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Influence of pH and ionic strengths on bilirubin binding to sheep serum albumin

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ABSTRACT

Despite great degree of similarity in the structure and conformation of different serum albumins, they often show marked difference in the ligand binding properties. These differences and similarities would be useful in understanding the molecular basis of ligand binding to serum albumins. In the present communication binding of bilirubin with sheep and bovine serum albumin have been studied by visible difference spectroscopy as a function of pH and ionic strength. The results suggest that the structure of bilirubin binding site in sheep serum albumin is similar to that in bovine serum albumin and that complex between sheep serum albumin and bilirubin is stabilized by electrostatic interactions.

1. Introduction

Binding of different ligands to serum albumin in the blood is believed to be an important primary step in the solubilization,[1] detoxification[2] or distribution of these ligands among different tissues.[3] Serum albumin obtained from different animal species has sequence homology and appear to be remarkably similar in their structural organization.[4] Yet they often show marked differences in their ligand binding properties[5] indicating that the structure and / or conformation of ligand binding sites in such cases may be different. Thus comparison of ligand binding properties of serum albumins of known sequences from different animal species would prove useful in understanding the molecular basis of ligand (or drug) interaction with serum albumin. In the present communication bilirubin binding properties of sheep, bovine and human are described.

2. Materials and Methods

Sephacryl S – 300 and Bilirubin were purchased from Sigma Chemical Co., USA. Bovine serum albumin was a product of Sisco Chemicals India. All other reagents used in this study were of analytical grade.

2.1. Optical Measurements

A Shimadzu double beam spectrophotometer, model UV-150 - 02, was used for the measurements of light absorption in the visible as well as in the UV range. Fluorescence measurements were performed on a Shimadzu spectrofluorometer, model RF -540 equipped with a data recorder, model DR – 03.

2.2. Determination of protein concentration

Protein concentration was routinely determined by the method of Lowry et al.[6] using bovine serum albumin as standard. The concentration of BSA standard was determined by meas uring absorbance at 279 nm and using an specific extinction coefficient of 6.67.[7]

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2.3. Isolation of sheep serum albumin

SSA was prepared from blood obtained from slaughter house by the salt fractionation method developed in this lab.[8] The purity of isolated SSA was checked by size exclusion chromatography on Sephacryl S – 300 and by polyacrylamide gel electrophoresis.

2.4. Interaction of bilirubin with albumins

Bilirubin solution was prepared fresh by dissolving 6 mg of bilirubin crystals in a 1 ml of 1M sodium carbonate solution containing 1 Mm EDTA and the volume was made upto 10 ml with Tris HCl buffer, Ph 8.0, I = 0.15 . The stock solution was further diluted as desired.

3. Results

The gel filteration and electrophoretic behavior of commercial BSA and isolat ed SSA are shown in Figure 1and Table 1. The apparent heterogeneity in commercial BSA is due to the well established presence of dimmers and other oligomer.[8] The isolated SSA appears free from dimmer and oligomer but contains a small amount of impurity in the form of other proteins. Together these impurities make about 5% of the total protein and were assumed inconsequential in bilirubin binding studies. The bovine and sheep serum albumins have nearly same elution volume on S-300 column indicating that they have identical Stokes' radii.

The visible difference absorption spectra of bilirubin albumin complexes at molar ratios of 0.5, 1.0 and 2.0 are shown inFigure 2 and Table 2. The difference absorption peak in both BSA and SSA comes out at 480 nm. The effect of ionic strength and pH on the binding of bilirubin to the two albumins was investigated at a molar ratio of 1.0 by monitoring the difference spectral change at 480 nm. The results are shown in Figure 3 and Table 3. It can be seen that in both the cases increase in ionic strength from 0.02 to 1.0 causes considerable decrease in the difference spectral change at 480 nm. Thus, clearly as in BSA (2), bilirubin to SSA is stabilized by electrostatic interactions. It has already been shown in earlier results that one or two lysine residues at the bilirubin binding site of HSA and BSA forms salt bridge with the carboxylate group (s) of bilirubin.[9,10] Thus the binding mechanism of SSA to bilirubin seems to be similar to that of BSA and HSA. This conclusion is further supported from the pH dependent bilirubin binding to SSA and BSA (see Figure 3) which is virtually identical. The sharp decrease in bilirubin binding above pH 11.5 is presumably due to alkali induced Denaturation in serum albumins.

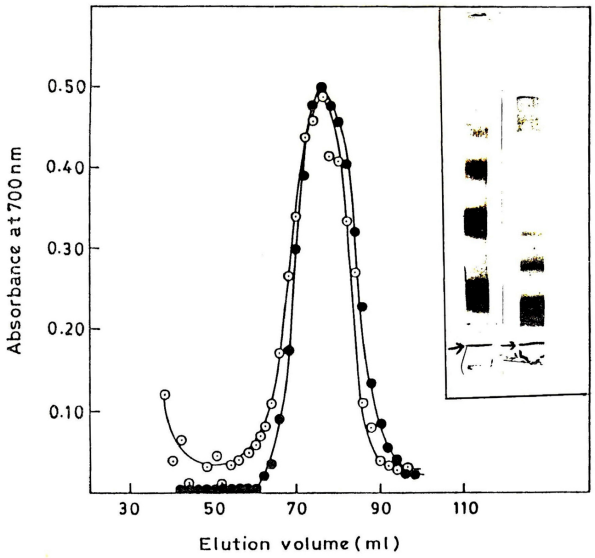

Fig. 1: Elution profiles of BSA (0-0) and SSA (● - ●) on Sephacryl S – 300 column equilibrated with 0.06 M sodium phosphate buffer, pH 7.0. The inset shows the PAGE pattern of BSA and SSA obtained in 0.02 M Tris – glysine buffer, pH 8.2

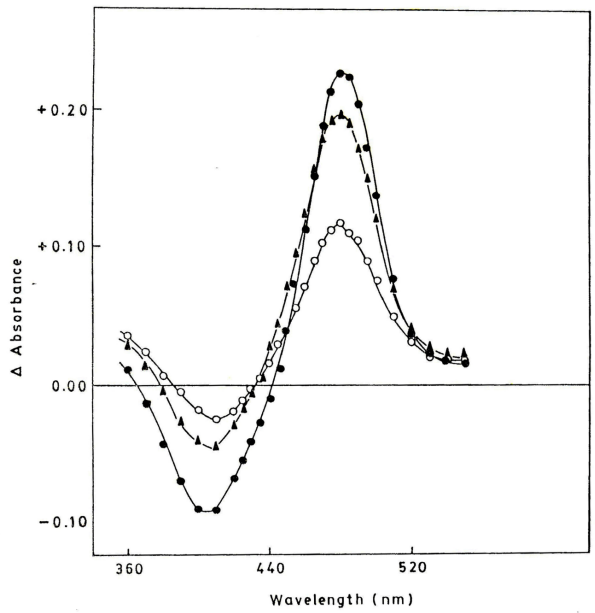

Fig. 2: Visible difference absorption spectra of bilirubin – SSA complex obtained in 0.1 M Tris – HCl buffer ph, 8.0, I 0.15 at different molar ratios of bilirubin over albumin as 0.5 (o), 1.0 (Δ) and 2.0 (●). The protein concentration was 8.0 u M.

Table 1: Gel filteration, electrophoresis and fluorescence data for serum albumins

Albumin preparation	Ve / Vo	Rm	Excitation maximum (nm)	Emission maximum (nm)
Bovine serum albumin	1.81	0.870	282	336
Human serum albumin	1.83	0.862	282	330
Sheep serum albumin	1.83	0.779	282	338

Table 2: Comparative bilirubin binding data of serum albumins at different molar ratios (0.5, 1.0 and 2.0).

Molar ratio (B /A)	ΔOD 1% at 480nm		
	BSA	HSA	SSA
0.5	1.431	0.998	2.20
1.0	2.109	1.996	3.69
2.0	3.370	4.105	4.25

Table 3: Comparative bilirubin binding data of serum albumins at different ionic strengths (0.02 to 1.0).

Serum albumin	Ionic strength	ΔOD 1% at 480 nm
Bovine serum albumin	0.02	3.516
	0.05	3.256
	0.20	3.004
	0.50	2.367
	1.00	1.913
Human serum albumin	0.02	1.748
	0.05	1.642
	0.20	1.613
	0.50	1.661
	1.00	1.613
Sheep serum albumin	0.02	3.845
	0.05	3.942
	0.20	3.468
	0.50	3.314
	1.00	2.927

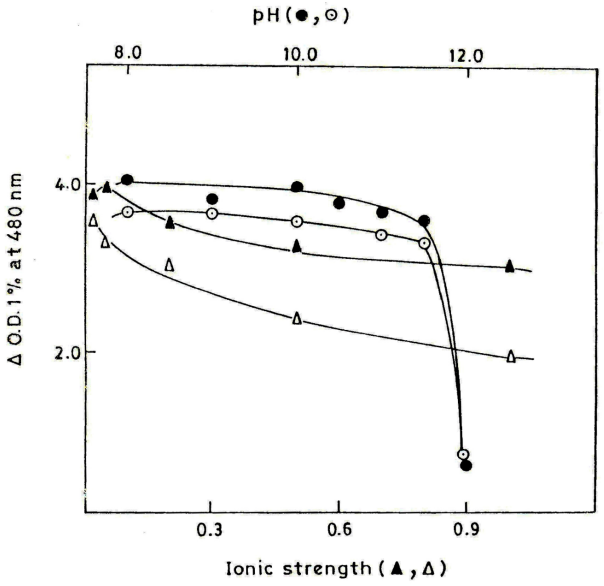

Fig. 3: Plot of ΔOD 1 % 480 nm of bilirubin – albumin complexes as a function of ionic strength and pH. Filled and unfilled symbols represent BSA and SSA respectively.

4. Discussion

From structural studies of serum albumins from different sources it seems that they have high degree of sequence homology[4] and have very similar overall conformation. Yet in several earlier studies[11] it has been found that different serum albumins may differ markedly in ligand binding properties.[12–14] The differences or similarity in the ligand binding properties of serum albumins would be helpful in understanding the structure and conformation of the ligand binding sites in albumin.[15] For instance from the present study it is strongly suggested that not only the overall conformation but the structure and geometry of bilirubin binding site in SSA is very similar to that in BSA and HSA.

5. Abbreviations

BSA – bovine serum albumin, **SSA** – sheep serum albumin, **HSA** – human serum albumin, **Tris** – Tris (hydroxymethyl) aminomethane, **UV** – ultraviolet**, ΔOD 1% at 480 nm** – change in absorption at 480 nm, **Ve/ Vo** – ratio of elution volume to void volume, **Rm** – Relative mobility.

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7. Source of funding

None

8. Conflict of interest

None.

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