

Enthalpy Changes of Dissociation of Hemoglobin Solution

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Heats of dilution of aqueous hemoglobin solutions were measured using a flow microcalorimeter at 298.15 ± 0.001 K. Thermodynamic quantities for the association-dissociation equilibrium of hemoglobin molecules were derived. In order to obtain values of the heats of dissociation ΔH and the dissociation constant K the treatment of data according to Eq. (5) was carried out using two different methods. In the first method ΔH value is estimated using the dissociation constant K as a reciprocal of the association constant previously determined by osmometry. In the second one the K and ΔH values are treated as adaptable parameters. The two sets of K and ΔH values so estimated are of comparable order. Furthermore, thermodynamic quantities for the association process of hemoglobin molecules were estimated and proved to be reasonable, compared with those reported by other investigators.

1. INTRODUCTION

It has been shown by a number of investigators¹⁻⁵⁾ that the hemoglobin molecule in very dilute aqueous solution dissociates into the $2\alpha\beta$ form from the original $\alpha_2\beta_2$ one, depending on the concentration of hemoglobin, temperature, and pH of the solution.

In our previous papers^{6,7)}, heats of solution and osmotic pressures were measured using a twin microcalorimeter and a membrane osmometer at 298.15 K, and thermodynamic quantities of association of hemoglobin molecules were reported. The equilibrium constant of association obtained was in good agreement with those reported by other investigators¹⁻⁵⁾ and all values of the heat of association were of comparable order. However, the value of the heat of association estimated from the heat of solution seems to be slightly large, compared with that characteristic for small molecules.

In order to check the reasonable values of the heat of dissociation of hemoglobin molecules, we constructed a flow microcalorimeter and measured the heat of dilution of an aqueous hemoglobin solution at 298.15 ± 0.001 K. We will report the enthalpy changes of dissociation of an aqueous

hemoglobin solution in this paper.

2. EXPERIMENTAL

2.1 Materials

The bovine hemoglobin sample, which is a mixture of methemoglobin and oxyhemoglobin, was purchased from Sigma Company (No. H-2500, U.S.A.). The solvents used are water and aqueous solutions containing 0.1 M NaCl and 0.1 M MgCl₂. The water was purified in the usual way.

2.2 Apparatus and Procedure

The calorimeter used in this study is a twin microcalorimeter similar to that of Wadsö⁸⁾ and the procedure was the same as that described for the LKB calorimeter.

3. RESULTS AND DISCUSSION

3.1 Heats of Dilution of Bovine Hemoglobin Solutions

Heats of dilution of bovine hemoglobin solutions were measured over the concentration range from 0.09 to 0.90 weight percent using a twin type microcalorimeter at 298.15 ± 0.001 K.

All systems proved to be endothermic, and the results obtained are shown in Table 1 and Fig. 1, where the heats of dilution per mole of hemoglobin are plotted against the concentration of hemoglobin. In Fig. 1, it is seen that the slope of the curve changes sharply in the concentration range

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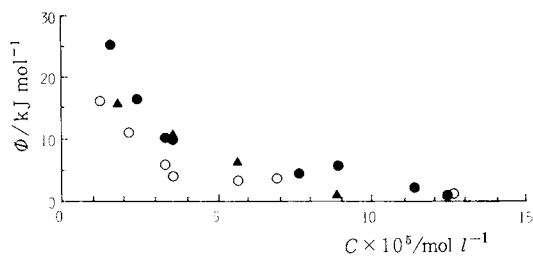


Fig. 1 Plots of the observed heats of dilution per mole of hemoglobin tetramer against the concentration of hemoglobin (mol l^{-1}).

○ : water ● : 0.1 M NaCl ▲ : 0.1 M MgCl_2

Table 1 Heats of dilution of bovine hemoglobin solution in various solvents at 298.15 K

solvent	(final conc.) $\times 10^5$ mol l^{-1}	Φ kJ mol^{-1}
water	1.30	16.38
	2.14	11.32
	3.30	6.32
	3.65	3.92
	5.75	3.29
	6.99	3.89
	12.66	1.42
0.1 M NaCl	1.66	25.41
	2.44	16.44
	3.70	9.93
	6.92	5.81
	7.79	4.69
	8.93	5.12
	11.55	2.22
	12.67	0.65
0.1 M MgCl_2	1.86	15.58
	3.58	10.64
	5.78	6.35
	9.09	0.89
	12.47	0.62

from 0.3 to 0.5 weight percent, indicating that the hemoglobin molecule may undergo a structural change in the form of a dissociation of the molecule. For the same dilute concentration regions a similar indication was obtained by osmotic pressure measurement⁶). Then, in the moderate concentration regions hemoglobin molecules can be considered to coexist in two forms, the original $\alpha_2\beta_2$ and $\alpha\beta$.

In this system, the heat of dilution is supposed to consist of two factors; the heat of dissociation

of hemoglobin molecules from $\alpha_2\beta_2$ to $2\alpha\beta$ upon dilution, and the heat necessary for diluting the hemoglobin solution. It is very difficult to measure the latter heat effect directly by calorimetry, since it is usually small compared with the heat effect going with a structural change such as a transition or a dissociation.

3.2 Thermodynamic Quantities

We shall derive the thermodynamic quantities of the association-dissociation equilibrium, assuming the hemoglobin molecule in the relatively moderate concentration range of 0.3 to 0.5 weight percent of hemoglobin to undergo an equilibrium as follows;



For this dissociation equilibrium, the equilibrium constant K is given by

$$K = \frac{[2(C - C_2)]^2}{C_2} \quad (2)$$

where C is a stoichiometric molar concentration and C_2 the concentration of $\alpha_2\beta_2$ (in $\text{mol} \cdot \text{l}^{-1}$). Then, from Eq. (2), C_2 can be expressed as

$$C_2 = \left(C + \frac{K}{8}\right) - \frac{1}{2} \left(CK + \frac{K^2}{16}\right)^{1/2} \quad (3)$$

On the other hand, the enthalpy change per mole of $\alpha_2\beta_2$ in solution, ΔH is given as;

$$\Phi = \frac{(C - C_2)}{C} \Delta H \quad (4)$$

where Φ is the observed heat of dilution and ΔH the heat of dissociation per mole of hemoglobin.

From Eqs. (2), (3) and (4), we can derive a following expression;

$$\Phi = \frac{1}{2C} \left[\left(CK + \frac{K^2}{16}\right)^{1/2} - \frac{K}{4} \right] \Delta H \quad (5)$$

According to Eq. (5), and using the value of K as the reciprocal of the association constant estimated from the osmotic pressure measurement previously⁶), ΔH values are adjusted so that the best fit between the observed heat and calculated one is secured, and are listed in Table 2. Φ versus C curves obtained by using the these K and ΔH values are shown as solid line in Fig. 2 and compared with experimental points. The agreement between the calculated and observed values is good

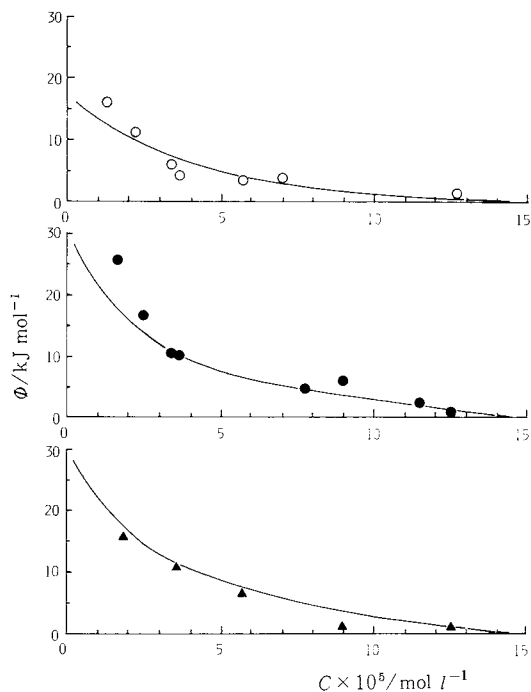


Fig. 2 ϕ curves calculated with Eq. (5) using the K value estimated from previous osmometric work⁶⁾.

○ : water ● : 0.1 M NaCl ▲ : 0.1 M MgCl₂

for concentrated hemoglobin solutions, but is not so good for dilute solutions.

In order to obtain a theoretical ϕ curve which reproduces the experimental points in the complete concentration region, K and ΔH are treated as adjustable parameters and adjusted so that the best fit between the calculated value and experimental points is obtained. The values of K and ΔH so obtained are also listed in Table 2 and the theoretical ϕ curves calculated with these K and ΔH values are plotted against the concentration of hemoglobin C as solid curves in Fig. 3.

It may be seen in Fig. 3 that the so calculated ϕ curves are in better agreement with the experimental data in the measured concentration range, than the theoretical ϕ curves calculated using the osmometric K value⁶⁾. As seen in Table 2, the K and ΔH values estimated by the two parameters fall in the same order of magnitude.

Table 3 shows the thermodynamic quantities for the dissociation-association equilibrium of hemoglobin molecules, where the free energy change ΔG and the entropy change ΔS for associ-

Table 2 Equilibrium constant K and enthalpy change ΔH for the association-dissociation equilibrium of hemoglobin at 298.15 K

solvent	K mol l^{-1}	ΔH kJ mol $^{-1}$
water	1.0×10^{-4} *	30**
	4.0×10^{-5}	40
0.1 M NaCl	3.3×10^{-5} *	60**
	5.0×10^{-5}	60
0.1 M MgCl ₂	6.7×10^{-5} *	50**
	6.0×10^{-5}	45

* This value expresses the reciprocal of the association constant obtained from osmometry previously⁶⁾.

** This value expresses the enthalpy changes calculated with Eq. (5) using the K value obtained from previous osmotic work.

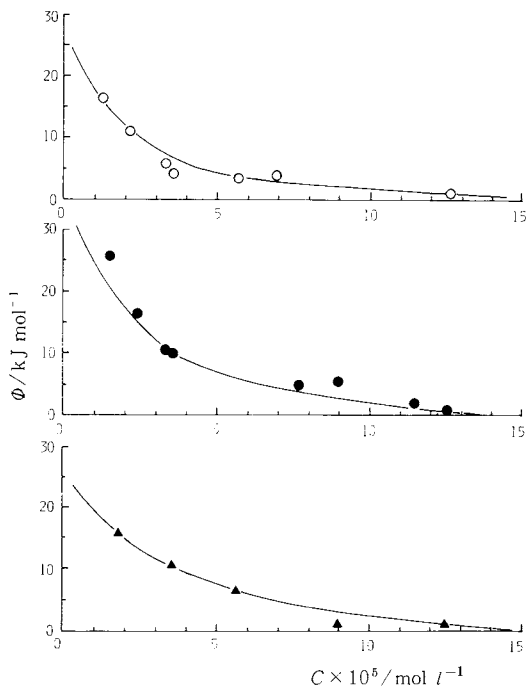


Fig. 3 ϕ curves calculated after fitting the data to Eq. (5).

○ : water ● : 0.1 M NaCl ▲ : 0.1 M MgCl₂

ation process have been calculated from the equations, $\Delta G = -RT \ln K$ and $\Delta S = (\Delta H - \Delta G)/T$ with the value of K and ΔH from the curve-fitting treatment described above.

Recently, these thermodynamic quantities were evaluated by treating of data of the heat of solu-

Table 3 Thermodynamic quantities of the association of hemoglobin molecule

Solvent	T K	K l mol ⁻¹	ΔG kJ mol ⁻¹	ΔH kJ mol ⁻¹	ΔS J K ⁻¹ mol ⁻¹	
water	298	1.0×10^4 ^a	-23 ^a	-30	-24	
	298	2.5×10^4	-25	-40	-50	
	298	3.0×10^4 ^b	-26 ^b	-30 ^b	-15 ^b	
0.1 M KCl	298	2.0×10^4 ^a	-25 ^a			
	298	3.0×10^4 ^b	-26 ^b	-30 ^b	-15 ^b	
0.1 M MgCl ₂	298	1.5×10^4 ^a	-24 ^a	-50	-88	
	298	1.7×10^4	-24	-45	-70	
	298	7.0×10^4 ^b	-28 ^b	-50 ^b	-75 ^b	
0.1 M NaCl	298	3.0×10^4 ^a	-26 ^a	-60	-115	
	298	2.0×10^4	-25	-60	-119	
	298	5.0×10^4 ^b	-27 ^b	-50 ^b	-77 ^b	
	294.5	6.0×10^4 ^c	-27 ^c	-121 ^c	-319 ^c	deoxyHb
0.2 M NaCl	293	2.0×10^5 ^d	-30 ^d	-55 ^d	-90 ^d	Hb.CO
1.0 M NaCl	298	1.0×10^5 ^b	-29 ^b	-50 ^b	-72 ^b	
	298	3.3×10^4 ^e	-26 ^e			Hb.CO
	293	5.0×10^4 ^f	-26 ^f			Hb.O ₂
3.0 M NaCl	298	1.0×10^6 ^b	-34 ^b	-50 ^b	-53 ^b	
0.1 M phosphate	288	2.0×10^5 ^g	-29 ^g	-55 ^g	-90 ^g	Hb.CO

a: ref. 6 b: ref. 7 c: ref. 1 d: ref. 3 e: ref. 4 f: ref. 5 g: ref. 2

tion as reported previously⁷⁾. The values from this evaluation are also included in Table 3. It is seen that agreement between K values from the heat of dilution estimated by two different methods in the presents works and that from the heat of solution reported previously⁷⁾ is fairly good, while, ΔH seems to scatter slightly between the different methods. Its values, however, are of comparable order of 10 kJ, and seem to be reasonable, compared with those reported by other investigators¹⁻⁵⁾.

On the other hand, the entropy changes for the association process have negative values in all cases as expected, and the considerable variation between these values may due to the salt effect surrounding hemoglobin molecule, which is difficult to make clear.

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