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Isothermal titration calorimetric studies of the pH induced conformational changes of bovine serum albumin

Robert Kun · Márta Szekeres · Imre Dékány

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Abstract Bovine serum albumin (BSA) is a soft globular protein that undergoes conformational changes through several identified transition steps in the pH range 2–13.5. The ability to change conformation makes BSA capable of complexing different ligands from fatty acids to cations or drugs and carries them in the bloodstream. Microcalorimetric titration of BSA with NaOH solution was performed to measure the enthalpy of conformational changes. Two exothermic enthalpy changes were found in the course of the titration between pH 3 and 9.5, which can be identified with the E–F, and the F–N transitions. The enthalpy change at pH 3.5 (transition from the E to the F form of BSA, folding of intra-domain helices in domain I) is independent of the protein concentration. The second transition (F–N, folding of domain III) was observed at pH 4.8 for the 0.1% BSA solution, but it shifted to higher pH values as the protein concentration increased to 0.2% and 0.3%. The tightening of the protein structure with increasing pH was verified measuring intrinsic fluorescence of tryptophan residues. At even higher pH value, pH 10.5, fluorescence measurements revealed protein expansion. The BSA conformational changes were also measured by dynamic light scattering. The hydrodynamic diameter was smaller at the i.e.p. of BSA (5–7 nm at pH ~5) and larger at the two ends of the pH range (17.5 nm at pH 2 and 8.3 nm at pH 10).

Keywords ITC · BSA · DLS · Calorimetry · Conformational change · Fluorescence

Introduction

Bovine serum albumin (BSA) is a globular protein with 66500 Da molecular mass, consisting of 583 amino acid residues. The protein net charge is -17 at neutral pH. Seventeen disulfide bridges help maintain the globular structure and form the three homologous structural domains, I, II and III. Serum albumins are the carrier proteins of blood. The soft globular structure easily undergoes conformational changes that makes easy for the protein to accommodate wide variety of solution species, and form complexes with fatty acids, lipids, dissolved small cations and different drug molecules and carry them in the bloodstream. Conformational changes in the albumin structure had been investigated using thermal denaturation processes [1, 2] and unfolding induced by urea and guanidine hydrochloride [3, 4] or changes in pH [5, 6]. The methods of investigation range from viscosity [6], light scattering [2, 4], thermoanalysis [2, 7–9], small angle X-ray scattering [10–12], NMR [13], photoluminescence [2, 14], circular dichroism [2, 15] to mention but a few.

It is generally accepted, that the soft BSA molecule undergoes multistep transformations, and the transformations are complex, because all the three domains of the protein unfold independently [16, 17]. Successive reversible transformations were identified between five isomeric forms of BSA [18] with increasing pH, from expanded (E), to fast (F), normal (N), basic (B) and aged (A) forms. The E–F transition is assigned to the folding/unfolding of the intra-domain α -helical structure of BSA domain I. The F–N transition is assigned to the folding/unfolding of the BSA

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domain III. The α -helix content of the different isomers decreases considerably at both below pH 2.7 and above pH 10. The molecular shape was found to be heart-shaped near the i.e.p. (around pH 4.8, N-form) and expanded with axial ratio of 9:1 in acidic media (pH < 4, E-form). Based on X-ray crystallographic data [19], the structure of albumin structure is 67% alpha-helical. Thus, two basic conformational change processes are possible: unfolding of the tertiary structure (for example, domain III), and unfolding of the secondary structure, i.e. unfolding α -helices (for example, intra-domain α -helix in domain I). The α -helix content decreases to 35% at pH < 2.7 and to 48% at pH > 8 [18].

In the present work we have studied the enthalpy changes induced by pH in BSA solution, when the acidic form at pH = 3 is gradually transformed to basic form (pH = 10) by NaOH titration in the measuring chamber of the isothermal titration microcalorimeter. Isothermal microcalorimetry is an invaluable experimental tool to get more information about the thermodynamics of different colloidal processes, such as adsorption, swelling, surface charging, wetting [20–26]. Thermodynamics of conformational changes of polyelectrolytes has also been widely studied [27]. In addition to the calorimetry, we have verified the changes in protein structure by using other experimental techniques such as dynamic light scattering (DLS) and fluorescence spectroscopy. The pH changes induce protonation–deprotonation of the dissociable groups of proteins, the carboxylic acid (aspartic acid and glutamic acid) and amine (lysine, arginine and histidine) side groups. Due to the charge density changes the electrokinetic properties of the proteins also vary with pH. Charge density of the protein at different pH values was determined by titration with polyelectrolytes in the Műtek Particle Charge Detector. We have measured the changes in the electrokinetic (zeta-) potential of the albumin, by using Malvern ZetaSizer Nano ZS. The electrokinetic and charging properties of the proteins together with size measurements and the microcalorimetric studies yield conclusions about the nature of the isomerization transitions. Finally, the results were supported by fluorescence experiments.

Materials and methods

Materials

Albumin from bovine serum (BSA, BioChemica-Fluka, M_w 66500 Da, fraction V), Poly(Sodium 4-styrenesulfonate) (PSS, PSS^-Na^+ , M_w 70000 Da, Aldrich), Poly(diallyldimethylammonium chloride) (PDDA, $PDDA^+Cl^-$, high molecular mass, average M_w 400000–500000 Da,

20 wt% in water, Aldrich), sodium hydroxide (NaOH, granules, puriss, Molar Chemicals Ltd., Hungary), hydrochloric acid (HCl solution, concentrated, 37 wt%, Reanal Hungary) were used. High purity ion exchanged water ($R > 18\text{ M } \Omega\text{cm}$, filtered through hydrophilic 0.22 μm Millipore syringe filter) was applied in all experiments as solvent or medium.

Methods

The actual specific charge (in mmol/g protein) of the BSA molecules at a certain pH value was determined by so-called charge-titration method using MűTEK PCD-02 particle charge detector. Ten millilitre of protein solution was introduced into the titration cell. Depending on the pH of the BSA solution, oppositely charged polyelectrolyte solution was added to the protein solution by 20 μL portions. Due to charge recombination, the so-called charge equivalent point of the BSA can be calculated from the titrant consumption at the charge equivalent point (0 mV charge state).

The pH determination was done by a Radelkis OP-211 type laboratory desktop pX/mV meter with a combined glass pH electrode type OP 0808P, purchased from Radelkis Budapest. Two-point calibration was taken prior to measurement with the electrode using pH 2.06 and pH 7.07 calibrating buffers. The electrode was activated as necessary, using 0.1 M HCl solution, and carefully cleaned against the protein deposition on the electrode surface.

The hydrodynamic mean diameter and electrokinetic-potential (ζ -potential) of the protein globules were determined by Malvern Zetasizer Nano Series, Nano-Zs apparatus, using a DTS 1060 folded polystyrene capillary cells. The measured data were evaluated by the Malvern BioSense software.

Thermometric titration (ITC) experiments were performed with a four-channel isothermal heat-flow microcalorimeter, ThermoMetric LKB 2277 Thermal Activity Monitor, equipped with a ThermoMetric 6120 Syringe Pump. The titration experiments were performed in a stainless steel measuring cell ($V_{\text{tot}} = 4\text{ mL}$), with constant stirring rate (0.8 1/s) of the protein solution. In a typical reaction 1.5 mL protein solution (pH = 3) was introduced into the stainless steel cell and titrated with 500 μL of NaOH solution of different concentrations in 25 μL portions (20 additions), 1 h time interval between the portions. The protein concentrations in the experiments were 0.1%, 0.3% or 0.5%. The pH of the protein solutions were set to 3 with HCl solution. The portions of the alkali were dosed by a 500 μL Hamilton syringe. The resulting pH values were measured outside the microcalorimeter, in identical titration experiments. Parallel to these experiments reference titrations were also done in which the total heat of neutralization ($\Delta H_{\text{neutr.}}$) and of the dilution ($\Delta H_{\text{dil.}}$) were determined. For

neutralization heat measurements 1.5 mL of 0.001 M HCl solution was titrated with dilute NaOH solution, and for heat of dilution measurements 1.5 mL of protein solutions were titrated with 500 μ L of ultra pure water. The heats of neutralization and dilution were subtracted from the raw enthalpies of the protein titration.

The fluorescence spectra of the protein solutions were recorded by a Horiba Jobin Yvon Fluoromax-4 apparatus using quartz cuvette. The fluorescence emission spectra were measured in the 300–450 nm wavelength range and 1 nm slit was used. For the excitation, $\lambda_{\text{ex}} = 280$ nm UV-light was applied.

Results and discussion

The effect of the pH on the specific charge and on the globulum size of the BSA molecules

Relationship between pH and the specific charge

The characteristic specific charge of the BSA molecules at different pH values were determined by streaming potential measurements. The raw titration curves can be seen on the Fig. 1. The isoelectric point (i.e.p.) of the BSA is around pH 4.5–5.5 [28–30]. Hence, below the i.e.p. value the BSA molecules draw positive-, and above the i.e.p. they get negative charge. The measurements were done around pH 3, pH 7 and pH 11 values. By acidic conditions (pH \sim 3) negatively charged poly(sodium 4-styrenesulfonate) was used as titrant. The concentration of the PSS solution was 1% m/v, and the specific charge of the PSS[−] polyelectrolyte was 4.85 mequ./g, which was determined previously. Knowing the concentration the PSS and the BSA solutions and the PSS consumption till the state of the 0 mV streaming potential, the specific charge of the BSA molecules can be calculated. The determined values are presented in Table 1.

Above the i.e.p. of the BSA, namely at pH \sim 7 and pH \sim 10 positively charged poly(diallyldimethylammonium chloride) was used as titrant. The specific charge of the PDDA⁺ polyelectrolyte is 6.10 mequ./g. As it can be seen from the Table 1, increasing the pH value from \sim 7 to \sim 10 the polyelectrolyte consumption (note that the same

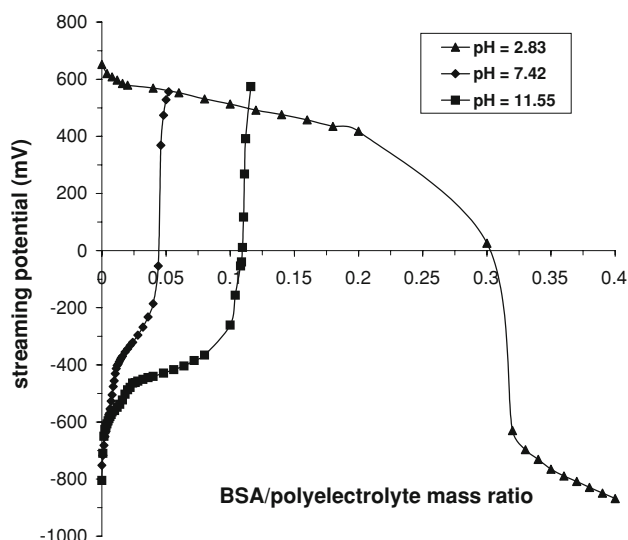


Fig. 1 Charge compensation titration curves of BSA under different pH conditions

polyelectrolyte concentration was used) till the charge equivalent point is increasing. It clearly shows that the amount of the negatively charged groups on the BSA molecule is increasing with the pH. As the pH is raised from \sim 7 to \sim 10 the determined specific charge of the BSA molecules is twice as much, i.e. 0.27 mmol/g protein and 0.66 mmol/g protein at pH \sim 7 and pH \sim 10, respectively (see Table 1). In summary, decreasing or increasing the pH value of the BSA solutions from the $\text{pH}_{\text{i.e.p.}}$, remarkably electronic charge can form along the biopolymer chain.

The calculated specific charge values in mequ./g BSA are plotted against the pH of the protein solutions on the Fig. 2. As it was discussed previously, increasing the pH from \sim 3 to \sim 10 the amount of the positively charged groups is decreasing afore (till $\text{pH}_{\text{i.e.p.}}$), followed by the persistent cumulation of the negatively charged groups along the protein chain.

The pH dependence of the globulum size, and of the electrokinetic potential

In general, changing of the pH of the medium, albumins undergo reversible conformational changes. This character

Table 1 Specific charge of the BSA molecules at different pH values

pH	c_{BSA} (%)	Type of polyelectrolyte ^a	Polyelectrolyte consumption (ml)	Specific charge (mmol/g BSA)
2.83	0.5	1.0% PSSNa	1.51	(+) 1.46
7.42	0.5	0.2% PDDA	1.11	(−) 0.27
11.55	0.5	0.2% PDDA	2.72	(−) 0.66

^a The specific charge of the polyelectrolyte was determined by simple charge equivalent titration using counter charged surfactants

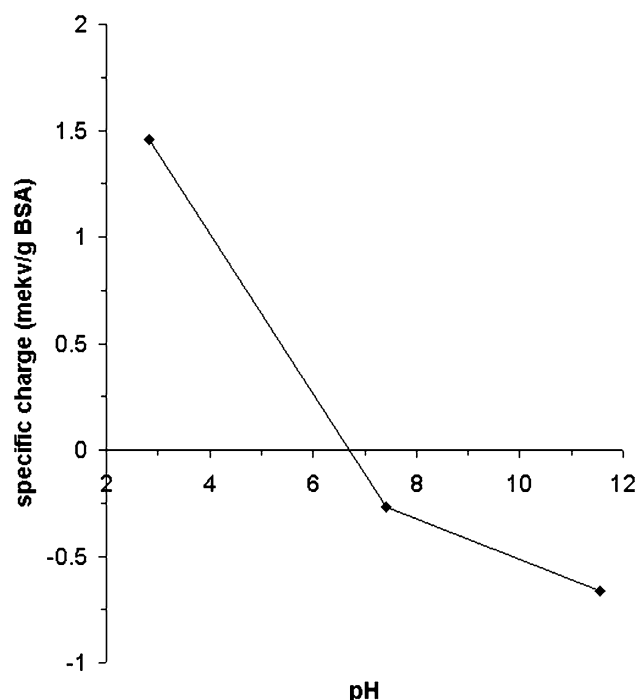


Fig. 2 Changing of the specific charge of the BSA molecules in the function of pH

is more pronounced in the pH 2–7 range, which was already ascertained previously [18, 31]. Because there is still no available crystallographic results on the BSA structure, thus the pH induced conformational changes of the protein can not be discussed in the atomic level.

In our DLS analyses it was studied how the mean hydrodynamic globulum size changes due to changing of the pH value of the medium. We assumed that close to the $pH_{i.e.p.}$, where the quantity of the of the negatively and the positively charged groups is definitionally the same, size contraction will occur around this pH value, because of the mutual attraction of the differently charged groups. While, above and below this narrow pH regime, globulum size expansion could occur due to the increasing amount of the presenting negatively (deprotonated) and conversely, the positively (protonated) charged functional groups, respectively. Afterwards it was corroborated by means of DLS measurements, and the results are presented in Fig. 3. It can be seen that drawing off from the $pH_{i.e.p.}$ the hydrodynamic mean diameter of the globules is increasing. In the same time of the size determination, electrokinetic potential (ζ -potential) measurements were also taken. The obtained results showed that under acidic circumstances excess positive, while in alkaline medium excess negative charge is forming on the biopolymer chain, according to the theory.

On the Fig. 4 the determined mean hydrodynamic diameter at certain pH is plotted against the protein concentration. It can be seen, that at pH ~5 and pH ~7 the

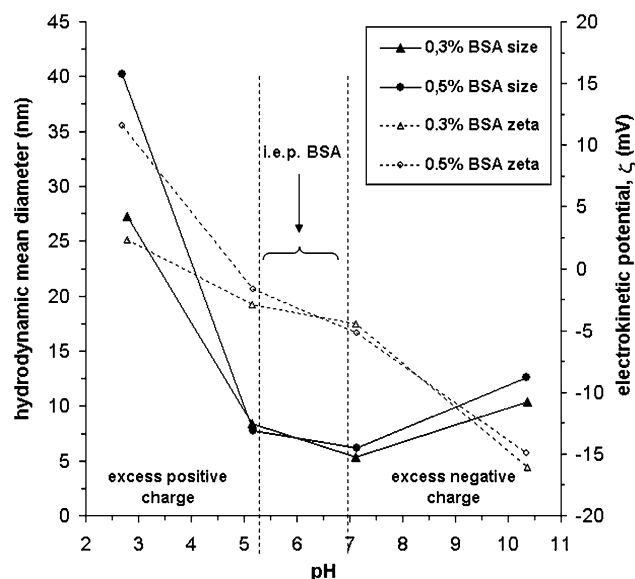


Fig. 3 The variation of the determined hydrodynamic mean diameter and of the electrokinetic potential with the pH and the protein concentration

mean diameters do not change significantly with the protein concentration. This phenomenon can be explained as follows, close to the isoelectric state ($pH_{i.e.p.} \sim 5$) the net charge of the BSA globules is nearly zero, thus a more contracted, rigid phase is forming due to the formation of ion-association complexes between the negatively charged $-COO^-$ (carboxylic) and the positively charged $-NH_2^+$ (amino) groups within the BSA chain, in this manner the volume fraction of the protein in the solution is rather low even in the higher concentration range. Whilst at pH ~3 and pH ~10 the globules are in an expanded phase (see results from DLS measurements) and the more easily accessible charged groups are highly solvated (i.e. hydrated) resulted in the increase of the volume fraction of the protein in the solution, which additionally decreases the diffusion constant upon increase of the protein concentration.

Knowing the actual mean hydrodynamic diameter of the particles (i.e. globules) at certain pH value and protein concentration, it is possible to calculate the diffusion constant (D_c) by means of the Stokes–Einstein equation (Eq. 1),

$$D_c = kT/6\pi\eta r \quad (1)$$

where k is the Boltzmann's constant, T is the thermodynamic temperature, η the viscosity of the medium, and r is the particle or globulum radius. The results are shown on Fig. 5, where the obtained D_c values are plotted against the protein concentration at pH 3 and pH 10.

It can be clearly seen on Fig. 5 that increasing the protein concentration the diffusion constant decreases monotonically at both applied pH values. Extrapolating into the

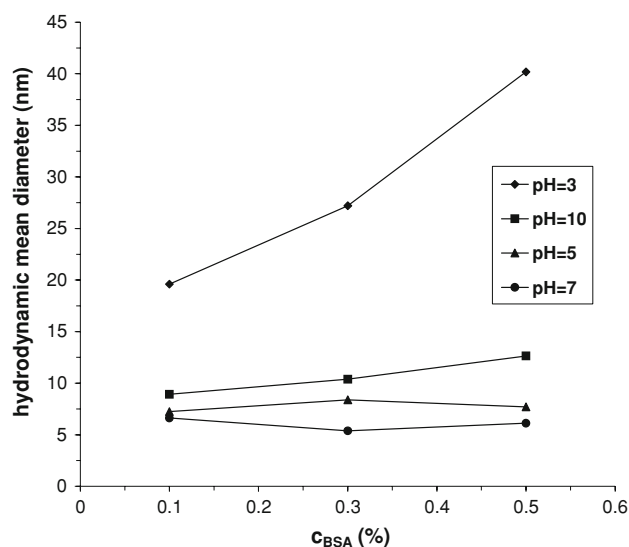


Fig. 4 The changing of the hydrodynamic mean diameter with the protein concentration determined at different pH values

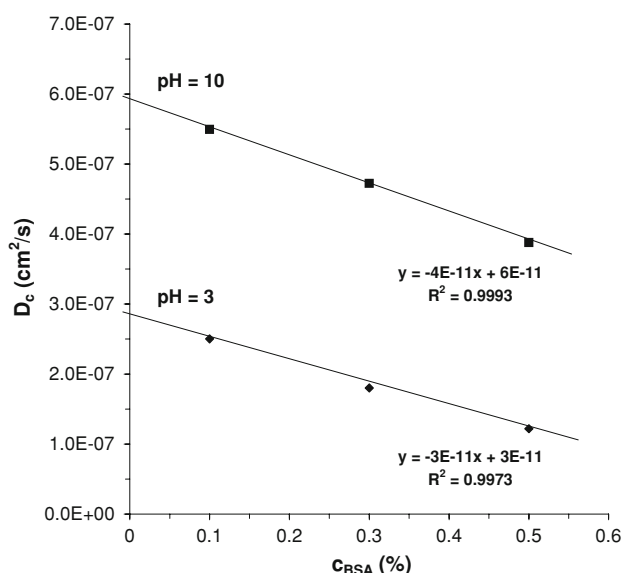


Fig. 5 The changing of the diffusion constant with the protein concentration determined at pH ~ 3 and pH ~ 10

$c_{BSA} = 0\%$, the diffusion constant of the perturbation-free state (D_0) can be determined. Using the D_0 values the real globulum sizes (intrinsic) were calculated; at pH 3 $d_{BSA} = 17.5$ nm (from $D_0 = 2.8024 \times 10^{-11} \text{ m}^2/\text{s}$) and at pH 10 $d_{BSA} = 8.3$ nm (from $D_0 = 5.9120 \times 10^{-11} \text{ m}^2/\text{s}$).

On the Fig. 6 the mean hydrodynamic diameter versus electrokinetic potential curves at three different protein concentrations are presented. It is well demonstrated that around the i.e.p., where the electrokinetic potential is zero, the size of the BSA globules reach minimum values due to possible formation of intra-chain ion association complexes, as it was discussed previously. In this potential

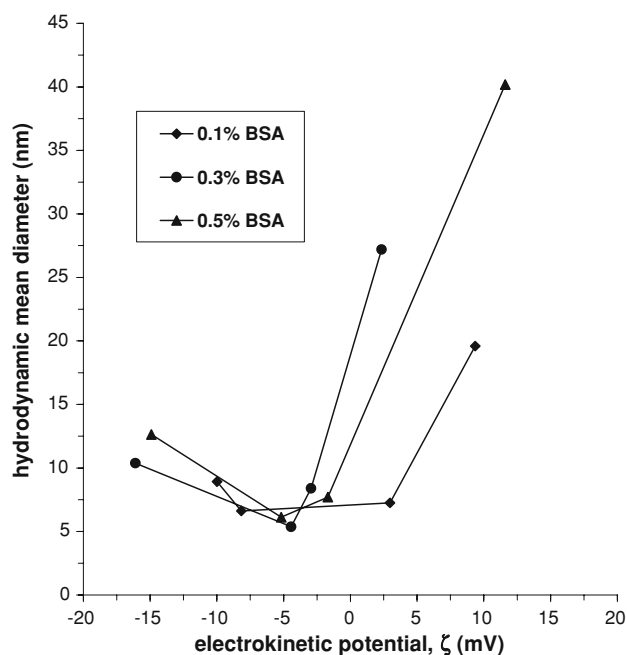


Fig. 6 The effect of the electrokinetic potential on the hydrodynamic mean diameter of the BSA globules

range the apparent particle size is nearly constant in the applied concentration range, so thus the apparent and the effective (intrinsic) globulum sizes are equal. In the negative and also in the positive electrokinetic potential regions the apparent globulum size is increasing, in addition it changes with the applied protein concentration. It can be seen that the effect of the concentration is more pronounced in the positive ζ -potential range, which probably originated from the higher solvation/hydration effects. In the case of both, positive and negative electrokinetic potential ranges the groups with the same charge—protonated and deprotonated—are repulsing each other along the protein chain, resulting in the expansion of the globules and the enlargement of the globules.

Microcalorimetry studies

In Fig. 7a the enthalpograms measured in the base titration of three different concentrations of BSA in acidic solution (pH = 3) are presented. All the measured heats were exothermic. It is apparent from the figure that the signals in the first part of the titration experiments are approximately identical at each titration step, and increase with increasing protein concentration. The parallel pH-measurements showed that the start of the decrease in the peak intensity coincides with the neutralization point, e.g., pH ~ 7 at each protein concentration. In the acidic range of the experiments (below pH 7) all the added OH is reacted, and the signal is proportional with the combined heat effect of

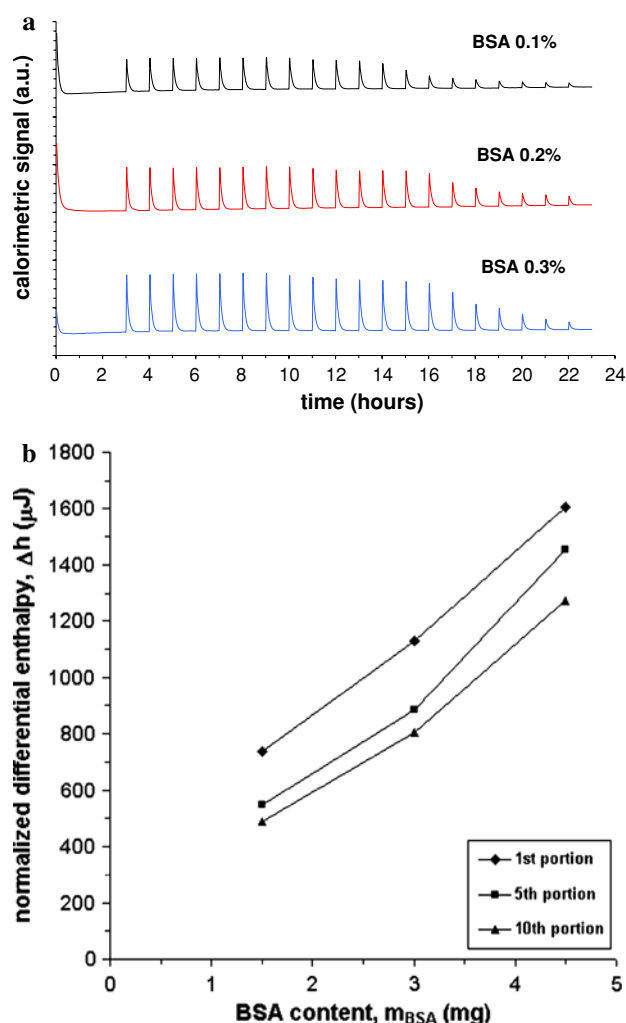


Fig. 7 **a** The raw enthalpograms obtained from the ITC measurement. **b** The extent, and the change with the protein amount of the corrected (i.e. normalized) differential enthalpies derived from the raw enthalpograms

deprotonation, desolvation, neutralization, dilution and any conformational changes. Among these processes, we were able to separate experimentally only the neutralization and the dilution effects, by subtracting the corresponding reaction heats from the background experiments, as described in the Experimental part (Methods). Corrected heats of the 1st, 5th and the 10th peaks are presented in Fig. 7b. As it can be clearly seen, the correction did not remove the concentration dependence of the measured heats of titration. This reveals the protein concentration dependence of the enthalpies of combined reactions of deprotonation, desolvation and conformational changes.

The corrected net enthalpies during the base titration of the albumin solutions are presented in Fig. 8 as a function of the added molar amount of base. The corrected enthalpies show several exothermic peaks, but with no observable trend. The added amount of NaOH was defined with

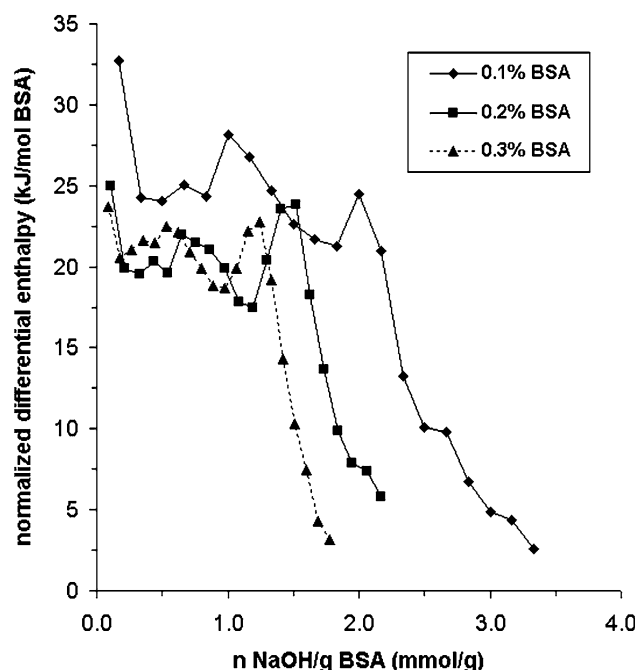


Fig. 8 The corrected net enthalpies as a function of the added molar amount of base

the help of parallel experiments to reach the same pH value of ~ 9.5 at the end of the titrations. As it can be seen from Fig. 8, the smallest specific amount of NaOH was needed in the case of the largest protein concentration. It suggests that the dissociation degree of BSA decreases with increasing protein concentration. The latter property is characteristic of dissociation of weak acids and bases, so it is for globular proteins. On the other hand, it was seen, that the net enthalpies increased with protein concentration. Thus, we may conclude, that the overwhelming part of the experimental exothermic heats originates not from the deprotonation, but rather from desolvation and conformational changes. Protein desolvation and conformational changes are bound to each other, since conformational changes necessary involve desolvation and desolvation induce conformational changes.

In Fig. 9, we see the presentation of the net calorimetric enthalpies as the function of pH, resulting after the addition of the corresponding portions of base. As it is seen, the enthalpy curves of the different protein concentrations in the function of pH show nice correlation. There are two definite exothermic peaks in each curve. The first peak can be observed at pH ~ 3.5 for each curve, but the second peak shifts to higher pH values with increasing protein concentration (from 4.5 to 5.2 and 6.0). We can speculate, that the concentration independent peak is most likely connected with the folding of α -helix structure of BSA domain I, which is responsible for the E to F transformation, below pH 4.3 [3]. Since this peak is independent of

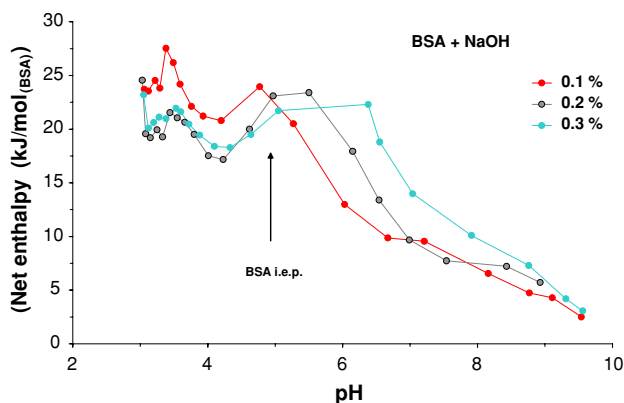


Fig. 9 The presentation of the net calorimetric enthalpies as the function of the pH

protein concentration, we can suppose, that the transformation is due to relatively strong interactions (large pK). In the case of the second exothermic peak, we see the signs of weak reactions, with relatively low affinity (low pK), since the change in the concentration of the reacting species is able to shift the equilibrium. This reaction should correspond with the F to N transformation (between pH 4.3 and 8), folding of domain III of BSA. The values of the net enthalpy change of the two kinds of transformations are not much different. All the peaks amount near 25 kJ/mol_(BSA). The latter observation again suggests, that the net enthalpy has a contribution from the desolvation-conformational changes, in addition to the protonation/deprotonation reaction of amino acid residues.

The changing of the fluorescence emission of BSA with the pH and the protein concentration

It is well known that the BSA molecules, due to the presenting tryptophan (W) residues in the polypeptide chain, emit $\lambda_{em} = 350$ nm light under UV-light excitation ($\lambda_{ex} = 280$ nm). This is frequently called as intrinsic fluorescence. The fluorescence emission intensity of the tryptophan residues is depending on the chemical surrounding of the involved groups. The BSA is a soft protein and it bears conformational changes in the course of pH changing, as it was discussed previously. The results of the pH dependence of the fluorescence emission are shown on Fig. 10. It was found that around the i.e.p. (see pH ~ 5 and ~ 7) the fluorescence emission of the BSA is maximal, but the decrease or increase of the pH value of the BSA solutions is resulted in the significant slack of the emission. These results are clearly confirming our previous observations, namely around the i.e.p. the BSA molecules have compact and contracted structure, while and they have expanded structure in both, acidic and alkaline medium. The fluorescence emission quenching was almost twice as

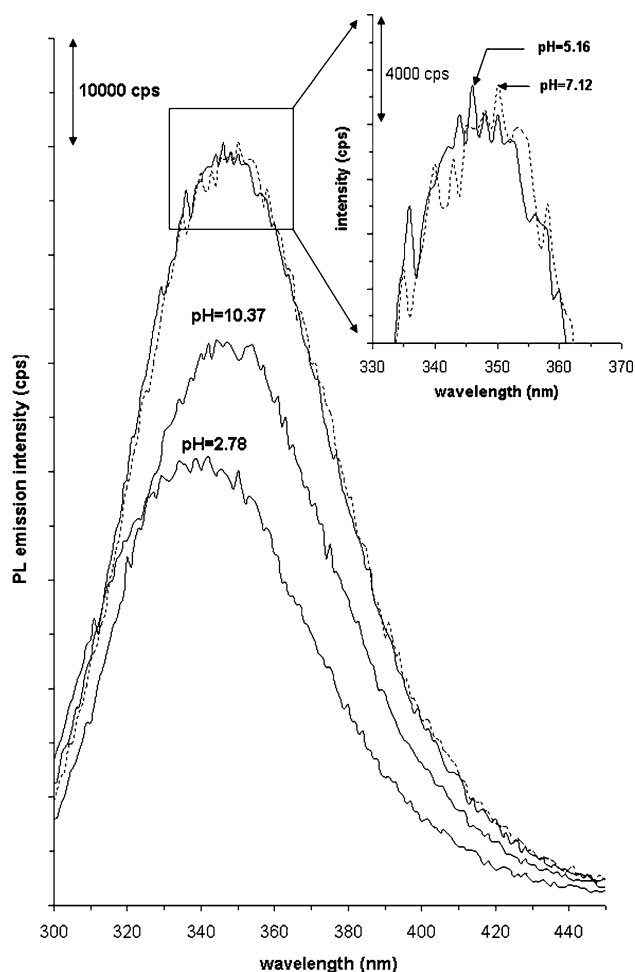


Fig. 10 The fluorescence emission spectrum of BSA at different pH values ($\lambda_{ex} = 280$ nm)

much at pH ~ 3 than at pH ~ 10 . So thus the acidic circumstances have much stronger fluorescence quenching effect on the tryptophan residues than that of the alkaline medium, because under acidic conditions the expansion of the BSA molecules is much significant according to the results from the DLS studies and to the literature [18].

It can be seen summarized on the Fig. 11, how the fluorescence emission intensity is changing with the pH of the protein solutions applying different BSA concentrations. In generally speaking, increasing the protein concentration the fluorescence emission intensity is decreasing at all the applied pH values. The explanation could be for this behavior that increasing the protein concentration the number of the presenting scattering centers in the solution is increasing, resulted in the more intense scattering out of the emitted light. The same phenomenon can be seen on Fig. 12, where the decrease of the fluorescence emission with the increase of the protein concentration at certain pH values is shown.

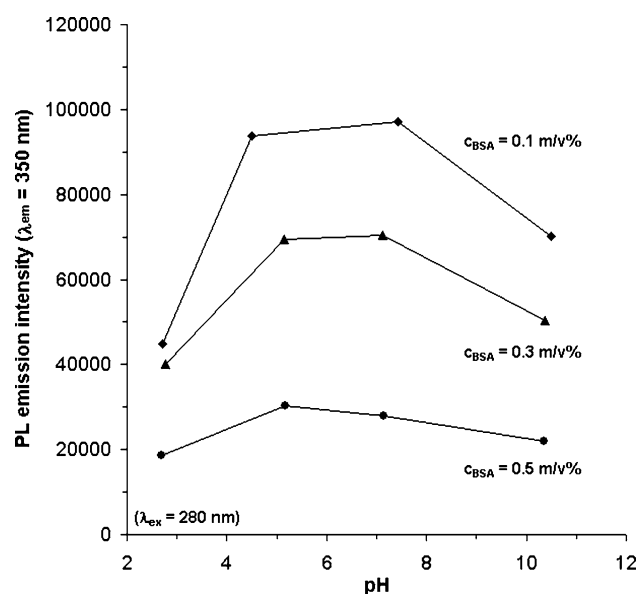


Fig. 11 The change of the BSA intrinsic fluorescence emission maximum in the function of the solution pH

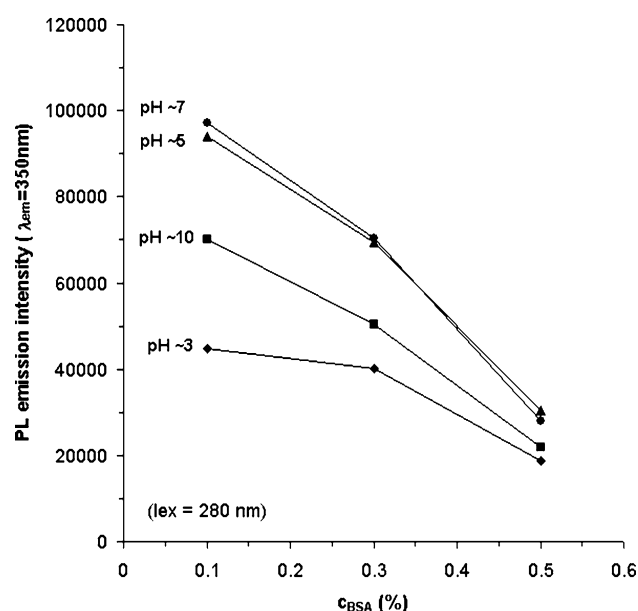


Fig. 12 The decrease of the fluorescence emission intensity maximum ($\lambda_{em} = 350$ nm) with the protein concentration at different pH values

Conclusions

Investigation of the conformational changes of BSA in solution due to pH-changes was conducted by using several experimental techniques: DLS, electrokinetic potential measurements, isothermal titration calorimetry and fluorescence spectroscopy. We concluded, that the pH-induced changes in conformation of BSA are in accord with earlier

results of Foster [18], and we could identify two transitions defined by Foster via calorimetric measurements, one of which is concentration independent, and the second is concentration dependent. BSA is a soft protein, and accordingly, profound changes in conformation were found both in the size and the fluorescence of BSA with changing the pH both to acidic and basic side from the pH of the i.e.p. at ~ 4.8 . Concentration dependence of the diffusion coefficient of the BSA at low and high pH values allowed us to determine the intrinsic protein size via extrapolation to zero protein concentration. All the pH-dependent conformational results were supported by the fluorescence spectroscopy results, revealing contracted structure at the i.e.p. and expanded at low and high pH values.

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References

1. Murayama K, Tomida M. Heat-induced secondary structure and conformation change of bovine serum albumin investigated by Fourier Transform Infrared Spectroscopy. *Biochemistry*. 2004; 43:11526–32.
2. Guo J, Harn N, Robbins A, Dougherty R, Middaugh CR. Stability of helix-rich proteins at high concentrations. *Biochemistry*. 2006;45:8686–9.
3. Vassilikou-Dova A, Grigorakakis S, Varotsos P, Kapetanaki A, Koutsikos D. Study of the denaturation process in albumin-urea solutions by means of the thermally stimulated depolarization currents technique. *J Phys Chem*. 1996;100:1914–7.
4. Adel A, Nadia M, Mohamed O, Abdelhafidh G. Study of thermally and chemically unfolded conformations of bovine serum albumin by means of dynamic light scattering. *Mater Sci Eng C*. 2008;28:594–600.
5. Li Y, Lee J, Lal J, An L, Huang Q. Effects of pH on the interactions and conformation of bovine serum albumin: comparison between chemical force microscopy and small-angle neutron scattering. *J Phys Chem B*. 2008;112:3797–906.
6. Tanford C, Buzzell JG, Rands DG, Swanson SA. The reversible expansion of bovine serum albumin in acid solutions. *J Am Chem Soc*. 1955;77:6421–8.
7. Michnik A, Michalik K, Drzazga Z. Stability of bovine serum albumin at different pH. *J Therm Anal Cal*. 2005;80:399–406.
8. Cao XM, Yang X, Shi JY, Liu YW, Wang CX. The effect of glucose on bovine serum albumin denatured aggregation kinetics at high concentration: the master plots method study by DSC. *J Therm Anal Cal*. 2008;93:451–8.
9. Wunderlich B. Thermal analysis of macromolecules: a personal review. *J Therm Anal Cal*. 2007;89:321–56.
10. Toft KN, Vestergaard B, Nielsen SS, Snakenborg D, Jeppesen MG, Jacobsen JK, et al. High-throughput small angle X-ray scattering from proteins in solution using a microfluidic front-end. *Anal Chem*. 2008;80:3648–54.
11. Zhang F, Skoda MWA, Jacobs RMJ, Martin RA, Martin CM, Schreiber F. Protein interactions studied by SAXS: effect of ionic strength and protein concentration for BSA in aqueous solutions. *J Phys Chem B*. 2007;111:251–9.

12. Santos SF, Zanette D, Fischer H, Itri R. A systematic study of bovine serum albumin (BSA) and sodium dodecyl sulfate (SDS) interactions by surface tension and small angle X-ray scattering. *J Coll Interf Sci.* 2003;262:400–8.
13. Orioni B, Roversi M, La Mesa C, Asaro F, Pellizer G, D'Errico G. Polymorphic behavior in protein-surfactant mixtures: the water-bovine serum albumin-sodium taurodeoxycholate system. *J Phys Chem B.* 2006;110:12129–40.
14. Lensun L, Smith TA, Gee ML. Partial denaturation of silica-adsorbed bovine serum albumin determined by time-resolved evanescent wave-induced fluorescence spectroscopy. *Langmuir.* 2002;18:9924–31.
15. Güzey D, Gülseren I, Bruce B, Weiss J. Interfacial properties and structural conformation of thermosonicated bovine serum albumin. *Food Hydrocoll.* 2006;20:669–77.
16. Dockal M, Carter DC, Ruker F. Conformational transitions of the three recombinant domains of human serum albumin depending on pH. *J Biol Chem.* 2000;275:3042–50.
17. Johanson KO, Wetlaufer DB, Reed RG, Peters T Jr. Refolding of bovine serum albumin and its proteolytic fragments: regain of disulfide bonds, secondary structure, and ligand-binding ability. *J Biol Chem.* 1981;256:445–50.
18. Foster JF. Some aspects of the structure and conformational properties of serum albumin. In: Rosenoer VM, Oratz M, Rothschild MA, editors. *Albumin structure, function and uses.* Oxford: Pergamon; 1977. p. 53–84.
19. Carter DC, Ho JX. Structure of serum albumin. *Adv Protein Chem.* 1994;45:153–203.
20. Tombácz E, Szekeres M, Klumpp E. Interfacial acid-base reactions of aluminum oxide dispersed in aqueous electrolyte solutions. 2. Calorimetric study on ionization of surface sites. *Langmuir.* 2001;17:1420–5.
21. Király Z, Findenegg GH, Klump E, Schlimper H, Dékány I. Adsorption calorimetric study of the organization of sodium n-decyl sulfate at the graphite/solution interface. *Langmuir.* 2001;17:2420–5.
22. Dékány I, Farkas A, Király Z, Klumpp E, Narres HD. Interlamellar adsorption of 1-pentanol from aqueous solution on hydrophobic clay mineral. *Coll Surf A.* 1996;119:7–13.
23. Szántó F, Dékány I, Patzkó A, Várkonyi B. Wetting, swelling and sediment volumes of organophilic clays. *Coll Surf.* 1986;18:359–71.
24. Li X, Wang C, Li J, Wang Z. Microcalorimetric studies on the interactions of lanthanide ions with bovine serum albumin. *J Therm Anal Cal.* 2007;89:899–905.
25. Zeiss D, Brandl AB. Isothermal titration calorimetry (ITC) method to study drug/ion exchanger interaction. *J Therm Anal Cal.* 2006;83:309–12.
26. Behbehani GR, Saboury AA. A thermodynamic study on the binding of magnesium with human growth hormone: consideration of the new extended coordination model solvation parameters. *J Therm Anal Cal.* 2007;89:857–61.
27. Bharadwaj S, Montazeri R, Haynie DT. Direct determination of the thermodynamics of polyelectrolyte complexation and implications thereof for electrostatic layer-by-layer assembly of multilayer films. *Langmuir.* 2006;22:6093–101.
28. Valstar A, Vasilescu M, Stilbs P, Almgren M. Heat-set bovine serum albumin-sodium dodecyl sulfate gels studies by fluorescence probe methods, NMR, and light scattering. *Langmuir.* 2001;17:3208–15.
29. Valstar A, Almgren M, Brown W. The interaction of bovine serum albumin with surfactants studied by light scattering. *Langmuir.* 2000;16:922–7.
30. Lu RC, Cao AN, Lai LH, Xiao JX. Effect of anionic surfactant molecular structure on bovine serum albumin (BSA) fluorescence. *Coll Surf A.* 2006;278:67–73.
31. Foster JF. Plasma albumin. In: Putnam FW, editor. *The plasma proteins*, vol. 1. New York: Academic Press; 1960. p. 179–239.