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A microcalorimetry and binding study on interaction of dodecyl trimethylammonium bromide with wigeon hemoglobin

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Abstract

The thermodynamic parameters for the binding of dodecyl trimethylammonium bromide (DTAB) with wigeon hemoglobin (Hb) in aqueous solution at various pH and 27 °C have been measured by equilibrium dialysis and titration microcalorimetry techniques. The Scatchard plots represent unusual features at neutral and alkaline pH and specific binding at acidic pH. This leads us to analyze the binding data by fitting the data to the Hill equation for multiclasses of binding sites. The best fit was obtained with the equation for one class at acidic pH and two classes at neutral and alkaline pH. The thermodynamic analysis of the binding process shows that the strength of binding at neutral pH is more than these at other pH values. This can be related to the more accessible hydrophobic surface area of wigeon hemoglobin at this pH. The endothermic enthalpy data which was measured by microcalorimetry confirms the binding data analysis and represents the more regular and stable structure of wigeon hemoglobin at neutral pH.

Keywords: Wigeon hemoglobin; Dodecyl trimethylammonium bromide; Equilibrium dialysis; Titration microcalorimetry; Ligand binding

1. Introduction

Hemoglobin (Hb), the circulating red pigment of blood, is a heme protein and has a long evolutionary history as an oxygen-transport protein [1,2]. The hemoglobin structure of different vertebrates are different, which may suggest the difference in their ability for oxygen affinity. Avian hemoglobins are functionally similar to mammalian hemoglobins. They are formed by four-heme containing units and four globin polypeptides as in mammals; the globin moieties are however different and migrate electrophoretically at different speeds than their other vertebrate counterparts [3,4]. Although, the primary structure of some avian's Hb and their function have been reported [5–9], there is a few report on avian's Hb denaturation.

The denaturation of the protein is a key study for obtaining structural information. Most of the protein denaturation studies by surfactants have been done using anionic surfactants like sodium *n*-dodecyl sulphate (SDS) [10–12]. There are also some studies using cationic surfactants [13–19]. The study of the nature of the interaction between protein and surfactant provides insight into action of surfactant as denaturant [20]. Proteins and surfactants both contain a balanced proportion of hydrophilic and hydrophobic groups. It has been suggested that the interaction between ionic

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surfactants and proteins involve initial binding of the surfactant molecules to charged groups of opposite sign on the surface of the protein followed by more extensive hydrophobic interactions until the critical micelle concentration (CMC) of surfactant is reached [20–22]. On the basis of this suggestion, it seems that we have to consider two classes of binding sites for analyzing the binding data [23–26].

In this paper, we have measured and analyzed the binding data for interaction of wigeon Hb as an avian Hb with dodecyl trimethylammonium bromide (DTAB) as a cationic surfactant. This leads to investigate the nature of interactions according to the one and two classes of binding sites and obtain some new aspects of wigeon Hb structure. The results have been certified by microcalorimetry data.

2. Experimental

2.1. Materials

Blood from wigeon was taken from brachial and pectoral veins in 3.8% sodium citrate which acts as an anticoagulant. Hb was isolated by Drabkin's method [27]. The plasma was removed and cells were washed with physiological saline (0.9% sodium chloride solution). The clear supernatant-containing Hb was separated from cell debris by centrifugation. The non-heme proteins was precipitated by centrifugation.

DTAB and orange II dye were obtained from Sigma. Visking membrane dialysis tubing (M_W cut-off 10,000–14,000) was obtained from SIC (East Leigh) Hampshire, UK. Buffer of sodium phosphate, 2.5 mM, pH of 6.4, $I = 0.0064$ and glycine buffer, 50 mM, pH of 3.2 and 10, $I = 0.0318$, have been used as buffers. All other materials and reagents were of analytical grade. Double distilled water was used in the preparation of solutions.

2.2. Methods

2.2.1. Equilibrium dialysis

The equilibrium dialysis experiments were carried out at 27 °C using 0.02% (w/v) wigeon Hb solutions, from which aliquots of 1 cm^3 were placed in dialysis bags and equilibrated, for over 96 h, with 2 cm^3 of DTAB solution covering the required concentration range, as reported previously [28]. All the measurements refer to DTAB concentrations below the CMC. The free DTAB concentration in equilibrium with the complexes were assayed by the orange II dye method [29]. All calculations were based on molecular weight of 65,000 Da for native wigeon Hb [30].

2.2.2. Microcalorimetric measurements

A four channel microcalorimetric system, Thermal Activity Monitor 2277 from Sweden Thermometric, interfaced with an IBM Pentium III and DIGITAM-3 software and a 1000 μl injection syringe for enthalpy measurements at 27.000 ± 0.005 °C were used. The enthalpy of Hb–DTAB interaction was measured by sequential injection 50 μl of 20 mM DTAB solution from a syringe to 2.5 ml of 0.1% (w/v) Hb solution in a 5 ml titration cell. The enthalpy of dilution and demicellization of the DTAB solution was measured as described previously in the absence of hemoglobin. The enthalpy of dilution and demicellization for surfactant micelles was subtracted from the enthalpy of wigeon hemoglobin–DTAB interaction. Heat of Hb dilution was negligible and system frequently calibrated electrically during experiments.

3. Results and discussion

3.1. Binding data analysis

Fig. 1 is the binding isotherms of wigeon Hb–DTAB interaction which shows the number of DTAB ions bound per molecule of wigeon Hb (ν) as a function of logarithm of the free DTAB concentration, $[S]_f$, at the specified conditions.

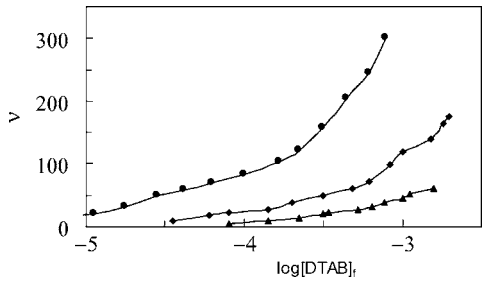

Fig. 1. Binding isotherms for DTAB interaction with wigeon Hb at 27 °C. Symbols: (●) pH = 6.4; (◆) pH = 10; (▲) pH = 3.2.

The Scatchard plot, $\nu/[S]_f$, versus ν was obtained from Scatchard equation, which can be used for analyzing the systems with one binding set [31]. The linear Scatchard plot indicates the identical and independent set of sites, whereas, the non-linear curves (upward or downward concave) indicate the non-identical and dependent set of binding sites [32]. The Scatchard plots for binding of DTAB to wigeon Hb at pH values of 3.2, 6.4 and 10 are shown in Fig. 2.

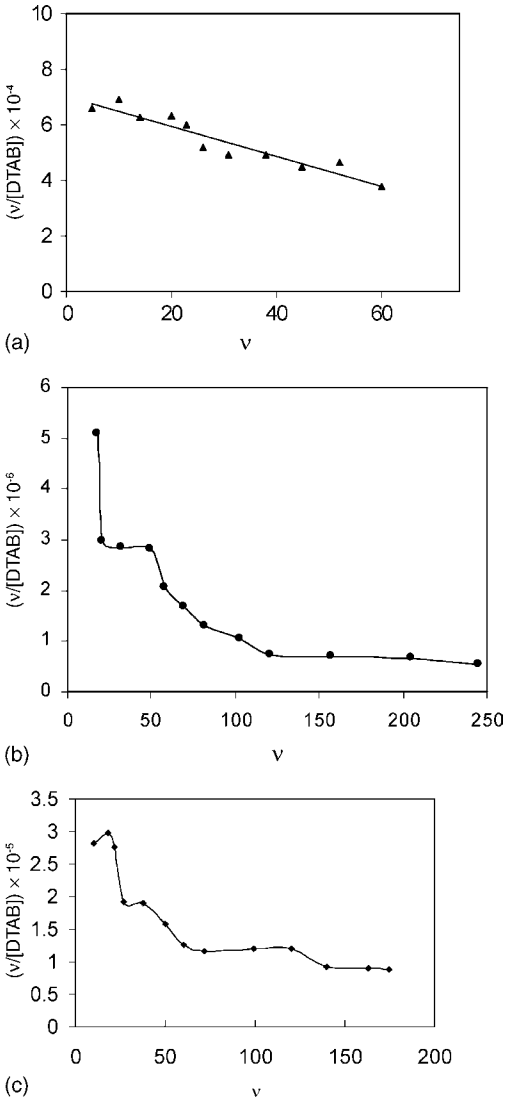

Fig. 2. Scatchard plots for binding of DTAB to wigeon Hb at 27 °C. Symbols: (a) pH = 3.2; (b) pH = 6.4; (c) pH = 10.

It is a linear plot with negative slope at pH 3.2, indicating the presence of one set of binding sites. The Scatchard concave plots with a tail at pH 6.4 and 10 are unusual, which can be interpreted by considering two binding sets with different cooperativity in each binding set [23–25].

The results of Scatchard plots can be used to analyze the binding data by fitting to the Hill equation for multiclasses of binding sites [33].

$$\nu = \sum_{i=1}^{N} \frac{g_i (K_{H_i}[S]_f)^{n_{H_i}}}{1 + (K_{H_i}[S]_f)^{n_{H_i}}} \quad (1)$$

where g_i, K_{H_i} and n_{H_i} are the numbers of binding sites, Hill binding constant and Hill coefficient for the ith binding class, respectively. The fitting was done using a computer program for non-linear least square fitting [34]. The best fitting was obtained with the equation for one class ($N = 1$) at acidic pH and two classes ($N = 2$) at neutral and alkaline pH. The fitting parameters of Hill equation are tabulated in Table 1. The values in this Table for pH 6.4 and 10 indicate that the interaction between ionic surfactant and protein corresponds to combination of hydrophobic and electrostatic interactions initially and pure hydrophobic interactions, subsequently. The values for pH 3.2 show a specific interaction, which corresponds to identical and independent sites with no cooperativity (Hill coefficient approximately equals to one). It can be due to this fact that the affinity between opposite charge groups of protein and DTAB as a surfactant is diminished at low pH, and the strength of initial interaction becomes close to the subsequent hydrophobic interactions. In this case, the system behaves as a one binding set. At higher pH, most of the acidic amino acids at the protein surface are deprotonated and the negative charge density is increased. Therefore, the difference between binding affinities of these two interactions are increased and it behaves as a two binding set system.

Table 1
Parameters derived from Eq. (1) for the binding of DTAB to wigeon Hb at 27 °C and various pH

pH	g_1	K_{H_1} (M^{-1})	n_{H_1}	g_2	K_{H_2} (M^{-1})	n_{H_2}
3.2	110	542	0.95	–	–	–
6.4	75	57553	1.15	420	875	1.50
10	39	9699	1.21	210	490	1.97

Comparison between the fitting parameters at pH 6.4 and 10 shows that the initial interaction is stronger at pH 6.4. It may be interpreted that the initial interaction is accompanying with hydrophobic interaction of surfactant tail and hydrophobic patches at the surface of protein. However, the neutralization of charges, which is occurred during this initial interaction, caused the balance of forces at the protein structure to be perturbed and the protein becomes unfolded. The unfolding causes more hydrophobic sites to become accessible for pure hydrophobic interactions with surfactant molecules. The difference between the binding parameters at pH 6.4 and 10 shows that the hydrophobic patches around the negative charged sites at the surface of wigeon Hb at pH 6.4 is more than that at pH 10. On the other hand, it can be suggested that the accessible hydrophobic surface area of wigeon Hb in native conformation at pH 6.4 is more than that at pH 10.

3.2. Thermodynamic analysis of binding

The Wyman binding potential, Π, has the property that [35]

$$\nu_i = \left(\frac{\partial \Pi}{\partial \mu_i}\right)_{T,P,\mu_{j\neq i}} \tag{2}$$

where ν_i and μ_i are the number of average bound ligands per macromolecule and chemical potential of component i, respectively.

An expression for the binding potential of the polyvalent macromolecule, P, in solution have been developed elsewhere [35,36]. For binding of ionic surfactant to protein, the binding potential is often given by

$$\Pi = RT\ln(1 + K_1[S]_f + K_2[S]_f^2 + \cdots + K_{g_1}[S]_f^{g_1} + K_{g_1+1}[S]_f^{g_1+1} + \cdots + K_{g_1+g_2}[S]_f^{g_1+g_2}) \tag{3}$$

where K_i is the phenomenological association constant for the following reaction

$$P + iS \Leftrightarrow PS_i \tag{4}$$

On the basis of Eq. (2), at constant pressure, temperature, and activities of all other components except ligand, we can write [37,38]

$$\Pi_{\nu_i} = 2.303RT\int_0^{[S]_f^{\nu_i}} \nu \, d\log[S]_f \tag{5}$$

where Π_{ν_i} and $[S]_f^{\nu_i}$ are the amount of binding potential and free concentration of surfactant at specified binding state, ν_i, respectively. Since the number of binding sites is very high in this system, analytical evaluation of Eq. (1) is really impossible. For solving this problem, we can assume that at any value of Π_{ν_i} the predominant component is PS_{ν_i}. Using this assumption, the approximate expression for Π_{ν_i} at any specified binding state (ν_i) is [39]

$$\Pi_{\nu_i} = RT\ln(1 + K_{\nu_i}^{app}[S]_f^{\nu_i}) \tag{6}$$

where $K_{\nu_i}^{app}$ is apparent macroscopic binding constant for ν_ith association reaction. Values of $K_{\nu_i}^{app}$ were determined by application of Eqs. (5) and (6) to determine values of

$$\Delta G_{\nu_i} = \frac{\Delta G(\nu_i)}{\nu_i} = \frac{-RT\ln K_{\nu_i}^{app}}{\nu_i} \tag{7}$$

where ΔG_{ν_i} is an approximate value of free energy change due to binding of one mole of surfactant to one mole of protein at specified ν_i. Fig. 3 shows the variation of ΔG_{ν_i} versus ν for binding of DTAB to wigeon Hb at pH 3.2, 6.4 and 10. It shows high affinity for binding at low values of ν. This arises from the fact that initial interactions (mixture of electrostatic and hydrophobic) is stronger than the subsequent one which is purely hydrophobic.

Fig. 4 shows the variation of calorimetric enthalpy per mole of DTAB, $\Delta H_\nu = \Delta H_{cal}/\nu$, versus ν, which was measured by titration microcalorimetry. This figure shows an endothermic process having a distinct maxima at pH values of 6.4 and 10, where ν at these points are approximately equal to the corresponding g_1 values. This represents the change in the type of

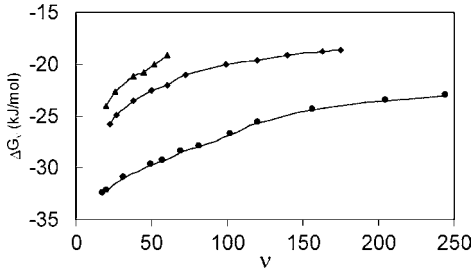

Fig. 3. Gibbs free energy of binding of DTAB to wigeon Hb as a function of ν at 27 °C. Symbols: (●) pH = 6.4; (◆) pH = 10; (▲) pH = 3.2.

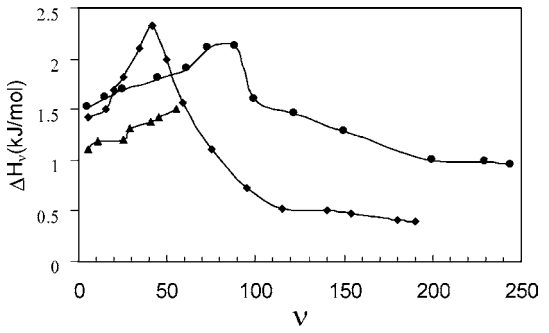

Fig. 4. Calorimetric enthalpy of interaction between wigeon Hb and DTAB as a function of ν. Symbols: (●) pH = 6.4; (◆) pH = 10; (▲) pH = 3.2.

binding at this point and confirms our binding data analysis on the basis of two binding sets. The change in enthalpy of interaction can also be due to denaturation of protein. Therefore, it can be concluded that the predominant unfolding occurs at this maximum point. Fig. 4 shows that this predominant unfolding has occurred at higher values of ν at pH 6.4. Such a behavior was not observed at pH 3.2, which indicates the diminishing of affinity for denaturation of DTAB at low pH. On the basis of this interpretation, it can be concluded that the initial electrostatic neutralization has the most important role in denaturation of protein by ionic surfactants.

4. Conclusions

The binding data analysis indicates that the binding of DTAB to wigeon Hb should be defined by a model with two independent classes of binding sites at neutral and alkaline pH. This behavior is similar to the many other ionic surfactant-protein systems [23–26] and confirms the proposed mechanism for such systems. Reduction of binding strength at alkaline pH represents the considerable role of hydrophobic interaction in the first binding class and more accessible hydrophobic surface area of wigeon Hb at neutral pH. Interpretation of calorimetric measurements confirms our binding data analysis and reveals the more conformational stability of wigeon Hb at neutral pH. This fact that the molecular constrains at the $\alpha\beta$ interface decreases by increasing pH [40], confirms our results. By comparison of ΔG_ν and ΔH_ν data, it can be concluded that the binding of DTAB to wigeon Hb is endothermic and predominately entropy driven. Comparison of the results of this study with our previous [12] shows a weaker interaction of DTAB with hemoglobin compared to SDS. Further, in comparison with DTAB, SDS shows an exothermic interaction with hemoglobin indicating its stronger initial electrostatic interaction.

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