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Spectroscopic and calorimetric studies of interaction of methimazole with human serum albumin

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ABSTRACT

The interaction of the anti-thyroid drug, 2-mercapto 1-methylimidazole (methimazole) with human serum albumin (HSA) has been examined by fluorescence and isothermal titration calorimetry (ITC) techniques. Fluorescence results indicate that in case of HSA–drug complex the quenching of fluorescence intensity is at 340 nm. The methimazole has an ability to quench the intrinsic fluorescence of HSA tryptophan through a static quenching procedure. The binding constant has been determined using Stern–Volmer modified equation and energy transfer mechanisms of quenching are discussed. The ΔG°, ΔH°, and ΔS° values are also calculated by ITC measurements. The experimental spectroscopic and thermodynamic parameters have been used for understanding the binding mechanism of anti-thyroid drug with HSA.

1. Introduction

Human serum albumin (HSA) is the major protein component of blood plasma but is also distributed to the interstitial fluid of the body tissues [1]. The protein binds a number of relatively insoluble endogenous compounds such as unesterified fatty acids, bilirubin, and bile acids and thus facilitates their transport throughout the circulation [1,2]. HSA is also capable of binding a wide variety of drugs [1–3] and much of the interest in this abundant protein derives from its effects on drug delivery. Drug binding to plasma proteins such as HSA can be an important determinant of pharmacokinetics, restricting the unbound concentration and affecting distribution and elimination. Structural studies have mapped the locations of primary drug binding sites on the protein [4]. Many drugs bind to one of the two primary binding sites on the protein, known as Sudlow's sites I and II [5]. Although examples of drugs binding elsewhere on the protein have been documented [5–7], most work has focused on the primary drug sites. Methimazole (MMZ) is an active metabolite of carboxy benzyl and is frequently used in the management of hyperthyroidism in humans [8–10]. It is proven to inhibit the production of new thyroid hormones and thus is effective in the treatment of hyperthyroidism. It is also taken before thyroid surgery or radioactive iodine therapy. The capability of serum albumins to bind aromatic and heterocyclic compounds depends largely on the existence of two major binding regions, namely Sudlow's site I and site II, [11,12] which are located within specialized cavities in sub domains IIA and IIIA, respectively [4,13]. These hydrophobic binding pockets enable the serum albumins to increase the apparent solubility of hydrophobic drugs in the plasma and modulate their delivery to the cells in vivo and in vitro [14]. It has been reported that MMZ has a very low affinity for serum proteins [15]. However, there is a report in literature [16] based on protein fluorescence quenching observations by excitation at 295 and 280 nm that reports the binding of MMZ to bovine serum albumin (BSA) and human serum albumin, respectively. In view of these contradicting reports on the binding of MMZ to proteins, we have employed a combination of isothermal titration calorimetry and fluorescence spectroscopy to study the possible binding mechanism of MMZ with HSA.

2. Experimental

Methimazole and fatty acid free human serum albumin were purchased from Sigma-Aldrich and used without further purification. All other reagents were of analytical grade. All the experiments were performed in sodium-phosphate buffer of pH 7.4 (20 mM). The concentration of protein was determined spectrophotometrically using $E^{1\%}_{280\ nm} = 5.3$ at 280 nm by using Perkin Elmer Lambda 25 spectrophotometer.

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2.1. Steady state fluorescence quenching measurements

Fluorescence emission spectra were recorded in range of 300–400 nm at three different temperatures 25, 30 and 37 °C on Schimadzu 5301PC fluorescence spectrophotometer equipped with water circulator (Julabo Eyela). Stock solutions of 27 mM methimazole in buffer (pH 7.4) were prepared at room temperature. Various solutions of Methimazole were prepared from the stock solutions by successive dilutions also at (25 ± 1 °C). A stock solution of HSA (67 μM) in 0.05 M sodium-phosphate buffer (pH 7.4) was also prepared at room temperature. All the solutions were kept in the dark and used soon after mixing the components. Both the excitation and emission slit widths were fixed at 10 nm. The excitation wavelength was set at λ_{exc}=295 nm to selectively excite the tryptophan residues, and the emission spectra were recorded in the wavelength range of (300–400) nm at a scan rate of 240 nm/min. The drug–protein binding information can be obtained from fluorescence quenching of protein.

The fluorescence data were analyzed according to the linear and modified Stern–Volmer equation [17]:

$$\frac{F_o}{F} = K_{sv}\,[Q] + 1 \tag{1}$$

where F_o and F were the fluorescence intensities in absence and presence of quencher (methimazole), respectively; K_{sv} is the Stern–Volmer quenching constant. Binding constants and binding sites were obtained from [18]

$$\log\left(\frac{F_o}{F} - 1\right) = \log K_b + n \log [Q] \tag{2}$$

where K_b is the binding constant and n is number of binding sites.

$$k_q = \frac{K_{sv}}{\tau_\circ} \tag{3}$$

where k_q is the bimolecular rate constant of the quenching reaction and τ_o the average integral fluorescence life time of tryptophan which is, $\sim 5.78 \times 10^{-9}$ s [19]. Whereas change in enthalpy (ΔH°) and entropy (ΔS°) at different temperatures were analyzed from van't Hoff Equation:

$$ln\, K_b = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{4}$$

ΔG° can be further resolved into separate terms for enthalpy change (ΔH°) and entropy change (ΔS°) in the equation:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{5}$$

2.2. Isothermal titration calorimetry (ITC)

Isothermal titration calorimetry was performed using a Microcal VP-ITC calorimeter (Northampton, MA). Drug titrants were dissolved in the 20 mM phosphate buffer of pH 7.4. The titrant and HSA samples were thoroughly degassed before titration. HSA samples ranging in concentrations from 0.015 mM were placed in a 1.4 ml reaction cell, and the reference cell was filled with 20 mM phosphate buffer of pH 7.4. All titrations were performed at 25 °C. After temperature equilibration, successive injections of an indicated titrant were made into the reaction cell in 10 μl increments at 180 s interval with stirring at 307 rpm to ensure a complete equilibration. The resulting heats of reaction were measured upto 28 consecutive injections. Control experiments to determine the heats of titrant dilution were carried out by making identical injections in the absence of HSA. The net reaction heat was obtained by subtracting the heat of dilution from the corresponding total heat of reaction. The titration data were deconvoluted based on a binding model containing either one or two sets of non-interacting binding sites by a nonlinear least-squares algorithm using the MicroCal Origin software. The binding enthalpy change (ΔH°), association constant (K_a), and the binding stoichiometry (n) were permitted to float during the least-squares minimization process and taken as the best-fit values.

3. Results

3.1. Fluorescence quenching of HSA by methimazole

The binding of methimazole to HSA was investigated by monitoring the intrinsic fluorescence of the HSA. Fig. 1 shows the fluorescence emission spectra of HSA in the presence of varying concentrations of methimazole following an excitation at 295 nm. It is seen that the addition of methimazole to HSA solution leads to a reduction in the fluorescence intensity indicating that the binding of methimazole to HSA quenches the intrinsic fluorescence of tryptophan. The intrinsic fluorescence of HSA excited at 295 nm is mainly contributed by the Trp residue alone, because the Phe residue has a very low quantum yield and the fluorescence of Tyr is almost totally quenched when it is ionized or nearby an amino group, a carboxyl group or a Trp [18]. Tryptophan, tyrosine and phenylalanine are the three aromatic fluorophores which are used for studying conformational changes on drug binding. However, among them contribution of tryptophan is maximum [20,21]. Fluorescence quenching of aromatic fluorophores in HSA by methimazole at three different temperatures *viz* 25, 30 and 37 °C were evaluated before and after addition of varying concentrations of methimazole. As shown in Fig. 1, HSA has the strong emission peak at 340 nm on excitation at 295 nm which decreases with gradual addition of methimazole at all three temperatures with concomitant blue shift, suggestive of internalization of Trp 214 in the hydrophobic environment of HSA. However, the fluorescence quenching of HSA was maximum at 25 °C followed by 30 °C and least at 37 °C. The fluorescence quenching data were analyzed according to the Stern–Volmer Eq. (1).

3.2. Binding affinity

The fluorescence quenching data of HSA–MMZ binding at different temperatures was presented as Stern–Volmer plot between F_o/F

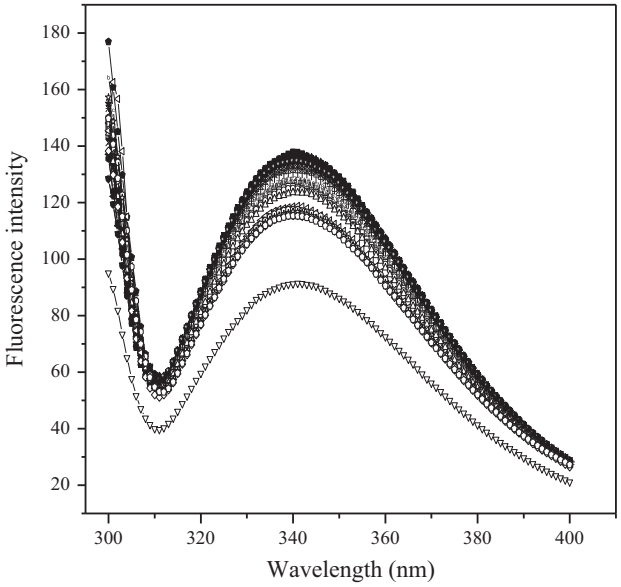

Fig. 1. Fluorescence quenching of HSA in the prescence of MMZ.