Journal of Molecular Liquids xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Intermolecular interactions and molecular dynamics in bovine serum albumin solutions studied by small angle X-ray scattering and dielectric relaxation spectroscopy

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ARTICLE INFO

Article history:
Received 30 October 2013
Received in revised form 12 May 2014
Accepted 17 May 2014
Available online xxxx

Keywords:
Bovine serum albumin
Intermolecular interactions
Pair correlation function
Collective dynamics
Small angle X-ray scattering (SAXS)
Dielectric relaxation spectroscopy (DRS)

ABSTRACT

We used small angle X-ray scattering (SAXS) and dielectric relaxation spectroscopy (DRS) to clarify static structure and molecular dynamics of bovine serum albumin (BSA) in solution. SAXS allowed us to access spatial correlations of the protein molecules in concentrated solutions at different ionic strength. Using a well-established Fourier inversion technique, called SQ-IFT, we deduced the effective pair correlation functions, $g(r)^{eff}$, from the effective (or experimental) structure factors, $S(q)^{eff}$, without assuming any potential models for protein–protein interactions. In terms of $S(q)^{eff}$ and $g(r)^{eff}$, the mean nearest-neighbor distance, d^*, the osmotic compressibility, κ_{osm}, and the coordination number, N_C, were evaluated.

The complex dielectric spectra of aqueous BSA solutions measured in the frequency range from 1 MHz to 10 GHz were able to be decomposed into three relaxation processes having different physical origins. The highest frequency process, whose relaxation time was ca. 8 ps, was assigned to cooperative rearrangement of H-bond network of bulk-water. We evaluated the effective hydration number of a BSA molecule, Z_{hyd}, using the bulk-water relaxation amplitude. This approach yielded ca. 1200 hydrated water molecules per a BSA molecule, corresponding to ca. 0.3 g hydrated water per 1 g protein. The lowest frequency process having a relaxation time of ca. 50 ns was assigned to rotational diffusion of the BSA molecule. We confirmed that an effective molar volume of BSA calculated with Stokes–Einstein–Debye equation was identical to that predicted from its molecular weight and specific density. According to the Kirkwood relationship, the dipole–dipole orientational correlation factor decreased with an increase of protein concentration, which indicated the operation of BSA–BSA interaction and favored antiparallel dipolar correlation between the protein molecules.

1. Introduction

Interactions between protein macromolecules in solution are of central importance in a number of areas, ranging from aggregation or segregation related to protein condensation diseases to protein crystallography. Small angle scattering of X-rays (SAXS) and neutrons (SANS) are highly efficient techniques to investigate protein–protein interactions in solution, and have been used for a number of globular proteins [1–9]. For example, Stradner et al. [1] discussed equilibrium cluster formation in concentrated lysozyme solution in view of the appearance of low-q (scattering vector) sub-peak position in their SANS structure factor. Conventional potential models, such as a hardsphere, a screened Coulomb [5,6], and a more complicated Derjaguin–Landau–Verwey–Overbeek (DLVO) potential model [2–4] have been applied to experimental scattering intensities of proteins in solution. However, in some cases, these potential models cannot fully explain rich behavior of proteins [7–9]. We infer that due to the complexity of structural features of proteins, e.g., irregular shape, distributed hydrophobic and hydrophilic patches, and inhomogeneous charge distributions, actual protein–protein interactions in solution may not fulfill an isotropic interaction assumed in these well-known potential models. Accordingly, we investigated spatial correlations of protein in solution at different ionic strengths varying concentration between 4.99 mg mL^{-1} and 263.6 mg mL^{-1} by means of SAXS. Using an extended version of a well-established indirect Fourier transformation (IFT) technique, the so-called SQ-IFT, the SAXS data was analyzed without assuming any potential models. Then, we tried to give an explanation of the structural quantities obtained with SQ-IFT.

Dynamical properties of protein solution are also of critical importance for understanding protein specific functions. Dielectric relaxation spectroscopy (DRS) observes collective dynamics of solute and solvent molecules. In the frequency range between 1 MHz and 10 GHz, two strong relaxation bands are observed for globular protein solutions, which are generally assigned to the reorientation of bulk-water and

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http://dx.doi.org/10.1016/j.molliq.2014.05.021
0167-7322/

rotational diffusion of the protein. In addition, an intermediate frequency process between these two is also resolved. However, its assignment to tightly bound hydrated water molecules is not yet very clear [10].

In this study, we investigated spatial correlations and molecular dynamics of bovine serum albumin (BSA) in solution. BSA is the most abundant plasma protein in bovine's blood stream. BSA is a counterpart of human serum albumin (HSA), exhibiting 75.8% identity. BSA architecture is predominately helical, consisting of three domains that create heart-like shape. A primary function of BSA is transportation of hydrophobic molecules, which is achieved by its nonspecific ligand binding capability [11]. BSA also serves as an adjuster of colloid osmotic pressure (COP) of blood [11]. HSA showing isoelectric point (pI) of 4.7 carries a net negative charge of about 18 electronic charges at physiological pH [12]. BSA is also strongly negatively charged at physiological pH because its isoelectric point is ca. 5.3 [13], which is rather close to that of HSA.

2. Materials and methods

2.1. Materials

For SAXS experiments, bovine serum albumin (BSA) was purchased from Sigma-Aldrich (product No. A7030). Two sets of several BSA samples in Millipore water and in 0.15 M phosphate buffered saline (PBS) solution (pH = 7.4, GIBCO®, Invitrogen) were prepared. Protein concentration, c, was calculated from weight fraction of BSA in solution and its partial specific volume (v = 0.733 and 0.741 cm^3/g for BSA in aqueous solution and in PBS solution, respectively, which were determined by densitometry using DMA5000 (Anton Paar)). For DRS experiments, the powdered BSA purchased from Wako Pure Chemical Industries (product No. STM1969) was used as received. The solid BSA was dissolved in Millipore water.

2.2. Small angle X-ray scattering (SAXS)

SAXS experiments on solutions of BSA were performed using a SAXSess camera (Anton Paar, Graz, Austria). A sealed tube anode X-ray generator (GE Inspection Technologies, Germany) was operated at 40 kV and 50 mA. A focusing multilayer optics and a block collimator provide an intense monochromatic primary beam (Cu K_α radiation, λ = 0.1542 nm) with a well-defined line shape (2.0 mm × 300 μm). A semi-transparent beam stop enabled a measurement of an attenuated primary beam at zero scattering vector. The samples were filled into a vacuum tight quartz capillary cell (ca. 1 mm ϕ) and set in a temperature controlled sample holder unit (TCS120, Anton Paar). Two-dimensional (2D) scattering intensity distribution recorded by an imaging-plate (IP) detector was read out by a Cyclone storage phosphor system (Perkin Elmer, USA). The 2D data was integrated into a 1D scattering intensity, $I(q)$, as a function of the magnitude of the scattering vector

$$q = \frac{4\pi}{\lambda}\sin(\theta/2) \tag{1}$$

where θ is the total scattering angle.

All $I(q)$ data were normalized so as to have a uniform primary intensity at $q = 0$ for transmission calibration. The back ground scattering contributions from capillary and solvent were corrected. The absolute intensity calibration was made by referring to water intensity as a secondary standard [14].

2.3. Data analysis

Small angle scattering of X-rays (SAXS) and neutrons (SANS) is a very efficient technique to study interparticle interactions in solution. The scattering intensity, $I(q)$, for a concentrated colloidal dispersion is given by the product of the form factor, $P(q)$, and the static structure factor, $S(q)$, as [15–17]

$$I(q) = nP(q)S(q) \tag{2}$$

where n is the particle number density.

$P(q)$ is a reciprocal-space function associated with the pair distance distribution function, $p(r)$, describing particle structure. These two functions are connected with each other via Fourier transformation [15,18,19] as

$$P(q) = 4\pi\int_0^\infty p(r)\frac{\sin qr}{qr}\mathrm{d}r. \tag{3}$$

On the other hand, the static structure factor, $S(q)$, is also given by Fourier transformation of the total correlation function, $h(r) = g(r) - 1$, as [20]

$$S(q)-1 = 4\pi n\int_0^\infty [g(r)-1]r^2\frac{\sin qr}{qr}\mathrm{d}r \tag{4}$$

where $g(r)$ is the pair correlation function describing spatial distribution of colloidal particles.

If we measure a scattering intensity at very low protein concentration, where intermolecular interactions can be neglected ($S(q) \approx 1$), the scattering curve exclusively represents intramolecular structure of the protein. In such a case, Eq. (2) is rewritten as

$$I(q) = nP(q)^{\exp} \tag{5}$$

where $P(q)^{\exp}$ is the experimental form factor.

Once we get $P(q)^{\exp}$, the effective structure factor, $S(q)^{\mathrm{eff}}$, can be deduced by dividing the concentration normalized intensity, $I(q)/c$, by $P(q)^{\exp}$ as [21]

$$S(q)^{\mathrm{eff}} = I(q)/cP(q)^{\exp}. \tag{6}$$

Using the relation given in Eq. (4), we transcribed $S(q)^{\mathrm{eff}}$ into the effective pair correlation function, $g(r)^{\mathrm{eff}}$, relying on indirect Fourier transformation (IFT) technique [15,18]. We call such an interaction potential model-free analytical technique of static structure factor SQ-IFT [21].

2.4. Dielectric relaxation spectroscopy (DRS)

To obtain specific information about states of the proteins and water in aqueous BSA solutions from a viewpoint of molecular dynamics, we determined complex dielectric spectra, $\varepsilon^*(\nu) = \varepsilon'(\nu) - i\varepsilon''(\nu)$, of aqueous BSA solutions at 25 °C in the frequency range from 1 MHz to 10 GHz using time domain reflectometry (TDR) [22], based on the Hewlett–Packard instrument (HP54120B).

3. Results and discussion

3.1. Spatial correlations of BSA

Using SAXS, we investigated spatial correlations of BSA at protein concentrations of $4.00 < c/\mathrm{mg\ mL^{-1}} < 264$ in water and in 150 mM PBS solution. These solvent conditions were chosen to minimize ionic strength and to achieve ionic strength and pH close to physiological conditions, respectively. Fig. 1(a) and (b) show the concentration normalized scattering intensities of BSA, $I(q)/c$, in water and in 150 mM PBS solution at 25 °C on absolute scale. It is often the case that the maximum q value of SAXS and SANS experiments is limited to 3–5 nm^{-1}. In this study, we have extended the maximum q-range to ca. 28 nm^{-1}, covering a wide-angle regime. With increasing BSA concentration, we observed a decrease of forward intensity and appearance of a

Please cite this article as: K. Yanase, et al., J. Mol. Liq. (2014), http://dx.doi.org/10.1016/j.molliq.2014.05.021