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PHYSICAL PROPERTIES OF THE RECONSTRUCTED GLOBULAR PROTEIN. A CASE OF THE BSA/COPPER ION COMPOSITE

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Abstract

Structural organization of serum albumins–the most abundant globula rproteins in plasma–gives rise to their extraordinary binding and functional capacity. Various classes of ligands,including the metal ions can be captured and transported by albumins. Metal binding to human serumalbumin, HSA, that is an essential multipurpose target for the modern biomedicine, to its bovine equivalent, BSA, and other mammalian analogs have been extensively explored in the context of metabolism of essential metal ions, like Cu^{2+} . Taking in to account structural similarity of human and bovine serum albumins, the later was selected as a relevant model in laboratory studies due to its low cost and wide availability. In the present work metal binding properties of BSA with copper ions (Cu^{2+})were explored using combined voltammetric and thermodynamic examinations. According to voltammetric data,addition of equal amount of BSA ($1.8x10^{-3}$)M to the solution(0.2MKCl) containing($1.8x10^{-3}$) M CuCl results that two pairs of redox peaks belonging to the Cu^{2+}/Cu^{+} (E=0,16V) and Cu^{+}/Cu^{0} (E=0.2V) electronic transformations disappear and a new weak single reductive peak,at Ep=0,55V attributable to the Cu^{2+}/Cu^{+} transition is shown.

Keywords: *copper ion, serum albumins, thermodynamic parameters, globular proteins.*

Introduction

The interaction of globular proteins with metal ions is one of the central topics in biophysics. Metal ions can bind to globular proteins through various non-covalent/ coordinative interactions. Binding of metal ions to proteins is governed by Gibbs free energy (ΔG)**.**

$$\Delta G=\Delta H-T\Delta S \qquad (1)$$

Where ΔH is enthalpy **,** which reflects bond formation strength and ΔS is entropy**.** In the present work we discuss the physical processes underlying copper binding to albumin**,** focusing on thermodynamic and charge transfer issues. For estimation of thermodynamic parameters we use differential scanning calorimetry (DSC) examinations of target protein in the presence and absence of Cu^{2+} ions.

The calorimetric enthalpy of thermal melting, ΔH_{cal}, 9determined as area under the calorimetric curves) in uncomplicated cases normally exhibiting an endothermic melting peak with a single transition temperature, Tm and can be approximately calculated according to [20-21]:

$$\Delta H_{\text{cal}} = \int_{T_1}^{T_2} C_{p(\text{prot})}\, dT, \qquad (2)$$

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where T is the absolute temperature, and T_1 and T_2 are the temperatures that correspond to the start and completion of heat absorption due to thermal melting ($T_1 < Tm < T_2$). *Cp(prot)* is partial heat capacity of protein.
The values of the counterpart parameter, melting entropy, **ΔS**cal, can be determined as follows[20-21]:

$$\Delta Scal = \Delta Hcal / Tm \quad (3)$$

Upon metal- protein binding process values of ΔS often decrease due to restriction of metal and protein movement upon binding.

In this study, two independent physical techniques—cyclic voltammetry (CV) and differential scanning calorimetry (DSC)—were employed in parallel to investigate the interaction between Cu^{2+} ions and bovin serum albumin.The copper–albumin system is a significant physiological example of metal protein interaction, especially because albumin (human serum albumin, HSA) plays a key role in copper transport and buffering in blood plasma.

Mammal albumins, including the bovine serum albumin (BSA), (with a molecular mass of ca. 66 500 Da), etc., earned much research attention since they are most abundant and multi-functional water-soluble monomer globular proteins in serum plasma [12,13,16], and their physiological function encompasses maintenance of osmotic pressure in serum, transporting of fatty acids, amino acids and metal ions, including Cu^{2+}, as well as scavenging of oxidants and reductants, including complex metal ions and drugs [2-4, 10,13,17,19]. Among other functions of serum albumins the binding, transportation and regulation of doubly charged metal ions such as Cu^{2+} should be mentioned. Among other issues, investigation of the interaction of copper (II) with serum albumins, evaluation of binding amplitude and mechanism of intera ction, have gained increased interest due to their application for numerous biomedical and bioanalytical issues, as well as for the design of metal-based drugs [1-3,5,7,8,11-14].

Fig. 1. Interaction between Cu ion and BSA

In the present work, taking into the account the importance of understanding of metal binding properties of serum albumins from one side and an exceptional ability of copper ions to form extremely versatile series of coordinated complexes, often with very unusual, even odd hence novel thermodynamic and/or kinetic patterns of electron transfer (exchange) from another side [9,18], we studied interaction/complexation of copper ions (Cu^{2+}) with a representative globular protein, BSA, using combined voltammetric and thermodynamic examinations. Voltammetric measurements [9,18] are of exceptional interest since offers opportunity of the direct instrumental detection of a current signal for the electron exchange between Cu^{2+} and the electrode. The combined voltammetric and thermodynamic (differential scanning calorimetry, DSC) examinations of target proteins in the presence and absence of Cu^{2+} ions, gives possibility to investigate the correlated impact of different factors on the stability and redox activity of BSA (HSA)-

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Cu^{2+}complexes which, in turn, will provide information on the role of conformational flexibility (dynamic properties), which, beyond the applied biomedical purpose, has the essential fundamental importance from the physiological and biophysical standpoints, as well.

Materials and methods.

Bovin Serum Albumin (BSA), copper oxide ($CuCl_2 \cdot 2\ H_2O$), potassium Chloride (KCl) were purchased from Sigma and were used without further purification. All solutions were prepared using MilliQ water.

Electrochemical experiments were performed with conventional three-electrode system. 2 mm Ø Glassy Carbon disc sealed in Teflon cylinders(BAS)was used as working electrode, platinum wire and Ag/AgCl/3M NaCl were used as the counter and the reference electrodes, respectively [9,18]. The working electrode was sequentially polished with 0.5 and 0.05μm Alumina water slurry and washed with water.

Electrochemical measurements were carried out with an Autolab PGSTATI2SN from Metrohm Autolab B.V., equipped with software for Windows (NOVA1.11). Mikrocallorimetric measurements were performed with DSC instrument DASM-4A connecting to PC via the Interface unit PCI-DASM 4-A.

Results and discussion.

Fig. 2, displays cyclic voltamperometric data, which demonstrate the reduction and oxidation (redox) behavior of Cu^{2+} ions in (1.8×10^{-3}) M $CuCl_2$ in 0.2 M KCl (pH was adjusted to 6.2, without using any buffer, to avoid the uncontrollable extra complexation of Cu^{2+} with the buffer components). Curve 1 clearly showing two pairs of redox peaks belonging to the Cu^{2+}/Cu^{+} at midwave potential E_0 = 0.16 V (Ep_k = 0.12V; Ep_a= 0.2V) and Cu^{+}/Cu^{0} at E_0 = –0.2V (Ep_k= –0.37V; Ep_a= –0.035V) electronic transformations [5,6]. Voltamperometric peak values can be calculated according to the eq 4 [22]

$$Ip = -0.4463\ nF(nF/RT)^{1/2} Co\ D^{1/2}\ v^{½} \qquad (4)$$

Where Ip is the peak current density, D is diffusion coefficient, n - number of electrons transferred in the course of redox reaction, R is gas constant, T- temperature, F- faraday constant, v- scan rate. Equation (4) at 25 ^{o}C reduces to the form [22]

$$Ip = -(2.69 \times 10^{5})\ n^{3/2} Co\ D^{1/2}\ v^{½} \qquad (5)$$

Addition of equal amount of BSA (1.8 x 10-3) M to the solution containing (1.8×10^{-3}) M $CuCl_2$ results that two pairs of redox peaks (belonging to the $Cu^{2+/}Cu^{+}$ and Cu^{+}/Cu redox transformations) disappear and a new weak single reductive peak, at Ep_k = –0,55V (curve 2) attributable to the Cu^{2+}/Cu^{+} transition is shown. Very dramatic shift of Cu^{2+} reduction process to much more negative potentials (for ca. 0.5 Volts (!)) is presumably due to the strong 1:1 BSA-Cu^{2+} complex formation. To our best knowledge, this is the first direct voltammetric (electrochemical) signal detection of complex formation between albumin and (Cu^{2+}). According to spectroscopic data [15] in the presence of electron donor (ascorbic acid or acrobat), the albumin-Cu^{2+}complex square planar geometry is distorted and the albumin connected to Cu(I) has linear geometry [15].

In the process of BSA-Cu^{2+} complex formation the “N-terminal” sequence of: Asp- Thr-His- is presumably forms the chelating environment for the entrapped Cu^{2+} ion. There is some published work [8] reporting that Cu^{2+} ions entrapped inside BSA (or HSA) lose their ability to exchange electrons with their proposed redox partners. It has been proposed [8] that the sulfuric group of the albumin’s Cys-34 residue that resides near the “N-terminal” site, having sufficient conformational flexibility, may provide additional ligation through the stabilizing electronic configuration that

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implies the partial charge-transfer to Cu^{2+}. This action may "lock" the copper ion in a redox inactive condition (hinder it's redox activity), unless the sulfur group is not oxidized by adding of some strong oxidant into the solution [8].

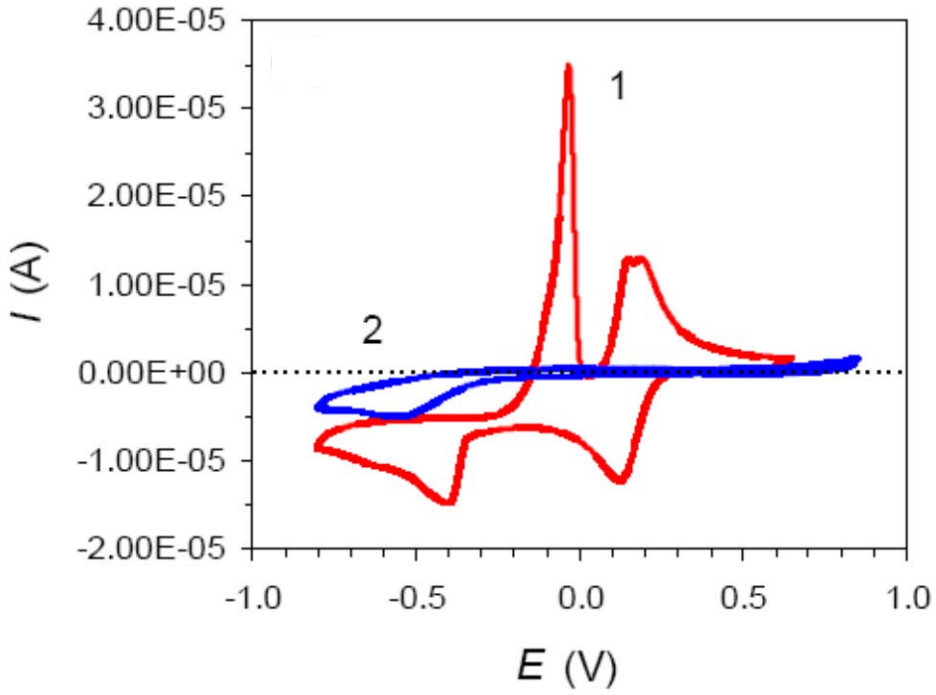

Fig 2. CV for Cu^{2+} alone (curve 1) clearly showing two pairs of redox peaks (belonging to the Cu2+/Cu+ and Cu+/Cu) electronic transformations) disappear upon the 1:1 complexation with BSA as indicated above (curve 2).

Fig. 3 displays the DSC data which additionally confirm the nearly 1:1 BSA-Cu^{2+} complexation for solutions containing the equal (1.8×10^{-3}) M concentrations of both, BSA and $CuCl_2$. It is clearly visible that there is small but distinct stabilization regarding the transition temperature, *Tm*, viz., 67.4 ± 0.5 ^{o}C for the BSA-Cu^{2+}complex (curve 2), versus 65.2 ± 0.5 ^{o}C for the BSA alone (curve 1); the over-all melting enthalpy, $\Delta Hcal$, also increased distinctly from 0.89 to 1.17 (given in arbitrary units), whereas the peak width (at the half height), ΔT, decreased from 8.0 to 6.6 ^{o}C, indicative of more cooperative character of the transition. Relatively minor stabilization caused by the BSA-Cu^{2+} complexation can be explained by the copper binding at the peripheral sight (see Fig. 1, panels (a) and (b)) that is remote from the central area connecting two largest domains of BSA (HSA). Indeed, the global thermodynamic stability is proposed to be determined by the interaction of these two largest domains (under certain pH conditions the global cooperativity of melting may be lost that shows up in splitting of a DSC peak). On the other hand, one can see that there exists some global conformational flexibility inside the protein matrix that is correlated with a global stability of the protein, both showing up through the extensive ($\Delta Hcal$) and intensive (*Tm*, ΔT) thermodynamic parameters associated to the protein's thermal denaturation (melting).

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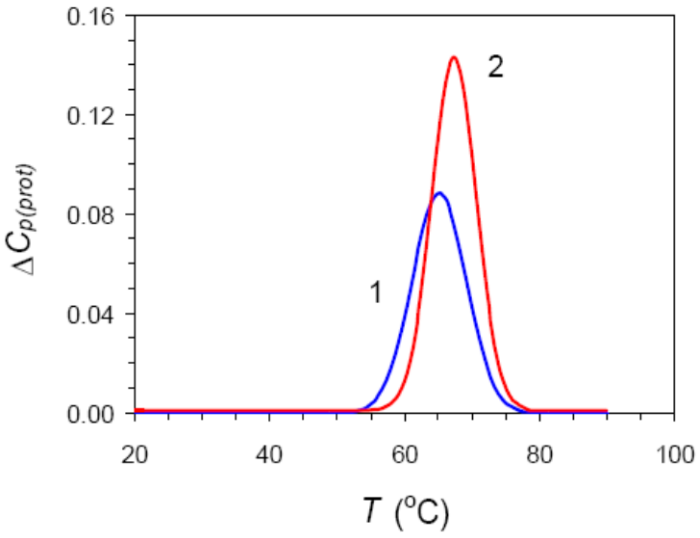

Fig. 3. DSC data for: the BSA alone (curve 1) and the BSA-Cu^{2+}complex (cuve 2).

In summary, The gained results provide direct experimental confirmation of a strong 1:1 complex formation between bovine serum albumin (BSA) and Cu^{2+} ions in aqueous solution, containing equal concentration of each component. The voltammetric measurements further revealed a significant suppression of the characteristic redox behavior of free Cu^{2+} ions. This combined electrochemical and calorimetric approach offers a robust basis for elucidating metal–protein interactions at the molecular level.

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