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Calorimetric investigation on the interaction of sodium taurodeoxycholate with human serum albumin

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ABSTRACT

The interaction between sodium taurodeoxycholate and human serum albumin was investigated in aqueous phosphate buffer at pH 7.2 through ITC titrations at 298.15 K and 310.15 K and TM-DSC measurements. The analysis of the ITC data required the previous determination of the thermodynamic properties of the self-associated oligomeric species of the bile salt in the buffer solution, according to a literature chemical model. Use of a computer program which allows the simultaneous determination of the stability constants and formation enthalpies of complexes indicated that albumin forms a strong 1–1 (salt to albumin) complex at low bile salt concentration and at least one larger complex at higher concentrations. In these larger complexes the protein seems to cooperatively bind a number of moles of the bile salt which increases with temperature. Thermodynamic data are provided for the formation of the most probable species which include a 4–1 complex at 298.15 K and an additional 9–1 complex at 310.15 K. TM-DSC data, while evidencing the partially reversible nature of the folding–unfolding process and the stabilization of the protein by interaction with the bile salt, supported the stoichiometry of the complexes found by analysis of ITC data.

1. Introduction

The interactions between proteins and small molecules, often leading to stable complexes, raised since long a noticeable interest in the scientific literature. The formation of these complexes is very important to reveal the enhanced stability of the proteins and also determine their higher capacity to act as carriers for specific drugs. Particularly important is the physicochemical characterization of these complexes in order to elucidate their structure and to evidence the nature and weight of their interactions (electrostatic, H-bond, van der Waals). Human serum albumin (HSA), the most prominent protein in plasma, displays a very high capacity to bind large amounts of a variety of molecules (drugs, fatty acids, metabolites), thus holding a key role in the pharmacokinetic behaviour and the bioavailability of many compounds [1]. Particularly, albumin is known to make adducts with surface active agents: very stable complexes with classical alkyl chain surfactants while much less stable complexes with natural surfactants as the bile salts (BS) [1].

In the present work we investigated the interactions in water of HSA with the BS sodium taurodeoxycholate. Sodium taurodeoxycholate (NaTDC), and most of bile salts as well, play an important role in many physiological systems, mainly because of their detergent-like and surface active properties, thus enabling to easily solubilize many water insoluble compounds like cholesterol.

Bile salts are characterized by the presence of a polar and a non-polar surface, allowing the formation of micellar-like aggregates. These aggregates, however, are small and their aggregation features strongly depend on the structure of the salt, its concentration and ionic strength [2]. Accurate X-ray, QELS and CD measurements [3] and EMF data [4,5] demonstrated that trihydroxy bile salts tend to form dimeric basic units [6] while analogous structural [5,7–9], and EMF [10] studies on dihydroxy salts (like NaTDC) indicate that the latter form trimers. Some authors, however, claim that the basic unit would be a dimer for both dihydroxy and trihydroxy salts (see Refs. [11,12], and references therein). The basic units tend to further aggregate with increasing BS concentration [1,10,13], leading to a series of stoichiometrically different species in which the negative charge of the TDC^- ions is fully or partially compensated by sodium and hydrogen ions [10]. In summary, we can say that what happens in BS solutions (and specifically in the case of sodium taurodeoxycholate) can hardly be described as a classical micellar transition but, actually, involves a complex system of multiple equilibria.

Human serum albumin deserved a strong interest in the scientific literature both as to its structure and to its capacity to interact with many different molecules. Studies based on SAXS technique, light scattering and spectroscopic data [14,15] show for the native

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protein in aqueous solution a folded form with a heart shaped conformation very similar to its crystallographic structure. In particular three domains have been recognized (I, II and III) which exhibit slight reciprocal movements [1]. A local electric charge −9, −8, and +2 has been assigned to these domains, respectively. Furthermore, many studies on the HSA binding properties have shown that the main binding regions on HSA are located in the hydrophobic subdomains indicated as IIA and IIIA [14]. The first site, indicated as Sudlow's site I, or Warfarin site, is known to bind very efficiently dicarboxylic acids and bulky heterocyclic molecules as well, with a negative charge localized in the middle of the molecule. This site is large enough to bind molecules as large as bilirubin. The second site, known as Sudlow's site II, or ibuprofen site, is preferred by aromatic carboxylic acids with a negatively charged acidic group at one end of the molecule and far away from its hydrophobic centre. This second site is topologically similar to site I, but seems to be smaller and less flexible since the binding is strongly affected by stereoselectivity. Besides these two main binding sites, there are many other different points of attack that can also be controlled by allosteric conformational changes [1].

Complexes of HSA with small molecules have been studied by many authors: with ibuprofen through SAXS, light scattering and spectroscopic techniques [16], with drugs and fatty acids by calorimetry [6,17,18], with naproxen by UV spectroscopy [19], with penicillins by thermodynamic properties [20], with hydrocarbon and fluorocarbon surfactants by thermodynamic and transport properties [21,22] and with ionic surfactants by spectroscopy [23,24] and calorimetry [25].

Interaction of HSA with several bile salts was studied by Roda et al. through an equilibrium dialysis method [26] and by Farruggia et al. through differential scanning calorimetry (DSC), fluorescence spectroscopy and circular dichroism [27,28]. La Mesa et al. investigated the interactions of bovine serum albumin (BSA) with NaTDC in dilute solution by transport and thermodynamic properties [29,30] and in concentrated solutions by DSC and NMR [31].

We here describe a series of ITC measurements at two temperatures, and a few TM-DSC experiments, on HSA-NaTDC aqueous solutions in phosphate buffer at pH 7.2. The experimental ITC data were analyzed through a chemical model which takes into consideration possible complexes of the general formula $Na_qTDC_r\ HSA_t$. Discussion of these data pointed to evidence the possible involvement of a plurality of binding sites of the protein, the nature and the stoichiometry of the species involved in its interaction with the bile salt, and the possible existence of cooperativity effects. TM-DSC data pointed to explore the effect of these interactions on the stability of the protein and test the reversibility of the process of thermal denaturation.

2. Experimental

2.1. Chemicals

Essentially fatty acid free human serum albumin (HSA) ≥96% and sodium taurodeoxycholate (NaTDC) ≥97% were obtained from SIGMA and used as such, after desiccation under vacuum. Sodium dihydrogen and monohydrogen phosphates used for buffer solutions were reagent grade products from Carlo Erba. Doubly deionized water was used as the solvent. Buffer solutions were obtained by titrating 10 mM Na_2HPO_4 solutions with 10 mM NaH_2PO_4 solution, in the presence of 11 mM NaN_3, to pH 7.2. All solutions were normally prepared by weight and their density measured by a vibrating tube densimeter Anton Paar DSA 5000. HSA solutions were allowed to stand overnight under gentle stirring before use. Their concentration was determined spectrophotometrically at 280 nm using $\varepsilon = 35{,}700\ l\ mol^{-1}\ cm^{-1}$ [32]. All aqueous buffered solutions were maintained at 4 °C and used within five days.

2.2. Isothermal titration calorimetry (ITC)

The isothermal titration calorimeter was a Thermal Activity Monitor 2277 (TAM) from Thermometric, equipped with a 612 Lund syringe pump. Titrations were performed at 298.15 K and 310.15 K by adding aliquots of a few microliters (1–10) of a concentrated buffered aqueous solution of one component into a 1 ml cell containing about 0.8 g of the aqueous buffered solution of the other component. Direct titrations mostly involved the addition of 0.1 M NaTDC to 6×10^{-5} M HSA (0.4%), covering a bile salt concentration range $0 < C_{BS} < 0.02\ mol\ l^{-1}$. Reverse titrations involved the addition of a 6×10^{-4} M HSA solution to a solution of NaTDC. Owing to the small additions of the titrant almost all measurements were made at the maximum sensitivity of the calorimeter (3 μW full scale) which in some case resulted in an increase of the experimental uncertainty in the measured heat (normally about 1%).

2.3. Temperature modulated differential scanning calorimetry (TM-DSC)

The equipment used for the temperature-modulated differential scanning calorimetry in the heating and cooling modes is home-built and has been described earlier [33–36]. The samples contained 1.5×10^{-4} mol of HSA per kilogram of phosphate buffer and variable concentration of NaTDC. Measurements were performed at 3.3 mHz (modulation period = 300 s) and 0.5 K modulation amplitude, while the sample was heated and subsequently cooled at an average rate of 0.2 K/min between 308.15 K and 353.15 K. In these conditions the complex heat capacity of the sample, C_p^*, and the apparent heat capacity, $C_{p,DSC}$, of the sample were determined at 1 K intervals, that is one data point for both measured quantity was obtained for each modulation period. From the measured heat flow to the sample to maintain the temperature scanning and modulation, the in-phase component, C_p' and the out-of-phase component, C_p'', of the complex heat capacity, $C_p^* = C_p' - iC_p''$, were calculated. From the same set of heat flow data $C_{p,DSC}$ was determined as follows:

$$C_{p,DSC} = \left(\frac{1}{\beta}\right)\left(\frac{dH(T,t,x_i)}{dt}\right) \quad (1)$$

where $(dH(T,t,x_i)/dt)$ is the measured rate of enthalpy change, x_i is the mole fraction of the HSA protein undergoing the unfolding process and β (=dT/dt) is the temperature scanning rate.

Eq. (1) can be written in a more general form by taking into account the two time dependent quantities T and x_i:

$$C_{p,DSC} = \left[\frac{\partial H}{\partial T} + \left(\frac{\partial H}{\partial x_i(T)}\right)\left(\frac{dx_i}{dT}\right)\right] + \left(\frac{1}{\beta}\right) \times \left[\frac{\partial H}{\partial t} + \left(\frac{\partial H}{\partial x_i(t)}\right)\left(\frac{dx_i}{dt}\right)\right] \quad (2)$$

where $\partial H/\partial T$ is the thermodynamic heat capacity. The first right end side term $[(\partial H/\partial x_i)(dx_i/dT)]$ in Eq. (2) refers to the contribution of the thermally reversible folding-unfolding process that may be fast enough that equilibrium is maintained at every instant of the temperature modulation period and consequently the original state of the system would be restored after one modulation cycle. [33] The second right end side term in Eq. (2) refers to the time dependent irreversible component of the enthalpy change and of the folding-unfolding process.

Comparison of the data obtained from the DSC and TM-DSC techniques is simple when $C_p'' = 0$. In this case the C_p^* determined by the modulated technique is not a complex quantity, as is the case in