Scientia Iranica, Vol. 14, No. 2, pp 87–95

SCIENTIA IRANICA

Review Article

An Overview of Drug Binding to Human Serum Albumin: Protein Folding and Unfolding

H.A. Tajmir-Riahi[1]

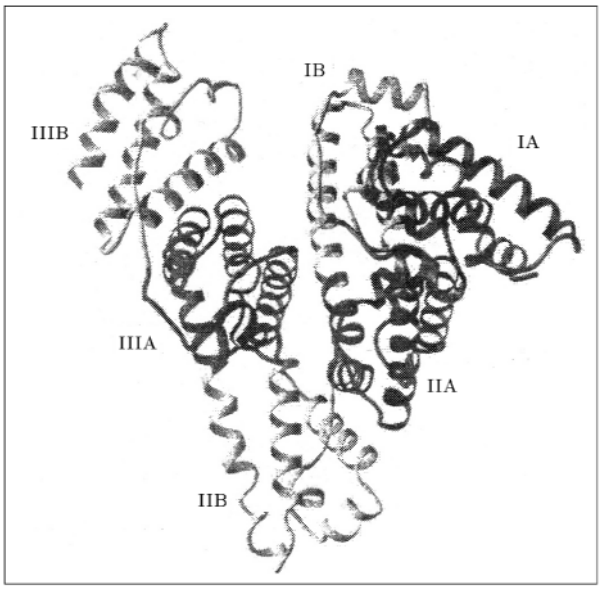
Human Serum Albumin (HSA) is a principal extracellular protein with a high concentration in blood plasma and a carrier of many drugs to different molecular targets. Drug binding to HSA can alter the protein biophysical and biochemical properties of protein. The structural analysis of human serum albumin complexes, with naturally occurring flavonoids quercetin (antioxidant), kaempferol (antioxidant), delphinidin (antioxidant), AZT (3′-azido-3′-deoxythymidine) (anti-AIDS), aspirin (anti-inflammatory), taxol (anticancer), cisplatin (anticancer), atrazine (herbicide), 2,4-D (herbicide), polyamines (biogenic), chlorophyll (antimutagenic), chlorophyllin (antitumor), poly(ethylene glycol) (polymer), vandyl cation and vanadate anion in aqueous solution are reported. Using capillary electrophoresis, FTIR (Fourier transform infrared), UV-Visible and CD (Circular dichroism) spectroscopic methods, the drug binding mode, the binding constant and the effects of drug complexation on protein secondary structure are determined. The concentrations of HSA used were 0.6 to 0.3 mM, while different drug concentrations were 1 μM to 1 mM. Structural analysis showed drugs are mostly located along the polypeptide chains, with both specific and non-specific interactions. The stability of drug-HSA complexes were in the order: $K_{VO}^{2+} = 1.2 \times 10^8 M^{-1} > K_{AZT} = 1.9 \times 10^6 M^{-1} > K_{del} = 4.7 \times 10^5 M^{-1} > K_{PEG} = 4.1 \times 10^5 M^{-1} > K_{kae} = 2.6 \times 10^5 M^{-1} > K_{que} = 1.4 \times 10^5 M^{-1} > K_{atrazine} = 3.5 \times 10^4 M^{-1} > K_{chlorophyll} = 2.9 \times 10^4 M^{-1} > K_{2,4\ D} = 2.5 \times 10^4 M^{-1} > K_{spermine} = 1.7 \times 10^4 M^{-1} > K_{taxol} = 1.43 \times 10^4 M^{-1} > K_{aspirin} = 1.04 \times 10^4 M^{-1} > K_{chlorophyllin} = 7.0 \times 10^3 M^{-1} > K_{VO3} = 6.0 \times 10^3 M^{-1} > K_{spermidine} = 5.4 \times 10^3 M^{-1} > K_{putrescine} = 3.9 \times 10^3 M^{-1} > K_{cisplatin} = 1.2 \times 10^2 M^{-1}$. At low drug concentration (1 μM), protein conformation was not altered (infrared and CD results), while, at high drug content (1 mM), a major reduction of α-helix from 60-55% (free HSA) to 49-40% and an increase of β-structure from 22-15% (free HSA) to 33-23% in the drug-protein complexes occurred. These observations indicated that low drug content induced protein stabilization (folding), whereas, at high drug concentration, a partial protein destabilization (unfolding) occurred in these drug-HSA complexes.

INTRODUCTION

Human serum albumin (Structure 1) with several high affinity binding sites is the major target for many organic and inorganic molecules. HSA is a principal extracellular protein with a high concentration in blood plasma (40 mg/ml or 0.6 mM) [1-3]. It is a globular protein composed of three structurally similar domains (I, II and III), each containing two subdomains (A and B) and stabilized by 17 disulphide bridges [1-6]. Aromatic and heterocyclic ligands were found to bind within two hydrophobic pockets in subdomains IIA and IIIA, namely site I and site II [1-7]. Seven binding sites are localized for fatty acids in subdomains IB, IIIA, IIIB and on the subdomain interfaces [7]. HSA also has a high affinity metal binding site at the N-terminus [2]. The multiple binding sites underlie the exceptional ability of HSA to interact with many organic and inorganic molecules and, thus, make this protein an important regulator of intercellular fluxes, as well as the pharmacokinetic behavior of many drugs [1-9].

In this report, the results of several studies on the interaction of human serum albumin with different drugs, such as quercetin, kaempferol, delphinidin, AZT, aspirin, taxol, cisplatin, atrazine, 2,4-D, polyamines, chlorophyll, chlorophyllin, poly(ethylene

1. *Department of Chemistry-Biology, University of Québec at Trois-Rivières, Québec, Canada.*



Structure 1. Structure of Human Serum Albomin (HSA).

glycol), vanadyl cation and vanadate anion are discussed. The data from FTIR, CD, UV-Visible spectroscopic methods and capillary electrophoresis, regarding drug binding sites, stability and protein secondary structural changes in an aqueous solution, are reported here.

MATERIALS AND METHODS

Stock Solutions

Human serum albumin was dissolved in an aqueous solution (40 mg/ml or 0.6 mM and 20 mg/ml or 0.3 mM) containing phosphate buffer 0.1 M and 0.05 M NaCl (pH 7.2). The different ligand solutions, 1 μM to 1 mM, were prepared in doubly distilled water.

FTIR Spectroscopic Measurements

Infrared spectra were recorded on a Perkin Elmer Spectrum 2000 FTIR spectrometer equipped with a nitrogen cooled HgCdTe detector and a KBr beam splitter. IR spectra were recorded on hydrated film, using AgBr windows with a resolution of 2-4 cm ^{1} and 100-500 scans.

CD Spectroscopy

Spectra were recorded with a Jasco J-720 spectropolarimeter. For measurements in the Far-UV region (195-280 nm), a quartz cell with a path length of 0.1 cm was used. Five scans were accumulated at a scan speed of 50 nm per minute, with data being collected at every nm from 195 to 280 nm. A sample temperature was maintained at 25°C using a Neslab RTE-111 circulating water bath connected to the water-jacketed quartz cuvettes. Spectra were corrected for the buffer signal and conversion to the Mol CD ($\Delta\varepsilon$) was performed with Jasco Standard Analysis software.

Absorption Spectroscopy

The absorption spectra were recorded on a Perkin Elmer Lambda 40 Spectrophotometer. Quartz cuvettes of 1 cm were used. The absorbance titrations were performed by keeping the concentration of HSA (12.5 μM) constant and varying the concentrations of the drug (1 μM to 1 mM).

DETERMINATION OF PROTEIN SECONDARY STRUCTURE BY FTIR AND CD SPECTROSCOPY

Analysis of the secondary structure of HSA and its drug complexes was carried out by CD [10,11] and FTIR spectroscopy on the basis of the procedure reported [12-14]. The protein secondary structure is determined from the shape of the amide I band, located at 1650-1660 cm ^{1}. Fourier self-deconvolution and second derivative resolution enhancement were applied to increase the spectral resolution in the region of 1700-1600 cm ^{1}. The second derivatives were produced using a point convolution of 11 or 13. The resolution enhancement resulting from self-deconvolution and the second derivative is such that the number and the position of the bands to be fitted are determined. In order to quantify the area of the different components of the amide I contour, revealed by the second derivative, a least-square iterative curve-fitting was used to fit the Gaussian line shapes to the spectra between 1700-1600 cm ^{1}. Before curve-fitting was done, a straight baseline, passing through the cordinates at 1700 and 1600 cm ^{1}, was subtracted. The baseline is then modified again by least-square curve-fitting, which allowed for a horizontal baseline to be adjusted as an additional parameter, in order to obtain the best fit. It is known that no meaningful curve-fitting can be performed by simple examination of the original infrared spectra, which is why the self-deconvolution procedure has to be carried out first. The curve-fitting was done using a number of 50 simulations. The resulting curve fitted is analyzed as follows: Each Gaussian band is assigned to a secondary structure, according to the frequency of its maximum; α-helix (1649-1660 cm ^{1}); β-sheet (1615-1640 cm ^{1}); turn (1660-1680 cm ^{1}); random coil (1641-1648 cm ^{1}) and β-antiparallel (1680-1692 cm ^{1}). The area of all the component bands assigned to a given conformation are then summed up and divided by the total area. The number obtained is taken as the proportion of the polypeptide chain in that conformation. These

assignments are consistent with the previous values determined theoretically [12] and experimentally [13,14]. The details of spectral manipulation have also been given in the author's previous report [15].

ANALYSIS OF DRUG-PROTEIN BINDING CONSTANT BY UV-VISIBLE SPECTROSCOPY

The values of the binding constants, K, were obtained, according to the method described earlier [16,17] By assuming that there is only one type of interaction between the drug and the protein in an aqueous solution, Equations 1 and 2 can be established:

$$\text{HSA} + \text{drug} \Leftrightarrow \text{HSA} : \text{drug}, \tag{1}$$

$$K = \frac{[\text{HSA} : \text{drug}]}{[\text{HSA}][\text{drug}]}, \tag{2}$$

where K is the binding constant for drug complexes:

Assuming $[\text{HSA} : \text{drug}] = C_B$,

$$K = \frac{C_B}{(C_{\text{HSA}} \quad C_B)(C_{\text{drug}} \quad C_B)}, \tag{3}$$

where C_{HSA} and C_{drug} are the analytical concentration of HSA and drug in solution, respectively.

According to the Beer-Lambert law:

$$C_{\text{HSA}} = \frac{A_0}{\varepsilon_{\text{HSA}}.\ell}, \tag{4}$$

$$C_B = \frac{A \quad A_0}{\varepsilon_B.\ell}, \tag{5}$$

where, A_0 and A are the absorbance of HSA at 280 nm, in the absence and presence of the drug, respectively. ε_{HSA} and ε_{B} are the molar extinction coefficient of HSA and the bound drug, respectively and ℓ is the light path of the cuvette (1 cm).

By displacing ε_{HSA} and ε_B in Equation 3 by Equations 4 and 5, Equation 6 can be deduced, as follows:

$$\frac{A_0}{A \quad A_0} = \frac{\varepsilon_{\text{HSA}}}{\varepsilon_B} + \frac{\varepsilon_{\text{HSA}}}{\varepsilon_B.K}.\frac{1}{C_{\text{drug}}}. \tag{6}$$

Thus, the double reciprocal plot of $1/(A \quad A_0)$ vs. $1/C_{\text{drug}}(1/[\text{drug}])$ is linear and the binding constant (K) can be estimated from the ratio of the intercept to the slope [16,17].

CAPILLARY ELECTROPHORESIS AND STABILITY OF DRUG-PROTEIN COMPLEXES

The binding constants for the drug-HSA complexes were determined using Scatchard analysis, following capillary electrophoresis [18,19]. The average number (R_f) of the drug bound per one binding site of the HSA was determined from the change of the peak area, due to the presence of the drug, by the following equation:

$$R_f = (m \quad m_o)/(ms \quad m_o), \tag{7}$$

where m is the change of the mobility shift measured for any added drug concentration, while m_o and m_s correspond to the mobility shifts of the free HSA and the drug saturated, respectively. Using the equation of the binding constant:

$$K_b = [\text{drug} \quad \text{HSA}]/[\text{HSA}][\text{drug}]. \tag{8}$$

The experimental drug binding constants, K_b, were then computed by fitting the experimental values of R_f and drug concentrations to the equation:

$$R_f = K_b[\text{drug}]/(1 + K_b[\text{drug}]). \tag{9}$$

The last equation provides a convenient form for Scatchard analysis:

$$R_f/[\text{drug}] = K_b \quad K_b R_f. \tag{10}$$

RESULTS AND DISCUSSION

Infrared and CD Analyses

The drug-HSA binding was characterized by infrared spectroscopy. The difference spectra [(protein solution + drug) − (protein solution)] showed major spectral changes for the protein amide I band at 1656 cm ^{1} (mainly C=O stretch) and amide II band at 1542 cm ^{1} (C-N stretching coupled with N-H bending modes) [12,13] upon drug interaction (Figure 1). Similarly, the infrared second derivative derivative and curve-fitting procedures were used [13,14] to determine the protein secondary structures in the presence of the drugs. The results are presented in Table 1. Information from CD spectroscopy was also used for comparative purposes to analyze the protein secondary structure in the presence of drug in aqueous solutions. The drug contents were of 1 μM to 1 mM, with a final HSA concentration of 0.3 mM.

The presence of negative features around 1651-1662 cm ^{1} (amide I) and 1537-1548 cm ^{1} (amide II) were observed in the difference spectra of the drug-HSA complexes (except for cisPt, AZT and aspirin complexes, in which positive features at 1557-1548 cm ^{1} were observed for the amide II band), which are attributed to intensity variations of the amide I band at 1656 cm ^{1} and amide II band at 1542 cm ^{1}, upon drug-protein complexation (Figure 1). The observed spectral changes were attributed to the interaction (H-bonding) of the drug with protein C=O and C-N groups [20-26]. The interaction of the drug with

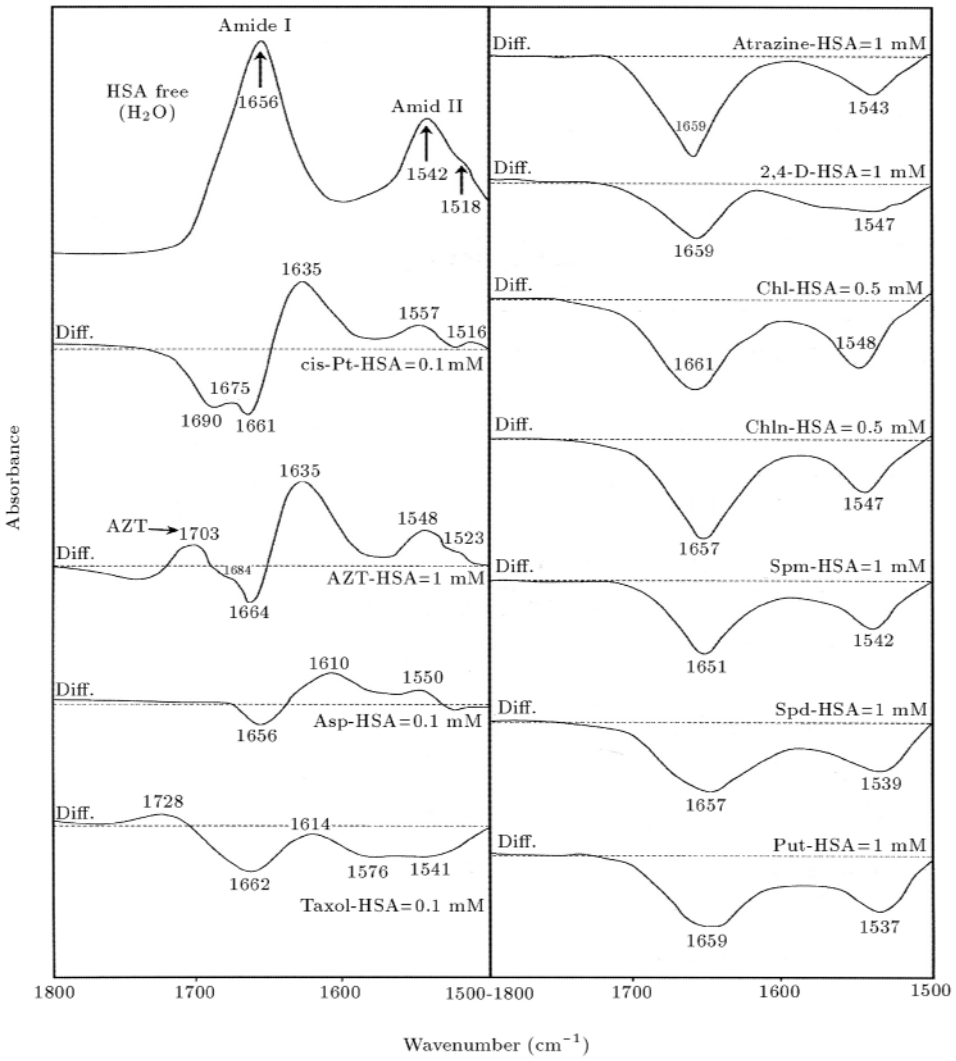


Figure 1. FTIR spectra (top first curve) and difference spectra [(protein solution + drug solution) - (protein solution)] (bottom eight curves) of the free HSA and its drug complexes, in aqueous solution at physiological pH with different drug concentrations of 1 μM to 1 mM and final HSA content 0.3 mM, in the region of 1800-1500 cm ^{1}.

the protein C-N group is also evident from the shift of the amide A band at 3303 cm ^{1} (peptide N-H stretching mode) [12] towards a lower frequency at 3290 cm ^{1}. Among these drugs, aspirin was found to bind to the ε-amino NH_3^+ group at low drug concentration, while causing acetylation of Lys-199 at high drug content [20]. Cisplatin was found to bind HSA at C-N and S-H groups [21]. The Cis-Pt drug binds also to the Cys-34 and to the Met-298 of human serum albumin, inducing major protein conformational changes [27], while the AZT binding site was located in the protein IIIA domain [4]. AZT showed two binding sites on HSA with strong and weak association constants [22]. 2,4-D exhibited two bindings with HSA subdomain III, while atrazine showed one binding site [24,28]. It was found that 90% of paclitaxel complexes serum albumin with specific binding [23,29,30]. Similarly, strong bindings were observed for chlorophyll and chlorophyllin adducts of human serum albumin [31]. The biogenic polyamines bind protein in a non-specific manner [32]. Spermine, spermidine and putrescine bind HSA through a H-bonding network [25]. Poly (ethylene glycol) binds protein via a H-bonding system [33,34]. Naturally occurring antioxidant flavonoids, such as quercetin, kaempferol and delphinidin, bind HSA strongly through a H-bonding network [35]. Vanadyl ions show a major affinity for binding to nitrogen and oxygen donors of biomolecules [36,37]. Vanadyl cation (VO^{2+}) binds strongly to two different binding sites of chloroperoxidase at histidine 496 (directly) and histidine 404 (indirectly) [38]. Vanadyl cation also complexes with HSA at two binding sites with two affinity constants (strong and weak), while vanadate anion (VO_3) shows

Table 1. Secondary structure determination for the free HSA and its drug complexes in H_2O at physiological pH with different drug concentrations and final protein content of 0.3 mM (at 25°C) [20-26]. The values in parentheses are from D_2O.

Amide I Components (cm ^{1})	HSA Free H_2O (%)	HSA-AZT 1 mM	HSA-cis-Pt 0.1 mM	HSA-Asp 0.1 mM	HSA-Taxol 0.1 mM	HSA-Chl 0.5 mM	HSA-Chln 0.5 mM	HSA-Atrazine 1 mM	HSA-2,4-D 1 mM	HSA-Spm 1 mM	HSA-Spd 1 mM	HSA-Put 1 mM
1692-1680 β-anti	12.0 ± 1	11.0	13.0	4.0	15.0	15.0	12.0	12.9	10.7	10.0	3.0	5.0
1680-1660 Turn	11.0 ± 1	10.0	10.0	16.0	13.0	9.0	19.0	14.8	12.1	20.0	23.0	22.0
1660-1649 α-helix	55.0 ± 3	49.0	45.0	49.0	46.0	41.0	40.0	49.3	44.8	40.0	43.0	40.0
1648-1641 Random	(11 ± 1.0)	-	(12.0)	(14.0)	(12.0)	-	-	-	-	7.0	7.0	-
1640-1615 β-sheet	22.0 ± 2	30.0	32.0	31.0	26.0	35.0	29.0	24.0	32.4	23.0	24.0	33.0

HSA: Human Serum Albumin; AZT: 3′-azido-3′-deoxythymidine; cis-Pt: Cisplatine; Asp: Aspirin; Chl: Chlorophyl; Chln: Chlorophyllin; 2,4-D: 2,4-dichlorophenoxyacetic; Spm: Spermine; Spd: spermidine; Put: Putrescine.

minor interaction towards the ε-amino NH_3^+ group with one binding constant [26]. As the drug concentration was increased, the intensity of the amide I band at 1659 cm ^{1} and amide II band at 1545 cm ^{1} decreased further in the spectra of drug-HSA complexes. The reduction in the intensity of the amide I is also related to the decrease of the α-helix content in favor of β-sheet and turn structures, which will be discussed below. It should be noted that a weak band at 1518 cm ^{1} of the free HSA is assigned to the tyrosine side chain vibration [39-41], which exhibited a minor intensity increase in the spectra of cis-Pt and AZT-HSA adducts but not in the other drug-HSA complexes, which is attributed to minor drug binding to tyrosine residues in these drug-HSA complexes.

A quantitative analysis of the protein secondary structure for the free HSA and its drug complexes in H_2O is given in Table 1. The free protein contained major α-helix 55%, β-sheet 22%, turn structure 11% and β-antiparallel 12%. The β-sheet structure is composed of three components at 1616 cm ^{1} (inter β-strand), 1625 cm ^{1} (intra β-strand) and 1635 cm ^{1} (hydrated), which are consistent with the recent spectroscopic studies of human serum albumin [42,43]. In D_2O solution, due to isotopic substitution (D/H), the presence of a component band at 1644 cm ^{1} in the spectrum of the free HSA was attributed to the contribution of the random structure (10%). Upon drug complexation, the α-helix structure was reduced from 55% to 43-40%, the β-pleated increased from 22% to 23-33%, the β-anti decreased from 12% to 11-3% and the turn structure increased from 11% to 20-24% at high drug concentration [20-26,28] (Table 1). The CD spectra of the free HSA and its drug complexes also exhibited reduction of the α-helix from that of 60% (free HSA) to 55-50% (drug complexes) at high drug concentration, which is consistent with the infrared results (Figure 2). The minor differences between the amounts of α-helix obtained by CD (60%) and infrared (55%) are due to the sample preparation (CD spectra were performed in aqueous solution, while infrared spectra were recorded on hydrated films).

It should be noted that there are some differences in the α-helix contents reported here and those of X-

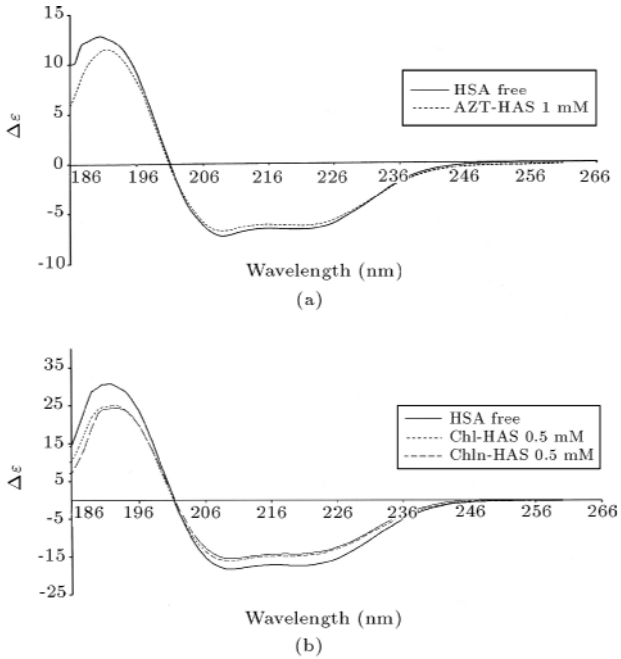
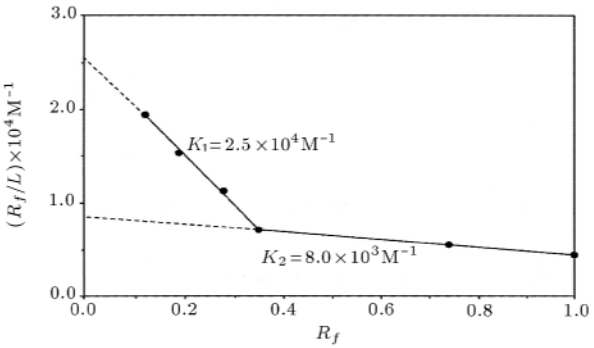


Figure 2. Circular dichroism of the free HSA and its drug complexes in aqueous solution with different drug concentrations of 0.5 mM to 1 mM and final HSA content 0.3 mM.

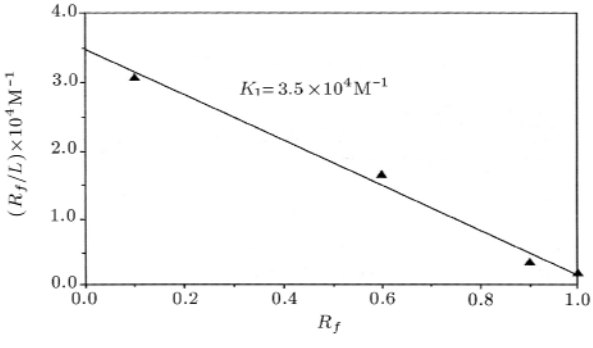
ray structural analysis of HSA in the solid state that showed α-helix 66% and 34% for other conformational components [5]. The differences in α-helix content can be due to the different structural arrangements of the protein in the solid state and aqueous solution. Structural differences were also observed for other proteins in the solid state and in aqueous solution [44-49]. The reduction of the α-helix in favor of the β-sheet and turn structures is indicative of a partial unfolding of protein in the presence of the drug at high concentration. Similar conformational transition from α-helix to β-sheet structure was observed for the protein unfolding upon protonation and heat denaturation [50-52].

STABILITY OF DRUG-HSA COMPLEXES BY UV AND CAPILLARY ELECTROPHORESIS

The drug-HSA binding constants were determined by capillary electrophoresis and UV-Visible methods (Figures 3 and Table 2). It showed the presence of strong and weak drug-protein interactions, both specific and non-specific bindings, with the order of $K_{VO}^{2+} = 1.2 \times 10^8 M^{-1} > K_{AZT} = 1.9 \times 10^6 M^{-1} > K_{del} = 4.7 \times 10^5 M^{-1} > K_{PEG} = 4.1 \times 10^5 M^{-1} > K_{kae} = 2.6 \times 10^5 M^{-1} > K_{que} = 1.4 \times 10^5 M^{-1} > K_{atrazine} =$ $3.5 \times 10^4 M^{-1} > K_{chlorophyll} = 2.9 \times 10^4 M^{-1} > K_{2,4\ D} = 2.5 \times 10^4 M^{-1} > K_{spermine} = 1.7 \times 10^4 M^{-1} > K_{taxol} = 1.43 \times 10^4 M^{-1} > K_{aspirin} = 1.04 \times 10^4 M^{-1} > K_{chlorophyllin} = 7.0 \times 10^3 M^{-1} > K_{VO3} = 6.0 \times 10^3 M^{-1} > K_{spermidine} = 5.4 \times 10^3 M^{-1} > K_{putrescine} = 3.9 \times 10^3 M^{-1} > K_{cisplatin} = 1.2 \times 10^2 M^{-1}$ (Table 2). The association constants calculated for the porphyrin-HSA complexes show a weak pigment-protein interaction (nonspecific binding), with respect to the other strong ligand-protein complexes, with binding constants ranging from $10^6 M^{-1}$ to $10^8 M^{-1}$ [53,54]. Similar weak interactions were observed in the cis-$Pt(NH_3)_2$-HSA and taxol-HSA complexes [21,23]. The larger stability of the Chl-HSA ($2.9 \times 10^4 M^{-1}$) over Chln-HSA complexes ($7.0 \times 10^3 M^{-1}$) is due to the formation of more stable five or six-coordination Mg(II) cation via protein C=O and C=N groups at axial positions (compared with the four coordinated Mg cation in chlorophyll), whereas the four coordination Cu(II) ion in Chln is more stable than the five or six-coordinated copper cation in the Chln-protein complexes [31]. Two



(a) HSA-2,4-D complexes



(b) HSA-Atrazine complexes

Figure 3. Scatchard plots for HSA-2,4-D and HSA-atrazine complexes with different drug concentrations of 6.25 μM to 250 μM and final HSA content 7.25 μM.

Table 2. Binding constants of drug complexes with Human Serum Albumin (HSA) of 0.3 mM final concentration and ligand content of 1 μM to 1 mM at pH 7 ± 0.2 and 25°C [20-26,31,33,35].

Drugs	n	$K_1(M^{-1})$	$K_2(M^{-1})$
AZT	2	1.9×10^6	2.1×10^4
Cisplatin	1	$*8.52 \times 10^2$	-
Aspirin	1	$*1.04 \times 10^4$	-
Taxol	1	$*1.43 \times 10^4$	-
Chlorophyl	1	$*2.9 \times 10^4$	-
Chlorophyllin	1	$*7.0 \times 10^3$	-
Atrazine	1	3.5×10^4	-
2,4-D	2	2.5×10^4	8.0×10^3
Spermine	1	$*1.7 \times 10^4$	-
Spermidine	1	$*5.4 \times 10^3$	-
Putrescine	1	$*3.9 \times 10^3$	-
PEG	1	$*4.1 \times 10^5$	-
VO^{2+}	2	1.2×10^8	8.5×10^5
VO_3	1	6.0×10^3	-
del	1	4.7×10^5	-
kae	1	2.6×10^5	-
que	1	1.4×10^4	-

n is the number of binding sites; and K_1 and K_2 are the corresponding association constants from capillary electrophoresis and *UV-visible methods;
AZT is 3′-azido-3′-deoxythymidine;
2,4-D is 2,4-dichlorophenoxyacetic;
PEG is poly(ethylene glycol); que is quercetin; kae is kaempferol; del is delphinidin.

major bindings were observed for the vanadyl cation, AZT and 2-4-D, while other drugs contained one binding (Table 2).

SUMMARY

Drugs bind HSA via specific and non-specific interactions with the order: VO^{2+} > AZT > del > PEG > kae > quer > atrazine > chlorophyll > 2,4-D > spermine > taxol > aspirin > chlorophyllin > VO_3 > spermidine > putrescine > cisplatin. The drug complexation induced major protein conformational changes with a reduction of α-helix from 55% (free HSA) to 49-39% and an increase of β-structure from 22% (free HSA) to 23-35%. The major reduction of the α-helix is characteristic of a partial protein unfolding in these drug-HSA complexes.

ACKNOWLEDGMENTS

This work is supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and FCAR (Québec).

REFERENCES

1. Carter, D.C. and Ho, J.X. "Structure of serum albumin", *Adv. Protein Chem.*, **45**, pp 153-203 (1994).
2. Peters, T., *All About Albumin. Biochemistry, Genetics and Medical Applications*, Academic Press, San Diego, USA (1996).
3. Sugio, S., Kashima, A., Mochizuki, S., Noda, M. and Kobayashi, K. "Crystal structure of human serum albumin at 2.5 Å resolution", *Protein Eng.*, **12**, pp 439-449 (1999).
4. He, H.M. and Carter, D.C. "Atomic structure and chemistry of human serum albumin", *Nature*, **358**, pp 209-215 (1992).
5. Peters, T. "Serum albumin", *Adv. Protein Chem.*, **37**, pp 161-245 (1985).
6. Curry, S., Brick, P. and Frank, N.P. "Fatty acid binding to human serum albumin: New insights from crystallographic studies", *Biochim. Biophys. Acta.*, **1441**, pp 131-140 (1999).
7. Petitpas, I., Grune, T., Battacharya, A.A. and Curry, S. "Crystal structure of human serum albumin complexed with momounsaturated and polyunsaturated fatty acids", *J. Mol. Biol.*, **314**, pp 955-960 (2001).
8. Grelamo, E.L., Silva, C.H.T.P., Imasato, H. and Tabak, M. "Interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants: Spectroscopy and modelling", *Biochim. Biophys. Acta.*, **1594**, pp 84-99 (2002).
9. Chuang, V.T.G. and Otagiri, M. "Flunitrazepan, A 7-nitor-1,4-benzodiazepine that is unable to bind to indole-benzodiazepine site of human serum albumin", *Biochim. Biophys. Acta.*, **1546**, pp 337-345 (2001).
10. Johnson, W.C. "Analyzing protein circular dichroism spectra for accurate secondary structure protein", *Struct. Funct. Genet.*, **35**, pp 307-312 (1999).
11. Gagnon, M.C., Williams, M., Doucet, A. and Beauregard, M. "Replacement of Tyr62 by Trp in the designer protein milk bundle-1 results in significant improvement of conformational stability", *FEBS Lett.*, **484**, pp 144-148 (2000).
12. Krimm, S. and Bandekar, J. "Vibrational spectroscopy and conformation of peptides, polypeptides and proteins", *Adv. Protein Chem.*, **38**, pp 181-364 (1986).
13. Byler, D.M. and Susi, H. "Examination of the secondary structure of proteins by deconvoluted FTIR spectra", *Biopolymers*, **25**, pp 469-487 (1986).
14. Bandekar, J. "Amide modes and protein conformation", *Biochim. Biophys. Acta.*, **1120**, pp 123-143 (1992).
15. Ahmed, A., Tajmir-Riahi, H.A. and Carpentier, R. "A quantitative secondary structure analysis of the 33 kDa extrinsic polypeptide of photosystem II by FTIR spectroscopy", *FEBS Lett.*, **363**, pp 65-68 (1995).
16. Zhong, W., Wang, Y., Yu, J.S., Liang, Y., Ni, K. and Tu, S. "The interaction of human serum albumin with a novel antidiabetic agent-SU-118", *J. Pharm. Sci*, **93**, pp 1039-1046 (2004).
17. Stephanos, J.J. "Drug-protein interactions. Two-site binding of heterocyclic ligands to a monomeric hemogolobin", *J. Inorg. Biochem.*, **62**, pp 155-169 (1996).
18. Klotz, I.M. and Hunston, D.L. "Properties of graphical representations of multiple classes of binding sites", *Biochemistry*, **16**, pp 3065-3069 (1971).
19. Klotz, I.M. "Numbers of receptor sites from Scatchard graphs: Facts and fantasies", *Science*, **217**, pp 1247-1249 (1981).
20. Neault, J.F., Novetta-delen, A., Arakawa, H., Malonga, H., and Tajmir-Riahi, H.A. "The effect of aspirin-HSA complexation on the protein secondary structure", *Can. J. Chem.*, **78**, pp 291-296 (2000).
21. Neault, J.F. and Tajmir-Riahi, H.A. "Interaction of cisplatin with human serum albumin. Drug binding mode and protein secondary structure", *Biochim. Biophys. Acta.*, **1384**, pp 153-159 (1998).
22. Gaudreau, S., Neault, J.F. and Tajmir-Riahi, H.A. "Interaction of AZT with human serum albumin studied by capillary electrophoresis, FTIR and CD spectroscopic methods", *J. Biomol. Struct. Dyn.*, **19**, pp 1007-1014 (2002).
23. Purcell, M., Neault, J.F., and Tajmir-Riahi, H.A. "Interaction of taxol with human serum albumin", *Biochim. Biophys. Acta.*, **1478**, pp 61-68 (2000).
24. Purcell, M., Neault, J.F., Malonga, H., Arakawa, H., Carpentier, R. and Tajmir-Riahi, H.A. "Interactions of atrazine and 2,4-D with human serum albumin studied by gel and capillary electrophoresis, and FTIR spectroscopy", *Biochim. Biophys. Acta.*, **1548**, pp 129-138 (2001).

25. Ahmed Ouameur, A., Mangier, E., Diamantoglou, S.R., Rouillon, R., Carpentier, R. and Tajmir-Riahi H.A. "Effects of organic and inorganic polyamine cations on the structure of human serum albumin", *Biopolymers*, **73**, pp 503-509 (2004).

26. Purcell, M., Neault, J.F., Malonga, H., Arakawa, H. and Tajmir-Riahi, H.A. "Interaction of human serum albumin with oxovanadium ions studied by FT-IR spectroscopy and gel and capillary electrophoresis", *Can. J. Chem.*, **79**, pp 1415-1421 (2001).

27. Ivanov, A.I., Christodoulou, J., Parkinson, A.J., Barnham, K.J., Tucker, A., Woodrow, J. and Sadler, P.J. "Cisplatin binding sites on human albumin", *J. Biol. Chem.*, **273**, pp 14721-14730 (1998).

28. Rosso, S.B., Gonzalez, M., Bagatolli, L.A., Duffard, R.O. and Fidelio, G.D. "Evidence of a strong interaction of 2,4-dichlorophenoxyacetic acid herbicide with human serum albumin", *Life Sci.*, **63**, pp 2343-2351 (1998).

29. Brouwer, E., Verweij, J., De Bruijn, P., Loos, W.J., Pillay, M., Buijs, D. and Sparreboom, A. "Measurement of fraction unbound paclitaxel in human plasma", *Drug. Metab. Dispos.*, **28**, pp 1141-1145 (2000).

30. Paal, K., Muller, J. and Hegedus, L. "High affinity binding of paclitaxel to human serum albumin", *Eur. J. Biochem.*, **268**, pp 2187-2191 (2001).

31. Ahmed Ouameur, A., Marty, R., and Tajmir-Riahi, H.A. "Human serum albumin complexes with chlorophyll and chlorophyllin", *Biopolymers*, **77**, pp 129-136 (2005).

32. Bougrah, A., Gingras, Y., Tajmir-Riahi, H.A. and Carpentier, R. "The effects of spermine and spermidine on the structure of photosystem II proteins in relation to inhibition of electron transport", *FEBS Lett.*, **402**, pp 41-44 (1997).

33. Ragi, C., Sedaghat-Herati, M.R., Ahmed Ouameur, A. and Tajmir-Riahi, H.A. "The effects of poly(ethylene glycol) on the solution structure of human serum albumin", *Biopolymers*, **78**, pp 321-236 (2005).

34. Shinji, A., Tsuboi, A., Izumi, T., Hirata, M., Dubin, P.L., Wang, B. and Kokufuta, E. "Formation of an interapolymer complex from human serum albumin and poly(ethylene glycol)", *Langmuir*, **15**, pp 940-947 (1999).

35. Kanakis, C.D., Tarantilis, P., Polissiou, M.G., Diamantoglou, S. and Tajmir-Riahi, H.A. "Antioxidant flavonoids bind human serum albumin", *J. Mol. Struct.*, **798**, pp 69-74 (2006).

36. Baran, E.J. "Oxovanadium (IV) and oxovanadium (V) complexes relevant to biological systems", *J. Inorg. Biochem.*, **80**, pp 1-10 (2000).

37. Nechay, B.R., Nanninga, L.B. and Nechay, P.S.E. "Vanadyl(IV) and vanadate(V) binding to selected endogenous phosphate, carboxyl, and amino ligands: Calculations of cellular vanadium species distribution", *Arch. Biochem. Biophys.*, **251**, pp 128-138 (1996).

38. Rokus, R, Wieger, H. and Ron, W. "Peroxidase and phosphatase activity of active-site mutants of vanadium chloroperoxidase from the fungus Curvularia inaequalis", *J. Biol. Chem.*, **275**, pp 11650-11657 (2000).

39. Olinger, J.M., Hill, D.M., Jakobsen, R.J. and Brody, R.S. "Fourier transform infrared studies of ribonuclease in H_2O and $2H_2O$ solutions", *Biochim Biophys. Acta*, **869**, pp 89-98 (1986).

40. Fabian, H., Schultz, C., Backmann, J., Hahn, U., Saenger, W., Mantsch, H.H. and Naumann, D. "Impact of point mutations on the structure and thermal stability of ribonuclease T1 in aqueous solution probed by Fourier transform infrared spectroscopy", *Biochemistry*, **33**, pp 10725-10730 (1994).

41. Yamamoto, T. and Tasumi, M. "FT-IR studies on thermal denaturation processes of ribonucleases A and S in H_2O and D_2O solutions", *J. Mol. Struct.*, **242**, pp 235-244 (1991).

42. Boulkanz, L., Balcar, N. and Baron, M.H. "FT-IR analysis for structural characterization of albumin adsorbed on the reversed-phase support RP-C_6", *Appl. Spectrosc.*, **49**, pp 1737-1746 (1995).

43. Bramanti, E. and Benedetti, E. "Determination of the secondary structure of isomeric forms of human serum albumin by a particular frequency deconvolution procedure applied to Fourier transform IR analysis", *Biopolymers*, **38**, pp 639-653 (1996).

44. Goormaghtigh, E., Cabiaux, V. and Ruysschaert, J.M. "Seconndary structure and dosage of soluble and membrane proteins by attenuated total reflection Fourier-transform infrared spectroscopy on hydrated films", *Eur. J. Biochem.*, **193**, pp 409-420 (1990).

45. Vandenbussche, G., Clercx, A., Curstedt, T., Johansson, J., Jornvall, H. and Ruysschaert, J.M. "Structure and orientation of the surfactant-associated protein C in a lipid bilayer", *Eur. J. Biochem.*, **203**, pp 201-209 (1992).

46. Murayama, K., Wu, Yuqing, Czarnik-Matusewics, B. and Ozaki, Y. "Two-dimensional/attenuated total reflection infrared correlation spectroscopy studies on secondary structural changes in human serum albumin in aqueous solution: pH- dependent structural changes in the secondary structures and in the hydrogen bonding of side chains", *J. Phys. Chem. B.*, **105**, pp 4763-4769 (2000).

47. Wu, Y., Murayaman, K. and Ozaki, Y. "Two-dimensional infrared spectroscopy and principle component analysis studies of the secondary structure and kinetics of hydrogen-deuterium exchange of human serum albumin", *J. Phys. Chem. B.*, **105**, pp 6251-6259 (2001).

48. Wei, Y., Lin, S.Y., Wnag, S., Li, M.J. and Cheng, W.T. "Fourier transfor IR attenuated total reflectance spectroscopy studies of cystein-induced changes in secondary conformations of bovine serum albumin", *Biopolymers (Biospectroscopy)*, **72**, pp 345-351 (2003).

49. Pelton, J.T. and McLean, L.R. "Spectroscopic methods for analysis of protein secondary structure", *Anal. Biochem.*, **277**, pp 167-176 (2000).

50. Parker, F.S., *Applications of Infrared, Raman and Resonance Spectroscopy in Biochemistry*, Plenum Press, New York (1983).

51. Surewicz, W.K., Moscarello, M.A., and Mantsch, H.H. "Secondary structure of the hydrophobic myelin protein in a lipid environment as determined by Fourier-transform infrared spectrometry", *J. Biol. Chem.*, **262**, pp 8598-8602 (1987).

52. Holzbaur, I.E., English, A.M., and Ismail, A.A. "FTIR study of the thermal denaturation of horseradish and cytochrome c peroxidases in D_2O", *Biochemistry*, **35**, pp 5488-5494 (1996).

53. Kragh-Hansen, U. "Structure and ligand binding properties of human serum albumin", *Dan. Med. Bull.*, **37**, pp 57-84 (1990).

54. Bousquet, Y., Swart, P.J., Schmitt-Collin, N., Velge-Roussel, F., Kuipers, M.E., Mejjer, D.K.E., Bru, Nicole, Hoebeke, J. and Breton, P. "Molecular mechanisms of the adsorption of a model protein (human serum albumin) on poly (methylidene malonate 2.1.2) nanaparticles", *Pharm. Res.*, **16**, pp 141-147 (1999).