Binding Data Analysis of the Interaction of Bovine Hemoglobin with Dodecyltrimethylammonium Bromide

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(Received September 29, 1995)

The interaction of dodecyltrimethylammonium bromide, as a cationic surfactant, with bovine hemoglobin, as a biopolymer, has been investigated at different temperatures by an equilibrium dialysis technique. The obtained binding isotherms have been analyzed and interpreted by the Wyman binding potential model and other thermodynamic parameters which have been extracted on the basis of this model. A new method of analysis for evaluating the binding isotherms and estimating the free energy change, ΔG_t, per mole of ligand has been proposed.

The unusual behavior of the Scatchard plot at 300 K was analyzed in terms of two sets of binding sites. The first set of binding sites was considered as electrostatic and the second as hydrophobic. The free energy of interaction ($\Delta G_{\bar{v}}$) from the Wyman model was also resolved according to electrostatic and hydrophobic binding free energies.

Protein function is modified by binding to other molecules. This kind of interaction is at the heart of many biochemical regulatory phenomena.[1)]

The binding of proteins with surfactants has been extensively studied.[1—6)] The widespread commercial use of surfactants has of necessity led to studies of their effects on biological systems; thus surfactant–protein interactions are basic to such investigations. On the other hand, as has been recently demonstrated, the pathway for surfactant-induced unfolding is very similar to that of thermal unfolding so that the study of surfactant-induced unfolding is a practicable approach towards understanding the problem of protein stability and unfolding.[7)]

The study of hemoglobin has gone beyond the interest in its physiological role as an oxygen carrier because it represents from all points of view an ideal model for investigating the properties of proteins in general, and enzymes in particular. Hemoglobins are heme proteins with four iron atoms per protein chelated to protoporphyrin IX.[8)] Hemoglobins are oligomers containing four tetrahedrally-arranged subunits in identical pairs (two α and two β chains). Both α and β chains appear to contain eight helical regions and have a short nonhelical region at the amino end, terminating with a helical region at the carboxyl end.

The purpose of this paper is to analyze the binding data for the interaction between bovine hemoglobin and dodecyltrimethylammonium bromide (DTAB) in terms of their Scatchard plots and to interpret the unusal shape of this plot, as well as to calculate the thermodynamic parameters of this interaction using the theoretical model of the Wyman binding potential. In the second part of this paper, we introduce new methods for the thermodynamic analysis of these interaction and the resolution of the obtained Gibbs' free energy as electrostatic and hydrophobic free energy of interaction. This study has revealed some structural changes of hemoglobin due to the increase in temperature.

Experimental

Materials. Bovine hemoglobin and DTAB were obtained from Sigma Chemical Co. and Merck, respectively. Visking membrane dialysis tubing (MW cut-off 10000—14000) was obtained from SIC (East Leigh), Hampshire, UK. Orange II dye was received from Sigma. 2.5 mM phosphate solution (1 M = 1 mol dm^{-3}), pH = 6.4, $I = 0.0069$, was used as a buffer. All other materials and reagents were of analytical grade. Double-distilled water was used in the preparation of solutions.

Methods. Equilibrium dialysis was carried out at 300 and 310 K using bovine hemoglobin solutions at a concentration of 0.02% w/v. From this, aliquots of 2 cm^3 were placed in dialysis bags and equilibrated with 2 cm^3 of DTAB solutions covering the required concentration range for longer than 96 h, as previously explained.[1)]

All reported measurements refer to DTAB concentrations below the CMC; free DTAB concentrations in equilibrium with the complexes were assayed by the orange II dye method.[9)]

The molecular weight of hemoglobin is approximately 65000.[8)]

Results and Discussion

Binding isotherms for the interaction of bovine hemoglobin with DTAB appear in Fig. 1 and show the number of DTAB ions bound per molecule of bovine hemoglobin ($\bar{v}$) as a function of the logarithm of the free DTAB concentration [S_f], measured by equilibrium dialysis at 300 and 310 K, 2.5 mM phosphate buffer pH = 6.4. Increased temperature results in higher free concentrations of DTAB.

Calculation of the apparent binding constant, K_{app} ($\bar{v}$), can be applied to the entire collection of binding isotherms. This is based on the Wyman binding potential model.[10)] The

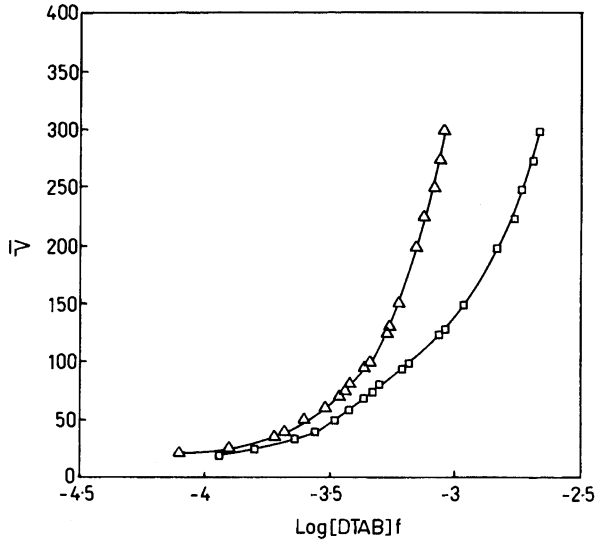

Fig. 1. Binding isotherms for the interaction of DTAB with bovine hemoglobin at pH = 6.4. (△) 300 K, (□) 310 K.

binding potential Π is calculated from the area under the binding isotherms according to equation (1):

$$\Pi = 2.303RT \int_{[S_f]_i=0}^{[S_f]_f} \bar{\nu} \mathrm{d} \log [S_f] \quad (1)$$

and is related to an apparent binding constant, K_{app} $(\bar{\nu})$, as follows:

$$\Pi = RT \ln (1 + K_{app}(\bar{\nu})[S_f]^{\bar{\nu}}), \quad (2)$$

values of K_{app} $(\bar{\nu})$ as a function of $\bar{\nu}$ were determined by application of Eqs. 1 and 2 to determine values of $\Delta G_{\bar{\nu}}$

$$\Delta G_{\bar{\nu}} = \frac{\Delta G(\bar{\nu})}{\bar{\nu}} = \frac{-RT \ln K_{app}(\bar{\nu})}{\bar{\nu}}. \quad (3)$$

Figure 2 shows $\Delta G_{\bar{\nu}}$ as a function of $\bar{\nu}$ at pH=6.4 and at 300 and 310 K. There are electrostatic and hydrophobic interactions in the binding of ionic surfactants to protein. Due to the fact that electrostatic interactions are stronger than hydrophobic interactions it is reasonable to assume that the initial interaction of surfactant molecules with protein is the electrostatic binding of the ionic head group of the surfactant with sites of opposite charge on the surface of the protein, which is followed by more extensive hydrophobic binding as the critical micelle concentration of the surfactant is approached. According to the basis of this interpretation, the limiting value of $\Delta G_{\bar{\nu}}$, at high values of $\bar{\nu}$, corresponds predominantly to hydrophobic binding which is temperature dependent. Due to the high value of the heat capacity change during this interaction, this matter is clear in the difference between the limiting values at two temperatures.

The enthalpy of the interaction of bovine hemoglobin and DTAB is shown in Fig. 3. This was obtained from the temperature dependence of the binding constant ($K_{app}(\bar{\nu})$) using the van't Hoff relation (4).

$$\Delta H(\bar{\nu}) = \frac{-R \mathrm{d}(\ln K_{app}(\bar{\nu}))}{\mathrm{d}(1/T)}, \quad (4)$$

Figure 3 shows a minima curve at $\bar{\nu} \cong 80$, which may correspond to the begining of an endothermic unfolding process and the end of exothermic electrostatic interactions. In order to analyze the binding data, the Scatchard equation was plotted and is shown in Fig. 4. The Scatchard equation is as follows:[11]

$$\frac{\bar{\nu}}{[S_f]} = K(n - \bar{\nu}), \quad (5)$$

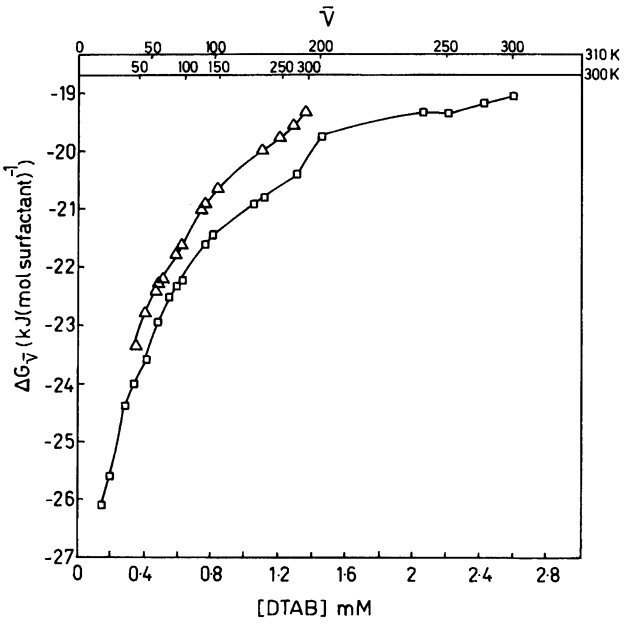

Fig. 2. Gibbs free energy of binding of DTAB to bovine hemoglobin as a function of total concentration of surfactant at pH=6.4. (△) 300 K, (□) 310 K. The upper axis shows the number of DTAB molecules bound per hemoglobin molecule ($\bar{\nu}$).

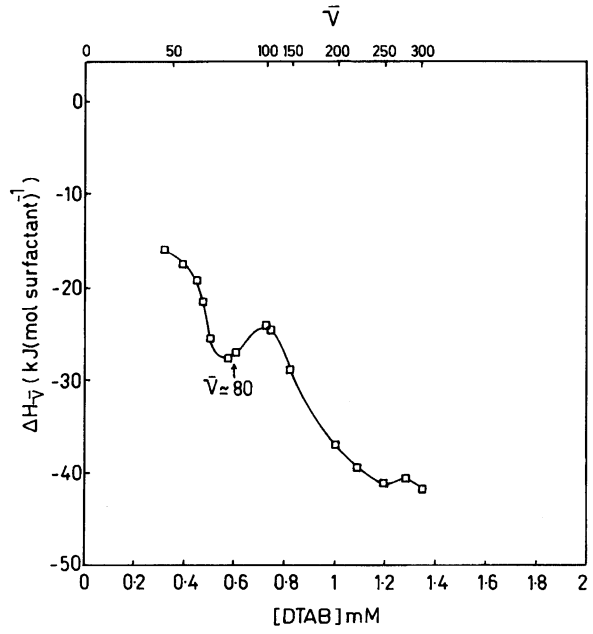

Fig. 3. Enthalpy of interaction between bovine hemoglobin and DTAB at pH=6.4, as a function of total concentration of DTAB. The upper axis shows the number of DTAB molecules bound per hemoglobin molecule ($\bar{\nu}$).

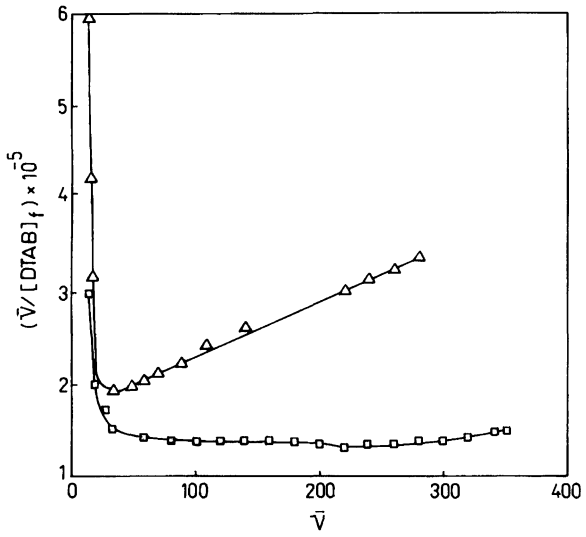

Fig. 4. Scatchard plots for the interaction of DTAB with bovine hemoglobin at pH=6. 4. (△) 300 K, (□) 310 K.

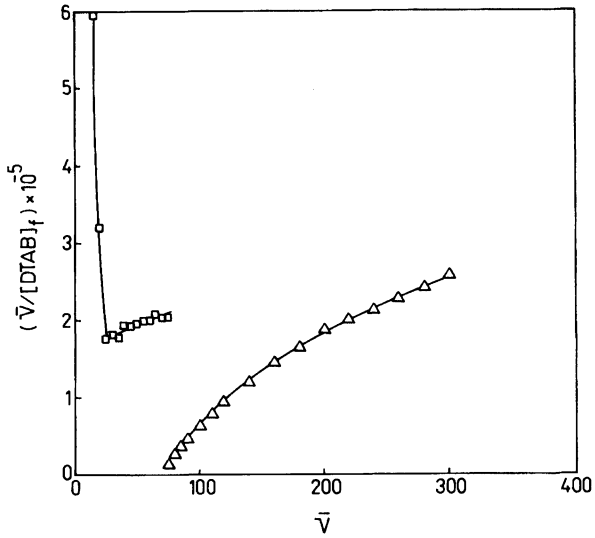

Fig. 5. Non-linear Scatchard plot of the same data resolved into two types of binding at pH=6. 4, 300 K. (□) The first binding sites; (△) The second binding sites.

where $[S_f]$ is the free DTAB concentration, K is the binding association constant, and (n) is the number of independent binding sites. If all the sites are identical and independent of each other, then this plot is linear with a slope of $-K$ and an intercept where $\bar{v}/[S_f]=0$ of (n). However, if these sites are not independent then the Scatchard plot is not linear. Figure 4 shows negative co-operativity at pH=6.4, at 310 K and an unusual concave plot at pH=6.4, at 300 K. This unusual behavior was observed previously for the interaction of sodium n- dodecyl sulfate with calf thymus histone H2B,[12] and was interpreted as a combination of negatively and positively co-operative binding sites with binding constants differing by several orders of magnitudes. Here we can apply the Hill equation to analyze our binding data as follows:[13]

$$\bar{v} = \frac{g(K[S_f])^{n_H}}{1+(K[S_f])^{n_H}}, \tag{6}$$

where g is the maximum value of $\bar{v}$, n_H is the Hill coefficient (the measure of co-operativity of the interaction), and K is the mean intrinsic binding constant. The data were analyzed using Eq. 7 which is based on the Hill equation for more than one term:

$$\bar{v} = \frac{g_1(K_1[S_f])^{n_{1H}}}{1+(K_1[S_f])^{n_{1H}}} + \frac{g_2(K_2[S_f])^{n_{2H}}}{1+(K_2[S_f])^{n_{2H}}}, \tag{7}$$

where g_1, K_1, and n_{1H} are the number of binding sites, binding constants, and the Hill coefficient for the first binding sets, respectively, and g_2, K_2, and n_{2H} are the corresponding parameters for the second binding sets. In order to fit the data to this equation, the values of g_1 and g_2 were estimated and then the binding data were fitted to this equation using a non-linear least squares program.[14] The results are listed in Table 1 and are also shown in Fig. 5.

In conclusion, a Scatchard plot with a minima at 300 K is the result of two kinds of binding sites having different co-operative coefficients. This combination must therefore have a large difference in co-operativity, $n_{1H}=0.98$ and $n_{2H}=6.44$. So, we can consider the first binding sites to be electrostatic and the second to be hydrophobic. Using this assumption, the number of electrostatic binding sites is equal to g_1 and the number of hydrophobic binding sites is equal to g_2; hence we can write that:

$$\Delta G(\bar{v}) = \Delta G_{elec}(\bar{v}) \quad \text{for} \quad \bar{v} \le g_1 \tag{8}$$

and

$$\Delta G(\bar{v}) = \Delta G_{elec}(\bar{v}) + \Delta G_{hyd}(\bar{v}), \quad \text{for} \quad g_2 \ge \bar{v} > g_1, \tag{9}$$

since

$$\Delta G_{elec}(\bar{v}) = \Delta G(g_1) \quad \text{for} \quad \bar{v} \ge g_1, \tag{10}$$

$$\Delta G_{\bar{v}} = \frac{\Delta G(\bar{v})}{\bar{v}} = \frac{\Delta G(g_1)}{\bar{v}} + \frac{\Delta G_{hyd}(\bar{v})}{\bar{v}},$$

$$\Delta G_{elec,\,\bar{v}} = \frac{\Delta G(g_1)}{\bar{v}}, \quad \text{for} \quad g_2 \ge \bar{v} \ge g_1, \tag{11}$$

$$\Delta G_{hyd,\,\bar{v}} = \Delta G_{\bar{v}} - \Delta G_{elec,\,\bar{v}}, \quad \text{for} \quad g_2 \ge \bar{v} > g_1, \tag{12}$$

where ΔG_{elec} $(\bar{v})$ and ΔG_{hyd} $(\bar{v})$ are the total electrostatic and hydrophobic Gibbs free energies, respectively, and $\Delta G_{elec,\bar{v}}$ and $\Delta G_{hyd,\bar{v}}$ are the electrostatic and hydrophobic Gibbs free energies per mole of ligand at $\bar{v}$, respectively. By application of these formulas, we can analyze the Gibbs free energies for electrostatic and hydrophobic interactions and this is shown in Fig. 6.

The difference in the process of the binding at 300 and 310 K can be interpreted in terms of the degree of difference in the strength of binding for electrostatic and hydrophobic forces. It seems the difference in the extent of these two forces is quite large at 300 K, which is imposed at two sets of binding sites as shown in Figs. 4 and 5. However, the difference in the scope of the two forces is less for hemoglobin conformation at 310 K, therefore it is imposed as one set of binding sites.

The last part of this article discusses the sigmodial saturated binding curve of $\bar{v}/g$ vs. the logarithm of [DTAB] at

Table 1. Hill Parameters for the Interaction of DTAB with Bovine Hemoglobin at pH=6. 4

T(K)	g_1	g_2	n_{1H}	n_{2H}	K_1	K_2	g	n_H	K
300	80	210	0.98	6.44	5219.4	150.8	—	—	—
310	—	—	—	—	—	—	450	0.97	537.46

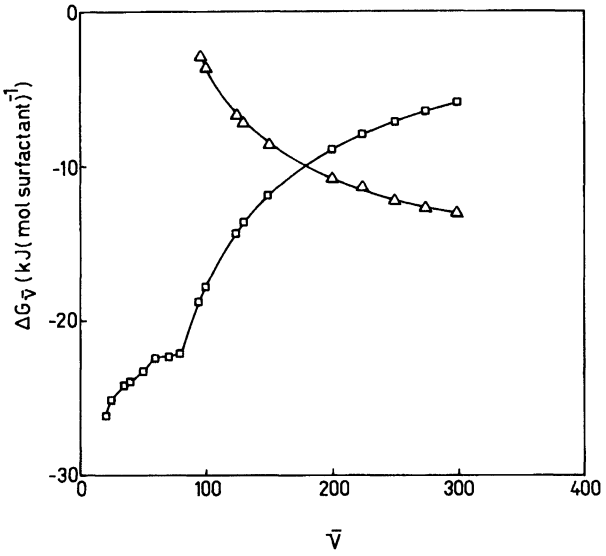

Fig. 6. (□) Electrostatic Gibbs free energy of binding, $\Delta G_{\mathrm{elec},\bar{v}}$ and (△) hydrophobic Gibbs free energy of binding, $\Delta G_{\mathrm{hyd},\bar{v}}$ in terms of kJ (mol surfactant)$^{-1}$, of bovine hemoglobin as a function of $\bar{v}$ at 300 K.

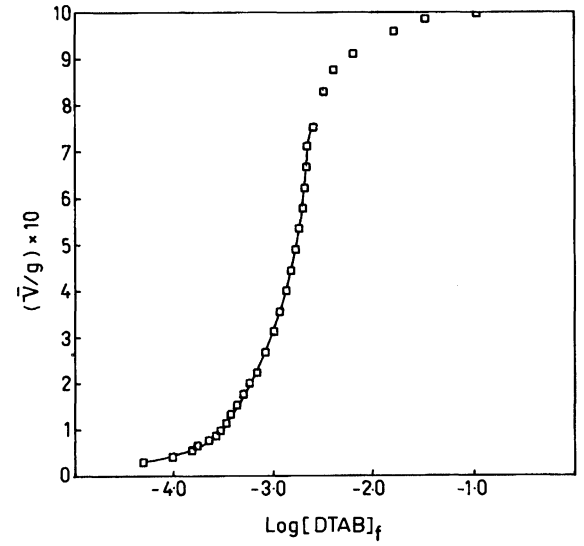

Fig. 7. Saturation curve for the interaction of DTAB with bovine hemoglobin at pH=6. 4 and 310 K. The disconnected points were simulated.

310 K which can be considered as a system with one set of binding sites and is shown in Fig. 7. The saturation plateau in the sigmodial curve was simulated symmetrically with the initial plateau of the sigmodial curve. This curve is sigmoidal corresponding to co-operative binding. A very useful concept in relation to this curve is that of median ligand activity $[S_m]$.[15] By this we mean that the value of $[S_f]$ is as follows:

$$\int_{[S_f]=0}^{[S_m]} \left(\frac{\bar{v}}{g}\right) d\ln[S_f] = \int_{[S_m]}^{[S_f]=\infty} \left(1-\frac{\bar{v}}{g}\right) d\ln[S_f], \quad (13)$$

which is related to the free energy change per mole of surfactant, ΔG_t, by the following equation:[15]

$$\Delta G_t = RT\ln[S_m]. \quad (14)$$

Applying this concept to the saturation curve of the interaction of bovine hemoglobin with DTAB gives ΔG_t equal to -20.9 kJ (mol surfactant)$^{-1}$ at 310 K. This value is comparable to the mean $\Delta G_{\bar{v}}$ in Fig. 2, therefore this method can offer an alternative way to estimate $\Delta G_{\bar{v}}$ for a binding isotherm with one set of binding sites.

The similarity of ΔG_t to $\Delta G_{\bar{v}}$ indicates an agreeable simulation for the sigmodial binding curve shown in Fig. 7.

We wish to thank Dr. Steve Connolly for editing the manuscript. This study was supported by a grant from the Research Council of the University of Tehran.

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