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Interactions of human serum albumin with doxorubicin in different media

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ABSTRACT

Interactions of human serum albumin (10 wt% H_2O and 0.3 wt% sodium caprylate) with doxorubicin hydrochloride (1 wt%) were studied alone or with addition of HCl (3.6 wt% HCl) using ^{1}H NMR spectroscopy. A model of hydrated HSA/12DOX was calculated using PM7 method with COSMO showing large variations in the binding constant depending on structural features of DOX/HSA complexes. DOX molecules/ions displace bound water from narrow intramolecular voids in HSA that leads to diminution of freezing-melting point depression of strongly bound water (SBW). Structure of weakly bound water (WBW) depends much weaker on the presence of DOX than SBW because a major fraction of DOX is bound to adsorption sites of HSA. Addition of HCl results in strong changes in structure of macromolecules and organization of water in hydration shells of HSA (i.e., mainly SBW) and in the solution (i.e., WBW + non-bound bulk water).

1. Introduction

Despite doxorubicin (DOX) has been used as an anti-cancer drug during a long period, it remains a popular medicine for chemotherapeutical applications to treat various cancers [1–3]. It is frequently used in conjugates with such compounds as human serum albumin (HSA) as a transport protein [4–12]. The DOX molecules (ions) can be strongly attached to the albumin macromolecules [13], and its desorption occurs during several hours [7]. The albumin molecules playing a typical role of a drug carrier in the delivery system promote fast penetration of the DOX molecules into cancer cells [6]. Polymeric and other types of the drug (including DOX) delivery systems are also effectively used in treatments of cancers [13–22]. However, the drug delivery system based on HSA is a natural one and, therefore, could be more preferable [23–26]. The HSA molecules (which are globular ones with sizes, structure and charge dependent on pH) have numerous and various active adsorption sites, at which transported molecules or ions can be located [13,23–29]. Note that compounds of different structures (nonpolar, polar, charged) can be located at different active sites of the HSA molecules. Localization of DOX molecules at different sites of HSA was well studied and analyzed in the literature [13,19,24]. The binding constant for DOX-HSA complexes can be relatively large (>10^4 M^{-1}) [13] that can be explained by the formation of several strong hydrogen bonds between each bound DOX molecule and HSA.

In native state, the HSA molecules are strongly hydrated. Water molecules form not only an outer hydration shell of HSA, but also fill intramolecular voids formed by polypeptide chain and side functionalities [30–32], as well as intermolecular voids in aggregates of macromolecules (see Fig. S1 in Electronic Supplementary Information (ESI) file). These voids have a mosaic hydrophilic-hydrophobic structure due to the presence of various side functionalities characterized by different polarity. Therefore, the water molecules bound to the HSA molecules differently interact (i.e., interaction energy differs) with a macromolecule surface and form various structures such as 2D and 3D clusters in intramolecular voids. Larger 3D structures (domains) of water molecules located in intermolecular space in the aqueous solution of HSA can undergo certain actions due to disturbing forces from strongly polar/charged HSA molecules. In other words, the bulk and bound waters in the aqueous solution of proteins are structurally non-uniform and can demonstrate a complex temperature behavior. To study these interfacial features of water bound to HSA or other biomacromolecules, cells, and other biosystems, low-temperature ^{1}H NMR spectroscopy with layer-by-layer freezing-out of bulk and bound waters can be effectively used [33]. Changes in the values of the chemical shift of proton resonance (δ_H) [34] of water molecules vs. temperature and concentration depending on the

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presence of various solutes are informative because they depend strongly on topological and structural features of surroundings and characteristics of the hydrogen bonds of water molecules with polar or hydrophobic functionalities [33,35–37]. The values of δ_H, numbers and intensity of different ^{1}H NMR signals of bulk and bound waters vs. temperature give information on the structure of bound water clusters and domains affected by kosmotropic and chaotropic solutes or functionalities of macromolecules and solids [33,37]. Clearly, strongly hydrated HSA molecules free and with adsorbed small molecules (e.g. drugs) can have different hydration shells and differently structured intramolecular water clusters. Information on these changes in water bound to biomacromolecules can be useful to analyze the HSA-drug systems. The solvation effects for proteins, as well as proton transfer and mobility in aqueous solution of proteins, are of importance [33,38–40] to understand the behavior of transport proteins used for drug delivery. Therefore, the aim of this study is the analysis of the hydration shells of the HSA molecules (including intramolecular water) alone and with bound DOX molecules/ions in the aqueous media pure and with addition of HCl modeling effects of acidic gastric juice on the HSA – DOX complexes.

2. Experimental and computational methods

2.1. Materials

Commercial human serum albumin (HSA, 66.5 kDa) prepared by fractionation of human plasma proteins (Biopharma, Kyiv) was used as an aqueous solution (10 wt% and containing 0.3 wt% sodium caprylate, i.e., sodium salt of octanoic acid, $C_8H_{15}O_2Na$) for infusion.

Commercial doxorubicin hydrochloride (DOX, IUPAC name (7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl] oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12-dione) was used in a powder form (Sinbias Pharma Ltd, India) added (1 wt%) into the HSA solution and carefully stirred to prevent sediment formation and equilibrated for several hours. Samples after addition of the HCl solution (10 wt% of 36% aqueous solution of HCl) were equilibrated for 0.5 h or longer period.

Microphotographs (Primo Star optical microscope, Carl Zeiss) of used samples show some structural features of them (see Electronic Supplementary Information (ESI) file).

2.2. ^{1}H NMR spectroscopy

^{1}H NMR spectra of static samples of HSA (placed into 4 and 5 mm NMR ampoules) strongly hydrated at h = 9 g H_2O per gram of dry albumin with DOX hydrochloride (Fig. S2b) as an adsorbate (1 wt%) and 36% solution of HCl (10 wt%) were recorded using a Varian 400 Mercury spectrometer (magnetic field 9.4 T, bandwidth 20 kHz) utilizing eight 90° pulses of 3 μs duration. Relative mean errors were less than ±10% for ^{1}H NMR signal intensity for overlapped signals, and ±5% for single signals. Temperature control was accurate and precise to within ±1 K. The accuracy of integral intensities was improved by compensating for phase distortion and zero-line nonlinearity with the same intensity scale at different temperatures. To prevent supercooling of samples, the beginning of spectra recording was at 200–210 K. Samples precooled to this temperature for 10 min were then heated to 285 K at a rate of 5 K/min with steps ΔT = 10 K or 5 K at a heating rate of 5 K/min for 2 min. They were maintained at a fixed temperature for 3–5 min for data acquisition at each temperature for 1 min [33].

The applications of the low-temperature ^{1}H NMR spectroscopy and NMR cryoporometry, based on the freezing point depression of liquids located in pores depending on the pore size, to numerous objects were described in detail elsewhere [33,41–43]. Note that high-molecular weight compounds do not contribute the ^{1}H NMR spectra recorded here due to a large difference in the transverse relaxation times of liquid (mobile) small compounds (such as water, HCl, *etc.*) and macromolecules or solids and due to a narrow bandwidth (20 kHz) of the spectrometer [33].

Water or other liquids can be frozen in narrower pores at lower temperatures that can be described by the Gibbs-Thomson relation for the freezing point depression

$$\Delta T_m = T_m(R) - T_{m,\infty} = \frac{2\sigma_{sl}T_{m,\infty}}{\Delta H_f \rho R} = -\frac{k_{GT}}{R} \quad (1)$$

where $T_m(R)$ is the melting temperature of a frozen liquid in pores of radius R, $T_{m,\infty}$ the bulk melting temperature, ρ the density of the solid, σ_{sl} the energy of solid-liquid interaction, ΔH_f the bulk enthalpy of fusion, and k_{GT} is a constant [33,41–43], and for water bound to HSA k_{GT} = 60 K nm. Differential size distribution of unfrozen water structures can be calculated as follows [33]

$$\frac{dV_{uw}(R)}{dR} = \frac{A}{k_{GT}}(T_m(R) - T_{m,\infty})^2 \frac{dC_{uw}(T)}{dT} \quad (2)$$

where $V_{uw}(R)$ is the volume of unfrozen water in pores of radius R, C_{uw} the amount of unfrozen water per gram of adsorbent as a function of temperature, and A is a constant.

The $f_V(R) = dV/dR$ function can be converted into the distribution function $f_S(R)$ with respect to the specific surface area in contact with unfrozen water or other liquids

$$f_S(R) = \frac{w}{R}\left(f_V(R) - \frac{V(R)}{R}\right) \quad (3)$$

where w = 1, 2 and 1.36 for slitshaped, cylindrical pores and voids between spherical particles packed in the cubic lattice respectively. Integration of the $f_V(R)$ and $f_S(R)$ functions at R < 1 nm, 1 nm < R < 25 nm, and 25 nm < R < 100 nm gives the volume and the specific surface area of nano-, meso- and macropores. The specific surface area (S_{uw}) of adsorbents in contact with bound water (assuming for simplicity that the density of unfrozen bound water ρ_{uw} = 1 g/cm^3) can be determined from the amount of this water C_{uw}^{max} (estimating pore volume as $V_{uw} = C_{uw}^{max}/\rho_{uw}$) at T = 273.15 K and pore size distribution $f(R)$ (used to estimate the average pore radius R_{av}) with a model of cylindrical pores

$$S_{uw} = \frac{V_{uw}}{2R_{av}} = \frac{2C_{uw}^{max}}{\rho_{uw}} \int_{R_{min}}^{R_{max}} f(R)dR \Big/ \int_{R_{min}}^{R_{max}} f(R)RdR \quad (4)$$

where R_{min} and R_{max} are the minimal and maximal radii of pores filled by unfrozen water, respectively. In the case of calculations of the structural characteristics of nanopores (R < 1 nm), mesopores (1 nm < R < 25 nm) and macropores (R > 25 nm), the R_{min} and R_{max} values are the boundary R values for the corresponding pore types (including R_{min} = 0.2 nm for nanopores), and the C_{uw}^{max}/ρ_{uw} value should be replaced by the corresponding values of the volumes of nanopores, mesopores, or macropores. The average melting temperature <T_m> was calculated using Eq. (5)

$$< T_m >= \int_{T_{min}}^{T_0} TC_{uw}(T)dT \Big/ \int_{T_{min}}^{T_0} C_{uw}(T)dT \quad (5)$$

where T_0 = 273.15 K, and T_{min} is the temperature corresponding to C_{uw} = 0.

2.3. UV–vis spectra

Ultraviolet–visible (UV–vis) spectra in a transmission mode (recalculated into the absorbance spectra) of the solutions studied