J. Serb. Chem. Soc. 72 (4) 331–337 (2007)
JSCS–3562

UDC 547.926.3+546.47:66.022.393+536.6
Original scientific paper

A DSC study of zinc binding to bovine serum albumin (BSA)

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(Received 20 March, revised 10 April 2006)

Abstract: The thermal denaturation of bovine serum albumin (BSA) is a kinetically and thermodynamically controlled process. The effects of zinc binding to bovine serum albumin (BSA), followed by differential scanning calorimetry (DSC), were investigated in this work, with the purpose of obtaining a better understanding of the albumin/zinc interaction. From the DSC curves, the thermodynamic parameters of protein denaturation were obtained, *i.e.*, the temperature of thermal transition maximum (T_m), calorimetric enthalpy (ΔH^{cal}), van't Hoff enthalpy (ΔH^{vH}), the number of binding sites (I, II), the binding constants for each binding site (K_{bI}, K_{bII}) and the average number of ligands bound per mole of native protein X_N. The thermodynamic data of protein unfolding showed that zinc binding to bovine serum albumin increases the stability of the protein (higher values of ΔH^{cal}) and the different ratio $\Delta H^{cal}/\Delta H^{vH}$ indicates the perturbation of the protein during thermal denaturation.

Keywords: bovine serum albumin, thermal denaturation, DSC, zinc, binding.

INTRODUCTION

Proteins are dynamic entities and can be denatured in the presence of certain organic small molecules, such as urea and guanidine hydrochloride (chemical unfolding), or by the effect of temperature (themal unfolding).

The serum albumins belong to the multigene family of proteins that includes α-fetoprotein (AFP) and the human group specific component (Gc) or vitamin D-binding protein. They are relatively large multi-domain proteins which, as major soluble protein constituents of the circulatory system, have many physiological functions.[1,2] Serum albumin is the most abundant protein in blood plasma and possesses the capacity of reversible binding of a great number of substances, including bilirubin, hormones, drugs and ions. The albumin–ion interaction might influence the absorption, transport, metabolism and excretion of ions.[1] A high homology between human serum albumin (HSA) and bovine serum albumin (BSA) has been found.[2,3] Albumin

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doi: 10.2298/JSC0704331O

has been used as a model protein for protein folding and ligand-binding studies. Bovine serum albumin (BSA) consists of three globular, helical homologous domains (I, II, III) and 17 paired disulfide bonds.[2,3] Each domain comprises two smaller subdomains, A and B. Roughly, 67 % of HSA is helical and the remainder is in turns and extended polypeptide. There are 10 principal helices in each domain (h l-h 10). The subdomains A and B share a common motif which includes h 1, h 2, h 3 and h 4 for subdomain A and h 7, h 8, h 9 and h 10 for subdomain B. The only exception is that the disulphide bridge connecting h 1 and h 3 does not exist in subdomain I A.[2] Stewart *et al.*[4] identified a potential five coordinate Zn site at the interface of domains I and II, consisting of N ligands from His-67 and His-247 (Nε of His-67 and Nδ of His-247) and O ligands from Asn-99 and Asp-249 (amide oxygen of Asn-99 and carboxyl oxygen of Asp-249) which are the same amino acid ligands as those in zinc enzymes calcineurin, endonucleotidase and purple acid phosphatase. Sequence comparisons showed that these four residues are completely conserved in all mammalian albumins sequenced to date.[4]

Zinc supports a healthy immune system and is needed for DNA synthesis. Zinc has structural and catalytic roles for many proteins and plays a fundamental role in expressing genetic potential, *i.e.*, in the synthesis and repair of the structural integrity of nucleic acids. Thus, zinc is indispensable for numerous physiological processes in humans, including growth, development, endocrinal functions, immune and nervous system.[5–8] Zinc is a small hydrophilic, charged ion, which can not cross biological membranes by passive diffusion.[5] Zinc transport is a temperature and pH-sensitive process.[9]

Although a protein is stable under certain conditions, the balance between folded and unfolded structures can be changed delicately by various factors, such as temperature, pH, inorganic salts, organic solvents, detergents and pressure.[1] DSC has proved to be a very useful method to estimate thermodynamic parameters and binding constants. During the process of thermal denaturation, the hydrogen bonding is weakened at higher temperature, but the hydrophobic interactions become strengthened. As heating is continued, some of the cooperative hydrogen bonds of the stabilized helical structure begin to break and expose the hydrophobic groups to the solvent unfolding the protein structure.[1] The denaturation of BSA and HSA might explain the transformation from the α-helix to the β-sheet.[1]

The goal of this study was to investigate the conformational stability of a model protein, bovine serum albumin (BSA) during the process of thermal denaturation in the presence of zinc ions, Zn (II), and to estimate the thermodynamic parameters of protein denaturation and zinc binding with the purpose of obtaining a better understanding of the albumin/zinc interaction.

EXPERIMENTAL

Bovine serum albumin (BSA) was purchased from Boehringer Mannheim (Fraction V) and used without further purification. $ZnCl_2$ and other chemicals were of *p.a.* grade and freshly pre-

pared. All protein solutions were degassed before being loaded into the calorimeter to prevent bubble entrapment in the cells. Calorimetric measurement were carried out on a MicroCal MC-2 sensitive differential scanning calorimeter (DSC) using the standard DA-2 software package for data acquisition and Origin software for DSC data analysis. A non two-state curve fitting model was used for estimating the thermodynamic parameters of protein unfolding: temperature of the transition maximum (T_m), calorimetric enthalpy (ΔH^{cal}), and van't Hoff enthalpy (ΔH^{vH}). All scans were performed in the temperature range from 283 K to 383 K at a scan rate of 90 K/h.

RESULTS AND DISCUSSION

The thermal denaturation process of BSA (0.05 mM) was studied under low ionic strength conditions (deionised water) and with the addition of 0.05 mM, 0.30 mM, 0.45 mM, 1.00 mM, 20.00 mM $ZnCl_2$, pH = 6.2. The obtained DSC curves showed that the thermal denaturation of the protein depended on the Zn(II) ion concentration. The higher values of the denaturation enthalpies (ΔH^{cal}) in the presence of zinc ions compared with those obtained in deionised water, as a reference, show that BSA was stabilized. The temperatures of the transition maximums (T_m) of protein unfolding were slightly decreased with increasing Zn(II) concentration. The shapes of the DSC curves (Fig. 1), presented as cooperativity measure $\Delta H^{cal}/\Delta H^{vH}$ (Table I), the number of independent domains undergoing denaturation, suggest the existence of perturbation during unfolding, which is coupled to the equilibrium betwen the folded and unfolded protein species.[10]

The ratio between $\Delta H^{cal}/\Delta H^{vH}$ with increasing zinc concentrations to 1mM Zn(II) (Table I) were greater than one, indicating that the denaturation mechanism is significantly different than a simple two state process,[11] showing that the transitions were less cooperative. It can suggest that the denaturation process of BSA in the presence of Zn(II) ions undergoes intermediate stages. The overall denaturation enthalpies, calorimetric enthalpy (ΔH^{cal}) and van't Hoff (ΔH^{vH}) and temperature transition maximum (T_m) of BSA unfolding are reported in Table I (higher values of ΔH^{cal} are in bold).

TABLE I. Thermodynamic parameters of BSA denaturation: temperature of transition maximum (T_m), calorimetric enthalpy (ΔH^{cal}) and van't Hoff enthalpy (ΔH^{vH})

$c(ZnCl_2)$ mM	T_{m1}/K	ΔH^{cal1} kJ/mol	ΔH^{vH1} kJ/mol	$\Delta H^{cal1}/\Delta H^{vH1}$	T_{m2}/K	ΔH^{cal2} kJ/mol	ΔH^{vH2} kJ/mol	$\Delta H^{ca12}/\Delta H^{vH2}$ kJ/mol	BSA:$ZnCl_2$ mole ratio
0	325.4	435.3	163.51	2.66	345.8	66.19	359.65	0.18	–
0.05	319.0	**802.28**	179.95	4.45	328.6	**434.29**	436.89	0.99	1:1
0.30	309.9	176.60	218.90	0.80	319.4	**426.14**	278.06	1.53	1:6
0.45	308.4	189.24	262.04	0.72	321.4	**847.26**	152.13	5.56	1:9
1.00	315.4	**655.80**	194.38	3.37	327.2	133.46	824.58	0.16	1:20
20.00	308.5	253.84	326.26	0.80	318.5	319.61	243.80	1.31	1:400

With increasing Zn(II) concentration in the protein solution, the thermal transitions become narrower, and stabilization effect of Zn(II) ions can be considered. Addition of different Zn(II) concentrations did not have a significant influence on the temperature of the transition maximum (T_m).

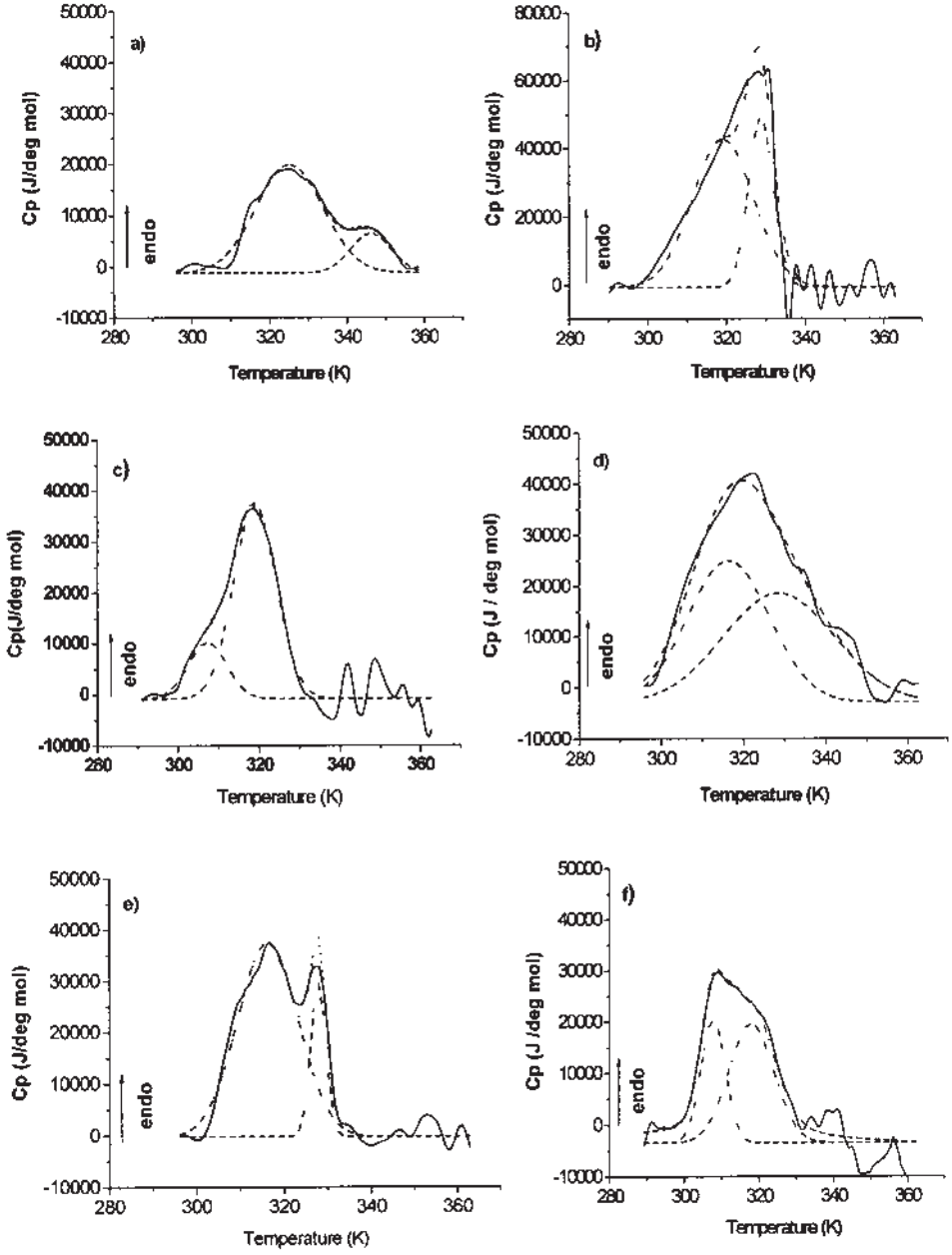

Fig. 1. DSC curves of BSA in deionised water and in the presence of different $ZnCl_2$ concentrations pH=6.2: a) deionised water; b) 0.05 mM $ZnCl_2$; c) 0.30 mM $ZnCl_2$; d) 0.45 mM $ZnCl_2$; e) 1.00 mM $ZnCl_2$; f) 20.00 mM $ZnCl_2$.

The curve profiles suggest two kinds of binding sites for Zn(II) on BSA, in agreement with the literature.[4,12] At each thermal transitions temperature (T_{m1} and T_{m2}), a binding constant (K_{bI} and K_{bII}) was calculated according to the equation (1).[13]

$$K_b = \exp(-\Delta H^{cal}/R(1/T-1/T_m) \quad (1)$$

K_b – binding constant at the reference temperature; ΔH^{cal} – enthalpy of denaturation; T_m – temperature of the transition maximum; R – universal gas constant.

The obtained binding constants of Zn(K_{bI}, K_{bII}) show that the binding of zinc ions occurs on two binding sites (site I and II), which is in agreement with the literature.[4,12] The binding constants (K_{bI}) for site I, and the binding constants (K_{bII}) for site II are presented in Table II and Table III, respectively.

TABLE II. Zn(II) binding constants (K_{bI}) for site II at the temperature midpoint T_{m1}

c/mM	T_{m1}/K	K_{b1}/K
0.05	319.0	7.0×10^{-9}
0.30	309.9	2.7×10^{-2}
0.45	308.4	4.7×10^{-2}
1.00	315.4	5.4×10^{-7}
20.00	308.5	3.6×10^{-2}

TABLE III. Zn(II) binding constants (K_{bII}) for site II at the temperature midpoint T_{m2}

c/mM	T_{m2}/K	K_{bII}/M
0.05	328.6	4.6×10^{-2}
0.30	319.4	1.0×10^{-3}
0.45	321.4	2.0×10^{-10}
1.00	327.0	5.0×10^{-3}
20.00	318.5	3.0×10^{-3}

As shown previously,[7,14,15] the average number of ligands bound per mole of native protein X_N, can be obtained from the heat capacity data by plotting the inverse transition temperature ($1/RT_m$) *versus* the logarithm of the total Zn (II) concentration (log [Zn(II)]). The average number of ligands bound per mole of native protein X_N can be determined from the slope of the plot (Fig. 2a and 2b).

The average number of ligands bound per mole of native protein X_N, as estimated from DSC data, was close to one for the first binding site ($X_{NI} = 0.77$), and seven for the second binding site ($X_{NII} = 7.3$). It can be assumed that the average degree of binding X_N, on site I (T_m) would be lower than on site II, which is in agreement with the obtained binding constants for site I (K_{bI}) and site II (K_{bII}) (Table III). The number of ligands bound per mole of native protein X_N on site II is higher, and regarding binding constants K_{bI} and K_{bII} (Tables II and III), it can be assumed that site II has a higher affinity for Zn(II).

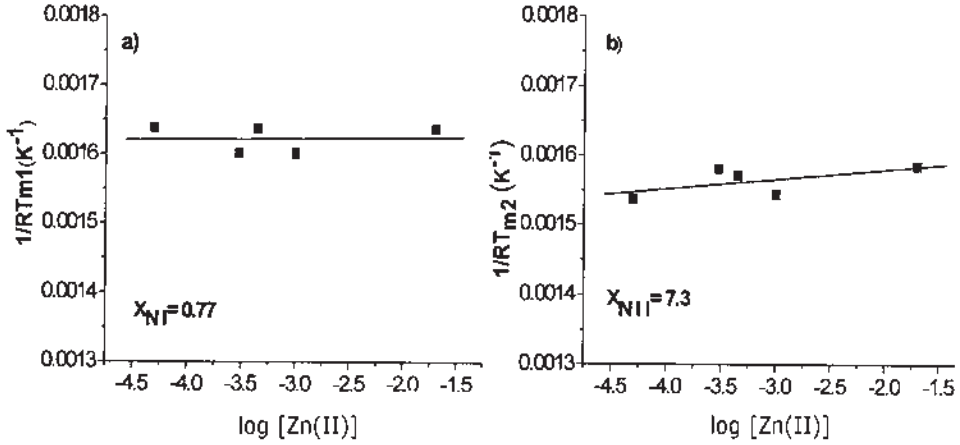

Fig. 2. Plot of the inverse transition temperature ($1/RT_m$) *versus* logarithm of the total Zn(II) concentration, log [Zn(II)]: a) determination of the average degree of binding X_{NI} at the transition temperature T_{m1}; b) determination of the average degree of binding X_{NII} at the transition temperature T_{m2} of BSA in the presence of different concentrations of Zn(II). Solid lines are the linear fits.

There are abundant binding data for Zn(II)–BSA interaction,[4,16–18] but there is still a disagreement about the affinity of the binding sites, because measurements have been performed by different methods and under different conditions, *i.e.,* different pH values, buffers, chelating agents, types of albumin *etc*., hence the results obtained can not be compared to literature data.

According to the results obtained in this work, it can be assumed that the interaction between BSA and Zn(II) is due to specific binding of Zn(II) on sites I and II.

CONCLUSION

The stability of proteins and their interaction with other molecules is a topic of special interest in biochemistry, because many cellular processes depend on ligand binding. These interactions have immediate consequences for protein stability, as shown by the varying thermodynamic properties of the system.[19]

This work has shown that the binding of zinc ions to the protein BSA in aqueous solution leads to conformational changes of the protein structure. It can be assumed that zinc binding to bovine serum albumin causes an increase in the stability of the protein, according to the values of ΔH^{cal} and the different $\Delta H^{cal}/\Delta H^{vH}$ ratios (Table I), which indicates perturbation of protein structure during thermal denaturation. The two obtained binding constants, K_{bI} and K_{bII}, show that the binding of zinc ions occurs on two binding sites (sites I and II) with the binding constants presented in Tables II and III. The average number of ligands bound per mole of native protein X_N, as estimated from DSC data, was close to one (0.77) for the first binding site (I), and seven (7.3) for the second binding site (II). From the obtained results it can be seen that the interaction between BSA and Zn(II) is due to specific

binding on sites I and II. The results obtained for the binding constants K_{bI} and K_{bII} (Tables II and III) suggest that the affinity for Zn(II) binding is higher at site II.

ИЗВОД

ПРОУЧАВАЊЕ ВЕЗИВАЊА ЦИНКА НА АЛБУМИН ГОВЕЂЕГ СЕРУМА ДИФЕРЕНЦИЈАЛНОМ СКЕНИРАЈУЋОМ КАЛОРИМЕТРИЈОМ (DSC)

САЊА ОСТОЈИЋ, ВИДА ДРАГУТИНОВИЋ, МИОДРАГ КИЋАНОВИЋ и БРАНИСЛАВ Р. СИМОНОВИЋ

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У овом раду ефекти везивања цинка на албумин говеђег серума праћени су методом диференцијалне скенирајуће калориметрије (DSC) ради испитивања цинк–албумин интеракције. Добијени термодинамички параметри термалне денатурације албумина говеђег серума су: температура максимума прелаза (T_m), калориметријска енталпија (ΔH^{cal}), Вант-Хофова енталпија (ΔH^{vH}), број места везивања (I и II), константе везивања за свако место (K_{bI} и K_{b2}) и средњи број лиганада везаних по молу нативног протеина (X_N). Добијене вредности константи везивања (K_{b1} и K_{b2}) и средњег броја лиганада везаних по молу протеина (X_N) показују да албумин говеђег серума има два места везивања цинка (I и II) и да је афинитет везивања цинка на другом месту (II) већи него афинитет везивања на првом месту (I).

(Примљено 20. марта, ревидирано 10. априла 2006)

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