Thermodynamic Study of Taurocholate Binding to Rat Serum Albumin

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Abstract. Taurocholate binding to rat serum albumin was studied by equilibrium dialysis. The bile salt-protein interaction was studied under different experimental conditions with respect to temperature; ionic strength; Cl^- concentration; pH and the presence of butanol in the medium. The results obtained suggest the existence of two binding sites for taurocholate on the albumin molecule, and indicate that both electrostatic and hydrophobic interaction play a role in the binding process.

Key words: Taurocholate — Binding — Albumin — Bile acid

Introduction

Bile salts transported in the blood stream are mainly bound to the plasma albumin (Rudman and Kendall 1957). This interaction may play an important role in the uptake and clearance of these substances by the liver. Elucidation of the nature and characteristics of the binding of bile salts to albumin is critical for an analysis of the mechanism of bile salts handling by the hepatocytes. The binding of taurocholate (TC) (a major bile salt in rat blood) to rat serum albumin (RSA) has, therefore been studied here from a thermodynamic approach, in order to estimate its thermodynamic parameters and to characterize the nature of the acting forces in this process.

Materials and Methods

Chemical: Sodium TC (Sigma Chemical Co., U.S.A.) was used without further purification, RSA was obtained from sera of adult Wistar rats by the Cohn's method, purified by chromatography on DEAE Sephadex A—25 (Pharmacia Fine Chem.) and dialysed 96 h against water. The purity of RSA was tested by acetate cellulose electrophoresis. All other reagents were of the highest grade commercially available.

Binding studies: TC binding to RSA was studied by equilibrium dialysis in 0.1 mol $.l^{-1}$ phosphate buffer, pH 7.40, ionic strenght 0.22 mol/kg; Donnan's effect was negligible under such conditions. Dialysis experiments were performed using semi-micro cells of 2 ml total volume. Cellulose membrane (Union Carbide, U.S.A.) which does not allow the passage of molecular weights over 5000, was used according to the manufacturers specifications and correcting the results for the adsorption of TC on the membrane.

Equilibrium was achieved after 24 h at 40 °C and after 48 h at 4 °C. The volume of the solution at both sides of the membrane did not show any significant variation during the dialysis. Free TC concentration was determined by the Minibeck reaction (Feher et al. 1973) measuring the absorbance of the final product at 390 nm in a Beckman DU spectrophotometer. To study binding, $1/r$ was plotted against $1/c$ (where r is the TC average mol number bound per albumin mol, and c is the free TC concentration at equilibrium). Plots of this kind are fitted by a model which postulates the existence of several independent groups of binding sites each having a respective association constant K_i and a respective number of sites n_i according to the following equation:

$$r = \sum \frac{n_i K_i c}{1 + K_i c}$$

The analysis of the data was performed using the computer program BMDPAR-derivative-free nonlinear regression (University of California).

The thermodynamic parameters $\Delta G°$, $\Delta H°$ and $\Delta S°$ were calculated by the usual thermodynamic equations (Mukkur 1984) from the K_i values determined at two temperatures. In order to characterize the nature of the interaction, binding was studied at different chloride ion concentration, ionic strength values, pH values and butanol concentrations in the incubation media.

Results

Binding isotherms and thermodynamic parameters: binding isotherms were obtained as stated in the Materials and Methods section at two different temperatures (4 °C and 40 °C). It has been demonstrated that the conformational structure of protein does not change in this temperature range (Brown et al. 1982). On the other hand, the differential spectra of the macromolecule were determined at different ligand/protein concentration ratios (1, 2, 4), to test any possibility of RSA unfolding induced by TC detergent action. There was no variation in the studied wavelength range (220—350 nm).

The non-lineality of Klotz plots (Fig. 1) suggested heterogeneity of the binding sites (Karush and Sonenberg 1949). The curves obtained were thus fitted by a model with two independent groups of binding sites, using a BMDPAR-derivative-free non-linear regression computer program. The values obtained for n_1, n_2, K_1 and K_2 by this method are shown in Table 1. We designate the sites having a higher association constant value as the primary group, and those with a lower association constant value as the secondary group. Table 1 also shows the $\Delta G°$, $\Delta H°$ and $\Delta S°$ values calculated for both classes of binding site.

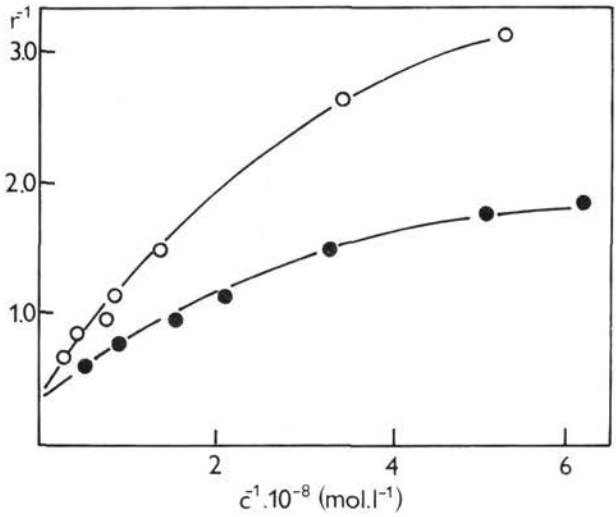

Fig. 1. Double reciprocal plots for the binding of TC to RSA in buffer phosphate 0.1 mol.l^{-1}, pH 7.40, ionic strength 0.22 mol/kg. Temperature: ● 4 °C, ○ 40 °C. RSA concentration 70 × × 10^{-6} mol.l^{-1}. Each point is the average of three measurements.

Table 1.

		n	K (l.mol^{-1})	$\overline{\Delta G°}$ (kcal/mol)	$\overline{\Delta S°}$ (e. U.)	$\overline{\Delta H°}$ (kcal/mol)
primary site	4 °C	1	7.0×10^4	−5.92	+14.5	−2.20
	40 °C		4.4×10^4			
secondary site	4 °C	5	6.8×10^2	−3.23	+1.1	−3.30
	40 °C		3.4×10^2			

$\overline{\Delta G°}$, $\overline{\Delta S°}$, $\overline{\Delta H°}$ are average values for the 4 °C to 40 °C temperature range.

Effect of chloride ions concentration on binding: the data of Fig. 2 show how TC binding to RSA (at pH 7.40 and at a TC/RSA concentration ratio for which the acting sites are mainly the primary ones) is reduced in the presence of chloride ions at increasing concentrations. During these experiments, the ionic strength of the media was maintained at a constant value of 0.22 mol/kg by compensatory variation of the sodium phosphate concentration. When the ionic strength of the media was varied by altering the sodium phosphate concentration, a net increase in bound TC was found for increasing ionic strength values.

Effect of butanol incorporation to the medium: by adding different amounts of butanol within the range from 0 to 0.2 mol.l^{-1} a decrease in the r values was obtained (Fig. 2) for systems where pH, ionic strength, chloride ion concentra-

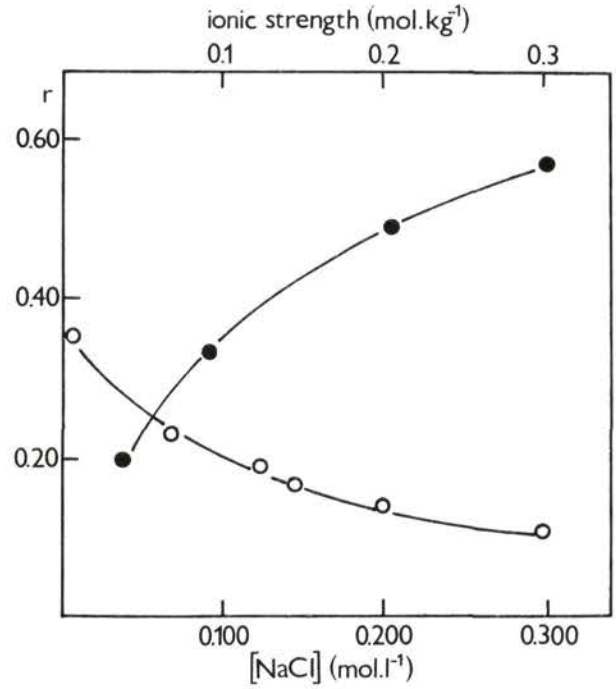

Fig. 2. ○ Effect of chloride ions on the binding of TC to RSA, pH 7.40. Temperature 4 °C, ionic strength 0.22 mol/kg. ● Effect of ionic strength on the binding of TC to RSA, pH 7.40, temperature 4 °C. RSA concentration 70×10^{-6} mol.l^{-1} ratio TC/RSA 1/1.

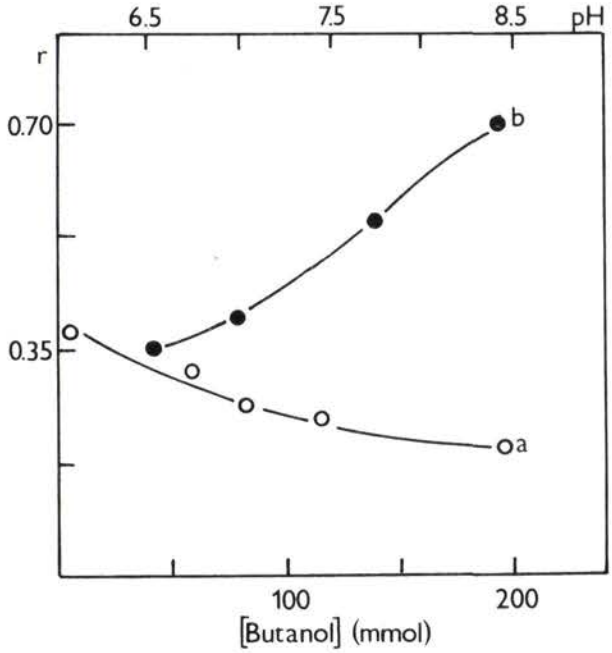

Fig. 3. ● Effect of pH on the binding of TC to RSA, buffer phosphate, temperature 4 °C, ionic strength 0.22 mol/kg. ○ Effect of butanol on the binding of TC to RSA buffer phosphate 0.1 mol.l^{-1}, pH 7.50, temperature 4 °C. RSA concentration 70×10^{-6} mol.l^{-1} ratio TC/RSA 1/1.

tion and TC/RSA ratio were maintained at constant values similar to those of the experiments represented by the data in Fig. 2.

Effect of pH: as presented in Fig. 3, r values were plotted as function of pH at constant ionic strength and chloride ion concentration, for a TC/RSA concentration ratio favouring the presence of primary sites. A net increase of bound TC was observed in response to increasing values of pH. It is important to note that pH values were in the range within which albumin undergoes the $N \leftrightharpoons B$ conformational transformation (Wanwimolruk and Birkett 1982). Nevertheless, this does not contribute to present observations of interaction, as indicated by the data presented above. On the other hand, the enthalpy changes are negative, while the unfolding process would require the breaking of several bonds and should result in an endothermic reaction of appreciable magnitude (Goto et al. 1978).

The positive entropy change probably results from the presence of a hydrophobic component of binding, due to its disordering effect produced by melting of the "iceberg" structure of water with the binding sites (Picconi et al. 1984). A higher negative enthalpic change and lower positive entropy change were observed for the binding of TC to the secondary binding sites, suggesting in this way the presence of a more important electrostatic contribution and an almost negligible hydrophobic component in this interaction.

The results obtained when binding was measured at increasing ionic strength and at constant ionic strength but increasing chloride anion concentration, must be analysed together. Firstly, the rise in the ionic strength which results in an increase of binding, is consistent with a hydrophobic interaction (Mitra and Chattoras 1978). Nevertheless, the possibility that increase actually could be the result of the sum of an increasing effect on the hydrophobic part of the interaction, and a decreasing one on the electrostatic component, has not been excluded.

Discussion

The non-lineal binding isotherms (Fig. 1) obtained for the TC-RSA interaction may be interpreted in terms of the heterogeneity of binding sites, or by the existence of an electrostatic interaction of negative cooperativity leading to this kind of curvature. To determine the origin of these non-linear binding curves, the binding values have been calculated taking into account the electrostatic effect as proposed by Karush and Sonenberg (1949). The non-lineal behaviour of the binding curves can not, however, be modified in this way, and heterogeity of the binding sites, also reported for other similar interactions (Goto et al. 1977) remains a viable explanation for the observed shape of the binding

isotherms. The binding was described in terms of two classes of binding sites. The primary class of sites showed value of 1 for the number of binding and the secondary class, showed a value of 5. It has been demonstrated (Ray et al. 1966), that the binding of some alkyl detergents to albumin produces a change of the adsorption spectrum of the protein, and this phenomenon has been related to conformational changes in the macromolecule. Since TC does not produce any change in the adsorption spectrum of RSA, it may be supposed that this ligand does not induce unfolding of this protein. In this sense there is a clear difference between the action of TC and the action of dodecyl sulfate, a strong denaturant of protein. An explanation of this difference could take into account the fact that dodecyl sulfate has a strong hydrophobicity, exhibiting strong hydrophobic binding to protein molecules, while the hydrophobicity and binding of TC are rather weak (Roda et al. 1983).

When binding was studied at increasing temperatures, the decrease observed in the binding constant was considered a characteristic for an exothermic reaction. This behaviour has been reported for many protein-ligand interactions of electrostatic nature (Goto et al. 1978), which is consistent with the anionic nature of TC, given by the taurine group (SO_3^-). The three highly polar hydroxyl groups could also be involved in this kind of process.

Notwhithstanding the former considerations, the molecular disorder factor, ($\Delta S°$), was positive for the primary site. A positive entropy change associated with many interactions involving proteins has been atributed to the unfolding of the protein molecule. On the other hand, it was found that binding diminished when chloride anion concentration was rised at a constant ionic strength. This competitive action of the chloride anion, which has been reported for other anion-albumin interaction (Coassulo et al. 1978), may be considered as an argument in favour of the electrostatic nature of this binding.

The incorporation of butanol to the medium, which decrease surface tension, also resulted in a decrease in binding. This observation is related to interactions where van der Waal's forces play an important role (Smeenk et al. 1983). Futhermore the effect of pH variation on binding was found within the pH range where the $N \leftrightharpoons B$ transition of albumin takes place. The B configuration (pH 8.5) exhibited a greater capacity for binding, which was interpreted as due to the exposure in this conformation of charged groups normally inaccessible to the ligand in the N form (Zurawski 1974).

The data obtained in the present experiments indicate therefore the existence of at least two froms of interaction between TC and RSA.

The characteristics of binding under different experimental conditions suggest that both coulombic (electrostatic) and van der Waal's forces are involved in the processes, particularly for the primary binding site (Lamers 1980).

The present results indicate that the affinity for RSA in higher than that

reported by Rudman and Kendall (1975). This fact emphasizes the role of RSA in the hepatic uptake of bile salts, since under physiological conditions most of the circulating TC would be bound to the albumin molecule, concentrations in the free fraction being almost negligible.

Further studies are necessary to achieve a better characterization of the present interactions and to compare the behaviour of TC with that of other bile salts.

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