Short communication

Bile Salts Binding-Induced Alterations in Ultraviolet Absorption Spectrum of Bovine Serum Albumin

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Generally, bile salts are hydroxy derivatives of β-5 cholanic acid. They are produced by the liver either by synthesis or by degradation of cholesterol. In plasma, bile salts are transported bound to serum albumin (Burke et al. 1971). The interaction between bile salts and serum albumin is responsible for the bile salts level and hepatic clearance. Previous studies carried out in our laboratory (Picó et al. 1987) showed two kinds of forces to be involved in the formation of the bile salts-albumin complex: electrostatic, between the amino groups of the protein and substituents of polar groups in the steroid ring of the bile salt molecule (hydroxyl- or oxo-groups), and hydrophobic, between the apolar groups of the bile salt steroid ring and the protein (Nerli et al. 1987).

The aim of the present work was to examine, by ultraviolet difference absorption spectrophotometry, the complex formation of hydroxy bile salts with bovine serum albumin to get insight into the molecular mechanism of this protein-bile salts interaction. The effects of hydroxy-(C), dihydroxy-(DC) and monohydroxy-(LC) bile salts (BS) on the ultraviolet absorption spectrum of bovine serum albumin (BSA), were tested.

Bovine serum albumin fatty acid free ($< 0.005\%$) and the following bile acids: cholic (C), deoxycholic (DC) and lithocholic (LC) were purchased from Sigma Chem. Co (USA) and used without further purification. Bile salts solutions (BS) were prepared from the free acids, LC was dissolved in methanol in view of the low solubility of this BS in aqueous media. All BS and BSA solution were prepared freshly before each experiment. The concentration of the BSA solution was calculated by absorbance at 280 nm.

All experiments were carried out in a phosphate buffer 0.02 $mol.l^{-1}$, pH 7.4 at 20 °C. All the other reagents used were the analytical quality.

BS titrations were performed by recording the basic line of BSA vs. BSA upon adding aliquots of the selected BS to the sample cuvette and an equal volume into the reference cuvette. Values of $\Delta\epsilon$ were calculated as the absorbance at max.

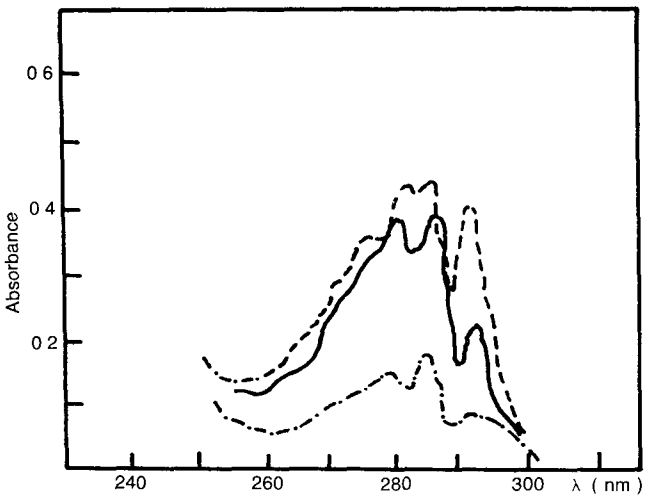


Figure 1. Ultraviolet difference spectra of BSA-BS. BSA concentration: 40×10^{-6} mol.l^{-1}. Medium: phosphate buffer 0.02 mol.l^{-1}. pH 7.4. Bile salt-protein ratio:1; C:— · —·, DC:—, LC:— — —.

following each addition of a BS aliquot, divided by the analytical concentration of BSA. Titration curves were prepared as a plot of $\Delta\epsilon$ vs. the accumulated BS concentration. BS-BSA binding parameters were calculated from the Scatchard plots using a non-linear computer program. All difference UV-BSA spectra were determined by a Perkin Elmer Lambda 5 spectrophotometer and the spectrophotometric titrations with a Shimadzhu 140–02 double beam spectrophotometer.

Table 1 shows the positions of the hydroxyl groups on the steroid ring of the BS tested. The addition of BS to BSA produced a modification in the UV spectrum of the protein (Fig. 1). All BS tested produced a positive change in the UV difference spectrum of the protein within a range of 270–300 nm. This change is only due to the effect of BS on the absorption by the protein as in the spectral range between 240 and 400 nm, BS does not absorb light. The UV difference spectra peaks were between 280 and 290 nm for all BS tested (see Fig. 1).

When the $\Delta\epsilon$ values were plotted against the total BS concentration, a curve was obtained reaching a plateau. This suggests that BS-BSA binding reaches saturation (Fig. 2).

According to Huang and Gabay (1974), $\Delta\epsilon$ may be considered as a measure of BS perturbation of the BSA molecule. To compare perturbation effects of the different BS, values of $\Delta\epsilon_{max}$ were calculated from the plateau of the $\Delta\epsilon$ vs. BS concentration curve. Table 2 gives the $\Delta\epsilon$ values obtained at the protein-ligand

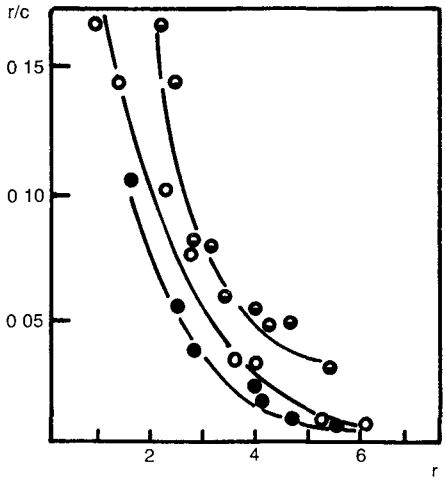


Figure 2. Plots of $\Delta\epsilon$ vs. BS total concentration: (•) cholate, (o) deoxycholate, (•) litocholate. Medium phosphate buffer 0.02 mol.l^{-1}, pH 7.4. BSA concentration: 40 × 10^{-6}mol.l^{-1}.

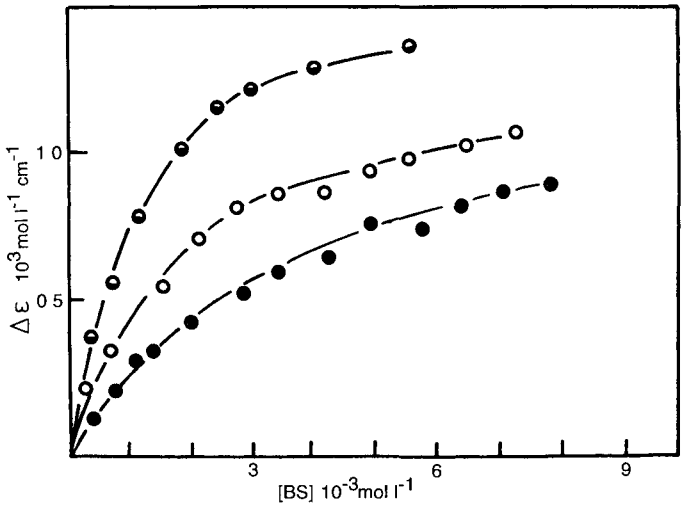


Figure 3. Scatchard plots for the binding of BS to BSA (Data taken from Fig. 2), (•)C, (o)DC and (•)LC.

saturation condition, and a sequence of LC>DC>C was obtained. This agrees well with the hydrophobic nature of BS molecules increasing in the same sequence.

Table 1.

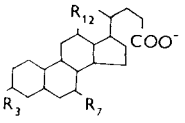
BS General formula	BS type	Position of the hydroxyl groups		
		R3	R7	R12
	C	OH	OH	OH
	DC	OH		OH
	LC	OH		

Table 2.

BS	$\Delta\epsilon$(280 nm) ($mol^{-1}.l.cm^{-1}$)	n_1	K_1 ($10^4 mol^{-1}.l$)	n_2	K_2 ($10^4 mol^{-1}.l$)
C	1.200	3.3	6.4	11	0.073
DC	1.700	2.6	6.7	9	0.099
LC	2.100	1.8	120.0	111	0.025

Scatchard plots (Fig. 3) were constructed from the same data as illustrated in Fig. 2. In all cases, non-linear plots were obtained suggesting a heterogeneity of the protein binding sites. The values of n and K were calculated from the Scatchard plots based on the two binding sites model (Table 2).

We have been the firsts to report that binding of hydroxy BS to BSA induces changes in UV spectrum. The observation might be of particular significance if it provided information about the nature of the binding sites. The induction of spectrum changes is indicative of perturbations of aromatic groups within the protein. The changes in $\Delta\epsilon$ are quite large for certain BS, and show a saturation effect with respect to BS concentration. Furthermore, this saturation is observed at relatively low BS concentrations, indicating a high binding affinity. The observations strongly suggested the presence of specific BS binding sites on the protein. It may therefore propose that the changes in the UV spectrum of the BS-BSA complex are due to direct interaction of BS with the tryptophanyl groups at the binding sites of the protein (Polet and Steinhardt 1968).

The interaction may involve a general effect on the protein conformation due to changes in the hydrophobicity of the BS binding sites. In the UV absorption region, the perturbation induced changes of the protein spectrum are dominated by spectral contributions of the tyrosyl and tryptophanyl residues (Harris W., 1985). When albumin is placed into a perturbing medium such as dimethyl sulfoxide, a negative difference spectrum is obtained at 280–295 nm corresponding to the tyrosine and tryptophan maxima. These changes are the result of the transfer of these groups from the hydrophobic environment burried within the protein to the aqueous medium. Similar results have been reported by Reynolds et al. (1968) for the binding of alkyl sulphates to albumin. These authors demonstrated that the perturbation effect in the UV region has a negative value with a maximum between

280–300 nm due to the proximity of the hydrocarbon tail of the alkyl groups to the aromatic groups of the protein.

On the other hand, Polet and Steinhardt (1968) reported a negative difference absorption spectrum between 280–300 nm for the alkyl sulphonate binding to albumin. A further analysis of these results by the same authors showed that the peak at 277 nm is due to the tyrosyl residues, the peak at 293 nm to the tryptophanyl residues, and that at 287 nm to additive effects of both residues. The negative change in the UV difference spectrum was associated with an increase in the specific viscosity and specific rotation of the BSA-alkyl sulphate complexes solution upon increasing the concentrations of the ligands in the solution. A comparison of the difference spectra of BSA-BS complexes to the solvent induced perturbation BSA spectrum and the BSA-alkyl sulphate difference spectrum shows similarities with respect to the peak at which maximum changes occur, indicating that the mechanisms underlying both phenomena are similar. The opposite direction of the absorption change produced by BS suggests an increase in the stability of the protein due to a conformational change produced by BS binding; the binding results in the hydrophobic groups of the protein being transferred into a hydrophobic environment.

It should be considered that BS increases the stability of albumin by increasing the hydrophobic nature of the binding sites through expelling solvent molecules from the proximity of the binding sites.

The increased intensity of the BSA difference spectrum induced by LC is consistent with the major hydrophobic area of this ligand.

According to the K values, the BSA affinity is reduced by the introduction of polar groups into the steroid ring, and this is associated with a lower stability of BSA-C as compared to BSA-LC. The results reported herein show that BSA has two types of binding sites for hydroxy BS: one with a high affinity ($K = 10^4$ mol^{-1}.l), and another with low affinity ($K = 10^4 - 10^5$ mol^{-1}.l) (nonespecific binding). Our results are in agreement with those reported by Scagnolari et al. (1984) who used dialysis equilibrium, and Nerli et al. (1987) similar results have also been obtained in studying the binding of other steroid derivatives to albumin (Attallah and Lata 1968).

Based on the present results it may be stated that difference spectrophotometry provides complementary information about the mechanism of BS-albumin complex formation and may be employed additionally to the widely used equilibrium dialysis or gel filtration techniques.

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