Food Chemistry 228 (2017) 265–269

Contents lists available at ScienceDirect

Food Chemistry

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

Multi-spectroscopic and molecular modeling studies of bovine serum albumin interaction with sodium acetate food additive

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ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 26 January 2017
Accepted 31 January 2017
Available online 2 February 2017

Keywords:
Sodium acetate
Bovine serum albumin
Fluorescence quenching
Molecular docking

ABSTRACT

Sodium acetate (SA) has been used as a highly effective protectant in food industry and the possible effect of this additive on the binding to albumin should be taken into consideration. Therefore, for the first time, the mechanism of SA interaction with bovine serum albumin (BSA) has been investigated by multi-spectroscopic and molecular modeling methods under physiological conditions. Stern–Volmer fluorescence quenching analysis showed an increase in the fluorescence intensity of BSA upon increasing the amounts of SA. The high affinity of SA to BSA was demonstrated by a binding constant value (1.09×10^3 at 310 °K). The thermodynamic parameters indicated that hydrophobic binding plays a main role in the binding of SA to Albumin. Furthermore, the results of UV–vis spectra confirmed the interaction of this additive to BSA. In addition, molecular modeling study demonstrated that A binding sites of BSA play the main role in the interaction with acetate.

1. Introduction

Sodium acetate is confirmed as food grade preservative and is a highly effective protectant, which has been used as acidulants, flavoring adjuvant, and sequestrants at various meat products, bakery industry and gelatin films (Gómez-Estaca, Gómez-Guillén, Fernán dez-Martín, & Montero, 2011; Smith, Daifas, El-Khoury, Koukoutsis, & El-Khoury, 2004).They have been evaluated by the US Food and Drug Administration (FDA) and commonly is considered as safe (Feedap, 2005). It is also used in cosmetics products, veterinary drugs and as plant preservation agents at concentrations typically less than 3000 ppm (Feedap, 2005). Recently, the influence of SA on the thermo-mechanical and physicochemical properties of edible films was investigated (Liu, Antoniou, Li, Ma, & Zhong, 2015).

The interactions between bio-macromolecules and various chemicals have attracted increasing research attention in recent years (Li et al., 2007). Among several bio-macromolecules, serum albumins are the most abundant proteins in the circulatory system of numerous organisms and have many numerous physiological functions. Various exogenous compounds carrying is the most prominent function of serum albumins (Malonga, Neault, & Tajmir-Riahi, 2006; Mancuso, Bonsignore, Capone, Stasio, & Pani, 2006). Serum albumins not only increase hydrophobic compounds solubility but also enhance delivery of these compounds to cells. Albumin plays a significant role in the absorption, distribution, metabolism, and repulse properties of materials. Therefore, the durability and toxicity of chemical substances can significantly influence the structure of serum albumin due to interaction with it (Flarakos, Morand, & Vouros, 2005). In addition, several studies results showed that primary and secondary structure of various proteins such as albumins change because of interaction with small molecules. Thus, the surveys on the interaction of chemicals substance with albumin are of great importance (Belatik, Hotchandani, Bariyanga, & Tajmir-Riahi, 2012; Dolatabadi, Hamishehkar, de la Guardia, & Valizadeh, 2014; Neault, Benkiran, Malonga, & Tajmir-Riahi, 2001). Bovine serum albumin (BSA) has been most widely studied albumin due to its structural similarity with human serum albumin (Fathi, Ezzati Nazhad Dolatanbadi, Rashidi, & Omidi, 2016). Because of that in this study, the interaction between BSA and SA for the first time was studied under phys-

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http://dx.doi.org/10.1016/j.foodchem.2017.01.149
0308-8146/

iological conditions using fluorescence and UV–vis spectroscopies and molecular docking.

2. Material and methods

2.1. Material

Bovine serum albumin (BSA) was purchased from sigma-Aldrich. All BSA solutions were prepared in the 0.1 M phosphate buffer solution containing NaCl (0.2 M, pH 7.4), BSA solutions stored in the dark at 4 °C. SA was purchased from Merck. All other chemicals were obtained from Sigma-Aldrich.

2.2. Stock preparation

SA and BSA solutions were prepared in the buffer solution adjusted to pH 7.4 with 0.01 M Na_2HPO_4 and NaH_2PO_4 in pure aqueous medium. BSA stock solution (3×10^{-5}M, based on its molecular weight of 66,000) was prepared in 0.01 M phosphate buffer of pH 7.4 and was kept in the dark at 277 °K. All other reagents were of analytical grade, and double-distilled water was used throughout all experiments.

2.3. Fluorescence experiments

Fluorescence measurements were carried out with a spectrofluorimeter, Jasco FP-750 (Kyoto, Japan) supplied with a 150 w Xenonlamp, using 1.0 cm quarts cell with a thermostat bath. The maximal fluorescence emission of BSA was located at 350 nm. Fluorescence spectra were recorded from 280 to 450 nm with the excitation wavelength at 290 nm. The concentration of BSA was constant (i.e.5.0×10^{-6} M) while varying the SA concentration from 0 to5.0×10^{-5} M. Experiments were measured at five temperatures (288, 293, 298, 303 and 310 °K).

2.4. Uv–vis spectrophotometry

Absorbance spectra were recorded using a UV–vis spectrophotometer, T 60, PG Instrument (Leicestershire, UK). Absorption titration experiments were carried out by keeping the concentration of BSA constant (5.0×10^{-6} M) while varying concentration of SA from 0 to 5.0×10^{-5} M. Absorbance values were recorded after each continuous addition of SA solution and equilibration.

2.5. Molecular docking study

Molecular docking study of acetate was performed by the open-source Windows version of Auto Dock Vina 1.1.2 software (Trott and Olson, 2010) which it was introduced by the Scripps Research Institute in order to improve the speed as well as accuracy of docking procedures. For survey the interaction of bovine serum albumin (BSA) and acetate by simulation study, the X-ray structure of BSA (PDB entry code 4F5S with 2.47 Å resolution) was selected from the RCSB protein data bank (http://www.pdb.org). The acetate molecular structure was prepared by drawing and energy optimizing with MM+ molecular mechanics and AM1 semiempirical procedures with HyperChem 8.0.8 software. In order to interaction study by molecular docking, the extra crystallographic structure with all water molecules were removed from the PDB structure. The BSA structure and compound were changed by adding the polar hydrogens. Also the Kollman charges as well as Gasteiger charges were added to the receptor and ligand, respectively. After developed the pdbqt files of BSA and acetate, ligand was inputted in determined six different binding area of BSA. The Grid box dimensions size at grid points in x * y * z directions was set to 24.375 Å with a grid spacing of 1 Å in Vina docking.

3. Results and discussion

3.1. Fluorescence spectroscopy study

Analysis of interaction of chemical substance to BSA can be evaluated by fluorescence spectroscopy. The fluorescence property of protein is caused by tyrosine, tryptophan, and phenyl alanine residues. Due to a very low amount of phenylalanine fluorescence yield and fluorescence quenching of tyrosine upon ionization, only tryptophan play major role in the intrinsic fluorescence of many proteins. Fluorescence quenching refers to any process that lead in fluorescence intensity decreasing of a given fluorophore due to interaction with various molecules. Quenching can occur through two mechanisms, which are generally categorized as dynamic quenching and static quenching; if both the fluorophore and the quencher come into contact during the transient existence of the exited state, quenching process will be dynamic and if complex formation occurs between fluorophore and quencher, the dominant quenching process will be static. In general, dynamic and static quenching can be distinguished through excited state lifetime and different dependence on temperature. In both cases, the fluorescence intensity is due to the quencher concentration. Hence, the quenched fluorophore can be used as an indicator for quenching agent.

As shown in Fig. 1, the fluorescence intensity of BSA was changed upon addition of SA. The fluorescence of BSA regularly decreased upon increasing concentration of SA indicating that intrinsic fluorescence of BSA was quenched due to interaction with SA. The fluorescence quenching data were described using the Stern–Volmer equation (Lakowicz, 2006).

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

where, F_0 and F illustrate the fluorescence intensities of BSA in the Absence and in the presence of quencher SA, respectively. K_{SV} is Stern–Volmer quenching constant, which is a measure of the fluorescence quenching upon addition of SA and [Q] is the concentration of the quencher (SA) (Dolatabadi & Kashanian, 2010; Kashanian & Dolatabadi, 2009).

As it is clear in Table 1, the increase of K_{sv} by rising temperature indicated that the most probable quenching mechanism of BSA fluorescence is a dynamic quenching procedure (Kashanian & Dolatabadi, 2009).

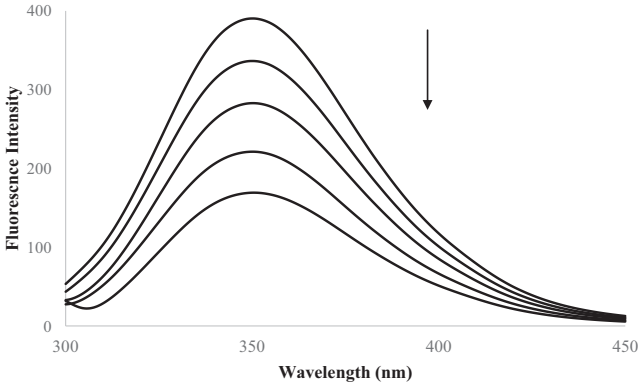

Fig. 1. Fluorescence spectra of BSA in the absence and the presence of the increasing SA concentration in double distilled water (pH = 7.4) at room temperature, [BSA] = 5×10^{-6} M and [SA] = 0, 1, 5, 10, 50×10^{-6} M.