Journal of Luminescence 146 (2014) 351–357

Contents lists available at ScienceDirect

Journal of Luminescence

journal homepage: www.elsevier.com/locate/jlumin

Characterization and antioxidant activity of bovine serum albumin and sulforaphane complex in different solvent systems

Xueyan Dong, Rui Zhou, Hao Jing *

College of Food Science and Nutritional Engineering, China Agricultural University, 17 Qinghua East Road, Haidian District, Beijing 100083, China

ARTICLE INFO

Article history:
Received 8 May 2013
Received in revised form
12 September 2013
Accepted 27 September 2013
Available online 15 October 2013

Keywords:
Bovine serum albumin
Sulforaphane
Fourier transform infrared spectroscopy
Fluorescence spectroscopy
Synchronous fluorescence spectroscopy
Antioxidant activity

ABSTRACT

Modes and influencing factors of bovine serum albumin (BSA) and sulforaphane (SFN) interaction will help us understand the interaction mechanisms and functional changes of bioactive small molecule and biomacromolecule. This study investigated interaction mechanisms of BSA and SFN and associated antioxidant activity in three solvent systems of deionized water (dH_2O), dimethyl sulfoxide (DMSO) and ethanol (EtOH), using Fourier transform infrared spectroscopy (FT-IR), fluorescence spectroscopy, synchronous fluorescence spectroscopy, DPPH and ABTS radical scavenging assays. The results revealed that SFN had ability to quench BSA's fluorescence in static modes, and to interact with BSA at both tyrosine (Tyr) and tryptophan (Trp) residues, while the Trp residues were highly sensitive, which was demonstrated by fluorescence at 340 nm. Hydrophobic forces, hydrogen bonds and van der Waals interactions were all involved in BSA and SFN interaction, which were not significantly changed by three solvents. The binding constant values and binding site numbers were in a descending order of dH_2O > DMSO > EtOH. The values of free energy change were in a descending order of dH_2O > DMSO > EtOH, which indicated that the binding forces were in a descending order of dH_2O > DMSO > EtOH. There was no significant difference in antioxidant activity between SFN and BSA–SFN. Moreover, three solvents had not significant influence on antioxidant activity of SFN and BSA–SFN.

1. Introduction

Bovine serum albumin (BSA) plays an important role in the disposition and transportation of various nutrients in blood plasma, and can interact well with many small molecules [1]. BSA has 583 amino acid residues in a single polypeptide chain, among them tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) residues are considered as intrinsic fluorophores of BSA. While in practical measurement, the intrinsic fluorescence of BSA is mainly contributed by Trp and Tyr residues, because the quantum yield of the Phe residue is very low [2]. The V-shaped BSA molecule consists of three homologous α-helical domains (I, II, and III), and each domain contains two subdomains (A and B), named IA, IB, IIA, IIB, IIIA and IIIB [3]. BSA contains 35 cysteine (Cys) residues, 34 of which are covalently linked to form 17 disulfide bonds whereas only one free thiol group exists at Cys-34 residue [4]. The thiol groups of the Cys residues could be involved in covalent bounding of BSA and electrophilic compounds, such as phenethyl isothiocyanate (PEITC) and sulforaphane (SFN), which was associated with their bioactivity [5]. There are 20 Tyr residues and two Trp residues in BSA. Trp-134 is in the IB domain, and is located on the surface of the BSA. Trp-212 is in the IIA domain, and is located within a hydrophobic binding pocket of BSA [6,7]. The Tyr and Trp residues are also important as the binding sites, due to their fluorescence significant [6]. Interaction and mechanism of SFN with Tyr and Trp residues need to be further explored.

Sulforaphane [1-isothiocyanato-4-(methylsulfinyl)-butane] is derived from glucoraphanin. Glucoraphanin, a major member of glucosinolate family, could be hydrolyzed by endogenous myrosinase to form isothiocyanates, mainly SFN [8,9]. There are over 120 glucosinolates in the various varieties of cruciferous vegetables such as broccoli or broccoli sprouts, cauliflower, cabbage and kale [10]. SFN can reverse preneoplastic lesions and has a promising anti-cancer effect on the cancers of lung, colon, prostate, breast, gastric and skin [11,12]. The mechanisms of SFN's bioactivity could be related to activate NF-E2-related factor-2 (Nrf2) and induce the expression of Nrf2-dependent phase-II enzymes such as heme oxygenase-1 (HO-1), NAD(P)H, quinone reductase 1, glutathione reductase and glutathione peroxidase [13]. SFN has two functional groups of S═O and N═C═S, which make it susceptible to degradation under oxygen, heating and alkaline conditions [14,15]. SFN was readily degraded in an aqueous solution at high temperatures of 50 and 100 °C. Its S═O group could

Abbreviations: BSA, bovine serum albumin; SFN, sulforaphane; dH_2O, deionized water; DMSO, dimethyl sulfoxide; EtOH, ethanol; FT-IR, Fourier transform infrared spectroscopy; Tyr, tyrosine; Trp, tryptophan; DPPH, 1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt

* Corresponding author. Tel./fax: 86 10 6273 7909.
E-mail addresses: h200521@cau.edu.cn, hao.haojing@gmail.com (H. Jing).

0022-2313/$ - see front matter Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jlumin.2013.09.078

participate in the formation of epoxide compound, 4-methylthio-4-hydroxybutyl isothiocyanate, which went through dehydration and produced 4-isothiocyanato-1-(methylthio)-1-butene. The N═C═S group of SFN could be hydrolyzed and released an amine, which reacted further with SFN to generate N,N′-di-(methylsulfinyl) butyl thiourea [14].

Stability of bioactive small molecule could be improved by interaction with protein. In presence of casein and whey protein, stability of bog bilberry anthocyanin extract (BBAE) had been improved, with less change in the absorbance and antioxidant activity of BBAE [16]. The stability and the antioxidant activity of quercetin, kaempferol and rutin were also preserved by interacting with BSA [17]. The interaction of bioactive small molecule and protein is influenced by many factors, such as pH, ionic strength, temperature, and also solvents [7,18]. Fluorescence quenching and associated binding constants of cochineal red A and BSA complex were significantly reduced by addition of ethanol [19]. The binding constant of isoniazid to BSA was increased with increasing DMSO concentrations from 0% to 10% (v/v), and then decreased when DMSO concentrations were increased from 10% to 20% (v/v). The maximum binding of isoniazid to BSA occurred at the concentration of 10% (v/v) DMSO [20]. While the influence of ethanol and DMSO on the interaction between BSA and SFN and associated contribution to the antioxidant activity of SFN were not clear.

The present study investigated the influence of dH_2O, DMSO and EtOH as solvent components on the characteristics of BSA and SFN interaction, especially interaction mode of SFN and BSA with Tyr and Trp residues, using FT-IR, fluorescence spectroscopy, synchronous fluorescence spectroscopy, DPPH and ABTS radical scavenging assays.

2. Materials and methods

2.1. Materials

BSA (fraction V, purity 98%) (A-0332) was purchased from AMRESCO (Amresco Inc., OH, USA). SFN (purity 95%, HPLC grade) was purchased from College of Life Science and Technology, Beijing University of Chemical Technology (Beijing, China). DPPH (D9132), ABTS (A-1888) and DMSO (purity 99.5%) were all purchased from Sigma-Aldrich, Inc. (St. Louis, MO). EtOH (purity 99.9%) was purchased from Xilong Chemical Co., Ltd. (Beijing, China). All other reagents were of analytical grade.

2.2. Preparation of the complex of BSA–SFN

The stock solution of BSA (1.5×10^{-3} mol L^{-1}) was prepared with deionized water (dH_2O, 18 MΩ). The stock solution of SFN (1.5×10^{-2} mol L^{-1}) was prepared with dH_2O. Both stock solutions were stored in the refrigerator at 4 °C prior to use. The BSA stock solution was diluted before use with dH_2O (pH 6.82), 10% DMSO (v/v, pH 6.28) and 10% EtOH (v/v, pH 6.26). The BSA–SFN complex was prepared by adding SFN stock solution into diluted BSA stock solution. The final concentration of BSA was 1.5×10^{-5} mol L^{-1}, and the concentrations of SFN were 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0, 13.5 and 15.0×10^{-4} mol L^{-1}.

2.3. FT-IR measurement

FT-IR spectra of pure BSA and BSA–SFN complexes were recorded using a Spectrum 100 FT-IR spectrometer (Perkin-Elmer, USA), equipped with a deuterated triglycine sulfate (DTGS) detector. The spectrometer was continuously purged with dried air. 2 mg of freeze-dried sample was mixed with 200 mg KBr and ground gently with an agate pestle and mortar under an infrared lamp and afterwards was pressed into a 13-mm diameter disc by applying 15 Torr pressure for 5 min. FT-IR spectra were obtained in the wave number range from 450 to 4000 cm^{-1} during 100 scans, with 2 cm^{-1} resolution.

2.4. Fluorescence measurement

The fluorescence intensities were recorded with an F-7000 fluorophotometer (Hitachi, Japan). The concentration of BSA was at 1.5×10^{-5} mol L^{-1}, and SFN concentrations were 0, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, 12.0, 13.5 and 15.0×10^{-4} mol L^{-1}. The fluorescence experiments have been carried out at 25 °C (298 K), 31 °C (304 K) and 37 °C (310 K). An excitation wavelength of 280 nm was selective in all cases for selective excitation of the Trp residues of BSA, and emission spectra are recorded from 280 to 440 nm.

The fluorescence quenching data can be analyzed using Stern–Volmer equation [21]

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

where F_0 and F are the fluorescence intensities of BSA without and with the existence of quencher, respectively. K_{SV} is the Stern–Volmer quenching constant with units of L mol^{-1}; [SFN] is in the concentration of mol L^{-1}. Stern–Volmer equation was applied to determine K_{SV} by linear regression of a plot of F_0/F against [SFN].

The binding constant (K) and binding sites (n) are calculated by the double-logarithm equation for static quenching [22]

$$\lg[(F_0-F)/F] = \lg K + n \lg[SFN] \quad (2)$$

Binding data at different temperatures were used to analyze the thermodynamic parameters using the van't Hoff equation [23]

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

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

$$\Delta S = (\Delta H - \Delta G)/T \quad (5)$$

where K is the association constant, T is the absolute temperature, R is the gas constant (8.3145 J mol^{-1} K^{-1}), ΔH is the enthalpy change, ΔS is the entropy change and ΔG is free energy change.

2.5. Synchronous fluorescence measurement

Synchronous fluorescence spectra of the samples were obtained after scanning them in the wavelength range of 260–320 nm and 240–320 nm for the difference between excitation and emission wavelengths ($\Delta\lambda$) of 15 and 60 nm, respectively. The final concentration of BSA in each sample was 1.5×10^{-5} mol L^{-1} while SFN concentrations were 0, 1.5, 3.0, 6.0, 9.0, 12.0, 15.0×10^{-4} mol L^{-1}.

2.6. Antioxidant activity assessment

The concentrations of SFN and BSA were set at 1.5×10^{-3} mol L^{-1} and 1.5×10^{-6} mol L^{-1}, respectively.

2.6.1. DPPH assay

The DPPH free radical scavenging activity was determined according to the method of Fang et al. [24]. Stock solutions of DPPH were prepared at 2.5 mmol L^{-1} and then diluted with ethanol to 0.15 mmol L^{-1}. Each sample (15 μL) was mixed with 0.05 mol L^{-1} (pH 7.4) Tris–HCl buffer (60 μL) and 0.15 mmol L^{-1} DPPH working solution (150 μL) in a 96-well plate. The mixture was shaken vigorously and then left to stand for 30 min in the dark. The absorbance (A_{sample}) at 517 nm was recorded using a microplate reader (model 680, Bio-Rad Laboratories, Inc., Hercules, CA).