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Drug-Biomacromolecule Interaction IX

Effects of pH and Ionic Strength on the Binding of Sulfaethidole to Bovine Serum Albumin Using Circular Dichroism

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Abstract □ Binding of sulfaethidole to bovine serum albumin(BSA) was studied by circular dichroism. The effects of pH and ionic strength on the binding of sulfaethidole to BSA were investigated It was found that one primary binding site on the BSA was capable of inducing optical activity in the presence of sulfaethidole. Enhancement of the induced ellipticity of sulfaethidole upon addition to BSA was not much affected by the change of pH and ionic strength. Taking the effects of pH and ionic strength into consideration, it seems that the binding of sulfaethidole to BSA was not much affected by electrostatic and ionic interactions. Therefore, it might be assumed that the binding was mainly due to the hydrophobic interactions. Sulfaethidole seems to be a reasonable CD probe for the study of hydrophobic drug interactions.

Keywords □ Circular dichroism, Protein binding, Bovine serum albumin, Sulfaethidole, pH, Ionic strength.

Proteins are well known of optically active compounds. When optically inactive compounds bind to optically active compounds, new circular dichroism bands are induced in the absorption bands of optically inactive compounds.[1] The binding sites on the protein responsible for the Cotton effects are known to contain a hydrophobic region and a hydrophilic region involving a cationic center or a center capable of forming a hydrogen bond. Since the Cotton effects can give information about conformational change of protein when drug attaches to protein, circular dichroism method is applicable to the study of drug-protein interaction.

In this study, the effects of pH and ionic strength on the binding of sulfaethidole to BSA were investigated by CD.

EXPERIMENTAL METHODS

Materials

Sulfaethidole, N^1-(5-ethyl-1, 3, 4-thiadiazol-2-yl) sulfanilamide, was purchased from E. Merck, Darmstadt, Germany. Bovine serum albumin(BSA), Fraction V was purchased from Sigma Co . The concentration of albumin solutions was determined from the absorbance of the peak at 280 nm. The molar concentration was calculated on the basis of $E^{1\%}_{1cm}=6.67$. All other chemicals used were of analytical reagent grade. Solutions were prepared with double distilled water from glass.

Method

All CD spectra were obtained with JASCO Model J-20C spectropolarimeter. All solutions were prepared in sodium phosphate buffer at various pHs and ionic strengths at 25°C. The concentration of BSA was fixed at 1.45×10^{-5} M. Sulfaethidole to BSA ratio was varied. Titration was carried out manually with an

auto-pipette. All solutions were scanned in 10 mm cells from 200 to 300 nm.

Data Treatment

Enhancement of the induced ellipticity of sulfaethidole upon addition to BSA was used to calculate the binding parameters for sulfaethidole. The induced ellipticities (θ) at two observed peaks were plotted at different ratios of sulfaethidole to BSA (D/P), where the induced ellipticity is the ellipticity of the drug-BSA mixture minus the ellipticity of the BSA alone at the same wavelength and is expressed in degrees. These data were analyzed according to the method of Rosen.[2] After the concentrations of bound and free sulfaethidole were obtained by using the method of Rosen, the Scatchard equation was applied to determine the binding parameters of the BSA-sulfaethidole interaction.[3]

$$\frac{V}{A}=nKa-VKa$$

where V is the number of moles of bound sulfaethidole per mole of BSA, A is the concentration of free sulfaethidole, n is the number of binding sites on the BSA molecule and Ka is the association constant of the sulfaethidole to the BSA.

RESULTS AND DISCUSSION

Nature of the binding of sulfaethidole to BSA was investigated at different pHs and ionic strengths through 250~300 nm region associated with the aromatic amino acid residues of the protein.

Fig. 1 shows the optical activity of BSA in the absence and presence of sulfaethidole in phosphate buffer(pH=7.4, I=0.2) in the wavelength region of 250~300 nm. The UV spectrum of sulfaethidole in phosphate buffer

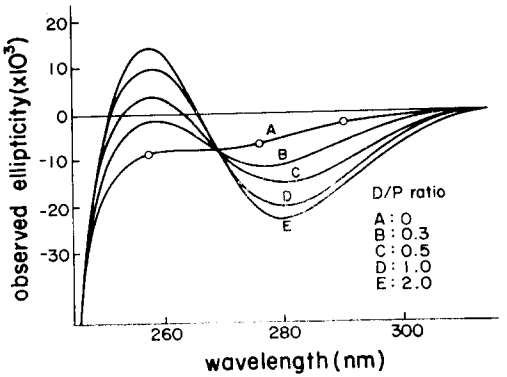

Fig. 1: CD spectrum of BSA alone(A) in pH 7.4 phosphate buffer (I=0.2) and CD spectra of BSA (B, C, D and E) in the presence of sulfaethidole. The concentration of BSA was 1.45×10^{-5}M; scale 5 mdeg/cm; 10mm cells.

results from at least two overlapping electronic excitations within tne sulfaethidole molecule. These electronic excitation transitions result in two induced CD peaks on binding to BSA, a positive peak at 257 nm and a negative peak at 280 nm. The two peaks in the induced CD curves probably result from excitations within both the sulfanilamide and the thiadiazole portions of the sulfaethidole molecule. For these investigations of induced optical activity, the concentration of BSA was fixed at 1.45×10^{-5} mole per liter and the molar ratios of sulfaethidole to BSA were varied from 0.3 to 4.

In Fig. 2, the induced ellipticities at both observed peaks versus D/P ratios were plotted at different pHs and ionic strengths. The curves in Fig. 2 were drawn by using polynomial curve fitting program in IBM computer. Drawing the tangent to the curve at zero drug concentration enables the appropriate intensive factor for the bound drug to be determined. This allows the determination of free and bound drug by the method of Rosen.[2] It is assumed that there is a proportionality between induced ellipticity and

the amount of bound drug. Such an assumption is valid only when all sites contribute equally to the ellipticity. However, this assumption is not satisfied when more than one class of binding site are observed, which contribute unequally to the ellipticity. If the binding constants of the primary and secondary sites are very different from each other and the ellipticities of secondary binding sites are small, then reasonable estimates of the primary binding constant can be obtained.

Fig. 3 shows the Scatchard plot for sulfaethidole at different pHs and ionic strengths. It can be suggested that only one primary binding site may be detected by CD, which provided no information about the secondary interactions. Binding constant and number of binding sites for sulfaethidole to BSA are identical at both wavelength 257 nm and 280 nm, respectively.

Conformational changes in the BSA itself and changes in UV absorption of sulfaethidole itself were investigated under the current experimental conditions. Conformational changes of the BSA at different pHs were not observed by CD technique at lower wavelngth than 250 nm. Also, the change of pH and ionic strength had no effect on the UV spectrum of sulfaethidole at lower wavelength.

CD spectra of sulfaethidole bound to BSA at different pHs and ionic strengths through wavelength 250~300 nm were slightly changed. However, binding constant and binding site for

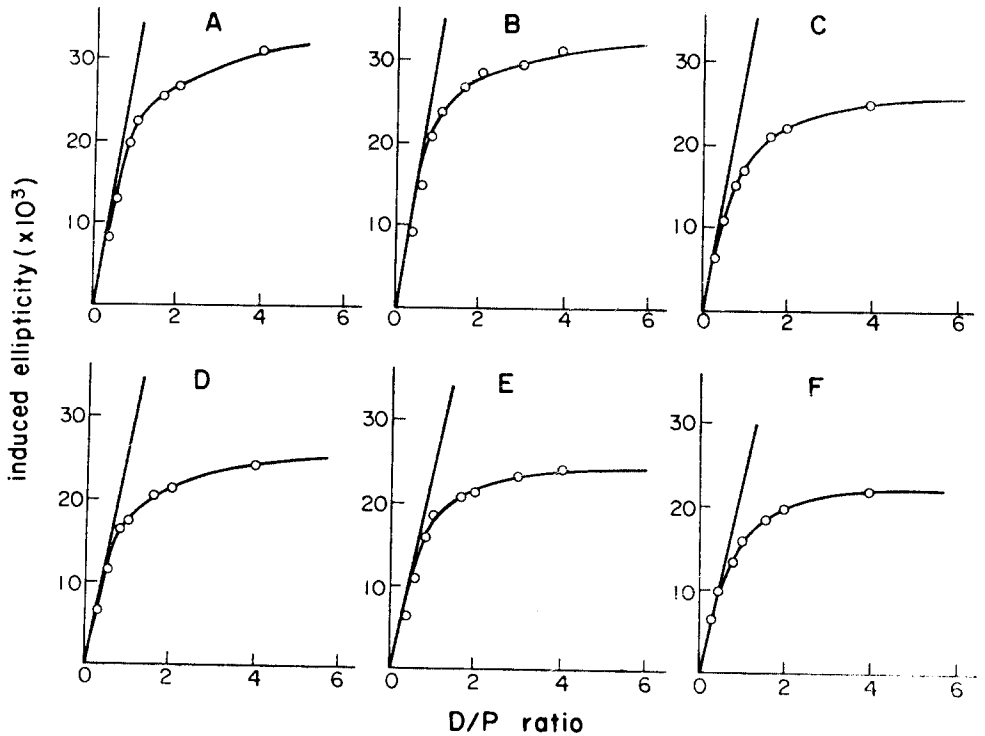

Fig. 2a: Plots of induced ellipticity versus D/P ratio at two absorption peaks and three different pHs.
A, (nm)=257, I=0.2, pH=6.0; D, (nm)=280, I=0.2, pH=6.0;
B, (nm)=257, I=0.2, pH=7.4; E, (nm)=280, I=0.2, pH=7.4;
C, (nm)=257, I=0.2, pH=8.5; F, (nm)=280, I=0.2, pH=8.5.

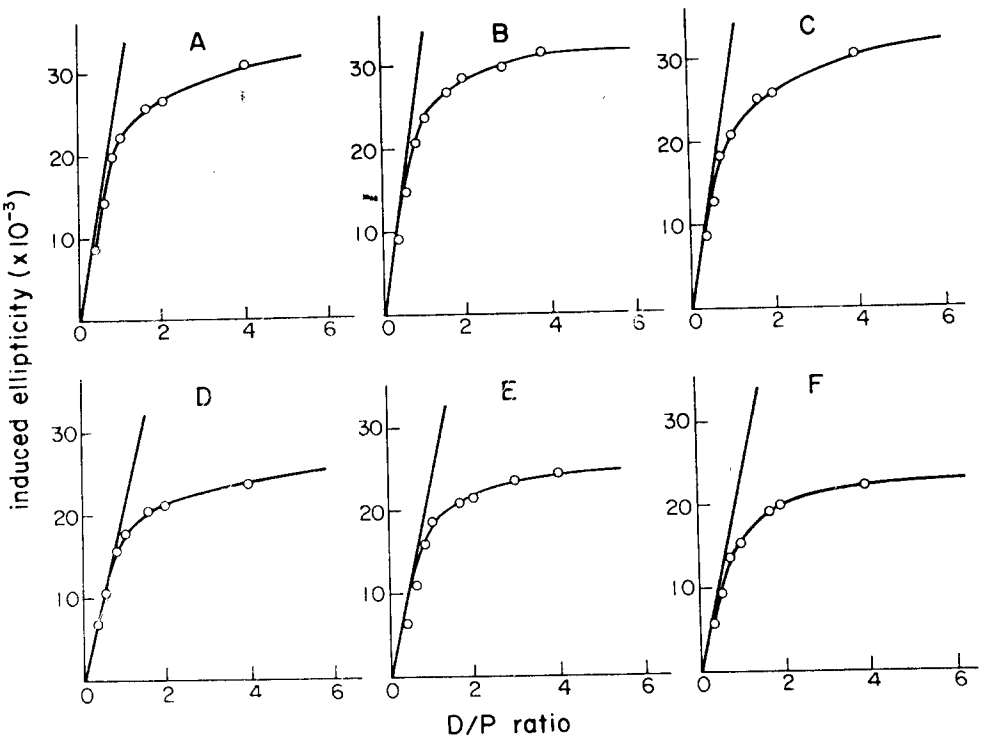

Fig. 2b: Plots of induced ellipticity versus D/P ratio at two absorption peaks and three different ionic strengths.

A, (nm)=257, pH=7.4, I=0.04; D, (nm)=280, pH=7.4, I=0.04;
B, (nm)=257, pH=7.4, I=0.2; E, (nm)=280, pH=7.4, I=0.2;
C, (nm)=257, pH=7.4, I=0.6; F, (nm)=280, pH=7.4, I=0.6.

Table I: Binding parameters of sulfaethiodole to BSA at different pHs and ionic strengths.

Wavelength	pH	I	Ka(M^{-1})	n
257 nm	6.0	0.2	5.6×10^5	1.05
	7.4	0.04	5.6×10^5	1.02
	7.4	0.2	5.4×10^5	1.03
	7.4	0.6	5.6×10^5	1.05
	8.5	0.2	5.7×10^5	1.03
280 nm	6.0	0.2	5.6×10^5	1.05
	7.4	0.04	5.6×10^5	1.01
	7.4	0.2	5.4×10^5	1.09
	7.4	0.6	5.6×10^5	1.01
	8.5	0.2	5.7×10^5	1.04

sulfaethidole to BSA were almost constant. Binding parameters for sulfaethidole to BSA at different pHs and ionic strengths are summarized in Table I.

Taking the effects of pH and ionic strength into consideration, it seems that the binding of sulfaethidole to BSA was not much affected by the electrostatic and ionic interactions. Therefore, it might be assumed that the binding was mainly due to the hydrophobic interactions. Sulfaethidole seems to be a reasonable CD probe for the study of hydrophobic drug interactions.

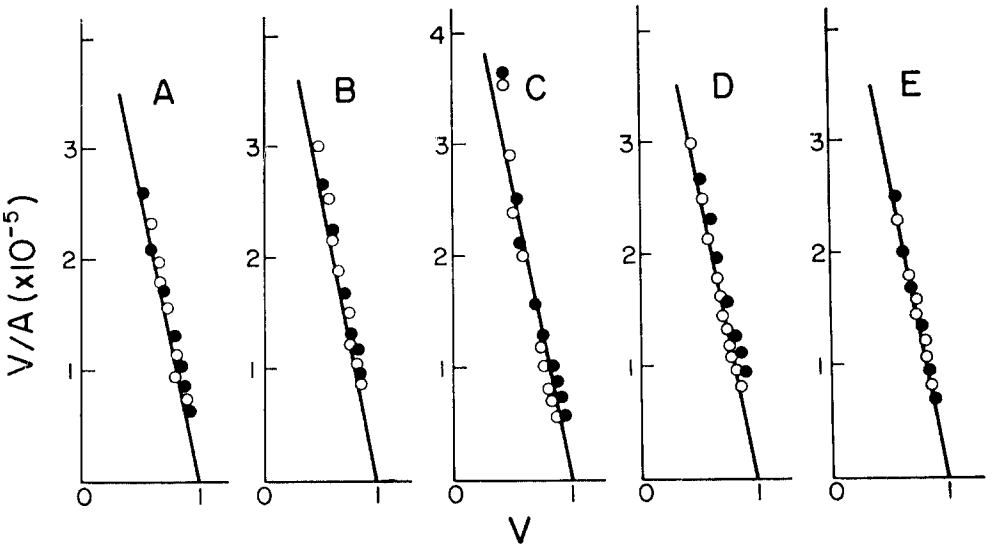

Fig. 3: Scatchard plots for the binding of sulfaethidole to BSA at different pHs and ionic strengths. (0) and (0) are the data obtained at 257nm and 280nm, respectively.
A, pH=6.0, I=0.2; C, ph=7.4, I=0.2; F, pH=8.5, I=0.2.
B, pH=7.4, I=0.04; D, pH=7.4, I=0.6;

ACKNOWLEDGEMENT

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