International Journal of Biological Macromolecules 111 (2018) 1–10

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: https://www.journals.elsevier.com/biomac

Behavior of bovine serum albumin in the presence of locust bean gum

Hongmei Zhang, Ying Wang, Yanqing Wang *, Shixin Sun, Jian Cao *

School of Chemistry and Environmental Engineering, Yancheng Teachers University, Yancheng City, Jiangsu Province 224002, People's Republic of China

ARTICLE INFO

Article history:
Received 16 November 2017
Received in revised form 23 December 2017
Accepted 25 December 2017
Available online 02 January 2018

Keywords:
Locust bean gum
Bovine serum albumin
Binding mechanism
Protein unfolding
Thermodynamics

ABSTRACT

In the present work, we have studied the structure and thermal stability of bovine serum albumin (BSA)-locust bean gum (LBG) mixture. It was found from the spectral results that the presence of LBG resulted in slightly decreasing the α–helical content and the partly unfolding of the skeleton of BSA. LBG binds to the neighboring amino acids of Trp and partly disturbs the microenvironment around Trp residues of BSA. The molecular docking confirms that there are more than one possible binding sites to bind with LBG by multi non-covalent forces. During the thermal unfolding process, LBG led to increase the secondary structure stability of protein by assembling each other. In addition, BSA even has good reversibility of the unfolding process in the presence of LBG promoting the thermal-induced tertiary structural degeneration of BSA. Collectively, our results provide evidence that LBG induces some behavior changes of BSA.

1. Introduction

In recent years, many studies about the interactions between aqueous biopolymers have been focused on in food hydrocolloids, which are complex systems containing many kinds of ingredients [1,2,3,4,5,6]. These studies play crucial roles in promoting research on development of the food industrial application [4,7,8,9]. Among the aqueous biopolymers used in food hydrocolloids, polysaccharides and proteins are wildly used and present together in many food products [10]. There have been many studies of protein-polysaccharide interactions [11,12,13,14]. For example, the effects of carrageenan on the properties of BSA were reported by Dickinson et al. [15]. Huang et al. have used multi-spectral methods to analyze the dextran–BSA conjugate structure with well thermal stability of BSA [16]. In addition, Huang et al. also have investigated the formation of a network-structured pectin allayer on the BSA surface. In our view, understanding the physico-chemical behavior of BSA binding with polysaccharide requires further study of protein–polysaccharide system at the molecular level [4]. Some investigations on the behavior of BSA in the presence of polysaccharide should be regard as the first step toward a comprehensive analysis of the complex behavior inside the functional food.

In this report, we focused on the behavior of BSA in the presence of locust bean gum (LBG). As one of polysaccharides, LBG is formed by linear chains of β-(1-4)-D-mannose with side units of α-(1-6) linked galactose (G). Its very high viscosity can be very attractive to be used as a thickening agent and a gelling agent used in food technology [17,18]. BSA has been often selected as a model globular protein to address critical problems about its binding interactions with polysaccharides in the food system [19,20]. In addition, such macromolecular crowding at the physiological level is well appreciated in the scientific community [21,22]. BSA and polysaccharides are important macromolecular crowding agents, which play a role in defining the features and dynamics of a host of cellular processes [23]. The different sizes and natures of macromolecular components also result in the heterogeneous of the distribution of viscosities in living cells [24]. Herein, multi spectral methods were used to obtain the mechanism proposed for the thermal stability of LBG-BSA mixture. CD studies have shown significant gain in secondary structure for BSA in the presence of LBG. Similar compaction effects have also been analyzed by UV–vis and DLS studies. Fluorescence spectral method has been used to monitor the fluorescence of LBG-BSA mixture. Molecular modeling was used to analyze the binding sites of LBG on BSA. The results of this study can offer some important information about LBG effects on protein conformations and functions in order to understand the intracellular environments of polysaccharides-protein system.

2. Experimental

2.1. Materials

LBG(mol. wt.310,000) is formed by linear chains of β-(1-4)-D-mannose with side units of α-(1-6) linked galactose (G) and was

* Corresponding authors.
E-mail addresses: wyqing76@126.com (Y. Wang), yctu_caojian@126.com (J. Cao).

https://doi.org/10.1016/j.ijbiomac.2017.12.139
0141-8130/

obtained from Aladdin Industrial Corporation Shanghai, China). BSA (A1933, lyophilized powder, ≥98%) was obtained from Sigma (St, Louis, MO, USA), respectively. Na_2HPO_4 and NaH_2PO_4 were of analytical purity and were used to make pH 7.4 potassium phosphate buffer.

2.2. Methods

2.2.1. Measurement of CD spectra

The CD spectra of BSA in the absence and presence of LBG were performed on a Chirascan spectrometer (Applied Photophyysics Ltd., Leatherhead, Surrey, UK) using quartz cuvettes of 0.1 cm path length. For the CD spectral experiments, the Far-UV spectra of the 5.0×10^{-6} mol/L BSA in the absence and presence of LBG were recorded from 200 to 260 nm with three scans averaged and scanning speed was set at 30 nm/min for each CD spectrum. The thermal stability of BSA-LBG mixture was measured by setting temperature from 20 to 90 °C, taking CD scan at 5 °C intervals, with 240 s increments. The secondary structural analysis of the CD spectra of BSA was done using the program CD spectra deconvolution program (CDNN) [25].

2.2.2. Measurement of UV–vis spectra

The UV–vis absorption spectra of BSA, LBG, and BSA-LBG system were measured on a SPECORD S600 spectrophotometer equipped with 1 cm quartz cells at room temperature.

2.2.3. Measurement of dynamic light scattering (DLS)

DLS was used to determine the hydrodynamic diameter of BSA and BSA-LGB system on a Malvern Zetasizer NanoS instrument with a 4 mW He–Ne 633 nm laser module. The hydrodynamic radius was calculated from the Stokes–Einstein equation where particles are assumed to be spherical [26,27].

2.2.4. Measurement of fluorescence spectra

The fluorescence spectra of the 5.0×10^{-6} mol/L BSA solution in the presence of LBG were recorded using a LS–50B Spectrofluorimeter equipped with 1.0 cm quartz cells and a thermostat bath. For the experiments, the excitation wavelength was set at 280 nm and the emission spectra were recorded from 300 to 500 nm. Both the excitation and emission slit widths were set at 5.0 nm. $\Delta\lambda(\lambda_{em}\text{-}\lambda_{ex})$ was set at 15 and 60 nm to obtain the synchronous fluorescence spectra(SFS) of BSA. Temperature dependence analysis of the stead-state fluorescence spectra of LBG-BSA mixture were recorded at 338 K with the time changes from 0 to 24 h. During the time- resolved fluorescence measurement, BSA and BSA-LBG system were excited at 280 nm and the decay was measured through a 50 ns time scale at a time resolution of 0.0122 s/channel.

2.2.5. Molecular modeling

The crystal structure of BSA (PDB ID 3V03) was obtained from the PDB for molecular modeling [28,29]. The geometries of LBG were optimized at density functional/B3LYP/6-311G $^{++}$(d, p) by Gaussian 09 [30]. Autodock 4.2.3 software was used to dock the binding mode of BSA with LBG [31]. The main calculation parameters were set. A grid box with $126 \times 126 \times 126$ grid points (0.357 Å) was used, and the Lamarckian Genetic Algorithm method was used as the searching algorithm. Finally, the Molegro Molecular Viewer software was used to analyze the predicting binding mode of BSA with LBG [32].

3. Results and discussion

3.1. Structure of BSA in the presence of LBG

Fig. 1 shows the UV–vis spectra of LBG (line a), BSA (line b), and BSA-LBG mixture (line c). It was found that the addition of LBG to BSA solution has brought appreciable reduction in the absorption intensity of protein at 220 and 278 nm. Characteristic absorption bands around

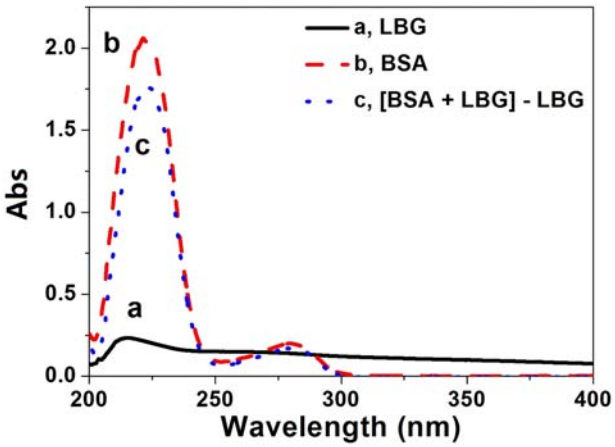

Fig. 1. The UV–vis spectra of LBG, BSA and BSA-LBG system. pH = 7.4, T = 298 K, c (BSA) = 5.0×1.0^{-6} mol/L, c (LBG) = 1.0 g/L.

wavelength of 220 and 278 nm were assigned to the n-π* and π-π* transitions of functional groups of BSA [33,34,35]. The observed spectral changes at 278 nm is directly related to modification in the microenvironment of aromatic rings amino acid residues in the BSA, indicting the interaction of LBG with BSA [36,37]. Furthermore, the outcome of the absorption spectral changes at 220 nm indicated the slightly decrease of α–helical content of protein and the skeleton of protein was partly loosened [38,39,40]. The secondary structure changes of BSA induced by LBG binding was also explored by using CD spectral method.

The far-UV CD spectra (Fig. 2A) were characterized for BSA and BSA-LBG mixture. The CD spectra of a-Helix are characterized by a negative

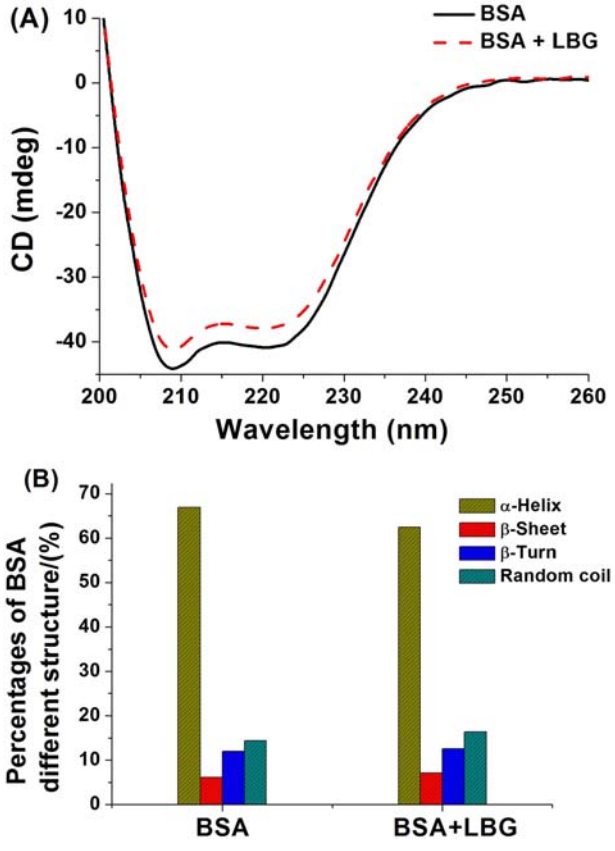

Fig. 2. The CD spectra of BSA, BSA-LBG system. pH = 7.4, T = 298 K, c (BSA) = 5.0×10^{-6} mol/L, c (LBG) = 1.0 g/L.