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Original Article

Study on the interaction of bioactive compound S-allyl cysteine from garlic with serum albumin

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ABSTRACT

Multispectroscopic techniques were used to investigate the interaction of S-allyl cysteine (SAC) from garlic with human serum albumin (HSA). UV–Vis absorption measurements prove the formation of the HSA–SAC complex. An analysis of fluorescence spectra revealed that in the presence of SAC, the quenching mechanism of HSA is considered static. The quenching rate constant K_q, K_{SV}, and the binding constant K_A were estimated. According to the Van't Hoff equation, the thermodynamic parameters enthalpy change (ΔH) and entropy change (ΔS) were calculated to be -1.00×10^5 J/mol and -255 J/mol/K, respectively. These indicate that hydrogen bonds and van der Waals forces are the major forces between SAC and HSA. The changes in the secondary structure of HSA, which was induced by SAC, were determined by circular dichroism spectroscopy. Energy transfer was confirmed and the distance between donor and acceptor was calculated to be 2.83 nm.

1. Introduction

Serum albumin is the principal extracellular protein of the circulatory system, and accounts for about 60% of the total plasma proteins, which corresponds to a total concentration of 42 mg/mL. It provides about 80% of the colloid osmotic pressure of blood [1,2]. Human serum albumin (HSA) is the most studied serum albumin because its primary structure is well-known and its tertiary structure has been determined by X-ray crystallography. It is a single-chain, nonglycosylated globular protein consisting of 585 amino acid residues and 17 disulfide bridges that assist in maintaining its familiar heart-like shape [3]. Crystallographic data show that HSA contains three homologous a-helical domains (I, II, and III): I (residues 1–195), II (196–383), and III (384–585), each of which includes 10 helices that are divided into six-helix and four-helix subdomains (A and B). A multitude of ligand-binding sites are scattered over the entire protein. The principal regions of ligand-binding sites in HSA are located in hydrophobic cavities in subdomains IIA and IIIA, called site I and site II, respectively.

There are nine distinct fatty acid-binding sites, four thyroxine-binding sites, several metal-binding sites including albumin's N terminus, and a site centered on residue Cys34 [4]. These multiple binding sites underline the exceptional ability of HSA to act as a major depot and transport protein, capable of binding, transporting, and delivering an extraordinarily diverse range of endogenous and exogenous compounds in the bloodstream to their target organs. Many studies to date have reported on the interaction between drugs and HSA.

Garlic (*Allium sativum*) has been used historically for medicinal purposes, particularly for treatment of diseases

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associated with aging [5]. Aged garlic extract (AGE) possess potent antioxidant activity and is prepared from natural garlic that is aged for 20 months, which reduces its harsh irritating taste and odor. However, this aged garlic has a greater concentration of organosulfur compounds such as S-allyl cysteine (SAC), which is a potent antioxidant and free radical scavenger [6–10]. Although numerous studies have demonstrated the antioxidant properties of AGE, only limited information is available on how SAC behaves when it is absorbed and transferred in blood [11–13].

Given that SAC is the most abundant compound in AGE, this paper focuses on binding characteristics of SAC and its binding with HSA. The binding of SAC with HSA at physiological pH was evaluated using steady-state fluorescence and circular dichroism (CD) spectroscopy measurements. Results pertaining to the binding parameters, the identification of binding sites, and the nature of forces in the interaction will be beneficial for understanding the SAC metabolism.

2. Materials and methods

2.1. Materials

Fatty acid-free HSA and phosphate buffer powder were procured from Sigma (St. Louis, MO, USA). SAC (purity > 98%) was obtained from Sinopharm Co. Ltd. (Beijing, China). HSA (1×10^{-5}M) was dissolved in 0.2M phosphate buffer solution as the stock solution. Double-distilled water was used in all our experiments. All other chemicals used in this work were obtained from Tianjin Damao Chemical Corporation (Tianjin, China). Deionized water was used throughout the work.

2.2. Methods

All the fluorescence measurements were performed on Shimadzu (5301PC) spectrofluorophotometer equipped with a constant temperature holder attached with Neslab RTE-110 water bath with an accuracy of 0.1 K. Intrinsic fluorescence was measured by exciting HSA at 280 nm, and the emission spectrum was measured between 290 nm and 410 nm. The decrease in fluorescence intensity was analyzed according to the Stern–Volmer equation

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

where F_0 and F are the fluorescence intensities in the absence and presence of quencher (SAC), respectively; K_{SV} is the Stern–Volmer quenching constant; K_q is the bimolecular rate constant of the quenching reaction; and τ_0 is the average integral fluorescence lifetime of tryptophan (Trp), which is 5.7×10^{-9} seconds. Binding constants (K_A) and binding sites (n) were obtained using the following equation:

$$\log\frac{F_0 - F}{F} = \log K_A + n\log[Q] \quad (2)$$

where K_A is the binding constant and n is the number of binding sites.

The thermodynamic parameters were calculated to characterize the forces involved in the binding process. Because there is no significant change in temperature, enthalpy change (ΔH) can be regarded as a constant. The values of ΔH and entropy change (ΔS) can be calculated using Eq. (3) and the value of free energy change (ΔG) can be obtained using Eq. (4).

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (3)$$

$$\Delta G = \Delta H - T\Delta S = -RT\ln K \quad (4)$$

where K is the binding constant at corresponding temperature and R is the gas constant (8.314 J/mol/K).

The Far–UV CD spectra were measured on a Jasco-720 CD spectrophotometer (Tokyo, Japan). The CD spectra of HSA were recorded in the absence and presence of SAC using a 1-mm path length cuvette within the wavelength range of 190–240 nm. The CD results have been analyzed in terms of mean residue ellipticity in deg cm^2/dmol.

3. Results and discussion

3.1. Fluorescence quenching of HSA by SAC and binding properties

The effect of SAC on fluorescence spectra of HSA is shown in Figure 1. The excitation and emission wavelengths were 280 nm and 338 nm, respectively. The results show that the fluorescence intensity of HSA decreased with the increasing concentrations of SAC, respectively, indicating that SAC can quench the intrinsic fluorescence of HSA. The quenching rate constant of biomacromolecule (K_q) was calculated using Eq. (1). The K_q values for SAC were 5.25×10^{12} /M/s, 3.65×10^{12} /M/s, and 2.39×10^{12} /M/s at 288 K, 298 K, and 308 K, respectively (Table 1 and Figure 2). The K_q values of HSA–SAC decreased with increasing temperature, and these K_{SV} values of HSA–SAC were all far greater than the maximal collisional quenching rate constant (2×10^{10} /M/s) of all classes of the biomolecule, which suggested that fluorescent quenching between HSA and SAC was caused by static quenching rather than by dynamic collisions [14,15].

A plot of log[(F_0/F)/F] versus log[Q] and its analysis will give us the binding constant and the number of binding sites. The result indicated a good linear relationship (Figure 3), suggesting that HSA interacts with SAC in a one-to-one ratio. The binding constant was calculated from the intercept as 1.58×10^4 /M at 298 K, which indicates an adequate binding of SAC to the protein. The calculated binding constants show a comparatively weak ligand–protein interaction, compared with other strong ligand–protein complexes [16–18]. It is important to note that natural products showed binding constants, which are in the order of magnitude smaller than 10^6 /M. Other natural products such as folic acid binds with an affinity of 10^4 /M and colorant binds with an affinity of 10^5 /M [19–21].

3.2. Thermodynamic parameters and nature of binding forces

The forces acting between a compound and a biomolecule may include hydrogen bonds, van der Waals forces, hydrophobic, and electrostatic interactions. The values of thermodynamic parameters of binding reaction are the major