade and double–distilled water was used during the experiment.

Apparatus

Fluorescence spectra were recorded on a Shimadzu RF-5301 fluorescence spectrophotometer (Tokyo, Japan) with a SB-11 water bath (Eyela) and 1.0 cm quartz cells. The emission and excitation slits were 10 nm and 10 nm, respectively. The synchronous fluorescence spectra were obtained by setting the excitation and emission wavelength interval ($\Delta\lambda$) at 15 nm and 60 nm. The absorption spectra were obtained from a Shimadzu UV-2501 spectrophotometer (Tokyo, Japan). Circular dichroism spectra were recorded on Chirascan (London, UK). The pH measurement was made with a Leici pHS-2 digital pH-meter (Shanghai, China) with a combinational glass calomel electrode.

Measurements of spectra

A 2.5 mL solution containing 1.00×10^{-5} mol L^{-1} BSA was titrated by successive additions of 0.614×10^{-6} mol L^{-1} PCL solution and the concentration of PCL varied from 0 to 5.53×10^{-6} mol L^{-1}. Titrations were done manually by using micro-injector. Fluorescence quenching spectra were measured in the range of 280–500 nm at the excitation wavelength of 280 nm. The fluorescence spectra were performed at three temperatures (294, 302 and 310 K).

The UV–Vis absorption spectra of PCL solution with the concentration of 1.00×10^{-5} mol L^{-1} was measured in the range of 240–500 nm at 294 K.

The CD measurements of BSA in the presence and absence of PCL were made in the range of 195–260 nm at 293 K. A stock solution of 0.20 μmol L^{-1} BSA was prepared in Tris–HCl buffer (0.05 mol L^{-1}) of pH 7.43 containing 0.10 mol L^{-1} NaCl. The molar ratios of PCL to BSA were varied from 0, 1:1 to 5:1 and the CD spectrum was recorded.

Results and discussion

Calculating method

Absorption of Phacolysin at the emission and excitation wavelength of fluorophore has an important effect on the fluorescence spectra, so the fluorescence intensity must be corrected. When the absorbance of drugs was lower than 0.3, the following equation can be used to correct the inner filter effects [15–17]:

$$F_{corr} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

where F_{corr} is the corrected fluorescence intensity, F_{obs} is the observed intensity. A_{em} and A_{ex} are the absorbance values of PCL at emission and excitation wavelengths, respectively. The fluorescence intensity used in this paper was corrected.

The fluorescence quenching spectra

Fig. 1 shows the emission spectra of BSA in the presence of various concentrations of PCL. It was observed that the fluorescence intensity of BSA decreased with the increasing concentration of

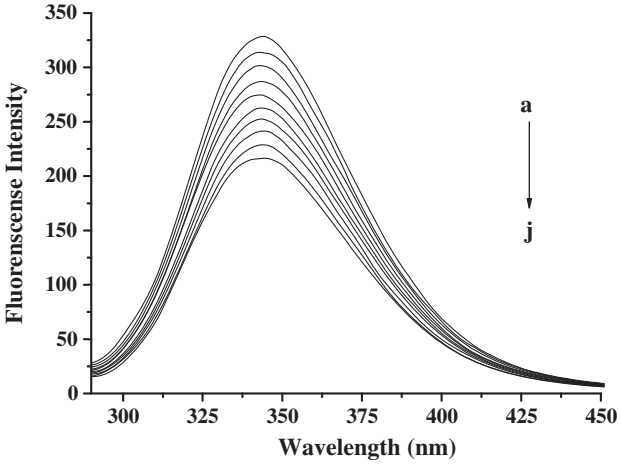

Fig. 1. The fluorescence quenching spectra of BSA by PCL at 310 K. λ_{ex} = 280 nm. [BSA] = 1.00×10^{-5} mol L^{-1}; [PCL] (*a–j*): 0, 0.614, 1.23, 1.84, 2.46, 3.07, 3.68, 4.30, 4.91, 5.53 ($\times 10^{-6}$ mol L^{-1}).