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Original Research Article Binding study of different drugs with serum albumins

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ABSTRACT

Objective: Besides bilirubin and fatty acids are more important among the physiological ligands transported by albumin, drugs constitute the major class of exogenous ligands transported by albumin. Considering the total number of ligands bound to albumin, it is inconceivable to think of the same number of ligand binding sites on albumin molecule, so it seems that same binding sites are shared by several compounds. Thus it is possible that some ligands may be displaced from albumin by other ligands. Various drugs are well known displacer of bilirubin from albumin.

Materials and Methods: In the present communication the binding of three drugs namely, Indomethacin, chlorpromazine and oxyphenbutazone to serum albumins (BSA and SSA) under different conditions of pH and ionic strength have been studied by fluorescence quenching technique in order to ascertain the role of various non – covalent interactions.

Results and Discussion: The results suggest that in case of indomethacin, a decrease in binding was observed on increasing the ionic strength, indicating the involvement of electrostatic interactions whereas in case of chlorpromazine and oxyphenbutazone, significant increase in binding was noticed on increasing the ionic strength was suggestive of the involvement of hydrophobic interactions. The extent of binding of these drugs was more pronounced in BSA compared to SSA.

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1. Introduction

Albumin molecule is synthesized as single polypeptide chain but usually shows heterogeneity during its life time which may also occur because of protein – protein association, ligand binding $^{1-5}$ and conformational isomerization. Albumin binds reversibly to an incredible variety of ligands and thus behaves as unique universal carrier^{6–12} having multiple and adaptable binding sites $^{13-15}$ Various ligands such as drugs and fatty acids are reported to displace bilirubin from albumin, leading to increase plasma bilirubin level. Drugs which displaced bilirubin from albumin are believed to act either in a competitive or noncompetitive manner so characterization of drug binding sites is therefore essential in order to understand the mode of their binding and displacing action.^{6,8,9,16–20} In the present communication interaction of drugs namely indomethacin, chlorpromazine and oxyphenbutazone with serum albumins (BSA and SSA) under different conditions of pH and ionic strength has been studied.

2. Materials and Methods

Sephacryl S – 300 and indomethacin, chlorpromazine and oxyphenbutazone were purchased from Sigma Chemical Co., USA. Bovine serum albumin was a product of Sisco Chemicals India and sheep serum albumin (SSA) was prepared according to the method of Tayyab and Qasim.²¹ All other reagents used in this study were of analytical grade.

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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.²² using bovine serum albumin as standard. The concentration of BSA standard was determined by measuring absorbance at 279 nm and using a specific extinction coefficient of 6.67.²³

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. The purity of isolated SSA was checked by size exclusion chromatography on Sephacryl S - 300 and by polyacrylamide gel electrophoresis.

2.4. Fluorescence quench titration method

Fluorescence emission and excitation spectra were obtained in 0.1 M tris HCl buffer, 8.0, I=0.10 at 25 0C. An excitation wavelength of 282 nm was fixed for recording the emission spectra of both albumins preparations (BSA and SSA). The excitation spectra were obtained by fixing the emission wavelengths at 336 nm for BSA and 338nm for SSA.

2.5. Drug- albumin interaction

Binding of three drugs namely Indomethacin, Chlorpromazine and Oxyphenbutazone to two different serum albumins (BSA and SSA) was studied by fluorescence quenching method. The single titration was performed in sodium phosphate buffer, pH 7.4 at 25 0C. To a fixed amount of protein solution (5 Um) taken in a series of tubes, desired volume of stock drug solution was added to obtain different molar ratios of drug to albumin in the range of 0.0 to 4.0. Fluorescence emission spectra were recorded in the wavelength range of 300 - 400 nm by exciting the protein solution at 280 nm. These binding studies were performed at varying ionic strengths.

3. Results

3.1. Drug- albumin interaction

Binding of three drugs namely Indomethacin, Chlorpromazine and Oxyphenbutazone to two different serum albumins (BSA and SSA) was studied by fluorescence quenching technique as the addition of these drugs caused quenching in fluorescence spectra of serum proteins. The fluorescence quench titration of different albumins namely BSA and SSA with these three drugs was performed at various ionic strengths of 0.02, 0.15, 0.50 and 1.0. Tables 1 and 2.

3.2. BSA -drug interaction

3.2.1. The interaction of indomethacin

The fluorescence emission spectra of BSA in absence and presence of increasing amounts of indomethacin (BSA/Indomethacin molar ratio of 0.2 to 4.0 at different ionic strengths of 0.02 to 1.0, pH, 7.4 sodium phosphate buffer. A significant increase in fluorescence quenching was observed on increasing the drug- albumin molar ratio at different ionic strengths but they differ in the magnitude of fluorescence quenching Table 1.

3.2.2. The interaction of Chlorpromazine

Like indomethacin, Chlorpromazine also caused significant quenching in the fluorescence emission spectra of BSA which increased on increasing the Chlorpromazine – BSA molar ratio. These data were transformed into percent quenching and binding results differ markedly on changing the ionic strengths Table 1.

The interaction of Oxyphenbutazone was also studied with BSA and data were transformed into percent quenching, Table 1.

3.3. SSA – drug interaction

Drug binding studies with SSA were performed in the similar manner as that of BSA using fluorescence quench titration method in sodium phosphate buffer, PH 7.4 and at different ionic strengths of 0.02, 0.15 and 1.0. Fluorescence quenching results of SSA obtained with Indomethacin, Chlorpromazine and Oxyphenbutazone and data were transformed in the form of percent quenching Tab 2.

4. Discussion

Serum albumins a universal bio carrier binds a large number of exogenous compounds including drugs in addition to physiologically important ligands such as bilirubin, fatty acids etc. as some of them share a common binding site on albumin. Considering this, the binding of three drugs namely indomethacin, chlorpromazine and oxyphenbutazone was studied using fluorescence quenching as all these drugs produced significant quenching in the emission spectra of albumins upon their addition. In order to study the role of electrostatic/hydrophobic interactions, the binding of these drugs to serum albumins (BSA and SSA) were studied at different ionic strengths from 0.02 to 1.0.

When the values of percent quenching (representative of binding) obtained with indomethacin at drug/albumin molar ratio of 1:1 were plotted against ionic strength, a

different ionic	strengths (0.02 tc	1.0).							
					Ionic Stre	ngths			
	MOIAF rotio	I	0.02	$\mathbf{I} = 0$).15	I = (0.50	I =	1.0
	rauo	Relative	%Quenching	Relative	% Quenching	Relative	% Quenching	Relative	% Quenching
		fluorescence		fluorescence		fluorescence		fluorescence	
Ind/BSA	0.0	100.00	0.0	100.00	0.0	100.00	0.0	100.00	0.0
	0.2	72.72	27.28	84.84	15.16	91.40	8.59	90.74	9.25
	0.6	39.33	60.61	43.93	56.06	56.25	43.75	69.21	30.79
	1.0	12.12	87.88	20.45	79.55	29.06	70.94	49.99	40.02
	2.0	90.6	90.91	15.15	84.85	28.13	71.87	49.21	50.78
	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	0.2	95.44	4.56	88.63	11.38	97.60	2.40	74.99	25.01
Chl/BSA	0.6	80.81	18.19	71.20	28.80	68.24	31.76	62.11	37.89
	1.0	72.11	27.89	52.11	47.88	47.61	52.39	36.36	63.64
	2.0	69.69	30.31	51.51	48.49	42.85	57.15	33.33	66.67
	0.0	100	0.0	100	0.0	100	0.0	100	0.0
	0.2	89.39	10.62	87.87	12.13	86.36	13.64	90.90	9.1
Oxy/BSA	0.6	68.17	31.83	60.60	39.40	59.09	40.91	59.09	40.91
	1.0	51.51	48.49	42.42	57.58	39.38	60.62	37.89	62.12
	2.0	51.20	48.79	39.39	60.61	36.35	63.64	36.32	63.68

Table 1: Comparative binding data of bovine serum albumin (BSA) to drugs (Indomethacin, Chlorpromazine and Oxyphenbutazone) by fluorescence quench titration at pH 7.4 at

Molar ratio Relati Ind/SSA 0.0 Relati 100.0 0.0 100.0 0.2 92.5 92.5 0.6 83.5 100.0 0.0 0.0 92.5 0.0 0.0 97.3 0.0 0.0 100.0 0.0 0.0 97.3 0.10 0.0 97.3 0.2 0.2 97.3 0.0 0.0 100.0 1.0 2.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.8			Ionic St	rengths			
Relati Ind/SSA 0.0 fluoresc 0.2 92.5 92.5 0.6 83.5 92.5 0.6 83.5 92.5 0.6 0.6 83.5 1.0 0.6 83.5 2.0 0.6 83.5 0.0 0.0 100.0 0.2 0.2 97.3 1.0 0.2 97.3 2.0 0.2 97.3 2.0 0.2 97.3 0.0 0.0 100.0 2.0 0.0 55.9 2.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9	I = 0.02	I = (.15	I=	0.50	I=	1.0
Ind/SSA 0.0 100.0 0.2 0.2 92.5 0.6 83.5 92.5 1.0 0.6 83.5 1.0 0.6 83.5 2.0 2.0 56.7 2.0 0.0 100.0 0.0 0.0 97.3 0.2 0.2 97.3 0.2 0.2 97.3 2.0 0.6 84.1 1.0 0.6 84.1 0.0 0.6 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 55.9 0.0 0.0 92.8	elative %Quenching	Relative	%Quenching	Relative	%Quenching	Relative	% Quenching
0.2 0.5 92.5 0.6 0.6 83.5 0.6 0.6 83.5 0.6 83.5 5.7 2.0 65.7 56.7 56.7 56.7 2.0 0.0 100.6 0.2 97.3 97.3 0.2 0.2 97.3 97.3 0.0 100.6 84.1 1.0 72.3 97.3 0.2 0.0 100.6 0.0 100.6 0.0 100.6 0.0 100.6 0.0 100.6 0.0 0.0 100.6 0.0 0.0 100.6 0.0 0.0 100.6 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	00.00 0.0	100.00	0.0	100.00	0.0	100.00	0.0
0.6 83.5: 1.0 65.5 2.0 56.7 2.0 56.7 0.0 100.6 0.2 97.3 97.3 97.3 97.3 2.0 72.3 2.0 55.9 97.3 92.8 92.8	92.54 7.47	94.57	5.43	92.50	7.50	91.35	8.65
1.0 65.5 2.0 56.7 2.0 56.7 0.0 100.0 0.2 97.3 97.3 97.3 97.3 97.3 97.3 2.0 72.3 2.0 55.9 92.8 92.8	83.58 16.42	83.76	16.23	83.75	16.25	85.79	14.20
2.0 56.7 0.0 100.0 0.2 97.3 0.2 97.3 72.3 97.3 1.0 72.3 2.0 55.9 0.0 100.0 0.2 92.8	55.55 34.45	66.19	33.80	71.87	28.12	72.83	27.16
0.0 100.0 0.2 97.3 97.3 97.3 0.6 84.1 1.0 72.3 2.0 55.9 55.9 0.0 100.0 1	56.72 43.29	56.74	43.26	53.11	46.87	59.87	40.13
0.2 97.3 Chl/SSA 0.6 84.1 1.0 72.3 2.0 559 0.0 100.0 0.2 92.8	0.00 0.00	100.00	0.0	100.00	0.0	100.00	0.0
ChVSSA 0.6 84.1 1.0 72.3 2.0 55.9 55.9 0.0 100.0 0.2 92.8	97.35 2.65	94.28	5.72	96.39	3.61	90.68	9.32
1.0 72.3 2.0 55.9 0.0 100.0 0.2 92.8	84.19 15.80	82.85	17.15	88.06	11.94	84.86	15.14
2.0 55.9 0.0 100.0 0.2 92.8	72.35 27.64	67.85	32.14	70.91	29.09	64.83	35.87
0.0 100.0 0.2 92.8	55.90 44.09	59.99	40.10	63.07	36.93	58.13	41.87
0.2 92.8	0.00 0.00	100.00	0.0	100.00	0.0	100.00	0.0
	92.85 7.15	91.90	8.09	93.75	6.25	92.90	7.10
Oxy/SSA 0.6 84.2	84.28 15.72	80.11	19.89	90.00	10.00	82.30	17.68
1.0 71.4	71.43 28.58	64.58	35.41	68.20	31.79	62.00	38.00
2.0 62.1	52.14 37.86	55.89	44.11	63.12	36.87	54.09	45.90



Fig. 1: Plot of percent quenching of indomethacin – albumin complex as a function of ionic strength (0-0) and $(\bullet - \bullet)$ represent SSA and BSA respectively



Fig. 2: Plot of percent quenching of chlorpromazine – albumin complex as a function of ionic strength (0-0) and $(\bullet - \bullet)$ represent SSA and BSA respectively



Fig. 3: Plot of percent quenching of oxyphenbutazone – albumin complex as a function of ionic strength (0-0) and $(\bullet - \bullet)$ represent SSA and BSA respectively

significant decrease in binding was observed on increasing the ionic strength in both BSA and SSA Figure 1, which was suggestive of involvement of electrostatic interactions in the binding process. Contrary to this when the percent quenching data obtained with two other drugs i.e. chlorpromazine and oxyphenbutazone were plotted against ionic strengths Figures 2 and 3, a significant increase in binding was noticed on increasing the ionic strength which was indicative of the importance of hydrophobic interactions in this binding. The results of indomethacin albumin interaction was also found to be similar to that of bilirubin - albumin interaction in terms of the involvement of electrostatic interactions which was found to be in good agreement with previous studies that indomethacin shares the same binding site on albumin where bilirubin binds.^{16,24-28} Based on the results of chlorpromazine albumin interaction obtained in this study and previous study of bilirubin displacing effect of chlorpromazine, it seems more probable that the binding sites for these ligands on albumin are different and the drug displaces bilirubin in a noncompetitive or allosteric manner. Binding results of oxyphenbutazone to serum albumins also suggested that the drug binding site is different from bilirubin binding site.

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6. Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Author biography

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