NEW ASPECTS OF THE INTERACTION OF COPPER (II) WITH SERUM ALBUMIN: VOLTAMMETRIC AND MICROCALORIMETRIC STUDIES

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Blood serum albumins of mammals, to begin with the most studied representatives such as human serum albumin (HSA), bovine serum albumin (BSA) etc. (with a molecular mass of ca. 66 500 Da), 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. It is natural that because of the extremely multifunctional physiological role of this class of proteins, exhaustive studies of a respective human prototype, HSA, play an outstanding role within the biomedical disciplines. Among other issues, investigation of the interaction of copper (II) with serum albumins, evaluation of binding amplitude and mechanism of interaction, 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]. It should also be noted that the structural and functional similarity of HSA (the undoubtedly most studied representative of this protein family) with most of its mammalian analogs, such as BSA, e.g. [3,13] (including occurrence of a high-affinity site for the Cu^{2+} capturing and transportation), provides a good basis for a many-sided modeling of HSA by its respective analogs. Indeed, this outstanding similarity makes BSA a perfect alternative for HSA in material-consuming laboratory studies with biomedical targeting owing to the relatively low cost and wide availability of BSA.

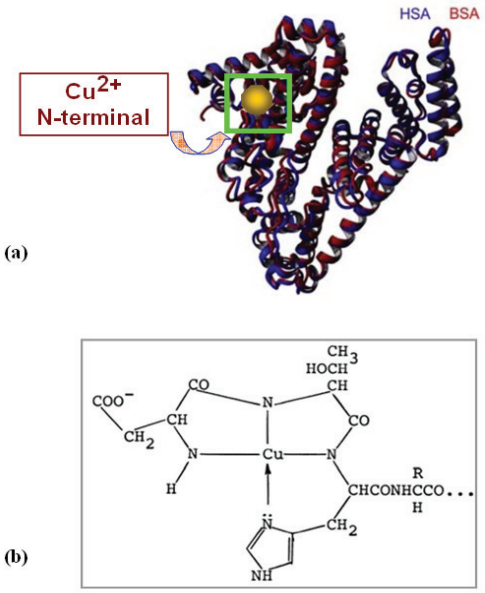

Fig. 1. (a) Structural alignment and comparative aspect for HSA and BSA, represented in blue and red colors, respectively. The Cu^{2+} (depicted by the yellow sphere) binding site is indicated by the bright green rectangle. (b) Model of suggested binding site for the "first" Cu^{2+} ion in BSA; within the "N-terminal" sequence of: Asp-Thr-His- (according to Ref. [3,13])

As a clarifying illustration, Fig. 1, panel (a) depicts the comparative tertiary structures of BSA and HSA, and panel (b) depicts the location of a Cu^{2+} ion within its binding site – so called N-terminal site in it [3,13].

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], in the present work 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)-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.

Material 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.

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, withou t 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 ($Ep_k = 0.12$V; $Ep_a = 0.2$V) and Cu^{+}/Cu^{0} at $E_0 = -0.2$V ($Ep_k =$

–0.37V; Ep_a = –0.035V) electronic transformations [5,6]. Addition of equal amount of BSA (1.8x10-3) M to the solution containing (1.8x10^{-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-Cu2+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- (see Fig. 1 (b)) 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 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].

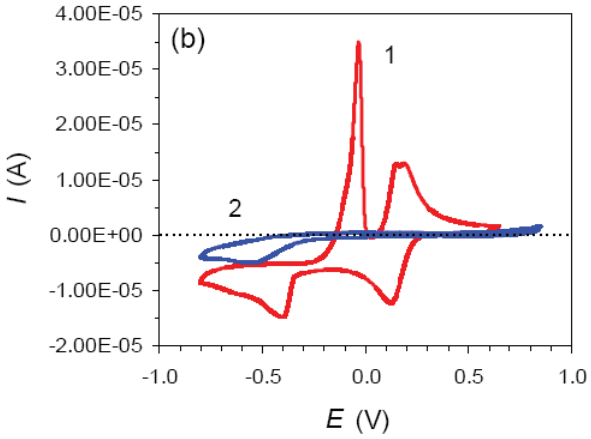

Fig 2. CV for Cu^{2+} alone (curve 1) clearly showing two pairs of redox peaks (belonging to the Cu2+/Cu+ and Cu+/Cu) electronic transformations) disappear upon the 1:1 complexation with BSA as indicated above (curve 2)

Fig. 3 displays the DSC data which additionally confirm the nearly 1:1 BSA-Cu^{2+} complexation for solutions containing the equal (1.8x10^{-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 °C for the BSA-Cu^{2+}complex (curve 2), versus 65.2±0.5 °C for the BSA alone (curve 1); the over-all melting enthalpy, D*Hcal*, also increased distinctly from 0.89 to 1.17 (given in arbitrary units), whereas the peak width (at the half height), Δ*T*, decreased from 8.0 to 6.6 °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 (D*Hcal*) and intensive (*Tm*, Δ*T*) thermodynamic parameters associated to the protein's thermal denaturation (melting).

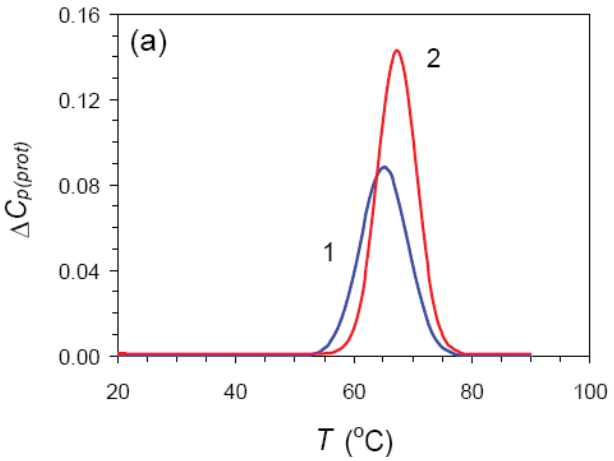

Fig. 3. DSC data for: the BSA alone (curve 1) and the BSA-Cu^{2+}complex (cuve 2)

Conclusions. A compbination of two independent physical methods, the CV (electrochemistry) and DSC (microcalorimetry), was applied for the first time to directly confirm the formerly proposed hypothesis about the strong 1:1 complexation of blood serum albumins with Cu^{2+} ions in solutions (containing in our case equal (1.8 x 10^{-3}) M concentrations of both, BSA and $CuCl_2$). In addition, the CV method allowed for a direct detection of blocking the "normal" redox activity of Cu^{2+} ions when presumably captured by the chelating site near the N-terminal group of BSA (HSA).

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SUMMARY

NEW ASPECTS OF THE INTERACTION OF COPPER (II) WITH SERUM ALBUMIN: VOLTAMMETRIC AND MICROCALORIMETRIC STUDIES

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Structural organization of serum albumins – the most abundant globular proteins in serum 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 serum albumin, 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 into 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 (0.2 M KCl) containing ($1.8x\ 10^{-3}$) M $CuCl_2$ results that two pairs of redox peaks belonging to the Cu^{2+}/Cu^{+} (E_0 = 0,16 V) and Cu^{+}/Cu^{0} (E_0 = –0.2 V) electronic transformations disappear and a new weak single reductive peak, at Ep_k=–0,55V attributable to the Cu^{2+}/Cu^{+} transition is shown. BSA- Cu^{2+} complex formation is presumably responsible for this dramatic shift of Cu^{2+} reduction process to much more negative potential. The chelating environment of "N-terminal" sequence of: Asp-Thr-His- of BSA, assisted by direct participation of the sulfuric group of a Cys-34 residue, is presumably responsible for the entrapment and "locking" the copper ion, in an "abnormal", redox inactive condition (showing virtually no voltammetric activity). Our DSC data confirmed the complex formation process in the solutions containing the equal ($1.8x10^{-3}$) M concentrations of both, BSA and $CuCl_2$ and clearly shows small but distinct conformational stabilization with respect of two thermodynamic parameters, the melting temperature and melting enthalpy.

Keywords: Serum albumins, interaction with copper (II) ions, voltammetry, redox properties, differential scanning calorimetry.

РЕЗЮМЕ

НОВЫЕ АСПЕКТЫ ВЗАИМОДЕЙСТВИЯ ИОНОВ МЕДИ (II) С СЫВОРОТОЧНЫМ АЛЬБУМИНОМ: ВОЛЬТАМПЕРОМЕТРИЧЕСКИЕ И МИКРОКАЛОРИМЕТРИЧЕСКИЕ ИССЛЕДОВАНИЯ

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Сывороточный альбумин (человеческий – ЧСА, бычий - БСА) представляет собой самую большую фракцию белков плазмы крови. Благодаря стуктурным особенностям, сывороточный альбумин связывает и транспортирует различные лиганды, лекарственные вещества, ионы металлов, в том числе ионы меди. Исследованию взаимодействия ионов металлов с альбумином, учитывая значимость проблемы, уделяется большое внимание. Исходя из того, что ЧСА и БСА имеют схожую структуру и учитывая широкую доступность и низкую цену последнего, БСА выбран в качестве модели для лабораторных исследований. Комбинированные вольтамперометрические и термодинамичеслие исследования проводились с целью изучения взаимодействия сывороточного альбумина с ионами меди (Cu^{2+}). Согласно вольтамперометрическим данным при добавлении в раствор хлористого калия (0.2M KCl), содержащего ионы меди (1.8×10^{-3}) M $CuCl_2$, равной концентрации БСА (1.8×10^{-3})M, пики, отражающие электронные переходы Cu^{2+}/Cu^{+} (E_0 = 0,16 V) и Cu^{+}/Cu^{0} (E_0 = –0.2 V) исчезают, вместо них при высоких отрицательных потенциалах (Ep_k =-0,55V) появляется слабо выраженный сигнал, соответствующий реакции Cu^{2+}/Cu^{+}. Исключительный сдвиг потенциала восстановления ионов меди (Cu^{2+}), по всей вероятности, связан с образованием комплекса сывороточного альбумина с ионами двухвалентной меди (БСА-Cu^{2+}). В процессе формирования данного комплекса, редокс активность иона меди(II), очевидно, “блокируется” совокупным эффектом его “захвата” N-терминальной хелатной группой Asp-Thr-His альбумина в купе с дополнительным взаимодействием с атомом серы группы Cys-34, переводя медь в частично восстановленное (неактивное) состояние. Данные калориметрических измерений подтверждают образование комплекса БСА-Cu^{2+} в растворах, содержащих равные концентрации (1.8×10^{-3}) М БСА и ионов меди.

რეზიუმე

ორვალენტიანი სპილენძის (II) იონების და შრატის ალბუმინის ურთიერთქმედების ახალი ასპექტები: ვოლტამპერული და მიკროკალორიმეტრული კვლევები

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[2]ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, რ. აგლაძის არაოგანული ქიმიის და ელექტროქიმიის ინსტიტუტი; [3]ი. ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ფიზიკის დეპარტამენტი, საქართველო

შრატის ალბუმინი (ადამიანის, ხარის) სისხლის პლაზმაში არსებული ცილებიდან რაოდენობრივად ყველაზე მეტია. შრატის ალბუმინის სტრუქტურული წყობა განაპირობებს მის განსაკუთრებულ თვისებას დაიკავშიროს სხვადასხვა ლიგანდები, სასიცოცხლოდ მნიშვნელოვანი მეტალთა იონები, როგორიცაა ორვალენტიანი სპილენძის იონები (Cu^{2+}) და მოახდინოს მათი ტრანსპორტირება. ალბუმინის და მეტალთა იონების ურთიერეთქმედების შესწავლას, მისი მნიშვნელობიდან გამომდინარე, დიდი ყურადღება ეთმობა. იმის გათვალისწინებით, რომ ადამიანის და ხარის შრატის ალბუმინის სტრუქტურები ძალიან ახლოსა არის ერთმანეთთან, ეს უკანასკნელი გამოყენებულია ლაბორატორიული კვლევებისათვის, მისი ხელმისაწვდომობისა და დაბალი ფასის გათვალისწინებით. ვოლტამპერული და მიკროკალორიმეტრული კვლევების გამოყენებით ავტორებმა შეისწავლეს ალბუმინის ურთიერთქმედება ორვალენტიანი სპილენძის იონებთან. მიღებული ვოლტამპერული მონაცემები ცხადყოფს, რომ 0.2 M KCl + $1.8\text{x}10^{-3}$ M $CuCl_2$-ის ხსნარში იგივე კონცენტრაციის ალბუმინის ($1.8\text{x}10^{-3}$) დამატებისას Cu^{2+}/Cu^{+} (E_0=0.16 V) და Cu^{+}/Cu^{0} (E_0 = –0.2 V) რედოქს რეაქციების შესაბამისი დენის პიკები ქრება და მათ მაგივრად ბევრად უფრო უარყოფით პოტენციალზე (Ep_k = –0.55 V) აღმოცენდება სუსტად გამოხატული ვოლტამპერული პიკი, რომელიც, სავარაუდო, მიეკუთვნება Cu^{2+}/Cu^{+} გადასვლას. გამოთქმულია მოსაზრება, რომ შრატის ალბუმინის Cu^{2+}-თან კომპლექსაციის დროს, სავარაუდოდ, “N-ტერმინალის” Asp-Thr-His-გარემოცვის პირობებში და ხელსაყრელ პოზიციაზე განლაგებული Cys-34-ჯგუფის გოგირდის ატომის უშუალო ჩართულობით ხდება სპილენძის იონების ნაწილობრივი “აღდგენა”, ანუ მისი ნორმალური ჟანგვა-აღდგენითი აქტივობის “ბლოკირება”, რის გამოც Cu^{2+}-ის იონების აღდგენის პოტენციალი, როგორც ჩანს, ბევრად უფრო მაღალი უარყოფითი მნიშვნელობებისაკენ გადაინაცვლებს. მიკროკალორიმეტრული კვლევები ადასტურებს შრატის ალბუმინის და Cu^{2+}-ის თანაბარი კონცენტრაციის (1.8x10-3) M შემცველ ხსნარებში კომპლექსაციის პროცესის არსებობას, რაც, თავის მხრივ, აისახება თერმული ლღობის ტემპერატურის, ასევე ენტალპიის მცირე, მაგრამ მკვეთრად გამოხატულ ზრდაში.