

Effect of Bile Salts on Proton Binding to Human Serum Albumin

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The binding of small molecules to proteins appears as the starting step in many processes of biological importance. This fact gives special interest to the study of the nature of such interaction. This is the case with the binding of hydrophobic anions (such as those of the bile salts) to serum albumin, an interaction which plays an important role in the transport of bile salts in circulation and their uptake by hepatocytes. Early studies of the complex of serum albumin with this kind of ligands showed that hydrophobic bonds, as well as electrostatic ones, are involved in the process (Picó et al. 1986). Since frequently prototropic sites of the protein are involved in the binding of small ions and the binding itself may be affected by the total protein charge (Janssen et al. 1981), it is logical to relate ion binding to the acid-base equilibrium of the protein. This may be done using the theory of multiple equilibria, which is suitable for the interpretation of both processes. The aim of this study is the identification of the proton groups involved in the binding process of the different ionic ligands. We describe the modification induced in the proton-human serum albumin equilibrium by the binding of bile salts to the macromolecule. This approach was used in an attempt to elucidate the nature of albumin-bile salts interactions.

Sodium salt of cholate (C), deoxycholate (DC) and dehydrocholate (DHC) (Sigma) were used without further purification. Human serum albumin (HSA) (Oesterreichisches Institut fuer Haemoderivate) was deionized through ionic exchange columns until constant conductance was obtained. Sodium hydroxide solution was prepared carbonate free. All the titrations were carried out under nitrogen atmosphere at constant temperature (20°C) and constant ionic strength (0.02). Sample dilution ionization of the bile salts were corrected according to Janssen et al. (1981). Albumin concentration was determined by adsorbance at 280 nm.

Titration of HSA and the bile salts-HSA complexes were carried out according to Janssen et al. (1981). The pH of the solution in the titration vessel was measured by means of a digital pH meter with combined glass electrode.

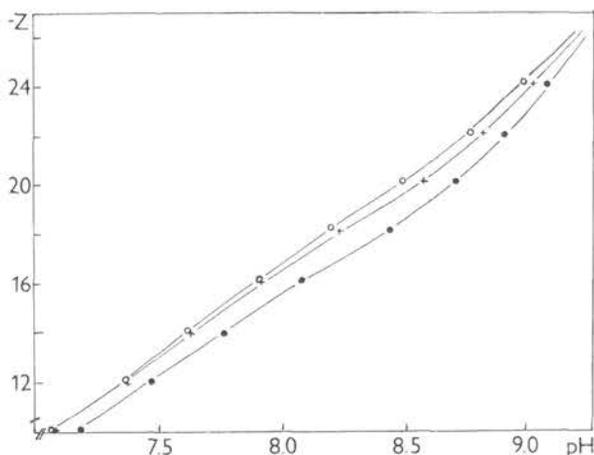


Fig. 1. Titration curves of human serum albumin in the absence (\times) and presence of bile salts: (\bullet) DC, (\circ) DHC. Total concentration of HSA $180 \times 10^{-6} \text{ mol.l}^{-1}$, that of NaCl $22 \times 10^{-3} \text{ mol.l}^{-1}$. Temperature 20°C . DC/HSA ratio 0.95, DHC/HSA ratio 1.0.

NaOH ($10 \times 10^{-6} \text{ l}$, 0.1 mol.l^{-1}) was added using a peristaltic pump (LKB) and the pH was measured after each addition, until stable pH values were reached. For the determination of the pK values for the bile acids, $2 \times 10^{-3} \text{ l}$ of the bile salts solution (about $2 \times 10^{-3} \text{ mol.l}^{-1}$) were made basic (pH = 11) with NaOH. This solution was then titrated with 0.1 mol.l^{-1} HCl as described above. Acid-base titration curves of a protein in the absence and presence of a ligand may be used to reveal pK shifts of certain groups of the macromolecule, caused by the binding of the ligand (Harmsen et al. 1971). If Z is the charge of the protein due to an isoionic solution of the protein only to the binding of proton, its value upon the addition of b equivalents of NaOH can be calculated as:

$$Z = -b \quad (1)$$

In the presence of a ligand which can bind to the protein and does not bind protons in the given pH range equation (1) also allows to calculate changes in protein charge during acid-base titration. The difference between the pH values at the same Z , (ΔpH) in the presence (pH) and absence of ligand (pH^0),

$$\Delta\text{pH} = \text{pH} - \text{pH}^0 \quad (2)$$

List of abbreviations used in this work: C—cholate, DC—deoxycholate, DHC—dehydrocholate, HSA—human serum albumin, volume of the titrated solution: $2 \times 10^{-3} \text{ l}$

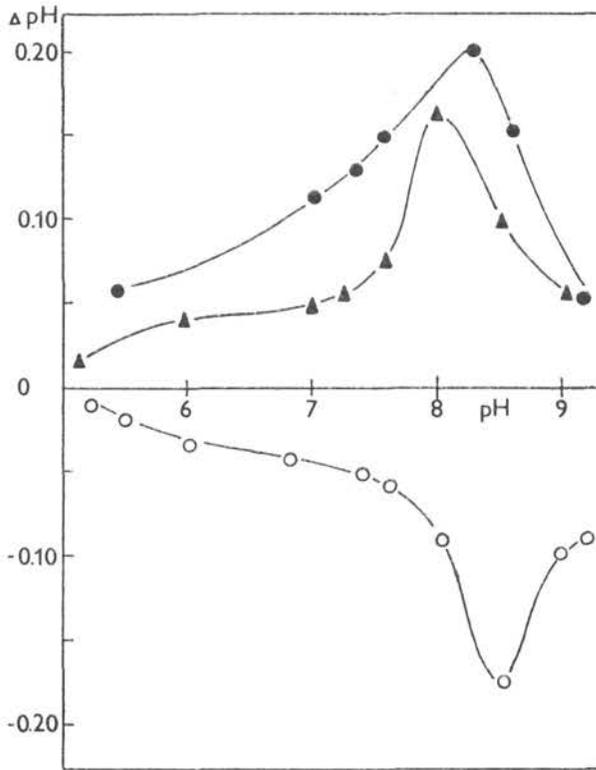


Fig. 2. Differential titration curves of HSA in presence of: (▲) C, (●) DC, (○) DHC. For details see legend to Fig. 1 and text.

will be due to a shift in the pK values of groups ionization of which determines the charge of the protein molecule (equation (2)).

Protein titration curves were obtained at constant ligand to protein ratio, a condition which does not imply a constant fraction of occupied sites for the different ligand used. Nevertheless, since the ligand/protein ratio was 1, we can assume that almost only primary sites were filled by the ligands, and the fraction of occupied secondary sites was negligible (Nerli et al. 1987). Our results, therefore are valid mainly for the binding of bile salts to HSA at the primary sites of the protein. The titration curves for HSA in the presence of DC and DHC, plotted as Z values vs. pH (Fig. 1) clearly different from the curves obtained in the absence of any ligand. DC caused the titration curves to shift to the right, while DHC produced a left shift.

To obtain further information about the influence of bile salts on the acid-base equilibrium of HSA, we replotted the data from Figure 1 according to equation (2) including the case of C. (not shown on Fig. 1) (Fig. 2). Positive ΔpH values were found for DC and C, a fact which implies that, at the same total charge, the ligand-protein complex has more bound protons than the free protein. Furthermore, the values of pH obtained showed a maximum of approxed 8. This can be interpreted as indicating that the ionized groups with pK values in this pH range (imidazol in this case) are involved in the binding process (Janssen and Wilgenburg 1978). On the other hand, the effect of DHC on the acid-base equilibrium of HSA differed those of C and DC: DHC produced negative ΔpH values and the free protein must have more protons bound than the ligand-protein complex. These results emphasize the important role electrostatic forces play in the binding of the ligands studied to HSA. The interaction seems to be driven mainly by electrostatic attraction between amine residues of the imidazole groups of the protein and hydroxyl, oxo or carboxyl of the bile salts molecules. It has been demonstrated (Picó et al. 1986) that the affinities of HSA for the bile salts increase in the order: C < DHC < DC < . Cholate has a very low affinity for HSA, while DC and DHC have relatively high affinities for the protein. Figure 2 shows that there is a close relation between the affinity constant of the bile salts and the total area under the curves. This observation also agrees with the participation of prototropic groups of HSA in the binding of bile salts. It is obvious that the presence of hydroxyl or oxo groups as substituent at C12 of the steroid ring plays a role in the binding of bile salts to HSA. These groups may contribute to the formation of complexes with a non protonated amine groups of the imidazol residues. On the other hand, when hydroxyl groups are present as substituents at C7 and C12 of the steroid ring, they may form complexes with the protonated amine groups, producing an increase in the pK values. A hydroxyl substituent at C7 produces a significant decrease in the affinity of C to HSA. This may be related to a steric hindrance in the binding sites. In summary, it seems that the acid-base titration method employed in our work is well suited to detect regularities in change in pK values of HSA amine groups produced by the action of congeneric ligands as bile salts, bound to the macromolecule; using this method the contribution of ionic forces to the total binding energy can be studied.

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Final version accepted March 8, 1989