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Short communication

Spectroscopic studies on the interaction between silicotungstic acid and bovine serum albumin

Yan-Qing Wang *, Hong-Mei Zhang, Gen-Cheng Zhang, Wei-Hua Tao, Zheng-Hao Fei, Zong-Tang Liu

Jiangsu Provincial Key Laboratory of Coastal Wetland Bioresources and Environmental Protection, Institute of Applied Chemistry and Environmental Engineering, Yancheng Normal College, Yancheng City, Jiangsu Province 224002, People's Republic of China

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Abstract

The interaction between silicotungstic acid and bovine serum albumin (BSA) was investigated using fluorescence and UV/vis. The experimental results showed that the fluorescence quenching of BSA by silicotungstic acid is a result of the formation of SiW–BSA complex; static quenching and non-radiative energy transferring were confirmed to result in the fluorescence quenching. The binding site number n, apparent binding constant K_A and corresponding thermodynamic parameters were measured at different temperatures. The process of binding SiW molecule on BSA was a spontaneous molecular interaction procedure in which entropy increased and Gibbs free energy decreased. Hydrophobic interaction force plays a major role in stabilizing the complex. The effect of silicotungstic acid on the conformation of BSA was analyzed using synchronous fluorescence spectroscopy.

Keywords: Silicotungstic acid; Bovine serum albumin; UV/vis spectroscopy; Fluorescence spectroscopy; Thermodynamic parameters

1. Introduction

Serum albumins are the major soluble protein constituents of the circulatory system and have many physiological functions [1]. The most important property of this group of proteins is that they serve as a depot protein and as a transport protein for many drugs and other bioactivity small molecules [2]. Bovine serum albumin (BSA) has been one of the most extensively studied of this group of proteins, especially of its structural homology with human serum albumin (HSA) [1]. BSA is made up of three homologous domains (I, II, III), which are divided into nine loops (L1–L9) by 17 disulfide bonds. The loops in each domain are made up of a sequence of large–small–large loops forming a triplet. Each domain in turn is the product of two sub-domains [3]. BSA has two tryptophans, Trp–134 and Trp–212, embedded in the first sub-domain IB and sub-domain IIA, respectively. HSA is a globular protein composed of 585 amino acid residues in three homologous α-helices domains (I–III). Each domain contains 10 helices and is divided into antiparallel 6 helix and four sub-domains (A and B) [4]. There is only one tryptophan located at position 214 along the chain, in sub-domain IIA of HSA.

The molecular interactions between proteins and many compounds have been investigated successfully including dyes and other some organic small molecules [3,5–10]. However, the binding of some inorganic ions to proteins has seldom been investigated [11,12]. Heteropoly acids, belonging to the heteropoly compounds, are sorts of inorganic acid radical anions [11]. Because of their unique combination of physical and chemical properties, heteropoly compounds have been widely used in analytical and clinical chemistry, catalysis (including photocatalysis), medicine (antitumonal, antiviral and even anti-HIV activity), biochemistry (electron transport inhibition) and solid-state devices [13]. The use of heteropoly acids for protein determination is well established [11,14]. However, other parameters such as mode of interaction, association constant and number of binding sites are important, when heteropoly acids are used as drugs [12]. Investigating the interaction of drugs

* Corresponding author. Tel.: +86 515 8336920; fax: +86 515 8233080.
E-mail address: wyqing76@126.com (Y.-Q. Wang).

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to serum albumins can elucidate the properties of drug–protein complex, as it may provide useful information of the structural features that determine the therapeutic effectiveness of drugs [15].

Fluorescence spectroscopy is essentially a probe technique sensing changes in the local environment of the fluorophore, various possibilities of structural rearrangements in the environment of the fluorophore may lead to a similar fluorescence signal; they can complicate interpretation of the experimental result and be exploited to obtain unique structural and dynamic information [16–18]. Silicotungstic acid (SiW) with the Keggin structure inhibits the replication of rubella [19], rubeola [20] and Moloney virus [21] as well as HIV [22,23]. In the present work, we used SiW as model drug and reporting the mechanism of binding of heteropoly acid with BSA. In order to attain these objectives, we planned to carry out detailed investigation of SiW–BSA association using fluorescence spectroscopy and UV/vis absorption spectroscopy. Through fluorescence resonance energy transfer (FRET), we planned to further investigate the energy transfer parameters of BSA for transfer to SiW. In addition, the conformational change of BSA is discussed on the basis of synchronous fluorescence spectra.

2. Materials and methods

2.1. Materials

Bovine serum albumin (Fraction V, approximately 99%) was purchased from Sigma (St. Louis, MO, USA) and used without further purification. Silicotungstic acid was obtained from ino-Biotechnology Company (Shanghai, China). The Tris buffer was purchased from Acros (Geel, Belgium), and NaCl, HCl, etc. were all of analytical purity. BSA solution (5.0 μmol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer solution (0.05 mol L^{-1} Tris, 0.1 mol L^{-1}NaCl). The SiW solution (2.5×10^{-4} mol L^{-1}) was prepared in pH 7.40 Tris–HCl buffer.

2.2. Equipments and spectral measurements

The UV/vis spectrum was recorded at room temperature on a GBC UV/vis916 spectrophotometer (Australia) equipped with 1.0 cm quartz cells and a slit width of 5 nm with a nominal resolution of 0.5 nm. All fluorescence spectra were recorded on LS–50B Spectrofluorimeter (Perkin-Elmer USA) equipped with 1.0 cm quartz cells and a thermostat bath, the widths of both the excitation slit and the emission slit were set to 5.0 nm with a nominal resolution of 0.5 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background of fluorescence.

2.3. Procedures

A 2.5 mL solution, containing appropriate concentration of BSA, was titrated by successive additions of a 2.5×10^{-4} mol L^{-1} stock solution of SiW (to give a final concentration of 8.0 μmol L^{-1}). Titrations were done manually by using micro-injector. The fluorescence spectra were then measured (excitation at 290 nm and emission wavelengths of 280–500 nm) at two temperatures (299, 309 K). The UV/vis absorbance spectra of SiW with concentration of 5.0 μmol L^{-1} were recorded at room temperature.

2.4. Principles of fluorescence quenching

The fluorescence intensity of a compound can be decreased by a variety of molecular interactions viz., excited-state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching [24]. Such decrease in intensity is called fluorescence quenching. Fluorescence quenching is described by the Stern–Volmer equation:

$$\frac{F_0}{F} = 1 + K_q\tau_0[Q] = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively. K_q, K_{SV}, τ_0 and $[Q]$ are the quenching rate constant of the bimolecular, the Stern–Volmer dynamic quenching constant, the average lifetime of the bimolecular without quencher ($\tau_0 = 10^{-8}$ s) and the concentration of the quencher, respectively. Obviously,

$$K_q = \frac{K_{SV}}{\tau_0} \quad (2)$$

Hence, Eq. (1) was applied to determine K_{SV} by linear regression of a plot of F_0/F versus $[Q]$.

2.5. Calculation of binding parameters

When small molecules bind independently to a set of equivalent sites on a macromolecule, the apparent binding constant K_A and binding sites n can be obtained from Eq. [25]:

$$\log\frac{F_0 - F}{F} = \log K_A + n\log[Q] \quad (3)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, $[Q]$ is the total quencher concentration. By the plot of $\log(F_0 - F)/F$ versus $\log[Q]$, the number of binding sites n and binding constant K_A can be abstain.

If the enthalpy change (ΔH) does not vary significantly over the temperature range studied, then the thermodynamic parameters ΔH, ΔS, ΔG can be determined from the following Eq. [26]:

$$\ln\frac{(K_A)_2}{(K_A)_1} = \frac{\Delta H}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \quad (4)$$

$$\Delta G = -RT\ \ln K_A \quad (5)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (6)$$

3. Results and discussion

3.1. UV/vis absorption spectroscopy

UV/vis absorption measurement is a very simple method and applicable to explore the structural change and to know the