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Comparative thermodynamic stability of bovine and pigeon haemoglobins by interaction with sodium *n*-dodecyl sulphate

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Abstract

The binding of sodium *n*-dodecyl sulphate (SDS) to pigeon and bovine haemoglobin has been studied at pH 6.4 and 25°C by equilibrium dialysis and titration microcalorimetry techniques. The thermodynamic parameters of these interactions have been determined by application of the Wyman binding potential concept and interpreted from a structural viewpoint. A comparison of the results shows that bovine haemoglobin has more thermodynamic stability than pigeon haemoglobin. From this, a reason for the high oxygen affinity of avian haemoglobin is proposed.

Keywords: Avian haemoglobin; Binding capacity; Binding isotherm; Titration microcalorimetry; Wyman binding potential

1. Introduction

Haemoglobins are haem proteins with four iron atoms per molecule attached to the protein and chelated to protoporphyrin IX [1]. Haemoglobin serves as the oxygen carrier in blood and also plays a vital role in the transport of carbon dioxide and hydrogen ions. Birds can breath normally at high altitude due to their special respiratory system [2, 3], but under the same conditions, sea-level vertebrates can not. The primary structures of haemoglobin for some mammals and birds have been reported [4–6] and it is known that although they are functionally similar, their amino

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acid sequences are different. However, there is no report on the thermodynamic stability of these proteins. It is possible to evaluate this stability by studying their interaction with sodium *n*-dodecyl sulphate (SDS). This interaction below the critical micelle concentration (c.m.c.) of SDS frequently leads to the disruption of the three-dimensional structure of the proteins and the formation of protein–SDS complexes [7–9]. The mechanism of denaturation by SDS involves the binding of SDS anions to sites with a positive charge on the surface of the protein molecule, which results in unfolding followed by more extensive hydrophobic binding [10, 11]. These systems have been studied extensively and there are several reviews on them [12, 13].

The aim of the present investigation is to compare the thermodynamic parameters for the interaction of pigeon and bovine haemoglobins with SDS and to interpret these parameters from the structural viewpoint.

2. Experimental

2.1. Materials

Bovine and pigeon haemoglobins and sodium *n*-dodecyl sulphate (especially pure grade) were obtained from Sigma Chemical Co. Visking membrane dialysis tubing (molecular weight cut-off, 10,000–14,000) was obtained from SIC (Eastleigh, Hampshire, UK). Rosaniline hydrochloride dye was used as supplied by BDH. Sodium phosphate buffer (2.5 mM), pH 6.4, $I = 0.0069$, was used. All solutions were prepared with double distilled water.

2.2. Methods

Equilibrium dialysis experiments were carried out to determine the free concentration of SDS in equilibrium with haemoglobin–SDS complexes at 25°C and hence the amount of SDS bound to the haemoglobin. Aliquots of haemoglobin solution (2 cm^3) at a concentration of 0.02% (w/v) were dialysed against 2 cm^3 aliquots of SDS solutions at different concentrations over 96 h.

The free SDS concentrations in equilibrium with the complexes were assayed by the rosaniline hydrochloride method [14]. The molecular weight of haemoglobin was taken to be 65,000 [1] for all calculations.

Titration microcalorimetry enthalpy measurements were performed at 25.0 ± 0.005°C using an LKB microcalorimeter (2277 Thermal Activity Monitor, Boromma, Sweden). The microcalorimeter was interfaced with an IBM PS/2 model 40–486 computer, and the software used was the Thermometric Digitam 3 program. A 1000-μl injection syringe was used.

For enthalpy measurements, the SDS solution was first placed in the syringe and the haemoglobin solution in the sample cell. The concentration of SDS in the syringe was 40 mM and that of haemoglobin in the cell was 0.05% w/v. The volume of haemoglobin solution in the cell was 2 cm^3 and the injection volume in each step was 20 μl. During the titration the enthalpy of demicellisation of SDS is corrected by measuring the

change due to injection of SDS solution to a buffer solution, and the heat of dilution of haemoglobin is negligible.

3. Results and discussion

Fig. 1 shows the binding isotherms for interaction of SDS with bovine and pigeon haemoglobin and also gives the number of SDS ions bound per molecule of haemoglobin ($\bar{v}$) as a function of the logarithm of the free SDS concentration [S_f] at specified conditions. Fig. 2 is the derivative of the binding isotherms and represents the change in the number of moles of SDS per mole of haemoglobin which accompanies a change in the logarithm of the free SDS concentration. This curve is a measure of the steepness of the binding isotherm and can be considered as the binding capacity [15]. However, it contains two distinict maxima for both binding isotherms and involves analysing this system with two sets of binding sites. An approximate approach for evaluation of such a system is the fitting of binding data to Eq. (1), a Hill equation [16], for more than one term

$$\bar{v} = \frac{g_1(K_1[S_f]^{n_1})}{1+(K_1[S_f])^{n_1}} + \frac{g(K_2[S_f])^{n_2}}{1+(K_2[S_f])^{n_2}} \tag{1}$$

where g_1, K_1 and n_1 are the number of binding sites, the binding constant and the Hill coefficient for the first binding set, respectively, and g_2, K_2, n_2 are the corresponding

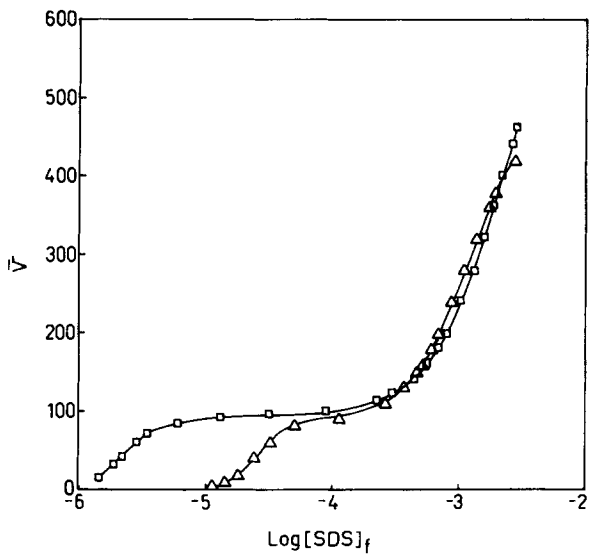

Fig. 1. Binding isotherms for sodium *n*-dodecyl sulphate on interaction with bovine (□) and pigeon (△) haemoglobin at pH 6.4, and 25°C.

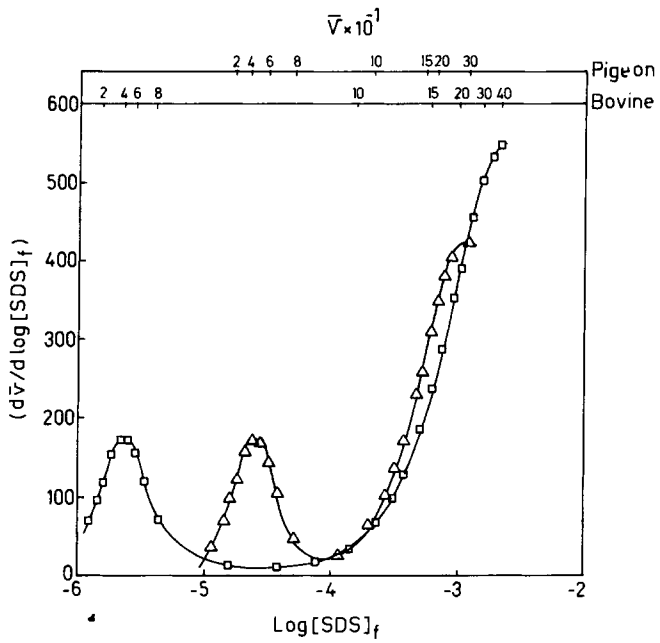

Fig. 2. Binding capacity for sodium *n*-dodecyl sulphate on interaction with bovine (□) and pigeon (△) haemoglobin at pH 6.4, and 25°C. The upper axes are for $\bar{v}$ at equilibrium.

parameters for the second binding set. The values of g_1 and g_2 can be estimated from the value of $\bar{v}$ at the maxima points in the binding capacity curve. The binding data for interaction of bovine and pigeon haemoglobin have also been fitted to Eq. (1) using a computer program for non-linear least-square fitting [17]. The results are shown in Table 1. It is well established that interaction between ionic surfactant and protein involves the initial binding of the surfactant molecules to charge groups with opposite sings on the surface of the protein, followed by more extensive hydrophobic binding as the critical micelle concentration (c.m.c.) of surfactant is approached [12, 18]. Therefore, the first binding set can be related to electrostatic binding and the second to hydrophobic binding. The results in Table 1 show that the number of electrostatic binding sites g_1 and the electrostatic binding constant. K_1 for bovine haemoglobin are more than for pigeon haemoglobin. The electrostatic interaction for bovine haemoglobin is more effective than for pigeon haemoglobin with SDS complexes.

The other approximate method for evaluation of the thermodynamic function of binding is application of the Wyman binding potential [19] to this system. The amount of binding potential at any specified $\bar{v}$, $\Pi\ \bar{v}$, can be calculated from the area under the binding isotherm according to the equation

$$\Pi_{\bar{v}} = RT \int_0^{\log[S]_f} \bar{v}_i d\ln[SDS]_f \tag{2}$$

Table 1
Parameters derived from the Hill equation for the binding of sodium *n*-dodecyl sulphate to bovine and pigeon haemoglobin at ph 6.4 and 25°C

	g_1	K_1/M^{-1}	n_1	g_2	K_2/M^{-1}	n_2
Bovine	90	426211.6	3.32	650	417	1.47
pigeon	85	39532.8	3.55	400	870	1.85

and is related to an apparent binding constant, $K_{app}(\bar{v})$, as a function of $\bar{v}$, as follows [13]

$$\Pi_{\bar{v}} = RT \ln(1 + K_{app}(\bar{v})[SDS]_f^{\bar{v}}) \quad (3)$$

By calculating the values of $K_{app}(\bar{v})$, we can determine the values of the total Gibbs free energy of binding, $\Delta G(\bar{v})$, and the Gibbs free energy of binding per mole of SDS, $\Delta G_{\bar{v}}$, at any specified $\bar{v}$, by the equation

$$\Delta G_{\bar{v}} = \frac{\Delta G(\bar{v})}{\bar{v}} = \frac{-RT \ln K_{app}(\bar{v})}{\bar{v}} \quad (4)$$

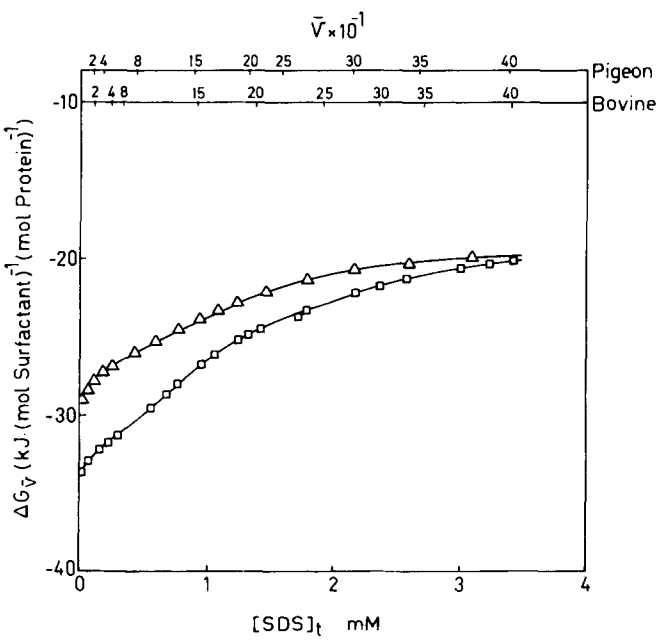

Fig. 3. Variation of Gibbs free energy of binding per mole of sodium *n*-dodecyl sulphate on interaction with bovine (□) and pigeon (△) haemoglobin as a function of total concentration of surfactant. The upper axes are for $\bar{v}$ at equilibrium.

Fig. 3 shows the variation of $\Delta G_{\bar{v}}$ as a function of the total concentration of SDS and $\bar{v}$. It shows that at lower concentrations the binding affinity of SDS to bovine haemoglobin is more than for pigeon haemoglobin. This confirms the results that electrostatic interaction in bovine haemoglobin is stronger than pigeon haemoglobin because most of the electrostatic interaction occurred at lower concentrations of surfactant. However, at higher concentrations of SDS, both curves approach the limiting value, which indicates that hydrophobic interactions for both haemoglobins are close to each other.

Fig. 4 shows the variation of calorimetric enthalpy per mole of SDS, $\Delta H_{\bar{v}} = \frac{\Delta H_{cal}}{\bar{v}}$, which was measured by titration microcalorimetry with respect to the total concentration of SDS. This figure shows a distinct minima for both kinds of haemoglobin. It represent an endothermic unfolding process within an exothermic binding process. However, the endothermic process occurred at lower concentrations of SDS for pigeon haemoglobin than for bovine haemoglobin. Therefore, bovine haemoglobin is more resistant to denaturation by SDS than pigeon haemoglobin. However, the interaction of SDS with bovine haemoglobin is more exothermic than with pigeon haemoglobin and, by considering the extent of the unfolding region, which is shown by arrows in Fig. 4, it seems that the unfolding enthalpy or the heat content of the three-dimensional structure of bovine haemoglobin is more than that of pigeon haemoglobin.

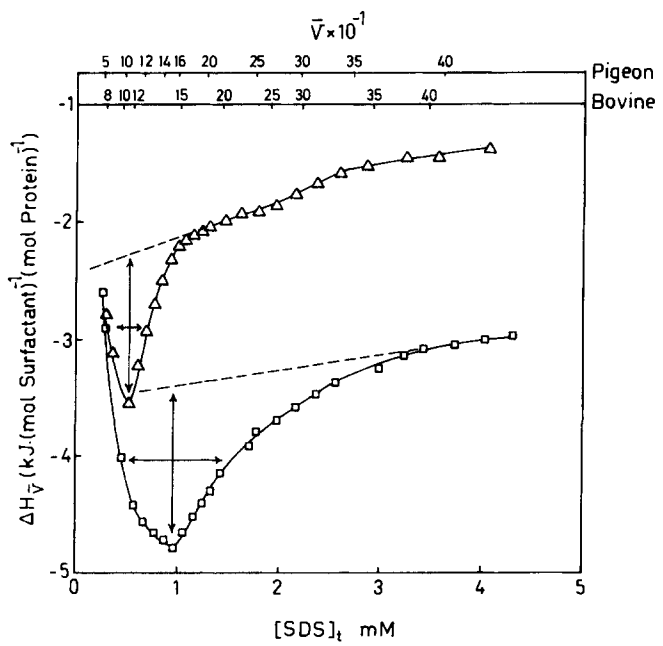

Fig. 4. Variation of calorimetric enthalpy per mole of sodium *n*-dodecyl sulphate on interaction with bovine (□) and pigeon (△) haemoglobin as a function of total concentration of surfactant. The upper axes are for $\bar{v}$ at equilibrium. The unfolding regions are shown by arrows.

On the basis of these result it can be concluded that bovine haemoglobin has more thermodynamic stability and probably a more packed structure than pigeon haemoglobin. However, the forces responsible for the stability of the three-dimensional structure are stronger than for pigeon haemolgobin. This conclusion has been confirmed by other reports in the literature [20]. In the corresponding primary structural analysis, it is known that the difference in the primary structure of avian haemolgobin compared with sea-level vertebrates is produced by the reduction of van der Waal's contacts at sub-unit boundaries [20]. Evalution of the interaction of the haem group with globin shows that this interaction is stronger in bovine haemoglobin than in pigeon haemoglobin, and the stability of bovine globin is more than that of pigeon globin. However, from our conclusions, the weaker structure of the pigeon haemoglobin loosens the constraints of the T-structure. So in the case of an induced-fit model [21], this may explain the high oxygen affinity of avian haemoglobin.

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