Process Biochemistry 48 (2013) 1686–1696

Contents lists available at ScienceDirect

Process Biochemistry

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

Interactions of bovine serum albumin with biological buffers, TES, TAPS, and TAPSO in aqueous solutions

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ARTICLE INFO

Article history:
Received 2 May 2013
Received in revised form 2 August 2013
Accepted 26 August 2013
Available online 3 September 2013

Keywords:
Protein stability
Molecular Docking
Buffers
BSA
Spectroscopy

ABSTRACT

The thermal stability of bovine serum albumin (BSA) in the aqueous solutions containing the biological buffers, *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES), *N*-[tris(hydroxylmethyl)methyl]-3-aminopropanesulfonic acid (TAPS), and *N*-[tris(hydroxymethyl)methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO), was studied by using dynamic light scattering (DLS) at various temperatures and concentration ranges of buffers. It is found that the increase of the buffer concentration enhanced the thermal stability of protein BSA, and the stabilization tendency follows the order of TAPSO > TES ≈ TAPS. In this study, we have also investigated the interactions of BSA with TES, TAPS, and TAPSO by using various techniques, such as UV–vis absorption, fluorescence, and molecular docking. It is revealed that the main interactions between the studied buffers and the peptide moieties of proteins are electrostatic including hydrogen bonds. The results obtained from this series of studies confirmed that the biological buffers, TES, TAPS, and TAPSO can serve as good stabilizers for the globular protein BSA, in the aqueous solutions.

1. Introduction

The protein stability in aqueous solutions is a commonly concerned issue in various biological fields, especially in the pharmaceutical field for development of therapeutic protein products. Each protein is physically as well as chemically unique; thus, different proteins show their own characteristics of stability behavior. However, a protein cannot be seen as beneficial to human health unless it is adequately stabilized.

Proteins remain in their native globular conformation at specific pH and temperature because this native state is thermodynamically favorable to the unfolded state. The conformational stability of the native state of the protein arises from different types of interactions such as hydrophobic, hydrogen bonding, van der Waals, electrostatic, and local peptide-peptide interactions [1]. Since the contribution of each type of interaction varies with temperature, protein molecules may unfold and lose their native conformation as temperature increases up to a certain value [2–5]. In lower temperature range, thermally induced denaturation may be reversible for some proteins, but it usually leads to irreversible denaturation at high temperatures. For example, the conformation of native BSA in aqueous solution at physiological pH is reversible below 50 °C, partially reversible above this temperature, and becomes completely irreversible at about 54–55 °C [6–9].

Since most proteins are biologically active only in their specific globular conformation, it is important to protect them from thermal denaturation in order to maintain their biological activity. Different types of stabilizers were reported by researchers in the past. These stabilizers, through various physicochemical mechanisms [10–14], modify the intramolecular interactions among proteins and the conformation of proteins and, thus, the functional properties. The stabilizer can influence protein molecules and their functional characteristics either directly by binding to the protein surface groups or indirectly by altering the structure and physicochemical properties of water shell surrounding the protein [13–16]. Generally, the nature of the interactions and their influence on protein functionality are governed by the type and the concentration of stabilizer presented [14,17].

Sugars and polyhydric alcohols are well known stabilizers for proteins [18–22]. Recently, stabilization of the protein structure and the activity in solution of ionic liquids were reported by many research groups [23–28]. Summers and Flowers [29] are the first to find that liquid organic salts tetra-ethyl or tetra-butyl ethyl-ammonium nitrate assist the protein renaturation. Later, Fujita et al. [30,31] have reported that dihydrogen-phosphate based biocompatible ionic liquids are promising solvents toward stabilizing the functional proteins. Lozano et al. [32] have investigated the interactions of various dialkylimidazolium and quaternary ammonium based ionic liquids with α-chymotrypsin as a model for

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http://dx.doi.org/10.1016/j.procbio.2013.08.018

protein. The stabilization of α-chymotrypsin structure in the presence of the ionic liquids was reported. The stabilization effect was observed to increase with the increasing chain length of the alkyl substituent on cation and with the increasing size of the anion of the ionic liquids. De Diego et al. [33] have also reported a similar stabilization effect of 1-ethyl-3-methylimidazoliumbis [(trifluoromethyl) sulfonyl] amide ionic liquid on the structure of α-chymotrypsin. Recently, Shu et al. [34] have studied the interactions of dibutylimidazolium chloride [Bbim][Cl], 1-butyl-3-methylimidazolium chloride [Bmim][Cl], and 1-butyl-3-methylimidazolium nitrate [Bmim][NO_3] ionic liquids with protein BSA. They investigated the effect of increasing hydrophobicity of the cation and the increasing size of the anion of the ionic liquids, on the interactions of respective ionic liquids with BSA. The magnitude of interactions of the selected ionic liquids with protein BSA follows the order of [Bmim][NO_3] > [Bmim][Cl] > [Bbim][Cl]. Based on the effect of the chain length of the alkyl group and the size of the anions, the magnitude of interactions is in agreement with the results of Lozano et al. [32]. However, the loss of secondary and ternary structures of BSA was also observed by using different amounts of these selected imidazolium-based ionic liquids. Singh et al. [35] have reported the denaturation effect of 3-methyl-1-octylimidazolium chloride [C_8mim][Cl] and 1-butyl-3-methylimidazoliumoctylsulfate [C_4mim][C_8OSO_3] ionic liquids on the structure of BSA. At low concentrations, both [C_4mim]$^+$ and [C_8mim]$^+$ interact with BSA electrostatically and induce protein unfolding. The magnitude of interactions was found to be stronger for [C_8mim]$^+$ as compared with [C_4mim]$^+$. Geng et al. [36] have studied the interactions of highly hydrophobic surfactants, like ionic liquid 1-tetradecyl-3-methylimidazolium bromide [C_{14}mim][Br], with BSA. They reported that, the [C_{14}mim][Br] binds to BSA electrostatically at the critical aggregation concentration (CAC) and hydrophobically at the critical micelle concentration (CMC), and leading to the protein denaturation above CMC. As discussed, different co-solute, like ionic liquids, can either stabilize or destabilize bio-macromolecules. Therefore, more studies on finding compounds to stabilize the bio-macromolecules such as BSA are still needed.

As a part of our research, we introduced some potential stabilizers for BSA in aqueous solution by using various techniques to find new possibilities for stabilizing BSA [37,38]. In this study, we have investigated the thermal denaturation process of BSA in 0.05 M, 0.5 M, and 1.0 M *N*-[tris-(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES), *N*-[tris(hydroxyl-methyl)-methyl]-3-aminopropanesulfonic acid (TAPS), and 0.05 M, 0.5 M, and 0.7 M *N*-[tris(hydroxymethyl)-methyl]-3-amino-2-hydroxypropanesulfonic acid (TAPSO) aqueous buffers solutions at pH = 7.0 with dynamic light scattering (DLS), in the temperature range of 25–85 °C. The results indicate that these common biological buffers are potentially applicable to support the native structure of BSA molecule. The selected biological buffers (TES, TAPS and TAPSO) are structurally related compounds, each one containing at least one TRIS groups. These buffers were first introduced by Good and co-workers [39–43] and thus are commonly known as Good's buffers, which are used worldwide in various biochemical studies because they fulfill most of the requirements of biological buffers.

Despite many efforts being made on the study of proteins in buffer solutions, relatively few information has been reported for the mechanisms of buffers–protein interactions. Therefore, in the present study, we investigated the interactions of these studied buffers with BSA by mean of spectroscopy (UV–vis and fluorescence) and molecular docking techniques.

We selected BSA as a model compound in this study because of its unique structural and functional characteristics. The molecular weight of BSA is 66 kDa and it contains 583 amino acid residues in a single polypeptide chain [44]. It is made up of three homologous domains (I, II, and III) and contains 17 disulfide bridges and one free SH group, which divide the protein into 9 loops (L1–L9) [6]. The presence of two tryptophan (Trp) residues: Trp 134 in the first domain and Trp 212 in the second domain, respectively, make this protein suitable for the study of interactions with different ligands [45] Trp 134 is situated on the surface of BSA and exposed to solvent while Trp 212 is found within a hydrophobic binding pocket of the protein. In addition, the BSA has 20 tyrosine residues and 27 phenylalanine residues. The interior of BSA is almost hydrophobic, while the charged amino acid residues and polar patches cover the interface [46]. BSA has negative charges at physiological pH [47] and is used as the major transport carrier for drugs, endogenous and exogenous substances. It is considered as a model protein for the investigation of protein-drug interactions *in vitro*. The globular proteins, especially BSA, are a functional constituent in pharmaceutical, food, and health care products. They are able to catalyze various biochemical reactions, to absorb on the surface of some substances, and to bind with other molecules and formed molecular aggregates [48]. Due to the unique functional ability of BSA, it is the most widely used in various applications [49–52]. However the proper functional efficiency of proteins depends upon several factors, such as molecular structure, chemical environment, and thermal stability [53,54].

2. Materials and methods

2.1. Materials

The buffers, TES (mass fraction purity > 0.99), TAPS (mass fraction purity > 0.99), and TAPSO (mass fraction purity > 0.99) were purchased from Sigma Chemical Co. The BSA/fraction V, pH = 7.0, obtained from Acros Organics. All materials were used as received. Deionized water used in preparation of aqueous solutions was obtained from NANO pure-Ultra pure water system with resistivity of 18.3 MΩ cm. All samples were prepared gravimetrically by using an electronic balance (R & D, Model GR-200) with a precision of ±0.1 mg.

2.2. Dynamic light scattering (DLS)

A DLS (Zetasizer Nano ZS90, Malvern Instruments Ltd., UK) was employed to measure the hydrodynamic diameter (d_H) and the size distribution of BSA in aqueous TES, TAPS, and TAPSO buffer solutions at temperatures from 25 °C to 85 °C. This instrument provided with a thermostatic sampling chamber, to maintain the desired temperature of the sample during measurements, within the temperature range of 0–90 °C. The source of light in the instrument is He–Ne laser light (4 mW) with a fixed wavelength, λ = 633 nm. The DLS measurements were conducted at a fixed scattering angle of 90°. The scattering intensity data were processed by using built-in software to determine the averaged d_H and the size distribution. The samples for DLS analysis were prepared with 25 mg cm^{-3} of BSA in (0.05, 0.5, and 1.0 M) TES and TAPS, respectively, and (0.05, 0.5, and 0.7 M) TAPSO buffer solution at pH = 7. Due to the solubility limit of TAPSO in water, the maximum concentration of TAPSO buffer solution investigated was up to 0.7 M at pH = 7 throughout this study. The analysis samples were filtered first with disposal filters (Millipore, 0.22 mm), then a bubble free sample of around 1.5 cm^3 was introduced in a square glass cuvette with round aperture (PCS8501) sample cell through a syringe. In order to protect the sample cell from dust, a Teflon-coated screw cap was placed at the mouth of the cell, forming an airtight sample cell. Prior to DLS measurements, the prepared BSA-buffer samples were kept about 4–5 h incubation at 25 °C to attain complete equilibrium. Then, the sample was scanned in the temperature range of 25–85 °C. At least three repeated measurements were made for each sample at a given temperature.

2.3. UV–vis spectra

Absorption spectra for BSA (2.5 mg cm^{-3} in 0.05 M, 0.5 M, 0.7 M and 1.0 M buffer solution) were measured at room temperature by using UV-visible spectrophotometer (JASCO, V-550) equipped with 1.0 cm quartz cell. The operating conditions were scan speed 100 nm min^{-1}, scan range 190–600 nm, slit width 2 nm, and Δλ = 0.1 nm.

2.4. Fluorescence spectra

The emission spectra for BSA (2.5 mg cm^{-3} in 0.05 M, 0.5 M, 0.7 M and 1.0 M buffer solutions) were measured at room temperature by mean of RF-5301PC Spectro-fluorophotometer (SHIMADZU), using 1-cm quartz cell. The spectra were collected at an excitation wavelength of 230 nm and 280 nm, with excitation and emission slit width of 5 nm for the emission range of 250–420 nm.