Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *86* (9) 795–807 (2021)
JSCS–5462
Original scientific paper
Published 28 May, 2021

Physicochemical characterisation of dihydro-alpha-lipoic acid interaction with human serum albumin by multi-spectroscopic and molecular modelling approaches

NIKOLA GLIGORIJEVIĆ[1]*, VLADIMIR ŠUKALOVIĆ[2], SIMEON MINIĆ[3#], GORAN MILJUŠ[1], OLGICA NEDIĆ[1#] and ANA PENEZIĆ[1]

[1]*Institute for the Application of Nuclear Energy, Department for Metabolism, University of Belgrade, Banatska 31b, 11080 Belgrade, Serbia,* [2]*Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia and* [3]*Department of Biochemistry and Center of Excellence for Molecular Food Sciences, University of Belgrade – Faculty of Chemistry, Studentski Trg 12–16, 11000 Belgrade, Serbia*

(Received 22 April, revised and accepted 27 May 2021)

Abstract: The binding of a popular food supplement and well-known antioxidant, dihydro-alpha-lipoic acid (DHLA) to human serum albumin (HSA) was characterised. The binding was monitored by several spectroscopic methods together with the molecular docking approach. HSA was able to bind DHLA with moderate affinity, $1.00\pm0.05\times10^4$ M^{-1}. Spectroscopic data demonstrated that the preferential binding site for DHLA on HSA is IIA (Sudlow I). Both experimental and molecular docking analysis identified electrostatic (salt bridges) and hydrogen bonds as the key interactions involved in DHLA binding to HSA. Molecular docking confirmed that the Sudlow I site could accommodate DHLA and that the ligand is bound to the protein in a specific conformation. The molecular dynamic simulation showed that the formed complex is stable. Binding of DHLA does not affect the structure of the protein, but it thermally stabilises HSA. Bound DHLA had no effect on the susceptibility of HSA to trypsin digestion. Since DHLA is a commonly used food supplement, knowledge of its pharmacokinetics and pharmacodynamic properties in an organism is very important. This study further expands it by providing a detailed analysis of its interaction with HSA, the primary drug transporter in the circulation.

Keywords: spectral analysis; molecular docking; protein–ligand interaction; protein stability, protein structure.

* Corresponding author. E-mail: nikolag@inep.co.rs
# Serbian Chemical Society member.
https://doi.org/10.2298/JSC210420041G

INTRODUCTION

Human serum albumin (HSA) is the most dominant protein in the circulation, with a concentration in the range from 35 to 50 g L^{-1} (522 to 746 μM). It has a molecular mass of 67 kDa.[1] Structurally, HSA is composed of three homologous domains (I, II and III), each divided into two subdomains, A and B. The dominant secondary structure motif of HSA is α-helix.[2]

HSA has numerous essential functions in the circulation. Due to its high concentration, HSA participates in the regulation of osmotic pressure.[3] Its free Cys34 thiol group (in healthy individuals 70–80 % of Cys34 thiol group is in a reduced form), makes HSA an important factor for plasma antioxidant capacity, contributing with 80 % to the total amount of plasma thiol.[4] HSA is also a general transporter of fatty acids, ions and many drugs. The structure of HSA enables it to accommodate and bind a large variety of small molecules with moderate to high affinities. Two main binding sites for many different molecules (excluding fatty acids) are located at the IIA subdomain or Sudlow I binding site, and IIIA subdomain or Sudlow II binding site. Warfarin and ibuprofen drugs are specific ligands for Sudlow site I and Sudlow site II, respectively.[5]

Lipoic acid (LA) is a naturally occurring molecule the main sources of which are potato, broccoli and spinach. Humans can also synthesise LA in small amounts. LA is readily absorbed from foods and its oral administration as a drug is a viable therapeutic option, including the treatment of patients with Covid-19 infection.[6] LA supplements are also commercially available, with LA concentrations of up to 600 mg per tablet. The antioxidant activity of LA is manifested through ROS scavenging, transition metal ions (*e.g.*, iron and copper) chelating, cytosolic glutathione and vitamin C level increases, and oxidative stress damage repair.[7]

Following cellular uptake, LA is reduced to dihydrolipoic acid (DHLA), which is a very potent reducing agent.[7] LA has several beneficial effects, such as antioxidant, improvement of glycaemic control, mitigation of toxicity by heavy metal poisoning and immunomodulatory effects.[7–9]

Although the ability of albumin to bind DHLA is well known,[10] no detailed analysis of this interaction has been reported so far. In the case of bovine serum albumin (BSA), DHLA was shown to bind at the IIIA subdomain.[11] However, no binding experiments in the presence of a specific ligand for this site were performed. Taking into account the structural similarity of DHLA and octanoic fatty acid, it was proposed that DHLA binds to subdomain IIA,[12] but IIIA subdomain was also considered.[11]

Considering that DHLA is a potent antioxidant used as a food supplement and that its usage can help in oxidative stress related conditions, it looked relevant to investigate its interaction with HSA, a universal transporter in the circulation. The properties of this interaction are still not fully defined in the literature. Hence, the

aim of the present study was to characterise DHLA–HSA binding, with the usage of both multi-spectroscopic and molecular modelling techniques.

EXPERIMENTAL

Materials

All chemicals used were purchased from Sigma (Burlington, MA, USA) and were of analytical grade. HSA was purchased from Sigma, (product number A-1653), and used without additional purification. A stock solution was made in 10 mM phosphate buffered saline (PBS), pH 7.4. The concentration of HSA was determined using a bicinchoninic acid (BCA) assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Stock solution (5 mM) of DHLA was prepared by suspending DHLA in 10 mM PBS following the addition of a small volume of 1 M NaOH until full clarification of solution was reached.[13] Trypsin was purchased from the Torlak Institute (Belgrade, Serbia) as a 0.25 % solution. Stock solutions of ibuprofen and warfarin were made in DMSO. In the experiments where they were used, the final DMSO concentration did not exceed 2 %. All experiments were performed in triplicate at room temperature, using 10 mM PBS, pH 7.4, unless otherwise stated. All results are presented as averaged of triplicates, and their *SD* never exceeded 5 % of the presented averages.

Analysis of HSA–DHLA complex formation by spectrofluorimetry

Determination of the binding constant (K_a) of the HSA–DHLA complex was realised by measuring the quenching of the intrinsic fluorescence emission of HSA (0.4 μM) in the presence of increasing concentrations of DHLA (from 5 to 35 μM) at 37 °C. Fluorescence spectra were recorded using FluoroMax®-4 spectrofluorometer (Horiba Scientific, Japan). HSA was excited at 280 nm and the emission spectra were recorded in the range from 290 to 450 nm. Each spectrum was corrected for the emission of the control that contained only DHLA at a particular concentration. The change of the emission intensity at 338 nm (HSA emission maximum) was used for the calculation of the binding constant. The emission intensity measured for HSA was first corrected for the small inner filter effect of DHLA using the equation:

$$F = F_0 10^{(A\mathrm{ex}+A\mathrm{em})/2} \quad (1)$$

where F is corrected fluorescence, F_0 is measured fluorescence, A_{ex} and A_{em} are absorbencies at excitation and emission wavelengths, which are 280 nm and 338 nm, respectively.

The binding constant between HSA and DHLA was calculated using the following equation:

$$\frac{F_0 - F}{F - F_c} = K_a c_L \quad (2)$$

where F_0 and F represent the intensities of the fluorescence emission signals of HSA in the absence and the presence of the ligand, F_c represents the fluorescence intensity of HSA completely saturated with the ligand, which was experimentally obtained by determination of the fluorescence emission intensity of the protein in the presence of 17 mM DHLA and c_L is the concentration of used ligand (DHLA).

For determination, if at the same time, dynamic (collision) and static (complex formation) types of quenching are present or only one of them, Stern–Volmer (SV) graph was plotted. From it, the SV quenching constant (K_{SV}) was calculated using the following equation:[14]

$$F_0/F = 1 + k_q \tau_0 c_Q = 1 + K_{SV} c_Q \quad (3)$$

where F_0 and F are the intensities of the emission signals without and in the presence of DHLA, k_q represents the biomolecule quenching rate constant, τ_0 is the average lifetime of the biomolecule without quencher (10^{-8} s), c_Q is the total concentration of quencher (DHLA). The slope from SV plot represents K_{SV}. K_{SV} was further used for the calculation of k_q.

Thermodynamic parameters of DHLA binding to HSA were calculated using the same experimental approach as for the calculation of K_a but at three different temperatures, 25, 30 and 37 °C. The calculated binding constants at three temperatures were then used to plot a van't Hoff graph. Enthalpy (ΔH) and entropy (ΔS) change were calculated from the graph applying the following equation:

$$\ln K_a = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \tag{4}$$

where T is temperature in K and R is a universal gas constant (8.314 J mol^{-1} K^{-1}). ΔH was calculated from the slope of van't Hoff graph and ΔS from the intercept.

For fluorescence emission changes, specifically originating from Tyr residues or the only Trp214 residue, synchronous fluorescence spectra were recorded on RF-6000 spectrophotometer (Shimadzu, Japan). The spectra were recorded in the range from 310 to 380 nm with $\Delta\lambda$ of 60 nm for Trp and in the range from 290 to 325 nm with $\Delta\lambda$ of 15 nm for the Tyr residues. Here, $\Delta\lambda$ represents λ of emission – λ of the excitation for each specific residue.

For the location of specific binding site(s), a competitive binding experiment was performed. To HSA, increasing concentrations of DHLA in the presence and the absence of site ligands (ibuprofen and warfarin) were added. HSA (0.4 μM) was first incubated with 0.4 μM of site specific ligands and the obtained mixture were titrated with DHLA. HSA was excited at 280 nm and emission spectra were recorded in 290–450 nm range. Calculation of HSA––DHLA affinity constants in the presence and absence of site-specific ligands was realized as already mentioned previously.

In order to confirm that DHLA binds HSA at the binding site specific of warfarin, a mixture of HSA (0.4 μM) and warfarin (20 μM) were titrated with DHLA (10, 20, 30, 40 μM). The warfarin fluorescence is enhanced in the presence of HSA and the excitation of the obtained complex was performed at 310 nm,[15] while recording of emission spectra was performed in the range 340–450 nm. The obtained spectra were corrected by subtracting the emission spectra obtained in the absence of HSA.

UV–Vis analysis of the HSA–DHLA complex

UV–Vis absorption spectra of HSA (9 μM), alone and in the presence of different concentrations of DHLA (9, 45 and 90 μM) were recorded at room temperature using a Ultrospec 2000 spectrophotometer (Pharmacia Biotech, Sweden). The spectra were recorded in the wavelength range from 250 to 300 nm. The spectrum of each mixture was corrected for the spectrum obtained for the appropriate concentration of DHLA alone. Furthermore, the UV––Vis spectrum of DHLA (90 μM) in the presence of HSA (9 μM) was recorded in the range from 300 to 450 nm and corrected for the spectrum obtained for HSA alone.

Structural analysis of HSA–DHLA complex using circular dichroism (CD) spectropolarimetry

The influence of DHLA binding on the structure of HSA was analysed using a CD-spectropolarimeter J-815 (Jasco, Japan) at room temperature by applying a scan speed of 50 nm min^{-1}. Different concentrations of DHLA were added (6, 15 and 30 μM) to HSA (3 μM). Both HSA and DHLA stock solutions were dissolved in 10 mM phosphate buffer, pH 7.4. The tertiary protein structure was analysed by recording near-UV CD spectra in the range from

260 to 320 nm using a cell with an optical path of 10 mm, while the secondary protein structure was monitored by recording far-UV CD spectra in the range 185–260 nm using a cell with 0.5 mm optical path length. The spectra of free ligand were subtracted from the spectra of corresponding protein–ligand mixtures.

Analysis of temperature stability of HSA–DHLA complex

Temperature stability of HSA (0.4 μM) alone and in the presence of DHLA (40 μM) was determined by recording the reduction of fluorescence emission at 338 nm (emission peak of HSA) and at 335 nm (emission peak of HSA–DHLA complex), using the same equipment as used in above. Reduction of protein emission was recorded in the temperature range from 37 to 87 °C with a temperature increase rate of 2 °C min^{-1}. At each temperature, the mixture was allowed to equilibrate for 1 min before the measurement. The obtained spectra were corrected by subtracting the spectra of DHLA recorded alone at each temperature. Results were fitted to sigmoid curves and from them inflexion points were obtained which represent melting temperatures of HSA (T_m).

Analysis of proteolysis of HSA by trypsin in the presence of DHLA

For the investigation of whether DHLA binding affects the susceptibility of HSA to trypsin proteolysis, the following experiment was performed at 37 °C. To solutions containing 4 μM HSA, alone and in the presence of DHLA (40 μM), 25 μL of 0.25 % trypsin solution was added. The final volume of reaction mixtures was 1 mL. At different time points (1, 5, 10, 20 and 30 min), 50 μL aliquots were taken from the reaction mixture and PMSF immediately added at the final concentration of 2 mM, thus stopping the reaction. Proteolytic fragments of HSA were analysed by reducing SDS-PAGE using a 12 % gel in a standard manner. The gel was stained using the Silver Stain Plus Kit (Bio-Rad, Hercules, California, USA).

Molecular docking and dynamic analysis

The methods used and the obtained results are given in the Supplement material to this paper.

RESULTS AND DISCUSSION

HSA–DHLA complex formation

The intrinsic fluorescence of HSA is quenched in the presence of DHLA, as shown in Fig. 1A. In addition, a small blue shift (3 nm) of the emission maximum of HSA is observed, in the presence of increasing concentrations of DHLA. The obtained results indicate that HSA is capable of binding DHLA and by doing so, the polarity in the vicinity of Tyr and Trp214 amino acid residues of HSA is not significantly changed. Fluorescence quenching can occur as a result of dynamic quenching (collisional) and static quenching (complex formation), whereby, both of them can be present simultaneously. To determine what applies for the present HSA–DHLA pair, an SV graph was plotted (Fig. 1B) and from its slope, K_{SV} was determined. Since the obtained SV plot is linear ($r^2 = 0.99$), it proves that only one quenching type occurs in our tested system. K_{SV} was determined to be 0.83×10^4 M^{-1} and from it, the quenching rate constant of the biomolecule, k_q, was determined to be 0.83×10^{12} M^{-1}. The obtained value of k_q is about two orders of magnitude higher than the diffusion rate of biomolecules (10^{10} M^{-1} s^{-1}),

strongly suggesting the presence of a static type of quenching or, in other words, complex formation between HSA and DHLA.[16] By using Eq. (2) and the plot from Fig. 1C, K_a was determined to be 1.00±0.05×10^4 M^{-1}, at 37 °C, showing that HSA can bind DHLA with a moderately strong affinity.

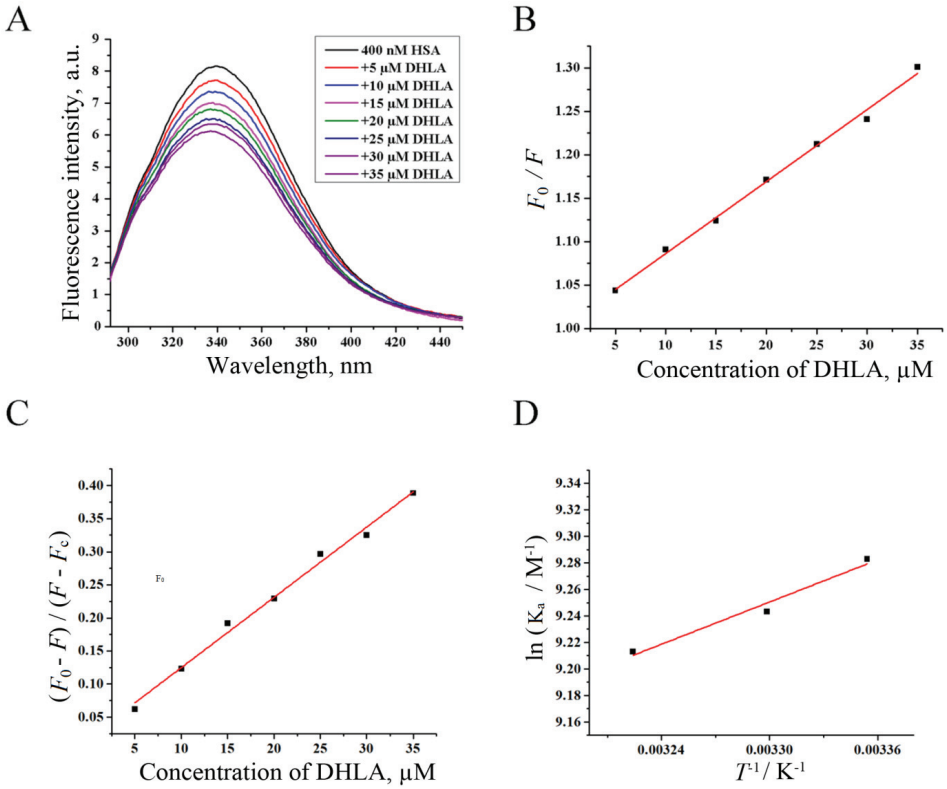

Fig. 1. Binding analysis of HSA and DHLA using spectrofluorimetry. Fluorescence emission spectra of HSA (excited at 280 nm) in the presence of increasing concentrations of DHLA (A). Stern–Volmer plot (B) and the plot used to determine the binding constant between HSA and DHLA (C) obtained using the fluorescence emission maximum of HSA at 338 nm. van't Hoff's graph, obtained by calculating the binding constants between HSA and DHLA at three different temperatures (D).

Calculation of K_a at three different temperatures showed that its value decreased as a consequence of temperature increase. This usually occurs when the static type of quenching is present since formation a complex is weaker at higher temperatures.[17] This result also confirms complex formation between HSA and DHLA. From the K_a values obtained at three temperatures, thermodynamic parameters were determined using a van't Hoff plot (Fig. 1D). A negative value of ΔH was obtained, –4.42 kJ mol^{-1} and positive value of ΔS, 62.27 J mol^{-1} K^{-1}.

These results indicate that electrostatic interactions are key interactions for HSA–DHLA complex formation,[18] which was also confirmed by molecular dynamic analysis.

Synchronous fluorescence spectra are used to provide information about the changes in the emission of Trp and Tyr amino acid residues. HSA has only one Trp residue that is located inside the binding site of subdomain IIA (Sudlow I binding site),[19] and information from synchronous spectra can provide insight into the binding site for a certain ligand. Increasing concentrations of DHLA significantly quenched emission spectrum of Trp (Fig. 2A), while quenching of emission spectrum of Tyr occurred to a very small extent (Fig. 2B). Bearing in mind the location of the only Trp residue in HSA, this result suggested that DHLA binds HSA at the subdomain IIA binding site (Sudlow I binding site).

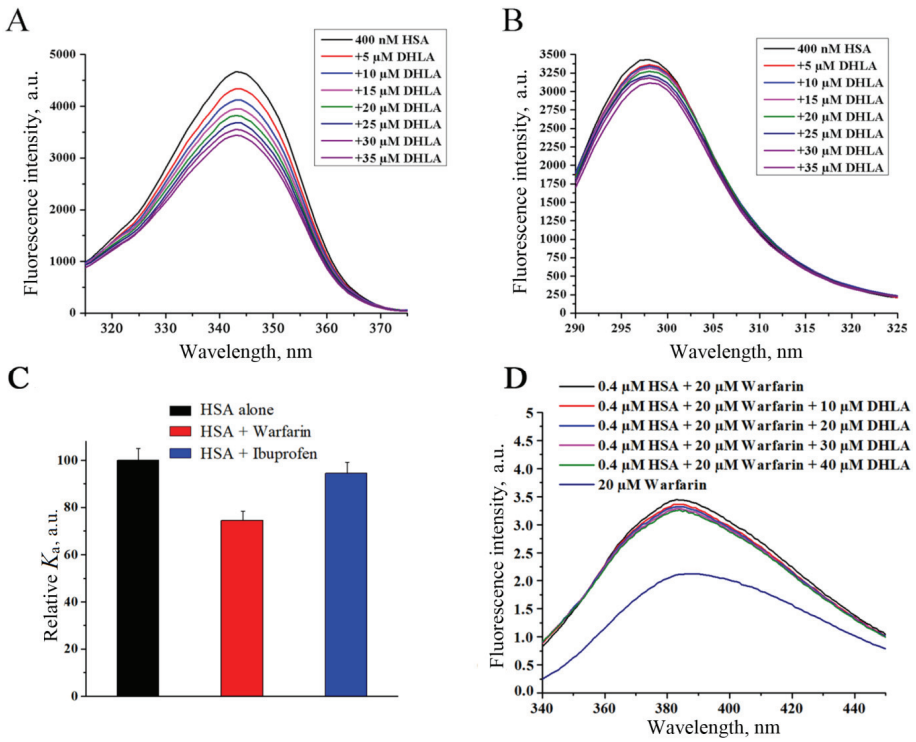

Fig. 2. Determination of a binding site of DHLA on HSA. Synchronous fluorescence spectra of HSA with $\Delta\lambda$ = 60 nm for Trp (A) and $\Delta\lambda$ = 15 nm for Tyr (B) in the presence of increasing concentrations of DHLA. Relative affinity constants for HSA–DHLA complex formation calculated for 0.4 μM HSA alone and in the presence of equimolar concentrations of warfarin and ibuprofen, respectively (C). Displacement of HSA-bound warfarin by increasing concentrations of DHLA, detected by specific excitation of warfarin at 310 nm and recording its emission in the range from 340 to 450 nm (D).

For determination the binding site, DHLA was added to HSA in the presence of warfarin and ibuprofen. The affinity constant of HSA–DHLA complex in the presence of these molecules was calculated as described previously. Binding of both warfarin and ibuprofen to HSA is well characterised with affinity constants of about 10^5 M^{-1} for warfarin,[20] and about 10^6 M^{-1} for ibuprofen.[21] As warfarin binds specifically to subdomain IIA binding site (Sudlow I) on HSA, while ibuprofen specifically binds to subdomain IIIA binding site (Sudlow II), they are used to block these sites in the studies that aim to locate the exact binding sites for other ligands.[5] The obtained results, Fig. 2C, show that in the presence of warfarin, the affinity constant for the HSA–DHLA complex is reduced by almost 25 %, while ibuprofen only marginally reduced this value, about 5 %. Therefore, although the binding constant of warfarin to HSA is one order of magnitude lower than ibuprofen, the presence of warfarin has a five times more pronounced effect on the decrease of DHLA binding affinity to HSA in comparison to the presence of ibuprofen. Hence, these results show that DHLA preferentially binds at subdomain IIA on HSA.

The fluorescence intensity of warfarin increases when bound to HSA, Fig. 2D. This usually happens when a ligand binds to a protein and by doing so it becomes located in a more hydrophobic environment and thus shielded from water.[22] A similar observation was noted for the binding of phycocyanobilin (PCB) to HSA, which happens at binding sites of both IIA and IB subdomains of HSA.[23] In the presence of increasing concentrations of DHLA, a reduction in the emission intensity of warfarin occurs, (Fig. 2D) indicating that DHLA displaces warfarin from its binding site, confirming that HSA binds DHLA at subdomain IIA.

The presence of aromatic amino acid residues enables proteins to absorb light in the UV region at about 280 nm and alterations in the absorption spectrum in this region may occur due to changes in the polarity of the environment that is in vicinity to these residues. The UV–Vis absorption spectrum of HSA does not change in the presence of increasing concentrations of DHLA, Fig. 3A, indicating that the binding of DHLA to HSA did not have any significant effect on the polarity or aromatic amino acids of HSA, which is in agreement with results obtained by spectrofluorimetry (Fig. 3A). The absorption spectrum of DHLA changes in the presence of HSA. Its peak is blue shifted and its intensity is reduced (Fig. 3B). Similarly, this was observed previously upon DHLA binding to fibrinogen.[24] Changes in the absorption spectrum of DHLA represent additional proof that the HSA–DHLA complex is being formed.

Influence of DHLA binding on the stability and structure of HSA

Often, ligand binding has effect on the protein structure, with some ligands inducing more ordered structures, others causing the protein to be more disordered,

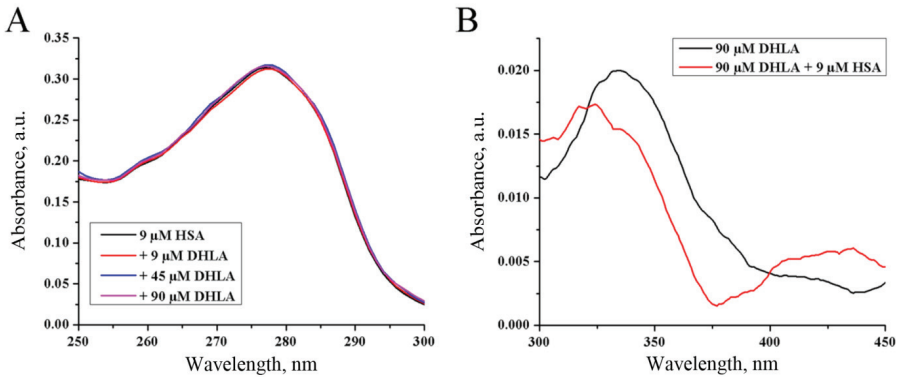

Fig. 3. The influence of complex formation on the UV-VIS absorption spectra of HSA and DHLA. UV absorption spectra of HSA alone and in the presence of increasing concentrations of DHLA (A). UV–Vis absorption spectra of DHLA alone and in the presence of HSA (B).

while some ligands have no effect. HSA has only α-helixes as the secondary structure motifs. Binding of amoxicillin and PCB to HSA increases its α-helical content,[25] while binding of safranal and crocin decreases it.[19] Far-UV CD spectra, obtained for HSA, (Fig. 4A), show a typical protein signal with α-helix as the dominant secondary structure motif. Its characteristic is a wide negative peak, ranging from 209 to 220 nm. From Fig. 4A, it can be seen that DHLA binding to HSA has no significant change in the secondary structure of HSA, even in the presence of ten times larger molar concentration of DHLA than HSA. The obtained near-UV-CD spectra for HSA alone and in the presence of all tested concentrations of DHLA are practically the same, showing that the tertiary structure of HSA upon DHLA binding is unaltered (Fig. 4B).

Besides the protein structure, other factors that affect the melting point of a protein may include the presence of bound molecules as well as their own structure. As a consequence of protein–ligand complex formation, new interactions are formed, and they may alter the temperature stability of the protein. Free HSA has a T_m of about 62 °C, while with bound fatty acids, its temperature stability increases, reaching a T_m from 64 to 72 °C.[26] Some ligands, such as embelin and PCB, are also able to increase the temperature stability of HSA.[25,27] The HSA used in this study had a T_m of 68 °C. When bound to DHLA, the T_m of the HSA is increased to 70 °C (Fig. 4C). Since DHLA did not have any effect on the structure of HSA upon its binding (Figs. 4A and 4B), it seems that newly formed interactions in the HSA––DHLA complex caused additional thermal stabilisation of HSA.

Since the T_m of HSA increased in the presence of DHLA, indicating that the protein is becoming more rigid, this in turn may affect its susceptibility to proteolysis. For a protein to be proteolysed, its peptide bonds need to be exposed enough to enable their proper placement in the active site of a protease. Some ligands, such

as bilirubin for example, can make HSA more resistant to cleavage by trypsin.[28] From the obtained results, it seems that DHLA, although it thermally stabilises HSA, has no significant effect on HSA proteolysis by trypsin (Fig. 4D). This suggests that the binding of DHLA to HSA will not have significant (if any) effect on the half-life of this protein in circulation, regarding proteolysis. The first and the most dominant fragment of HSA resulting from its proteolysis by trypsin is the one at about 5 kDa. Other, less abundant fragments, with lower molecular masses appear later. This finding is in agreement with literature data.[25]

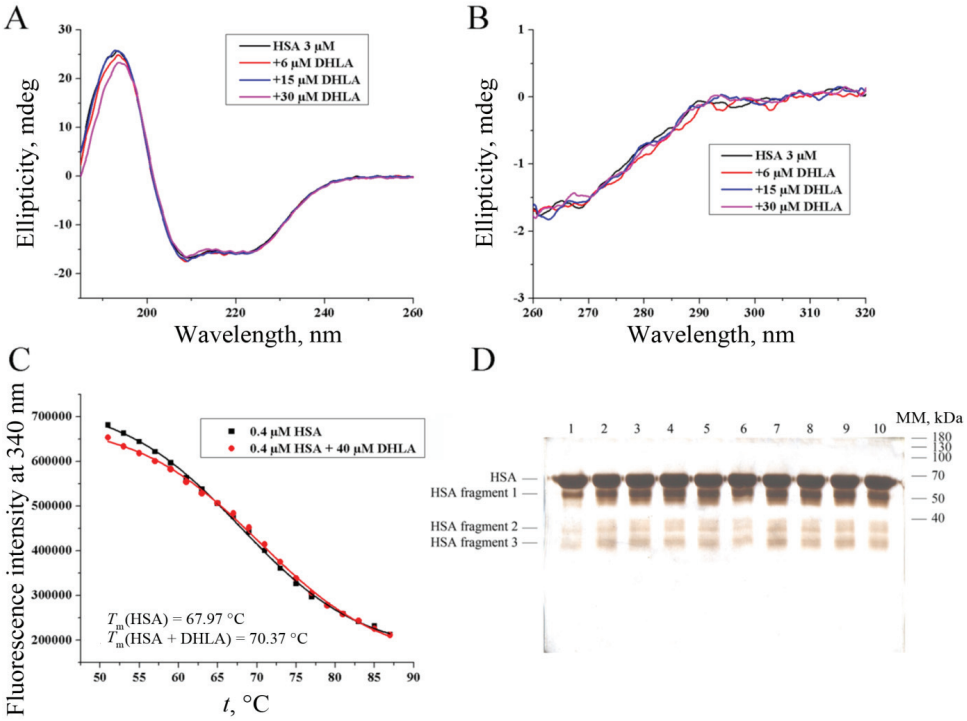

Fig. 4. Analysis of structural alterations of HSA due to DHLA binding. Far-UV CD (A) and near-UV CD (B) spectra of HSA alone and in the presence of increasing concentrations of DHLA. Temperature stability of HSA alone and in the presence of DHLA (C). Analysis of HSA digestion by trypsin in the absence (lanes 1–5, samples were taken after 1, 5, 10, 20 and 30 min of proteolysis) and in the presence of DHLA (lanes 6–10) by reducing SDS-PAGE on 12 % gel (D). MM stands for molecular weight markers.

HSA can be oxidised, and the small structural changes that arise as a consequence of this chemical modification can lead to an impairment of HSA functions, including its important ligand-binding ability.[29] The binding properties of HSA can also be influenced by the redox state of its free Cys34.[30] Considering the high concentration of HSA and its capacity to bind a large range of small

molecules, changes in its binding properties may have significant consequences on pharmacokinetic and pharmacodynamic (PKPD) properties of prescribed drugs. The ability of DHLA to protect albumin from non-enzymatic glycosylation[10] and methylglyoxal modification[31] was previously shown. Additionally, DHLA could protect Cys34 from oxidation.[12] Thus, by complex formation with HSA, DHLA can prevent the oxidation of HSA, and also preserve the antioxidative and binding properties of HSA. Considering its popular usage as a food supplement, detailed knowledge of the PKPD properties of DHLA is very important, including information on its binding proteins in the circulation.

CONCLUSIONS

The results of this paper characterise, in more detail, complex formation between DHLA and HSA, which was lacking in the literature. The obtained results showed that preferential binding on HSA of DHLA is located at subdomain IIA (Sudlow I binding site). The ability of the Sudlow I site to bind DHLA and the stability of the formed complex was confirmed by molecular docking analysis and dynamics. The structure of HSA is not significantly altered on DHLA binding, however, HSA is stabilised. The presence of DHLA did not alter the susceptibility of HSA towards trypsin proteolysis and hence, no change of half-life of HSA in circulation due to proteolysis, is expected. These results add to the knowledge of the PKPD properties of DHLA and offer a future perspective for further studies that deal with the usage of DHLA as a food supplement.

SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/index, or from the corresponding author on request.

Acknowledgement. This research was funded the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos: 451-03-68/2020-14/200019, 451-03-68/2020-14/200026 and 451-03-68/2020-14/200168). There are no potential conflicts-of-interest to declare.

ИЗВОД

ФИЗИЧКО–ХЕМИЈСКА КАРАКТЕРИЗАЦИЈА ИНТЕРАКЦИЈЕ ДИХИДРО-ЛИПОИНСКЕ КИСЕЛИНЕ И АЛБУМИНА ИЗ СЕРУМА ЉУДИ ПРИМЕНОМ МУЛТИ-СПЕКТРОСКОПСКИХ МЕТОДА И МОЛЕКУЛСКОГ МОДЕЛОВАЊА

НИКОЛА ГЛИГОРИЈЕВИЋ[1], ВЛАДИМИР ШУКАЛОВИЋ[2], СИМЕОН МИНИЋ[3], ГОРАН МИЉУШ[1], ОЛГИЦА НЕДИЋ[1] и АНА ПЕНЕЗИЋ[1]

[1]Институт за примену нуклеарне енергије, Одељење за метаболизам, Универзитет у Београду, Банатска 31б, 11080 Београд, [2]Институт за хемију, технологију и металургију, Универзитет у Београду, Његошева 12, 11000 Београд и [3]Катедра за биохемију и Центар изврсности за молекуларне науке о храни, Универзитет у Београду – Хемијски факултет, Студентски трг 12–16, 11000 Београд

У раду су описане карктеристике везивања дихидро-липоинске киселине (DHLA), познатог суплемента у исхрани и антиоксиданса, за албумин из серума људи (HSA).

Процес везивања је праћен применом већег броја спектроскопских метода и молекулским моделовањем. HSA везује DHLA умереним афинитетом, $1{,}00\pm0{,}05\times10^4$ M^{-1}. Спектроскопски резултати су показали да је везујуће место IIA (Sudlow I) главно место везивања DHLA за HSA. Експериментални, као и резултати молекулског моделовања, су идентификовали електростатичке (сони мостови) и водоничне везе као главне типове интеракција. Резултати молекулског моделовања су потврдили да и место Sudlow I може везати DHLA, која је у том случају у специфичној конформацији. Симулација молекулске динамике је показала да је формирани комплекс стабилан. Везивање DHLA не утиче на структуру протеина, али повећава његову термалну стабилност. Везани DHLA не утиче на подложност HSA трипсинској дигестији. Како је DHLA уобичајен суплемент у исхрани, знање о његовим фармакоконетичким и фармакодинамичким особинама је веома важно. Ово испитивање допуњује досадашња знања детаљном анализом интеракције DHLA са HSA, примарним транспортним протеином лекова у циркулацији.

(Примљено 22. априла, ревидирано и прихваћено 27. маја 2021)

REFERENCES

1. R. E. Wang, L. Tian, Y.-H. Chang, *J. Pharm. Biomed. Anal.* **63** (2012) 165 (https://doi.org/10.1016/j.jpba.2011.12.035)
2. A. D. McLachlan, J. E. Walker, *J. Mol. Biol.* **112** (1977) 543 (https://doi.org/10.1016/S0022-2836(77)80163-0)
3. P. Lee, X. Wu, *Curr. Pharm. Des.* **21** (2015) 1862 (https://doi.org/10.2174/1381612821666150302115025)
4. I. D. Pavićević, V. B. Jovanović, M. M. Takić, A. Z. Penezić, J. M. Aćimović, L. M. Mandić, *Chem. Biol. Interact.* **224** (2014) 42 (https://doi.org/10.1016/j.cbi.2014.10.008)
5. M. Fasano, S. Curry, E. Terreno, M. Galliano, G. Fanali, P. Narciso, S. Notari, P. Ascenzi, *IUBMB Life* **57** (2005) 787 (https://doi.org/10.1080/15216540500404093)
6. R. I. Horowitz, P. R. Freeman, *Med. Hypoth. J.* **143** (2020) 109851 (https://doi.org/10.1016/j.mehy.2020.109851)
7. C. Zuliani, L. Baroni, in *Bioactive Nutraceuticals and Dietary Supplements in Neurological and Brain Disease*, R. R. Watson, V. R. Preedy, Eds., Elsevier Inc., Amsterdam, 2015, p. 341 (https://doi.org/10.1016/B978-0-12-411462-3.00035-7)
8. S. Salinthone, V. Yadav, D. N. Bourdette, D. W. Carr, *Endocrine, Metab. Immune Disord. Targets* **8** (2008) 132 (https://doi.org/10.2174/187153008784534303)
9. A. R. Smith, S. V. Shenvi, M. Widlansky, J. H. Suh, T. M. Hagen, *Curr. Med. Chem.* **11** (2004) 1135 (https://doi.org/10.2174/0929867043365387)
10. T. Kawabata, L. Packer, *Biochem. Biophys. Res. Commun.* **203** (1994) 99 (https://doi.org/10.1006/bbrc.1994.2154)
11. G. Suji, S. A. Khedkar, S. K. Singh, N. Kishore, E. C. Coutinho, V. M. Bhor, S. Sivakami, *Protein J.* **27** (2008) 205 (https://doi.org/10.1007/s10930-008-9126-3)
12. P. Atukeren, S. Aydin, E. Uslu, M. K. Gumustas, U. Cakatay, *Oxid. Med. Cell. Longev.* **3** (2010) 206 (https://doi.org/10.4161/oxim.3.3.11786)
13. N. Perricone, K. Nagy, F. Horváth, G. Dajkó, I. Uray, I. Zs.-Nagy, *Arch. Gerontol. Geriatr.* **29** (1999) 45 (https://doi.org/10.1016/S0167-4943(99)00022-9)
14. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer US, New York, 2006 (https://doi.org/10.1007/978-0-387-46312-4)
15. J. M. Vasquez, A. Vu, J. S. Schultz, V. I. Vullev, *Biotechnol. Prog.* **25** (2009) 906 (https://doi.org/10.1002/btpr.188)

16. S. K. Chaturvedi, M.K. Siddiqi, P. Alam, M. Zaman, R.K. Khan, *RSC Adv.* **6** (2016) 85860 (https://doi.org/10.1039/C6RA10487H)
17. N. Zaidi, E. Ahmad, M. Rehan, G. Rabbani, M. R. Ajmal, Y. Zaidi, N. Subbarao, R. K. Khan, *J. Phys. Chem., B* **117** (2013) 2595 (https://doi.org/10.1021/jp3069877)
18. P. D. Ross, S. Subramanian, *Biochemistry* **20** (1981) 3096 (https://doi.org/10.1021/bi00514a017)
19. A. A. Salem, M. Lotfy, A. Amin, M. A. Ghattas, *Biochem. Biophys. Reports* **20** (2019) 100670 (https://doi.org/10.1016/j.bbrep.2019.100670)
20. Q. Li, W. Yang, L. Qu, H.-Y. Qi, Y. Huang, Z. Zhang, *J. Spectrosc.* **2014** (2014) Article ID 834501 (https://doi.org/10.1155/2014/386586)
21. A. Ploch-Jankowska, D. Pentak, *Pharmaceuticals* **13** (2020) 205 (https://doi.org/10.3390/ph13090205)
22. L. Liang, H.A. Tajmir-Riahi, M. Subirade, *Biomacromolecules* **9** (2008) 50 (https://doi.org/10.1021/bm700728k)
23. S. L. Minic, M. Milcic, D. Stanic-Vucinic, M. Radibratovic, T. G. Sotiroudis, M. R. Nikolic, T. Ć. Velickovic, *RSC Adv.* **5** (2015) 61787 (https://doi.org/10.1039/c5ra05534b)
24. N. Gligorijević, V. Šukalović, A. Penezić, O. Nedić, *Int. J. Biol. Macromol.* **147** (2020) 319 (https://doi.org/10.1016/j.ijbiomac.2020.01.098)
25. M. Radibratovic, S. Minic, D. Stanic-Vucinic, M. Nikolic, M. Milcic, T. C. Velickovic, *PLoS One* **11** (2016) e0167973 (https://doi.org/10.1371/journal.pone.0167973)
26. B. E. Lang, K. D. Cole, *Biotechnol. Prog.* **31** (2015) 62 (https://doi.org/10.1002/btpr.1996)
27. D. P. Yeggoni, A. Rachamallu, R. Subramanyam, *J. Photochem. Photobiol., B* **160** (2016) 248 (https://doi.org/10.1016/j.jphotobiol.2016.04.012)
28. T. Sjödin, R. Hansson, I. Sjöholm, *Biochim. Biophys. Acta* **494** (1977) 61 (https://doi.org/10.1016/0005-2795(77)90135-0)
29. A. Kawakami, K. Kubota, N. Yamada, U. Tagami, K. Takehana, I. Sonaka, E. Suzuki, K. Hirayama, *FEBS J.* **273** (2006) 3346 (https://doi.org/10.1111/j.1742-4658.2006.05341.x)
30. K. Oettl, R. E. Stauber, *Br. J. Pharmacol.* **151** (2007) 580 (https://doi.org/10.1038/sj.bjp.0707251)
31. I. Sadowska-Bartosz, S. Galiniak, G. Bartosz, *Molecules* **19** (2014) 4880 (https://doi.org/10.3390/molecules19044880).