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NOTE

Electrochemical Potentials in Bovine Serum Albumin (BSA) and Coomassie Brilliant Blue (CBB) Interaction

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Coomassie brilliant blue (CBB) is used extensively as a dye for the detection and staining of proteins[1]. However, its interaction with bovine serum albumin (BSA) appears to be quite complex and mechanism of this interaction is still the subject of active discussion.

CBB shows a characteristic absorption spectrum in aqueous solutions which by addition of BSA shows changes in its chromicity and a very specific isobestic point at 450 nm suggesting stoichiometric interaction between BSA and CBB. We have attempted to calculate the binding parameters n (binding sites) and k' (the association constant) for this interaction by plotting the Scatchard[2] plots from the absorbance data at 565 nm, and the electrochemical potential values (μ) under various experimental conditions have been calculated using these values of the association constant (k').

Results and Discussion

A characteristic absorption spectrum is shown by CBB in aqueous solution which by addition of BSA shows changes in its chromicity and a very specific isobestic point at 450 nm (Fig. 1) suggesting a stoichiometric interaction between BSA and CBB. The results of the binding interaction between CBB and BSA in aqueous solution are shown in Fig. 2. The points obtained at higher ratios of CBB concentration are correlated by best possible straight line drawn according to least square method which is extrapolated to the abscissa to obtain the value of apparent binding sites (n) in terms of number of dye molecules bound per molecule of BSA per free dye molecule. The value of apparent association constants (K') were obtained by calculating the slope of this line. The value of n and K' for this interaction obtained from this graph are 6.2 and 2.625×10^7 mol dm^{-3}; the corresponding electrochemical potential is 439 mV. The binding thus appears to be quite strong in the region. Some tendency to show curved lines is noticed, at higher BSA concentrations this tendency is more prominent.

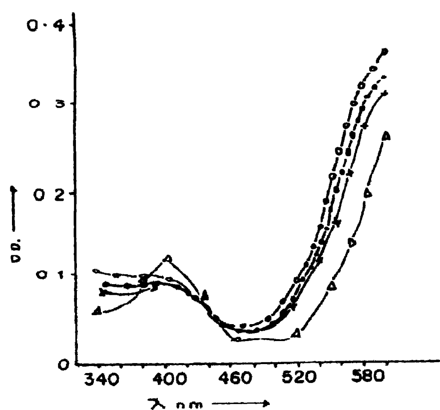

Fig. 1. Spectra of CBB and CBB + BSA (0.25 mg CBB in 10 ml aqueous solution : (O) CBB alone, (•) CBB + 2.5 mg BSA, ( ) CBB + 5.0 mg BSA and (Δ) CBB + 7.5 mg BSA.

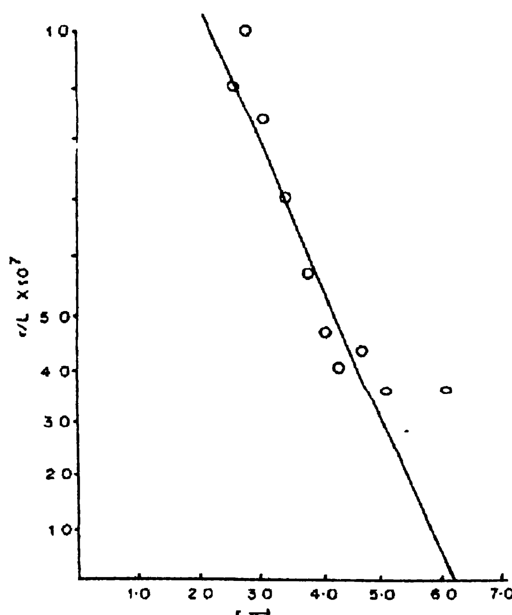

Fig. 2. Scatchard plots for the interaction between BSA and CBB in aqueous solution : $r/L = \frac{(NL)/P}{L} \times 10^7$ against $r = \frac{(NL)}{P}$.

Several workers have attempted to explain this behaviour. Muller and Crothers[3] suggested that the binding may be cooperative or there might be overlapping of potential binding sites. Possibility of a second weaker binding is also mentioned. Murakini[4] reported such distinct binding classes for the interaction between bromophenol blue and BSA at 20° and pH 7.0. Some workers suggested that CBB exists in various forms depending upon the experimental conditions and several specific and non-specific interaction between CBB and BSA are supposed to contribute to the curved nature of the plots. Stutter *et al.*[5] pointed to limitations of the Scatchard plots. Thus several

interpretations of the curved nature of the plots are possible and in the absence of precise explanation, our results need to be viewed with this background.

We have further attempted to obseved the effects of experimental factors on these apparent binding parameters by using various physicochemical modifications of the BSA solution (Table 1). Significant increase in binding energy and reduction in binding sites are observed in salt solutions, suggesting strong influence of electrovalent and specific interactions. Bradford[6] did not observe such effect. He reported that there are changes in the form of the dye with the change in salt concentration. This is accompanied by shifts in the wavelength of maximum absorption. Thus the influence of ions is probably shadowed by this shift. However, in the drawing of Scatchards plots for observations at specific absorption wavelength (565 nm) the influence of salts seems to make the effect more apparent.

TABLE 1—ELECTROCHEMICAL POTENTIALS OF CBB-BSA INTERACTIONS

Sl. no.	Experiment	n	$10^7 K'$ mol dm^{-3}	μ mV
1.	CBB-BSA (aq. pH 3.5, temp.25°)	6.2	2.625	439
2.	CBB-BSA (0.5 M NaCl)	3.4	30	502
3.	CBB-BSA (0.15 M $MgSO_4$)	3.5	12	478
4.	CBB-BSA (pH 7.4)	6.5	3	442
5.	CBB-BSA (5 M urea)	3.5	5.77	459
6.	CBB-BSA (aq., temp. 37°)	4.3	4.1	460
7.	CBB-BSA (aq., temp. 45°)	3.7	17.0	487

Changes in pH from acidic to neutral (7.4) do not show appreciable changes in the binding parameters. In 5 M urea, there is considerable reduction in the binding sites but a slight increase in the binding affinity. With increase of temperature also progressive decrease in binding sites with increase in binding strength is observed. This suggests that distruption of tertiary organised structure of BSA does not affect the strong binding between CBB and BSA drastically.

Thus we can conclude the following. (i) At higher ratios of CBB/BSA concentration there is strong interaction between them corresponding to an electrochemical potential of 0.439 V. Although this interaction is stoichiometric and specific, other cooperative interactions and overlapping of potential binding sites influence this binding. (ii) Observation under various experimental conditions shows that with the changes in physicochemical parameters the binding sites available are significantly affected but there is overall increase in binding affinity with the loss of BSA tertiary structure.

Experimental

BSA (Sigma) and CBB (Loba-Chemie) were used as such. The dye solution was prepared by the reported method[7]. The stock BSA solution was prepared in double-distilled water (2 mg ml^{-1}). The required modifications for studying the various physicochemical factors were obtained by using the corresponding solutions, instead of water, for preparing the BSA solutions.

The actual complexes between BSA and CBB were prepared by a method similar to that described in literature[8] keeping the dye concentrations constant throughout (0.25 mg).The absorbance values of these solutions were determined at 565 nm on a Bosch and Lamb spectronic-20 spectrophotometer. The values of bound and free dye were calculated from these absorbance values by the method of Irvin and Irvin[9],

$$\text{Fraction of dye bound } (NL) = \frac{\text{Absorb. of sample} - \text{Absorb. of dye}}{\text{Max. diff. in absorb. values between completely bound and free dye forms}}$$

$$\text{Fraction of free dye } (L) = 1-(NL)$$

The values of r and r/L were further calculated as follows :

$$r = \frac{(NL)}{\text{Total amount of BSA in solution (P)}}$$

$$r/L = \frac{(NL)/P}{L}$$

From these values the Scatchard plots[2] were drawn and apparent values of n and association constant (K') were calculated (n = intercept on the abscissa and K' = slope) by the least-square method for the points which could be best fitted on a straight-line. The corresponding electrochemical potentials were calculated by using the expressions[10] : $\Delta G = \Delta G° + RT \ln K'$ and $\Delta G = -nFE$.

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