Inorganica Chimica Acta, 107 (1985) 133–137

Preferential Solvation of Bovine Serum Albumin in Cobalt Nitrate Solutions

M. TRUEBA*, A. VALLEJO, A. ZATÓN** and C. ABAD+

Departamento de Bioquímica, Facultad de Ciencias, Universidad del País Vasco, Apartado 644, 48080 Bilbao, Spain

Received January 3, 1985

Abstract

Interaction between bovine serum albumin and Co(II) cation was studied by equilibrium dialysis-differential refractometry, viscometry and dilatometry at different cobalt salt concentrations. Preferential absorption parameters and specific viscosity were determined from refractometric and viscometric measurements. This interaction produces structural changes in bovine serum albumin depending on the metal ion and protein concentrations. The results obtained by refractometric and viscometric techniques can be correlated with those deduced from dilatometric studies.

Introduction

Bovine serum albumin, BSA, is a single-chain globular protein with a known aminoacid sequence [1, 2]. Its structure has been determined by X-ray crystallographic analysis [3]. Optical [4], hydrodynamic [5], electrophoretic [6], dilatometric [7, 8] and ^{13}C NMR spectra [9] techniques have been used to follow the denaturation of BSA under several conditions.

A pH-dependent conformational change for this protein around physiological pH has been described [10–12]. Zurawski and Foster [13] showed evidence for the existence of two conformational states and a transition from a neutral (N) to a basic (B) form as pH increases. This transition is also affected by calcium ions [14–16].

In a previous paper [17] we correlated volume changes accompanying lysozyme–metal ion interactions with the solvation and conformation of this protein. It has been demonstrated that techniques such as dilatometry, viscometry and differential refractometry make up a good set in order to establish conformational changes in proteins. In the present work a similar study is made for BSA–cobalt nitrate systems. The evolution of the λ solvation parameter as a function of cobalt salt concentration is related to volume changes and specific viscosity under the same conditions.

Experimental

Albumin used in this study was crystallized and lyophylized bovine serum albumin A-4378, lot n° 38-C-8160, obtained from Sigma Chemical Co. and used without further purification. The purity of the preparation was checked by polyacrylamide gel electrophoresis.

Molecular weight of the albumin was taken as 66 000. Analytical reagent grade metal nitrates and potassium chloride were obtained from Merck, Darmstadt. All solutions were carefully degassed before use.

Volume changes at 30.0 ± 0.01 °C were measured according to the procedure previously described [17]. The volume change produced by mixing both components is referred to as ΔV. ΔV values were measured 5 to 15 min after mixing. Values reported here are averages of two or three experiments and could be reproduced to ±0.07 μl, which leads to an uncertainly of 5 ml/10^5 g of protein.

Blank experiments were run in which the Co(II) solutions were mixed with distilled water. Volume changes observed in these experiments were subtracted from those obtained by adding the protein uncertainty of 5 ml/10^5 g of protein.

Differences in refraction index were measured with a Brice-Phoenix differential refractometer model BP-2000 V at 546 nm and 30 °C, and employing a sealed-type differential cell with ground-glass stoppers to prevent solvent losses. Calibration was made with aqueous solutions of highly purified KCl, taking the data of Kruis [18] as reference; the temperature difference between the bath and the cell did not exceed 0.02 °C. Values of $(dn/dc)_\kappa$ were determined by the method of Hertz and Strazielle [19] as described previously [20]. Values of $(dn/dc)_\mu$ were obtained from the refractive index differences between protein solutions and their dialysates.

*Author to whom correspondence should be addressed.

**Present address: Departamento de Bioquímica, Colegio Universitario de Alava, Apartado 450, Vitoria, Spain.

+Present address: Departamento de Bioquímica, Facultad de Ciencias Químicas, Universidad de Valencia, Burjasot, Valencia, Spain.

0020-1693/85/$3.30

The membranes used for equilibrium dialysis were Wisking tubing from Union Carbide Corporation, Chicago, and were pretreated as described by Cooper and Wood [21].

Viscometric measurements were made using a modified Ubbelhode suspended level viscometer, thermostatted at 30 °C. Solutions were subjected to dialysis equilibrium and experimental measurements were carried out comparing flow times of each dialysed solution and the corresponding dialysed solvent.

A Phillips pH meter model PR-9403-01 with a semimicro combination electrode was used for pH determinations and a Beckman spectrophotometer model UV-5260 for spectrophotometric measurements at 510 nm.

Results

By means of dialysis equilibrium and differential refractometry, the preferential adsorption (or solvation) parameter λ was obtained for the BSA–Co(II) system at a protein concentration of 7.57×10^{-5} M. This technique has been used by other authors [22–24] as well as by ourselves in studies dealing with proteins in dilute urea solutions [20] and lysozyme in cobalt or copper nitrate solutions [17]. The evaluation of λ has been previously explained in detail [20]. Positive λ values represent the preferential solvation of water (component **1**) by protein (component **3**), and negative values the preferential solvation of metal ion (component **2**).

Table I summarizes the experimental data needed for λ determinations. v_{02} is the volume fraction of component **2** in the solvent mixture, without protein and before dialysis. $(dn/dc)_\kappa$ and $(dn/dc)_\mu$ are variations of refractive index with protein concentration before and after dialysis respectively, and dn/du_2 is the refractive index variation with the volume fraction of component **2** without protein. An absolute error of ±0.03 ml/g was estimated for all the λ measurements.

For all cobalt concentrations (or volume fractions of component **2**) λ values are always positive (Table I), indicating that albumin is preferentially solvated by water (preferential hydration) in the ternary system water **1**–cobalt **2**–albumin **3**. Maximum water solvation occurs at 0.024 M total Co(II), and the minimum at 0.048 M Co(II) concentration.

The λ parameter was compared with the intrinsic viscosities obtained by the viscometric technique, as described previously [17]. Relative errors calculated for $(\eta_{sp})/c$ values are 1–2%. When comparing the results in Fig. 1 it can be stated that the $(\eta_{sp})/c$ variation as a function of cobalt salt concentration is similar to the evolution of λ. At a total cobalt nitrate concentration of 0.048 M a

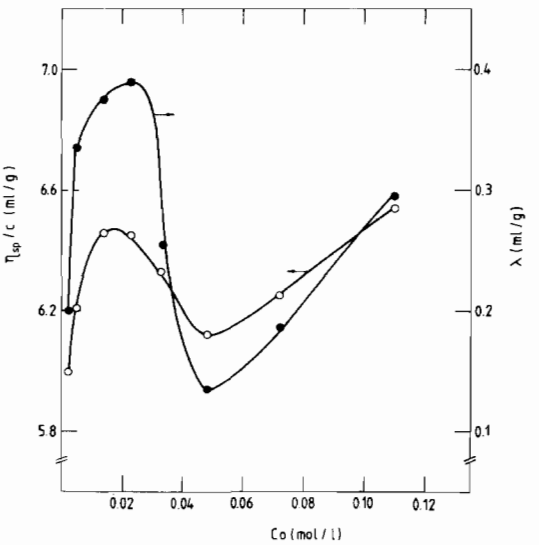

Fig. 1. Plot of $(\eta_{sp})/c$ and λ against Co(II) concentrations. (○): $(\eta_{sp})/c$ is indicated with an arrow toward the left ordinate; (●): λ is the preferential adsorption parameter and is indicated with an arrow toward the right ordinate. Protein concentration is 7.6×10^{-5} M.

TABLE I. Values Obtained for Albumin–Cobalt(II) System from Equilibrium Dialysis-differential Refractometric Measurements. $dn_0/du_2 = 0.024$; $\rho_{Co(NO_3)_2} = 1.8725$ g/ml.

Equilibrium concentration (M)	$v_{02} \times 10^2$	$(dn/dc)_\kappa$ (ml/g)	$(dn/dc)_\mu$ (ml/g)	λ (ml/g)
0.002	0.004	0.192	0.188	0.197
0.005	0.007	0.197	0.189	0.331
0.014	0.022	0.195	0.186	0.378
0.024	0.037	0.198	0.189	0.390
0.033	0.052	0.195	0.189	0.255
0.048	0.074	0.188	0.185	0.135
0.072	0.112	0.191	0.197	0.185
0.110	0.171	0.193	0.186	0.295

minimum in $(\eta_{sp})/c$ as well as in λ is observed. Similarly a maximum value in both parameters is attained at total cobalt nitrate concentrations between 0.014 and 0.033 M.

It is clear that λ is a relative measure of the composition of the binary system in the proximity of the macromolecule in relation to the bulk solvent. Consequently albumin interacts with the components of the binary system water–cobalt, but preferentially with only one of them. For this reason cobalt binding to albumin has also been studied using difference spectroscopy, according to Ahlfors [25]. Albumin binding was found to enhance the extinction coefficient for cobalt salt at 510 nm. The absorption (510 nm, 30 °C) of a solution containing 1.5×10^{-6} M Co(II) and 1.5×10^{-4} M albumin was used in order to determine $\epsilon_1 \times \epsilon_2$, calculated from the slope of the corresponding absorbance against metal salt concentration calibration. At several cobalt nitrate concentrations the unbound Co(II) was calculated at several protein concentrations and association constants, K_a, were evaluated from Scatchard plots [26].

Figure 2 shows the results obtained for $\bar{\nu}$ expressed as mol of bound ligand/10^5 g protein at different

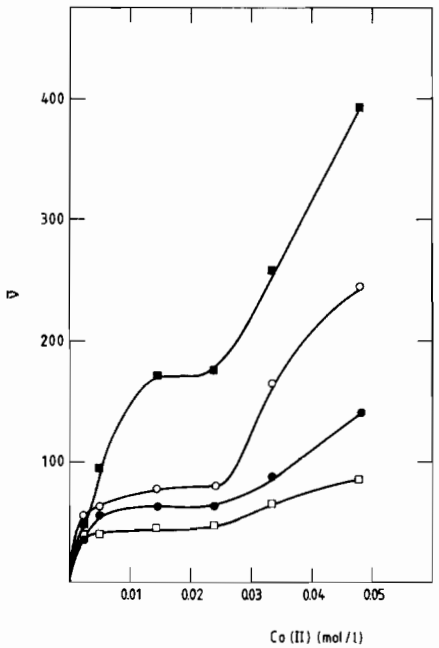

Fig. 2. Plot of $\bar{\nu}$ against total cobalt(II) concentrations. $\bar{\nu}$ values indicate mol Co(II) bound to albumin/10^5 g protein. (△): 1.9×10^{-5} M; (○): 3.8×10^{-5} M; (●): 7.6×10^{-5} M and (□): 1.5×10^{-4} M of BSA respectively.

protein concentrations, as a function of total metal ion concentration (free + bound). $\bar{\nu}$ increases at low salt concentrations up to about 0.014 M; after a plateau it increases again markedly between 0.024 and 0.048 M. This effect is more pronounced at low protein concentrations. From $\bar{\nu}$ results and plotting $\bar{\nu}/\nu \times c$ *vs.* $\bar{\nu}/c$ (being ν, unbound cobalt concentration and c protein concentration, both expressed as molarities) the association constants, K_a, may be evaluated (Fig. 3). K_a determined in this way are 2.0 $\times 10^{-3}$ M^{-1} and 2.9×10^{-3} M^{-1} for albumin concen-

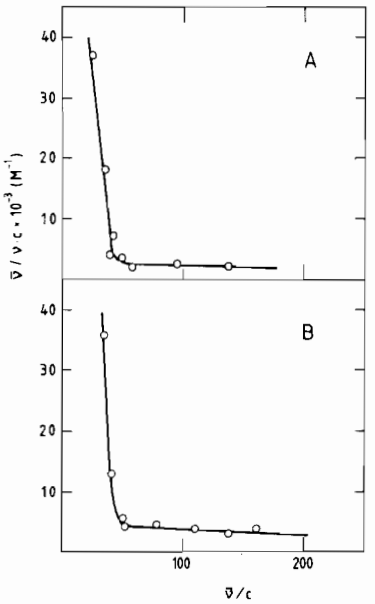

Fig. 3. Scatchard plots for albumin–Co(II) systems. (A): 7.6×10^{-5} M and (B): 3.8×10^{-5} M of BSA respectively.

trations of 7.6×10^{-5} M and 3.8×10^{-5} M respectively, and are close to those described by other authors [27, 28].

On the other hand, viscometric results have been compared to those obtained by the dilatometric technique. Intrinsic viscosities of proteins are proportional to the corresponding hydrodynamic volumes, and volume changes can be used to determine the partial molar volume of protein [29, 30]. Consequently both may be compared from a qualitative point of view.

Volume effects for albumin–Co(II) interactions at protein concentrations of 7.6×10^{-5} M and 3.8×10^{-5} M are shown in Fig. 4. The experimental procedure is based on those described by Katz and Ferris [31] and Katz and Roberson [32] and has been used by us as described previously [17]. Volume effects are always positive and are a function of metal concentration. It is observed that δV increases with increasing metal concentration until a maximum is attained at 0.033 M, which corresponds to 251 ml/10^5 g of protein for 7.6×10^{-5} M albumin and 243 ml/10^5 g of protein for 3.8×10^{-5} M albumin. For higher metal ion concentrations, δV decreases reaching a minimum at 0.048 M. Qualitatively, the variation of δV as a function

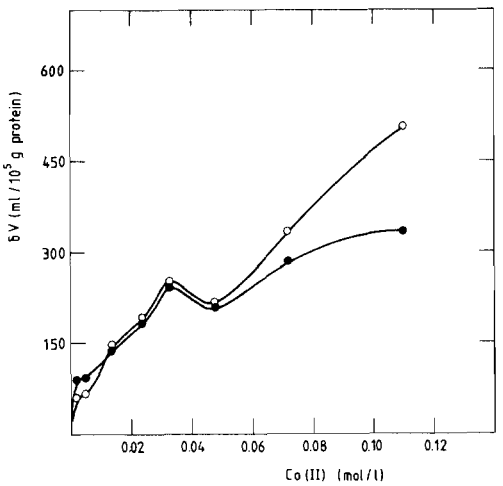

Fig. 4. δV isotherm produced by the reaction of albumin with cobalt(II). (○): 3.8×10^{-5} M and (●): 7.6×10^{-5} M of BSA respectively.

of cobalt nitrate concentration (Fig. 4) is similar to the variation of $(\eta_{sp})/c$ and λ under the same conditions (Fig. 1).

Discussion

Albumin is present in many biological fluids and binds a wide variety of organic and inorganic ligands [33]. The behaviour of bovine serum albumin in cobalt nitrate solutions has been examined in terms of the preferential solvation parameter, λ, deduced from dialysis equilibrium-differential refractometry, and compared with the results of intrinsic viscosity and dilatometric experiments.

The dependence of preferential solvation of BSA upon concentration of cobalt nitrate salt is given in Fig. 1 and Table I. λ determination at 30 °C indicates that BSA solvates preferentially water in all the metal ion concentration range used, in contrast with dilute urea solutions [20]. A striking feature of the results is the large amount of preferential water binding that occurs with BSA in relation to lysozyme in the same range of metal concentrations [17]. On the other hand the λ isotherm exhibits a maximum at ≃0.03 M total metal concentration and a minimum at 0.05 M (Fig. 1). Changes in λ seem to be related to variations in binding of the other component, the metal ion (Fig. 2). As the preferential solvation gives a measure of the difference between solvent composition in the protein domain and outside it, the decrease in the preferential solvation of one component (*i.e.* water in the metal concentration range between 0.03 and 0.05 M) implies a selective binding of the other component to the protein, metal ion (Figs. 1 and 2).

Previous studies have shown that viscometric [17, 30] as well as dilatometric techniques [7, 34, 35] are a good tool to detect conformational changes. The addition of metal ions, such as copper(II) to lysozyme [17, 36] or copper(II) to albumin [30, 37] causes a change in volume effects. These changes increase as the protein concentration diminishes [17, 30, 36] and the more pronounced changes were interpreted as being due to protein structural alterations. The dilatometric studies reveal that the volume effects produced by protein-cation interaction are determined primarily by the protein [30, 32, 36] and the type and concentration of cation involved [17, 32]. Inspection of data in Fig. 4 show that volume effects produced by albumin–cobalt(II) interaction differ substantially from those obtained for albumin copper(II) [30] and lysozyme–cation interaction [17]. Volume changes are higher than those obtained for lysozyme–Co(II) systems [17] and lower than those detected for BSA–Cu(II) ones [30]. The δV at 0.05 M Co(II) are in good agreement with those described by Katz and Roberson [32]. The interaction of Co(II) with albumin produced a volume increase with increasing cation concentration up to 0.03 M, which may be correlated with an increase in λ. Furthermore, the decrease observed in δV between 0.03 and 0.05 M is accompanied by a diminution in λ. That is, volume changes are a consequence of the high or low water solvation, as has been already observed for lysozyme [17].

As in the case of copper(II) [38, 39] cobalt(II) can complex carboxylate residues, nitrogen donor atoms, and the peptide backbone. Carboxylate groups have more hydrogen-bonded water than do nitrogen donor atoms such as lysine. Similarly, the carbonyl oxygens in the peptide backbone characteristically have more water bonded than to N–H groups [40]. These data lend credence to the hypothesis that coordination to an oxygen donor atom will release more water than the corresponding process involving nitrogen donor atoms [37].

On the other hand, the analysis of the dilatometric isotherms provide information about conformational changes of proteins in solution [7, 34, 41]. The present results show a change in the sign of the slope of the δV isotherm between 0.03–0.05 M, signifying that BSA undergoes a structural change. BSA upon dilatometric titration gave evidence for at least three structural transitions as a function of pH. Although the protein ligand interaction determines the character of consequent volume effects [35], pH and the ionic environment plays a small but significant role in the overall ΔV process. It may be noticed that buffers and supporting electrolytes were avoided to preclude competitive binding effects [37], but these studies were conducted outside the pH range where pH transitions were detected. It is tempting to conclude that the observed

changes in pH solutions (between 6.1 and 5.4) are not related to the present structural change. The pH contribution to the volume change results only in a quantitative correction of an 1–3% of the parameter evaluated for the protein–metal interaction.

It has been described also that the uptake of Co(II) by the carboxyl sites of BSA tends to induce conformational changes [42] and that the coordination of Cu(II) to BSA at pH 7.4 produces a cooperative binding isotherm and a curvilinear volume isotherm [37], indicating that cupric ions can produce a structural transition similar to the alkaline-induced neutral transition [15] at salt copper concentration below 5×10^{-5} M. The present experiments show a gross transition between 0.03 and 0.05 M of metal ion. Studies with copper(II) [30] or lysozyme [17] indicate a similar metal ion-dependent conformational change. A salt can produce structural alteration or denaturation by direct interaction with the macromolecule, or indirectly by altering solvent properties [23]. The foregoing evidence suggest that structural alteration observed in BSA is not due to a direct salt binding but rather to a general effect of the salt on the solvent because, although the lower magnitude, it occurs over the same range of metal ion concentration than is observed for lysozyme [17] or for BSA itself in copper nitrate solutions [30, 36].

References

1 T. Peter, *J. Clin. Chem., 14*, 1147 (1968).
2 J. R. Brown, *Fed. Proc., 34*, 591 (1975).
3 R. J. McClure and G. M. Craven, *J. Mol. Biol., 83*, 551 (1974).
4 P. Callagham and N. H. Martin, *Biochem. J., 83*, 144 (1962).
5 S. Takashima, *Biochim. Biophys. Acta, 79*, 531 (1964).
6 T. G. Ferris and S. Katz, *Nature (London), 211*, 586 (1966).
7 S. Katz, M. E. Shaw, S. Chillag and J. E. Miller, *J. Biol. Chem., 247*, 5228 (1972).
8 S. Lapanje and J. Skerjanc, *Biochem. Biophys. Res. Commun., 56*, 338 (1974).
9 Y. Inoue, S. Sase and R. Chujo, *Biopolymers, 18*, 373 (1979).
10 W. J. Leonard, K. K. Vijai and J. F. Foster, *J. Biol. Chem., 238*, 1984 (1963).
11 D. E. Coldsack and P. M. Waern, *Can. J. Biochem., 49*, 1267 (1971).
12 K. K. Wallevik, *J. Biol. Chem., 248*, 2650 (1973).
13 V. R. Zurawsky and J. F. Foster, *Biochemistry, 13*, 3465 (1974).
14 S. Katz and I. M. Klotz, *Arch. Biochem. Biophys., 44*, 351 (1953).
15 B. J. M. Harmsen, S. H. De Bruin, L. J. M. Janssen, J. F. Rodrigues de Miranda and G. A. J. Van Os, *Biochemistry, 10*, 3217 (1971).
16 J. Wilting, M. M. Weideman, A. C. J. Roomer and J. H. Perrin, *Biochim. Biophys. Acta, 579*, 469 (1979).
17 C. Abad, M. Trueba, A. Campos and J. E. Figueruelo, *Biophys. Chem., 14*, 293 (1981).
18 A. Kruis, *Z. Phys. Chem., Abt. B:, 34*, 13 (1936).
19 M. Hertz and C. Strazielle, *Makromol. Chem., 175*, 2149 (1974).
20 C. Abad, M. Trueba, A. Zatón and A. Campos, *Anal. Quim., 75*, 993 (1979).
21 P. F. Cooper and G. C. J. Wood, *Pharm. Pharmacol. Suppl., 20*, 150 S (1968).
22 J. Span, S. Lenarcic and S. Lapanje, *Biochem. Biophys. Acta, 395*, 311 (1974).
23 M. E. Noelken, *Biochemistry, 9*, 4122 (1970).
24 J. Span and S. Lapanje, *Biochim. Biophys. Acta, 295*, 371 (1973).
25 C. E. Ahlfors, *Anal. Biochem., 110*, 295 (1981).
26 G. Scatchard, *Ann. N.Y. Acad. Sci., 51*, 660 (1949).
27 A. K. Nandadhar, P. K. Basn and F. Friedberg, *Bioinorg. Chem., 2*, 149 (1973).
28 I. V. Kolosov and A. P. Bondareva, *Koord. Khim., 4*, 427 (1978).
29 S. Lapanje and J. Skerjanc, *Biochem. Biophys. Res. Commun., 43*, 682 (1971).
30 C. Abad, M. Trueba, J. M. Macarulla and A. Campos, *Anal. Quim., 76*, 171 (1980).
31 S. Katz and T. G. Ferris, *Biochemistry, 5*, 3246 (1966).
32 S. Katz and L. C. Roberson, *Bioinorg. Chem., 6*, 143 (1976).
33 W. W. Sukow, H. E. Sandberg, E. A. Lewis, D. J. Eatough and L. D. Hansen, *Biochemistry, 19*, 912 (1980).
34 S. Katz, J. R. Crissman and J. A. Beall, *J. Biol. Chem., 248*, 2440 (1973).
35 S. Katz, J. E. Crissman and L. C. Roberson, *J. Biol. Chem., 249*, 7892 (1974).
36 C. Abad, M. Trueba and J. M. Macarulla, *Anal. Quim., 74*, 1318 (1978).
37 S. Katz, R. G. Shinaberry, E. L. Heck and W. Squire, *Biochemistry, 19*, 3805 (1980).
38 R. Nakon and R. J. Angelici, *J. Am. Chem. Soc., 96*, 4178 (1974).
39 S. Katz and R. G. Shinaberry, *Bioinorg. Chem., 8*, 215 (1978).
40 J. D. Watenpaugh, T. N. Margulis, L. C. Sicker and L. H. Jensen, *J. Mol. Biol., 122*, 175 (1978).
41 S. Katz, J. E. Miller and J. A. Beall, *Biochemistry, 12*, 710 (1973).
42 F. R. N. Gurd, in 'Physical Principles and Techniques in Protein Chemistry, Part B', Academic Press, New York, 1970, p. 365.