Studies of conformational changes induced in a carrier protein: Bovine serum albumin

N. El Kadi*, N. Taulier, W. Urbach[§±], and M. Waks*
*Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7623, Laboratoire d'Imagerie Paramétrique (LIP), Paris F-75006, France, kadpont@yahoo.fr
and Université Pierre et Marie Curie-Paris6, Paris F-75005, France; [±]UFR Biomédicale, Université René Descartes, 75006 Paris, France; and [§] Laboratoire de Physique Statistique, UMR, 8550 CNRS, Ecole Normale Supérieure, 75231 Paris Cedex 05, France

ABSTRACT

In the present work we have used bovine serum albumin (BSA), the most abundant macromolecule in the circulatory system. It is a carrier protein for ligands and for drugs. The aim of our work is to investigate the correlation existing between the protein conformation and its compressibility measured by an ultrasonic technique developed in the laboratory (LIP). We have examined the behaviour of BSA during its unfolding and its refolding at low pH, with the help of osmolytes. We have studied also the binding of amphiphile molecules to the protein. All together, our results demonstrate the very close correlation existing between these methods and circular dichroïsm. They also underline the importance of hydration in protein design, for example of de novo drug carriers, modelled on albumin.

Keywords: albumin, amphiphiles, adiabatic compressibility, circular dichroïsm, conformational transitions, hydration.

1 INTRODUCTION

Serum albumin is the most abundant protein in the circulatory system. At neutral pH, the crystal structure of the 66-kD protein reveals a heart-shaped molecule with a high α-helix content (70%) and a large number of disulfide bonds [1]. The sequence of bovine serum albumin (BSA) is 76% identical to that of human serum albumin (HSA) [1, 2]. Serum albumin carries a large number of metabolites, fatty acids, hormones, and drugs [3]. The reversible conformational modification, induced by decreasing pH in the 2 range, was described several decades ago by Foster [4]. Since no albumin crystal structure is so far available at low (or high) pH values, the conformational changes induced at these pH values are not known at atomic level resolution. However the conservation of the above transition in a number of animal species suggests for it an important physiological role, probably linked to the ligand/drug release and distribution mechanisms [2], making it an important problem to explore.

Any protein transition should therefore be reflected by corresponding changes in system volume and compressibility. Recent developments in acoustic techniques have made possible high precision ultrasound velocimetry measurements of small volume samples, leading to estimation of protein compressibility [5]. The variation in partial specific volume and adiabatic compressibility associated with the pH-induced conformational transition have been determined. Furthermore, to investigate the reversal of the conformational transition and its effect on the protein compressibility, we have carried out the refolding of BSA induced by sorbitol at pH 2. In each case of our study, the compressibility variation have been correlated with the change of the α-helix content using circular dichroism (CD) under similar conditions. Our aim is to investigate the possible correlation between compressibility variation and the partial unfolding and refolding of BSA at acid pH. In addition we have studied the binding of amphiphile molecules to the protein. We have investigated in the first time the BSA adiabatic compressibility as a function of CTAC concentration and in the second time we have studied the behaviours of albumin during the fixation of its physiological ligand: myristic acid. These results are of value for the design and the developpement of new, tailor-made drug carriers, an important area for future research.

2 MATERIALS AND METHODS

2.1 Materials

BSA was purchased from Sigma (St. Louis, MO) (A-0281, 99% pure minimum, fatty acid free) and used without further purification. Sorbitol, crystallized 99% pure, was from FLUKA (Buchs, Switzerland). All the other chemicals were analytical grade. Water was of MILLIQ purity.

2.2 Sample preparation

BSA was dried under vacuum for at least 24 h and the samples prepared by weighing the dry protein in volumetric flasks (class A60.04 ml) on a model AG 245 Metler Toledo balance (Greitensee, Switzerland) with a precision of 60.03 mg. The protein concentration of the samples used for measurements was ~ 3 mg/ml. The solvents used to make up the volume at 20°C, (water or 0.02 M phosphate buffer) were brought to the required pH value with 0.1 M HCl. The pH of the solutions was determined on a PHMB 82 Radiometer (Copenhagen, Denmark) pH meter.

2.3 Volumetric measurements

The densities of solvents and protein solutions were determined at 25°C ± 0.01°C, using a vibrating tube Anton Paar DMA 58 digital density meter (Graz, Austria). The precision in density is better than 10^{-5} g/ml. The averaged value of 10 measurements has been used to calculate the protein apparent specific volume φ_v using the well-known relationship:

$$\varphi_v = \frac{1}{\rho_0} - \frac{\rho - \rho_0}{\rho_0 C} \quad (1)$$

where (ρ) and (ρ_0) are the relative densities of the protein solution and of the reference solvent, respectively, and c is the protein concentration.

2.4 Ultrasound velocity measurements

The method used is based on measured resonance peaks of an ultrasonic resonator [6], with precision higher than 10^{-5} m/s permitted by recent instrumental and computational advances.

2.4.1 The principle

Ultrasonic resonators consisting of a liquid sample (volume, 1 ml) enclosed by two planar piezoelectric transducer plates [7] with a central frequency of 5 MHz. The relative specific sound velocity increment is given by:

$$[u] = \frac{u - u_0}{u_0 C} \quad (2)$$

where u and u_0 are the ultrasound velocity in the solution and the solvent, respectively.

2.4.2 The system

We have used two identical cells to avoid temperature fluctuations [5]; experiments are carried out at 25°C, in a temperature-controlled room. The setup allows sequential determination of the ultrasonic frequency difference between a reference and a measuring cell. First, the two cells are filled with the reference liquid and measurements are carried out. In a second step the measuring cell is drained, rinsed, and refilled with the liquid under investigation.

2.5 Compressibility calculation

By using Laplace's equation:

$$\beta = \frac{1}{\rho u^2} \quad (3)$$

we can determine the solution adiabatic compressibility, β, equations 1, 2, and 3 were used to calculate the partial specific apparent adiabatic compressibility, φ_k, of BSA using the following relationship:

$$\varphi_k = \beta_0 \left(2\varphi_v - 2[u] - \frac{1}{\rho_0} \right) \quad (4)$$

where β_0 is the coefficient of adiabatic compressibility of the solvent [8].

2.6 Circular dichroism

CD spectra were recorded using an AVIV model 62A (Aviv Associates, Lakewood, NJ) spectropolarimeter. The temperature of the sample was controlled at 25°C ± 0.1°C. In the far ultraviolet (UV), a 0.1-cm path length cuvette was employed. The results are expressed as mean residue ellipticities. Secondary structure analysis was carried out using the CD Pro suite of programs [9].

3 RESULTS AND DISCUSSION

3.1 Unfolding and compressibility

The curves measured by compressibility (on the left) and ellipticity difference $\Delta[\theta]_{220}$ (on the right) superimpose well (Fig. 2 b), indicating that the main conformational change associated with changes in hellicity (in the 5–2 pH range) is responsible for the change in compressibility or, in other words, the two methods probe the same phenomenon. We observe that the adiabatic compressibility decreases continuously with decreasing pH, indicating again a parallelism between the pH dependence of ellipticity at 220 nm and compressibility changes. But an interesting phenomenon, occurring in the range of pH between 7 and 4, concerns the progressive increase in BSA compressibility ($\Delta\varphi_k$ = 1.0 x 10^{-3} m^3 kg^{-1} Pa^{-1}) shown in Fig. 1. This is due to an unexpected sigmoidal rise of the (214) tryptophan fluorescence, reaching a 2.4-fold increase in intensity at pH 4, as had been previously reported in the pioneering work of Foster [10]. This effect was explained by a change of the tertiary structure of the protein leading to a

transition of the fluorophore (located in domain II of HSA) from an aqueous environment to a more hydrophobic one and by the expulsion of water in contact with the aromatic residue.

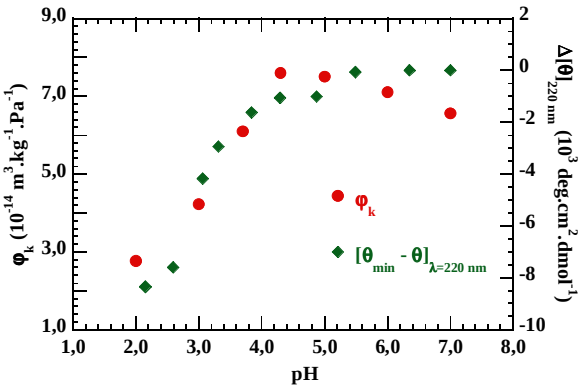

FIGURE 1 Plot of the ellipticity difference on the right (♦). $\Delta[\theta]_{220}$ represents the difference in ellipticity measured at pH 7 and at any pH value below 7. On the left, the partial specific adiabatic compressibility φ_k of BSA versus pH (○).

3.2 Compressibility and refolding

3.2.1 The sorbitol-induced transition

The osmolytes are known to induce the refolding of proteins and to stabilize them [11]. We have used here sorbitol, an electrically neutral, low molecular weight polyol to induce refolding of BSA at low pH unfolded state. As a basis for comparison, we first examined the behavior of the BSA native state at pH 7 as a function of sorbitol concentration in the 0–3 M range. At neutral pH, we observe a decrease of volume and compressibility of BSA, these results were interpreted as the consequence of water release from the protein interior followed by the collapse of its cavities [12]. At pH 2, both volume and compressibility of BSA increase as a function of sorbitol concentration (Fig. 2, a and b). Thus whereas the α-helix content of BSA is progressively restored and the protein refolded up to 80% of its initial ellipticity value at 220 nm, compressibility and volume both reach values close to those of the native protein. Furthermore at pH 2, once the protein is partially refolded, it behaves toward sorbitol as the native protein at pH 7. This fact could explain the decrease of compressibility at sorbitol concentrations higher than 1 M (Fig. 2, a and b).

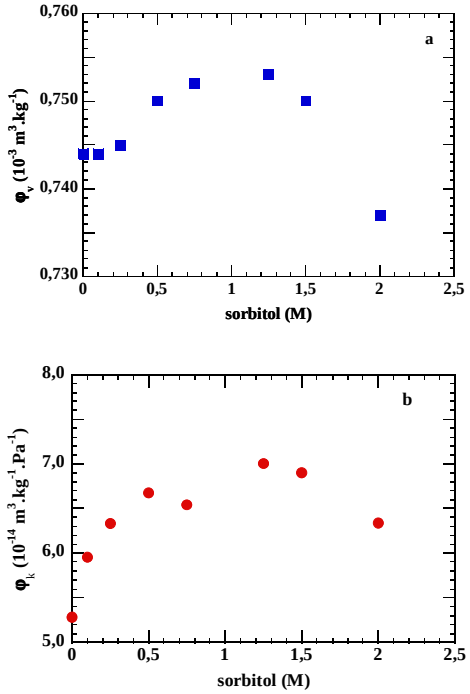

FIGURE 2 BSA at pH 2 (a) Plot of φ_v, the apparent partial specific volume, as a function of sorbitol molarity (M). The standard deviation in volume measurements is ±0.003 X 10^{-3} m^3 kg^{-1}. (b) Plot of the adiabatic compressibility φ_k as a function of sorbitol (M).

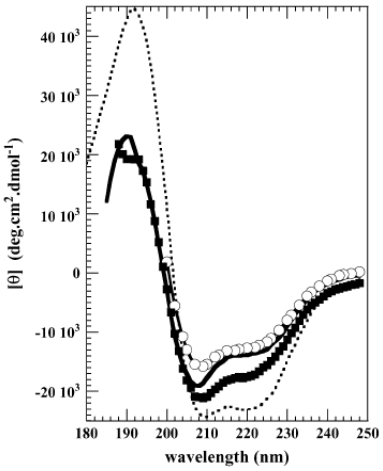

FIGURE 3 CD spectra of BSA in the far UV. At pH 7 (dashed line). At pH 2, in water (○), in 0.5 M sorbitol (solid line), and in 1 M sorbitol (■).

3.3 Interaction effect of CTAC on the BSA molecule

CTAC (Cetyltrimethyl ammonium chloride) is a cationic surfactant, is known to bind strongly globular proteins, induces conformational changes. We have investigated the BSA adiabatic compressibility as a function of CTAC

concentration during the binding of this amphiphile molecule to the BSA. Tabak [13] had shown that the protein binds as a first step CTAC in a molar ratio of 5:1 CTAC/protein. From our results we did not observe any change in conformation even to a report of CTAC/BSA = 8. By contrast, the maximum of apparent adiabatic compressibility and specific volume correspond to the ratio CTAC/BSA = 20, we can conclude as a first approximation, that the increase of partial specific volume and adiabatic compressibility correspond to the binding of CTAC and the release of water molecules. Up to molar ratio CTAC/BSA = 20 the decrease of partial specific volume and adiabatic compressibility correspond to the unfolding of the protein, which we are observing with CD (Fig. 4).

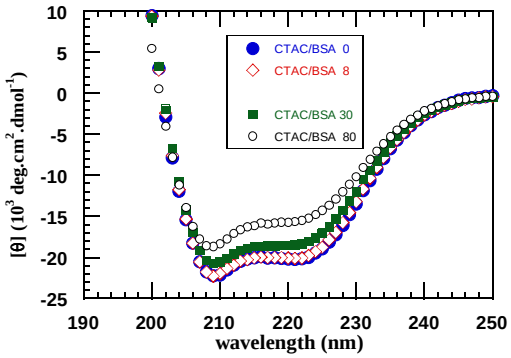

FIGURE 4 CD spectra: ellipticity variation of BSA as function of molar ratio CTAC/BSA.

3.4 Interaction effect of myristic acid on the BSA

Albumin serves as a transport vehicle for several endogenous compounds and non-esterified fatty acids, delivered to tissues according to the metabolic demand; the multiplicity of hydrophobic sites on the BSA, increases the solubility of long chain fatty acids. The crystallographic studies of complex HSA-fatty acids have revealed the presence of 7 binding sites on protein [14]. Our volumetric measurements of this binding reveal a maximum at a myr/BSA molar ratio of 7 (Fig. 5), which corresponds precisely to the maximum number of binding sites e.g. maximum number of fatty acids (high and low affinity) molecule bound by BSA as reported by recent NMR studies [15]. At higher ratio values, a very slow decrease of the compressibility is observed, which might be interpreted by the formation of myristate micelles.

These results shows once again the extremely high sensitivity and accuracy of ultrasonic techniques, which are used to reach valuable results. The high sensitivity of ultrasonic techniques provides a wide range of applications in multiple industries and economics processes. At the industrial level, the ultrasonic measurements used to monitor the conformational transition of a synthesized protein. Another important feature of ultrasonic techniques is its ability to be used for probing a broad range of samples including opaque materials unlike the spectroscopic measurements; the technique does not require optically transparent solutions.

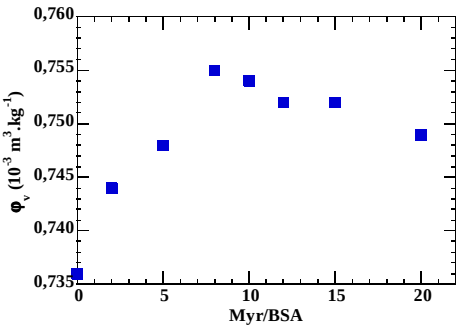

FIGURE 5 Variation of partial specific volume as function of molar ratio Myr/BSA, the standard deviation in volume measurements is ±0.003 X 10^{-3} m^3 kg^{-1}.

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