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Probing the binding ability of vitamin B_1 with bovine serum albumin: Calorimetric, light scattering, spectroscopic and volumetric studies

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ABSTRACT

Various techniques like spectroscopy (UV-visible absorption, fluorescence, ^{1}H-NMR), dynamic light scattering (DLS), isothermal titration calorimetry (ITC), and volumetry (density and sound velocity) have been used to throw light on the interactional behaviour of vitamin B_1 *i.e.* thiamine hydrochloride (TH) with bovine serum albumin (BSA). The spectroscopic results revealed the preservation of tertiary structure of BSA after TH binding. The affinity value of the order of 10^4 M^{-1} has been deduced from both absorption and fluorescence titrations. Metal ion (Ca^{2+}, Mg^{2+}, K^+, Mn^{2+}, Na^+, and Ni^{2+}) effect on the interactional behaviour has also been monitored. The increase in the hydrodynamic size and decrease in the emission intensity through static quenching mechanism of BSA along with its non-specific nature of interactions with TH have been observed. The ionic interactions at lower TH concentrations and hydrophobic interactions at higher concentrations along with hydrational shell structural changes have been visualized from initial increase of both V and K_s values at lower while decrease at higher concentrations of TH. Calorimetric binding enthalpograms also show the initial exothermic binding process followed by endothermic. The involvement of ethylthiazolium part of TH in hydrophobic interactions whereas substituted pyrimidine ring part in hydrophilic interactions has been depicted from ^{1}H-NMR measurements.

1. Introduction

Proteins are the bio-macromolecules that consist of one or more long chain polypeptide subunits. These polypeptide subunits are folded either into globular or fibrous form and are required for the growth and maintenance of life systems. Medical science as well as various biological systems (immunology, pharmacology, and biology *etc.*) considers the interaction of bio-macromolecules with small molecules like drugs, fatty acids, hormones *etc.*. Nowadays, the binding investigations of plasma proteins with drugs become an interesting and vast field in these days to modulate the pharmacokinetic and pharmacodynamic properties of drugs [1,2]. The designing of a drug primarily depends upon its interactions with bio-macromolecules in the body and hence these studies play fundamental role in the drug discovery and development process. The functioning of protein was also found to be affected after their interaction with drug molecules because of drug induced structural changes [3–5]. Among serum albumins, bovine serum albumin (BSA, Fig. 1(A)), an extracellular globular protein synthesized by the liver, is a single polypeptide chain with 583 amino acids, 17 disulphide bridges, two tryptophans (Trp), and a free thiol group [6–8]. It is a highly soluble, easily available at low cost, highly abundant protein which exhibits unusual ligand binding properties [9,10]. At physiological pH, it is found to exist in anionic form having a −18 surface charge and capability to bind reversibly to a variety of exogeneous and endogeneous ligands [11,12]. The principle binding sites on BSA for variety of drugs are found to be in subdomain IIA (Sudlow site I) and IIIA (Sudlow site II) which are recognized as the warfarin and benzodiazepine binding sites, respectively [13–16]. The structure of BSA shows 76% similarity with human serum albumin (HSA) [17,18]. The interactional process among drugs and proteins is reversible in nature and has contributions from various weak interactions like van der Waals, ionic, hydrophobic, and hydrogen bonding [19,20].

In addition, the metal ions in the body can also interfere in drug-protein binding interactions and hence, modulate the therapeutic effect of a drug by reducing its free concentration [21,22]. The metal ions were either present already inside the body or taken from outside *via* any means. Some proteins offer several binding sites to these metal ions with different specificities and behave like their sequestration agents [21,22]. Hence, it seems imperative to rationalize the binding of drugs with proteins in the presence of different metal ions.

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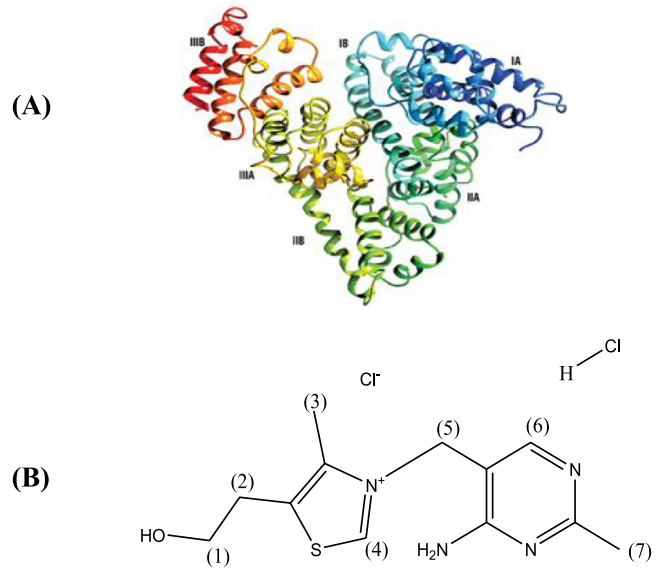

Figure 1. Structure of (A) bovine serum albumin (BSA) presenting its different subdomains and (B) thiamine hydrochloride (TH). Each proton of TH has been numbered for interpreting the results of ^{1}H-NMR measurements.

The 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-5-(2-hydroxyethyl)-4-methelthiazolium, which is known as thiamine hydrochloride (TH, Fig. 1(B)) or aneurin, is a water soluble vitamin B_1 having antioxidant properties. Several foods like cereal grains, rice bran, beans, nuts, banana *etc.* contains vitamin B_1 [23]. It is also used in some foods and drinks for the formation of enriched products for special purposes [23,24]. For numerous enzyme complexes like pyruvate dehydrogenase and transketolase, TH acts as a coenzyme or a precursor and hence, it plays crucial role in genetic regulatory processes and in carbohydrate metabolism [24,25]. It is found to be useful in increasing the immunity of the body against AIDS as well as in the treatment of heart diseases, diabetic pain, cerebellar syndrome and vision related problems (cataracts and glaucoma) [26]. The shortage of TH in the body results in optic neuropathy, a disease called beriberi that affects the peripheral nervous system and Wernicke encephalopathy disease [26]. Hosseinzadeh and Khorsandhi [27] reported the interaction of TH at subdomain IIIB of BSA mainly through hydrophobic and hydrogen bonding interactions. The current work aims to study the effect of TH on hydrodynamic size and hydrational structure of protein, to estimate the binding affinity value, to monitor structural variations in protein and the mode of interaction. For this, the techniques like isothermal titration calorimetry (ITC), dynamic light scattering (DLS), UV-visible absorption, fluorescence, and ^{1}H-NMR spectroscopy in combination with volumetric techniques like density and sound velocity have been used. Effect of different metal ions (Ca^{2+}, Mg^{2+}, K^+, Mn^{2+}, Na^+, and Ni^{2+}) on the binding of TH with BSA have been envisaged from UV-visible absorption spectroscopy in the present report.

2. Experimental

2.1. Materials

The chemicals used and sources of their procurement along with mass fraction purity and purification methods have been given in Table 1. Milli-Q water having resistivity of 18.2 MΩcm was used in the preparation of 0.01 mol kg^{-1} potassium phosphate buffer (PPB, pH 7.4) which was further used to prepare solutions of protein and drug. The 0.01 mol kg^{-1} PPB of pH 7.4 was prepared from the stock solution of 0.1 mol kg^{-1} PPB (pH 7.4). To prepare 100 mL 0.1 mol kg^{-1} PPB (pH 7.4), 8.02 mL of 1 mol kg^{-1} solution of dipotassium hydrogen orthophosphate (K_2HPO_4) and 1.98 mL of 1 mol kg^{-1} potassium dihydrogen orthophosphate (KH_2PO_4) were mixed and diluted to 100 mL with Milli-Q water. To weigh all the chemicals for solution preparation by mass, Mettler Toledo AB 265-S weighing balance with an accuracy of 0.01 mg was employed. The solutions of protein are prepared by weighing it followed by addition of solvent by mass. The molar mass 66463 g mol^{-1} of BSA has been used for preparing the solutions. All the analytical grade reagents were stored *in vacuo* in the desiccator over anhydrous $CaCl_2$ before use. Thereafter, the mass fraction of water in salt hydrates $CaCl_2{\cdot}2H_2O$, $MgCl_2{\cdot}6H_2O$, $MnCl_2{\cdot}4H_2O$, and $NiCl_2{\cdot}6H_2O$ has been determined by Karl Fischer titration which comes out to be 0.022, 0.029, 0.017, and 0.019, respectively. Hence, during concentration calculations of these salt hydrates, this water content has also been accounted.

Table 1
Sources and purities of chemicals used in the current work.

Chemical	Source	Purification method	Mass fraction Purity[a]
Bovine serum albumin	Sigma Aldrich	Used as received	≥0.98
Thiamine hydrochloride	SRL	Stored in desiccator	≥0.985
$CaCl_2{\cdot}2H_2O$	SRL	Stored in desiccator	≥0.995
$MgCl_2{\cdot}6H_2O$	Loba Chemie	Stored in desiccator	≥0.98
KCl	SRL	Stored in desiccator	≥0.995
$MnCl_2{\cdot}4H_2O$	SRL	Stored in desiccator	≥0.99
NaCl	SRL	Stored in desiccator	≥0.999
$NiCl_2{\cdot}6H_2O$	SRL	Stored in desiccator	≥0.99
KH_2PO_4	Qualigens	Stored in desiccator	≥0.995
K_2HPO_4	Qualigens	Stored in desiccator	≥0.99

[a] As stated by the supplier.

2.2. Methods

2.2.1. UV-visible absorption spectroscopy

Shimadzu-1800 UV-visible spectrophotometer was used to monitor the absorption titrations using 1 cm wide quartz cuvettes fitted with Teflon stopper. The 0.005 mmol kg^{-1} concentrated solution of BSA was placed in the sample cuvette followed by consecutive additions of highly concentrated solution of TH into it so that the change in the concentration of BSA should be negligible. The reference cuvette was filled with the similar concentrated solutions of TH in buffer as present in sample cuvette. The data obtained after taking the average of at least three measurements was further analyzed to evaluate the binding affinity values. During absorption study to see the effect of metal ions on the binding process of TH with BSA, the solutions of BSA (0.005 mmol kg^{-1}) were prepared in the respective metal ion solutions. The molality of the solutions of Ca^+, Na^+, K^+, and Mn^{2+} ions is 0.005 mmol kg^{-1} and of Mg^{2+} and Ni^{2+} ions is 0.01 mmol kg^{-1} with an uncertainty of 2.3×10^{-3} mmol kg^{-1}. The whole process of recording the absorption spectra before and after TH titrations was same as above explained without metal ions.