Microcalorimetric Studies of Hemoglobin

S. J. Gill*

This paper reviews calorimetric results obtained on ligand binding reactions to various hemoglobins. A special gas-liquid microcalorimeter has been developed for these studies. Both liquid-liquid and gas-liquid titrations can be performed with the system. Application of this method has been made to determine the heats of CO (g) and IHP (aq) binding to a variety of hemoglobins under various conditions. Results for human hemoglobins normal A and M Iwate show differences due to allosteric changes and proton release values. The heats measured are sensitive to buffer and counterion species. Proper cognizance of these effects is needed in order to interpret the measured heat values. Calorimetric gas titrations have also been made to examine the heat of CO (g) ligation as a function of extent of reaction. In the case of Hb A, no discernible differences are found between 5 to 95% degree of saturation. However, hemoglobin Trout I shows a marked dependence of the heat of reaction with degree of saturation. A full analysis of the calorimetric results of Trout I in conjunction with binding curve data has allowed an optimal determination of stepwise equilibrium constants and enthalpies of reaction for this hemoglobin.

I. INTRODUCTION

The numerous biochemical studies of respiratory proteins is not accidental. Oxygen binding proteins are widely distributed in a vast number of biological species. This broad availability is ideal for general comparative function-environment studies. However, it is the reversible oxygen binding capability that enables precise thermodynamic studies to be made in ways that are unparalleled in biochemistry. Because the chemical potential of gases is accurately measurable, binding curves can be determined with high precision. Such data led to the allosteric concept[1] and linked function principles[2,3]. Several reviews have outlined many results[4,5], but calorimetric studies of basic reactions have only recently started to add to the overall thermodynamic picture.

The allosteric regulatory properties of hemoglobin have motivated many physical studies of this molecule. As a "molecular transistor" chemical information is controlled through the chemical potentials of oxygen, hydrogen ion, carbon monoxide, diphosphoglycerate, and possibly other chemical species as well. The mode of information transmission between various binding sites is a key question in the function of hemoglobin. Oxygen binding studies on single α and β chains of Hb A[6] in comparison with the tetramer ($\alpha_2\beta_2$) of Hb A[7] show that the first steps of binding have greatly reduced affinities in the tetramer form. The tetramer also shows cooperative binding and Bohr effect behavior. Structurally linked interactions depend upon the state of ligation between various binding sites. This result is born out in ligand linked dissociation of hemoglobin tetramers to dimers[8,9] and the direct influence of such linkage upon oxygen binding curves at different concentrations[10] where $\alpha_2\beta_2$ or $\alpha\beta$ forms are emphasized. Thermodynamic studies of subunit assembly[9] reveal extremely strong enthalpy and entropy dependences upon the state of oxygenation. Proton release values[11] for similar assembly processes also show a strong dependence on the state of ligation. Such detailed studies on the consequences of interactions between the component chains provide insight into the nature of ligand linked interactions.

Certain species of hemoglobin show extreme effects of subunit interactions through ligand linked association of chains and aggregation of tetramers. Lamprey hemoglobin exists[12] in the oxygenated form primarily as single-chain mole-

* Department of Chemistry
University of Colorado
Boulder, Colorado 80309

cules, and in the unligated forms as dimers and tetramers. A particularly important example of this effect is known in Hb S gelation upon deoxygenation. The effect of aggregation on Hb S oxygen binding curves is a complex function of concentration[13]. The heat of gelation at various temperatures of deoxygenated Hb S[14] has revealed the hydrophobic nature of this reaction process.

The cooperative binding properties of heme proteins can be analyzed in most detailed terms for non-dissociating, non-aggregating systems. In such cases the ligand binding process can be described in stoichiometric stepwise reactions. Equilibrium constants were first obtained by Roughton *et al.*[15] for sheep hemoglobin as a function of temperature and stepwise enthalpies estimated. The extensive studies by Japanese workers[7,8,16,17] have also permitted analysis in terms of stepwise reactions. The temperature dependence[18] and the pH dependence[19] of equilibrium constants provide details about the stepwise heats and proton release values of the reaction with oxygen for Hb A. Calorimetric measurements on Hb A[20,21] have not revealed the enthalpic differences for the heats of successive binding steps evaluated by van't Hoff procedures[18]. Direct proton release measurements[22] as a function of oxygen binding are not consistent with values derived from pH dependence of successive equilibrium constants[19]. This situation is particularly troubling since details of the stepwise reactions provide crucial information on allosteric phenomena. The high cooperativity places extreme demands on the various experiments. Furthermore, in the case of calorimetric studies of the ligand binding reaction, the heat effect is a function of species concentrations and proton release values coupled with buffer heat effects. Thus comparison with heats derived from temperature dependence of equilibrium constants would generally require accurate information about stepwise Bohr effects.

The motivation for applying microcalorimetric techniques to study the processes associated with ligand binding to hemoglobin is to obtain direct information about the energetics of these processes. Special microcalorimeters have been required. The selection of various hemoglobins has enabled unusual properties to be characterized. Not only do the results provide insight into the molecular complexity of various oxygen carrying molecules, but the results also can often reveal the reason for the physiological advantage that certain species of hemoglobin have for particular environments and functions.

II. GAS-LIQUID MICROCALORIMETRIC SYSTEM

A gas-liquid microcalorimeter[23] in our laboratory has undergone various improvements over ten years[21,24] and is shown in its present form in Fig. 1. The operation is briefly described as follows. The reaction cell is thermally isolated by dynamically controlled adiabatic shields and is maintained at a temperature constant to within a microdegree. Any heat effect in the cell is compensated and recorded electronically. The cell is disk shaped and has a volume of approximately 15 cm^3. This volume is connected by stainless steel tubes, which pass through the inner shields, to an electronic differential manometer (Validyne Engineering Corp., Northridge, California) and to the gas inlet valve. The manometer has a sensitivity approaching one microbar. The valve is shown in the configuration for loading the cell with hemoglobin solution. To start a gas binding reaction, the reactant gas space is filled with gas and the valve rotated to bring that space over the filling tube. The gas then diffuses down the filling tube to the cell and consumption is measured as a pressure decrease. This pressure response is then calibrated by a precision syringe.

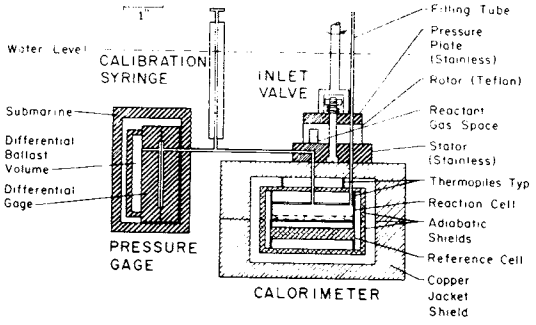

Fig. 1. Gas-liquid microcalorimetric system showing thermostated elements including calorimeter, inlet valve, calibration syringe, and differential manometer. This diagram shows essential instrument features and does not include electrical or gas tubing details.

The instrument can be run in either a constant pressure mode by adjusting the precision syringe as the gas reaction proceeds or in a constant volume mode by following the pressure change of the manometer.. In general the latter is more precise and convenient. In the constant volume mode the measured heat is the internal energy change ΔE and the enthalpy change ΔH is given by $\Delta H = \Delta E - fRT$, where f is the fraction of the total gas volume inside the reaction cell ($f = 0.9$).

Special care is taken to equilibrate the reacting gas with water vapor in the diffusion chamber connected to the calorimeter cell. When CO was used it was bubbled through alkaline dithionite solution in order to remove traces of oxygen[25]. An auxiliary electrical heater inserted into the calorimeter was used to check the linearity and accuracy of the calorimetric system. The calibrations showed that quantities of heat observed in the gas reaction experiments could be measured to within 1% accuracy. In addition the calorimeter has been calibrated to this accuracy by tests with the CO_2 reaction with NaOH.

The reacting gas is allowed to flow continuously into the calorimeter cell once the reaction begins. The resulting heat effect and electrical compensation are not simultaneous with the observed pressure changes. This lag in time between the measurement of heat and the pressure change is not of any consequence when total heats and total moles of reacting gas are measured, but it is important if we want to compare the heats of reaction per mole of gas reacted along the course of the reaction. In order to bring the heat effect into proper time correspondence with the pressure effect it is necessary to know the time response of the calorimeter to an instantaneous heat burst. The response was found to rise very rapidly and then decay quite slowly with a single exponential with a time constant $\tau = 20-30$ s. It can then be shown that an arbitrary input function of heating rate $I(t)$ is simply related to the observed output heating rate $\theta(t)$ by:

$$I(t) = \theta(t) + \tau \, \mathrm{d}\theta(t)/\mathrm{d}t \qquad (1)$$

Thus, the input heating rate caused by the reacting gas can be determined from the output heating rate as a function of time. From $I(t)$ values the amount of heat can be determined for a particular time interval of a measured pressure change (or moles of gas reacted). In this way a table of heats per mole of gas reacted can be generated from the experimental data.

Although this instrument was originally designed for gas-liquid studies it has proven to be conveniently adapted for liquid-liquid reactions. A teflon tube for the addition of titrant was inserted through the filling tube shown in Fig. 1. A long tube filled with nitrogen vented the system so that atmospheric oxygen could not diffuse into the reaction cell during the titration time nor would there be any pressure increase upon titrant addition.

The titrant contained in that part of the teflon tube which was inside the calorimeter was thermally equilibrated through the physical contact of the teflon and filling tubes. It was observed that as much as 20 mm^3 of solution could be added to the reaction chamber without a noticeable heat effect due to insufficient thermal equilibration. The size of a typical aliquot of titrant was 10 mm^3 and the time between additions was two minutes, which is the time for the heat effect to be fully measured. In order to prevent the reaction from starting at the time the teflon tube was inserted into the hemoglobin solution, the end of the tube contained a gas lock of 2 mm^3 of nitrogen. It was observed that the introduction of nitrogen in this manner produced no measurable heat effect.

III. APPLICATION OF THERMAL TITRATIONS

1. Human hemoglobins

Calorimetric results obtained with normal human hemoglobin A and the mutant hemoglobin M Iwate serve to illustrate several factors that contribute to heats of reaction of small molecules, specifically carbon monoxide and inositol hexaphosphate to hemoglobin. The mutant hemoglobin M Iwate has proximal histidines in the α chains replaced by tyrosine[26,27]. This substitution renders the heme groups on the α chains readily oxidized and under normal conditions only the two β chain heme groups combine with oxygen or carbon monoxide[28-32]. Proton nmr[33] and X-ray crystallographic[34] studies of this half oxidized hemoglobin suggest that it exists in a T-like state and

that ligand linked structural changes are slight. The similarity of the enthalpy changes for the binding of carbon monoxide to hemoglobin M Iwate and non-allosteric heme proteins supports this idea of minor ligand linked structural changes[21].

The overall reactions can be conducted in several ways as represented in Fig. 2 for hemoglobin A. The four reactions of this scheme yield measurable enthalpy changes that permit a test of cyclic thermodynamic consistency. The general reaction scheme for binding two molecules of CO and one molecule of IHP to Hb M Iwate is similar to Fig. 2 but appropriately modified to account for two heme reaction sites. Again the results of this set of four calorimetric experiments can be tested for thermodynamic consistency. Typical IHP titration results are shown in Fig. 3 for Hb A and Hb M Iwate. The heats of titration are sensitive to the state of CO. Virtually no heat is observed for CO ligated Hb A, whereas a near normal heat of IHP binding is seen for CO ligated Hb M Iwate. This serves to indicate that the IHP allosteric binding site is strongly changed in Hb A but not in Hb M Iwate upon CO ligation. In terms of T and R formalism the IHP binds to the T form but not the R. Thus in Hb M Iwate the T form persists even upon CO ligation. Crystal structural studies by Greer[33] led to a similar conclusion some years ago, but the thermal titrations confirm that the T form persists in solution as well as solid.

The CO(g) thermal titrations complete the set of reactions of Fig. 2. Typical data for the Hb M Iwate case is shown in Fig. 4 where the heat per mole of CO(g) reacted is plotted at different extent of reactions. There is no indication that the heat changes upon degree of saturation. Similar

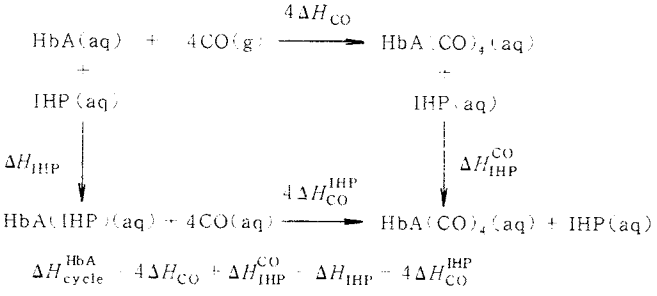

Fig. 2. Cyclic reaction equations for the respective CO IHP binding experiments to Hb A.

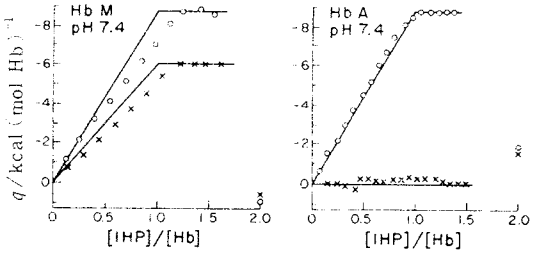

Fig. 3. Results of calorimetric titrations of Hb M Iwate and Hb A with IHP. Hemoglobin concentration 1–5 millimolar heme. 25°C. Buffer is pH 7.4 bis-tris 0.2 M, pH adjusted with $HClO_4$. Circles and crosses represent the baseline corrected heat of titration per mole Hb tetramer in absence of CO and in the presence of CO, respectively. The magnitude of the baseline corrections are shown at (IHP)/(Hb) = 2 by × with CO and ○ without CO.

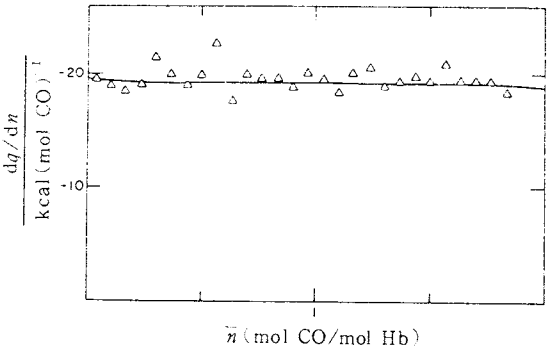

Fig. 4. Plot of differential heat dq/dn of CO binding by the $\alpha_2^{Mmet}\beta_2$ form of hemoglobin M Iwate as a function of the average number $\bar{n}$ of moles of CO bound per mole of hemoglobin. Measurements were performed at 25°C and pH 7.4 in 0.2 M bis-tris perchlorate buffer. The sample was 6 ml of solution ~1.7 mM in heme[20].

Table I.

Heats of Reaction of CO(g) and IHP(aq) with Hb A at 25 °C

pH	Buffer (0.2M)	ΔH_{CO}/kcal mol^{-1}	ΔH_{IHP}^{CO}/kcal mol^{-1}	ΔH_{IHP} kcal mol^{-1}	ΔH_{CO}^{IHP}/kcal mol^{-1}	$\Delta H_{cycle}^{Hb\ A}$/kcal mol^{-1}
8.5	Tris perchlorate	−21.4 ± 0.4	0 ± 0.2	+0.3 ± 0.2	−22.2 ± 0.4	+2.9 ± 3.6
7.4	Bis-tris perchlorate	−19.4 ± 0.4	0 ± 0.2	−8.8 ± 0.6	−17.3 ± 0.4	+0.4 ± 4.0
7.4	None (deionized)	−22.6 ± 0.4	+3.8 ± 0.2	−14.5 ± 0.5	−17.4 ± 0.4	−2.3 ± 4.0
7.4	None (0.1 M NaCl)	−19.8 ± 0.4	+2.8 ± 0.2	−6.5 ± 0.8	−17.0 ± 0.4	−1.6 ± 4.0

Heats of Reaction of CO(g) and IHP(aq) with Hb M Iwate ($\alpha_2^{Mmet}\beta_2^{deoxy}$) at 25 C

pH	Buffer (0.2M)	ΔH_{CO}/kcal mol^{-1}	ΔH_{IHP}^{CO}/kcal mol^{-1}	ΔH_{IHP}/kcal mol^{-1}	ΔH_{CO}^{IHP}/kcal mol^{-1}	$\Delta H_{cycle}^{Hb\ A}$/kcal mol^{-1}
8.5	Tris perchlorate	−18.4 ± 0.5	+0.7 ± 0.1	+1.1 ± 0.1	−18.7 ± 0.5	+0.2 ± 2.2
7.4	Bis-tris perchlorate	−19.7 ± 0.5	−5.7 ± 0.2	+8.7 ± 0.4	−18.7 ± 0.5	+1.0 ± 2.6

observations are found with Hb A[20] over the accessible range of 5 to 95% saturation. The average heat per mole CO(g) can be determined from the total heat and total moles reacted. In Table I are summarized data taken for Hb A and Hb M Iwate at pH 7.4 and 8.5. One notes that cyclic consistency is realized within experimental error. Furthermore the heat of IHP reaction to CO ligated Hb A is not detectable. The pH dependence of the results is complicated by the presence of different buffers and the actual pH used.

The observed heats of ligation (ΔH_{obs}) are sensitive to the amount of protons (ΔN_H^+) released in a particular reaction through the heat released for the buffering reaction where ΔH_{buff} is the heat of protonization of buffer. The heat of reaction with changes in bound ions is ΔH_{ion}. The sum of these effects determines the solution corrected heat of reaction (ΔH_{BC}) by

$$\Delta H_{obs} = \Delta H_{BC} + (\Delta N_H^+)\,\Delta H_{buff} + \Delta H_{ion} \quad (2)$$

Plots of ΔH_{obs} versus ΔH_{buff} serve to identify the magnitude of proton release and ion heat effects involved in various ligation processes.

An extensive set of experiments similar to those detailed in Table I on Hb A has been performed to explore these various contributions for reactions of CO(g) and IHP. Results at pH 7.4 in 0.1 M NaCl for three buffered and one unbuffered solution are shown in Fig. 5a and 5b. The solid line is drawn on the basis that the slope is determined by proton release values as given by Kilmartin[35] and Brygier *et al.*[36] and the reference state is the 0.1 M NaCl unbuffered hemoglobin solution. The deviation from the theoretical line indicates differential ion heats. One sees that in the cases of bis-tris and tris buffers these are negligible. The maleate and phosphate buffers make noticeable contributions particularly upon IHP binding (12–14 kcal mol^{-1}). Separate experiments of CO ligation to deionized hemoglobin show that ΔH_{ion} for 0.1 M NaCl is 3 kcal (mol CO)$^{-1}$.

For the case of IHP ligation value of −24 kcal (mol IHP)$^{-1}$ is found for $\Delta H_{BC} + \Delta H_{ion}$ at pH 7.4 in 0.1 M chloride solution. A value of ΔN_H^+ is estimated from heats in different buffers as +2.1 mole proton/mol IHP. The value of ΔH_{ion} due to the 0.1 M chloride is +8 kcal (mol IHP)$^{-1}$ under these conditions. This gives ΔH_{BC} as −30 kcal (mol IHP)$^{-1}$. In terms of the number of protons absorbed in this reaction process the heat per mole of protons [−14 kcal (mol protons)$^{-1}$] is approaching that found for the heat of protonization of amine and imidizole groups. This suggests that organic phosphates such as diphosphoglycerate and IHP owe their specific and strong binding to the allosteric site of hemoglobin by involvement of the proton reaction to amine groups through the large endothermic effect.

2. Trout hemoglobin

An unusual absence of pH and salt effects, as noted for human hemoglobins, was discovered by Binotti *et al.*[37] for the first chromatographically separated component of *Salmo irideus* designated as hemoglobin Trout I. Practically no effect of temperature was found upon the value of the mid-saturation pressure. This combination of properties

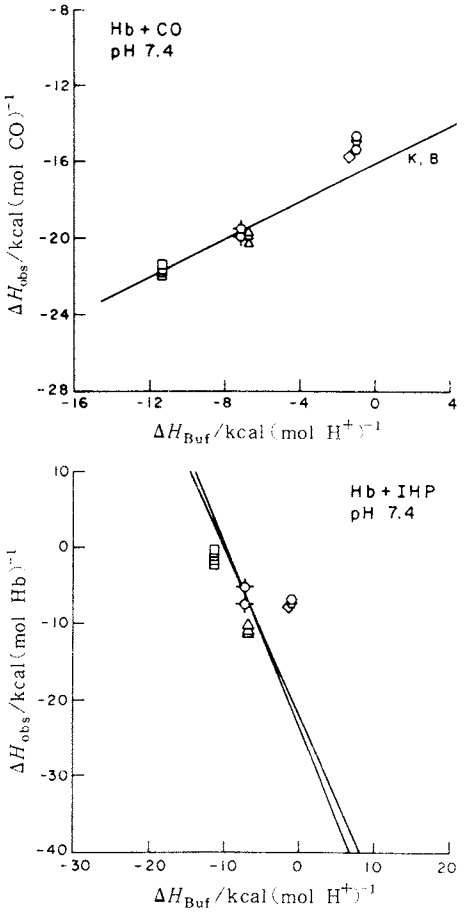

Fig. 5. Plots of heats of reaction vs. heat of protonization of buffers at 25 °C, pH 7.4 and 0.1 M Cl^-: a. Hb + CO, b. Hb + IHP. Buffer symbols are Tris- □, bis-tris-△, 0.1 M NaCl -◇-, maleate- ◇, and phosphate- ○. The lines are based on data from Kilmartin[35] and Brygier[36]; slopes are labeled K and B.

has led to a collaborative effort to obtain both detailed calorimetric and binding curve measurements on this unusual hemoglobin[38]. Carbon monoxide was used as the gaseous ligand in order to minimize oxidation effects and to optimize the calorimetric titrations by use of a high affinity reaction. The results of the heat produced at different extents of reaction are illustrated in Fig. 6. Identical thermal titrations were obtained using buffers of bis-tris or maleate. The striking feature of these measurements is the extreme sensitivity of the heat effect to the extent of reaction. The reaction is initially endothermic. No

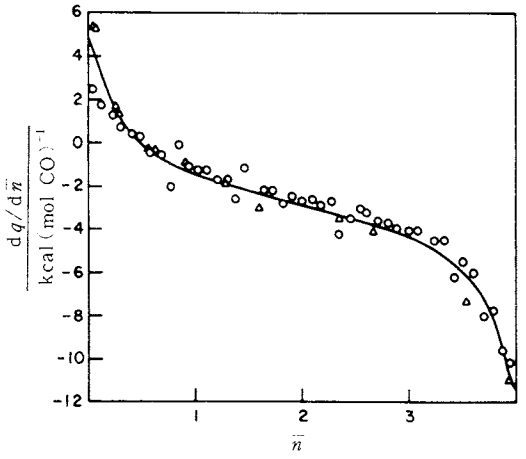

Fig. 6. Differential heat of CO binding to Trout Hb I at 25 °C as function of average number $\bar{n}$ of CO molecules bound per tetramer. Circles and triangles denote experiments in 0.05 M bis-tris chloride, pH 7.4, and 0.2 M sodium maleate, pH 7.1, buffers respectively. The smooth curve results from simultaneous analysis of all data, both isotherm and calorimetric.

other gaseous ligand binding reaction to hemoglobin has been observed to absorb heat. At approximately fifteen percent bound carbon monoxide the heat of reaction is zero. From that point on the reaction is increasingly exothermic.

The heat of reaction $\Delta\bar{H}$ determines the temperature dependence of the equilibrium free ligand concentration, x, at any given degree of saturation $\bar{y}$:

$$\left(\frac{\partial \ln x}{\partial T}\right)_{\bar{y}} = -\frac{\Delta\bar{H}}{RT^2} \qquad (3)$$

One sees that where the reaction is endothermic the binding curve will be shifted to larger x values upon lowering the temperature and where the reaction is exothermic the binding curve will be shifted to smaller x values upon lowering the temperature. In other words the binding curve should become steeper as the temperature is lowered. At the point of zero heat of reaction the binding curves at different temperatures should cross. The results of a set of experiments by Giardini and Brunori[36] in Rome indeed verified this as shown in Fig. 7. The combination of the binding curve and calorimetric data was first analyzed by simple curve generation on the basis

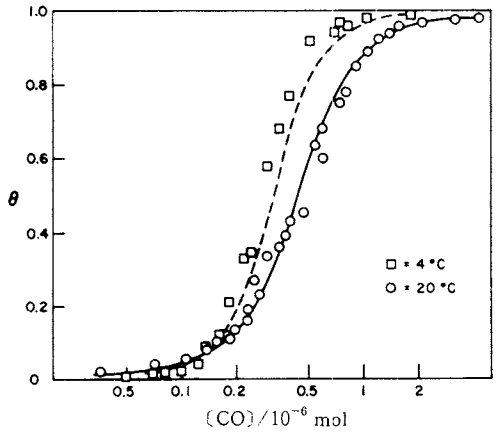

Fig. 7. CO-binding isotherms of Trout Hb I in 0.1 M sodium phosphate buffer, pH 6.8, at 4°C and 20°C. The smooth curves result from the overall data fitting procedure.

of the MWC[39] theory and showed that the allosteric transition enthalpy must be nearly 30 kcal endothermic. The data has also been subject to a rigorous non-linear least square treatment[40] based upon either the Adair four step reaction process[41] or the MWC allosteric reaction scheme.

The results of this analysis for the ith Adair reaction,

$$Hb(CO)_{i-1}(aq) + CO(aq) \rightarrow Hb(CO)_i(aq) \qquad (4)$$

are shown in Table II. The four free energy changes and four enthalpy changes were resolved to satisfactory error limits. The enthalpy and entropy changes largely compensate for each other. Initially the reaction is entropically driven and finally is enthalpically driven.

Analysis using the MWC model depicted as

$$\begin{array}{ccccccccc} Hb^T & \xrightarrow[\Delta H_T]{k_T} & Hb^T X & \rightarrow & Hb^T X_2 & \rightarrow & Hb^T X_3 & \rightarrow & Hb^T X_4 \\ \downarrow L & \Delta H & \updownarrow & & \updownarrow & & \updownarrow & & \updownarrow \\ Hb^R & \xrightarrow[\Delta H_R]{k_R} & Hb^R X & \rightarrow & Hb^R X_2 & \rightarrow & Hb^R X_3 & \rightarrow & Hb^R X_4 \end{array} \qquad (5)$$

yields the results shown in Table III. The allosteric transition involves a large endothermic enthalpy change that is largely compensated by large positive entropic effects. As an aside the fit to the six parameter MWC case was statistically inferior to fit obtained with the eight parameter Adair case. The free energy diagram for the MWC case is shown in Fig. 8.

The thermodynamic data available for this system allows prediction of the temperature dependence of the slope of the binding curve at any given extent of reaction. The most familiar parameter related to the slope is the Hill coefficient[42] at fifty percent saturation. Values of this parameter have been calculated from the thermodynamic quantities of the MWC and Adair schemes and are shown plotted in Fig. 9. The two are virtually equivalent until high temperatures (~75°C) are reached where the Adair scheme allows for the non-cooperative situation of a Hill coefficient less

Table II. Adair Stepwise Thermodynamic Parameters for Reaction of Hemoglobin Trout I with CO at 25°C

Adair Step	$\Delta G°$/kcal mol^{-1}	$\Delta H°$/kcal mol^{-1}	$T\Delta S°$/kcal mol^{-1}	$\Delta S°$/cal K^{-1} mol^{-1}
1[a]	−8.42 ± .07	4.85 ± .35	13.27 ± .36	44.5 ± 1.2
2	−8.30 ± .09	.10 ± .74	8.40 ± .75	28.2 ± 2.5
3	−8.97 ± .09	−5.57 ± .76	3.40 ± .77	11.4 ± 2.6
4	−8.80 ± .05	−11.36 ± .39	−2.56 ± .39	−8.6 ± 1.3
1[a,b]	−7.60 ± .07	4.85 ± .35	12.45 ± .36	41.8 ± 1.2
2	−8.06 ± .09	.10 ± .74	8.16 ± .75	27.4 ± 2.5
3	−9.21 ± .09	−5.57 ± .76	3.64 ± .77	12.2 ± 2.6
4	−9.62 ± .05	−11.36 ± .39	−1.74 ± .39	−5.8 ± 1.3

[a] The Adair reaction i cited here is defined as
$Hb(CO)_{i-1}(aq) + CO(aq) \rightleftarrows Hb(CO)_i(aq)$

[b] In these reactions the statistical entropic term equal to $-R \ln(\frac{5-i}{i})$ has been subtracted from all appropriate quantities.

Table III. Monod-Wyman-Changeux Thermodynamic Parameters for Reaction of of Hemoglobin Trout I with CO at 25°C

Reaction	ΔG°/kcal mol^{-1}	ΔH°/kcal mol^{-1}	$T\Delta S^\circ$/kcal mol^{-1}	ΔS°/cal K^{-1} mol^{-1}
$T_o \rightleftarrows R_o$	4.29 ± .18	29.44 ± 2.15	25.15 ± 2.16	84.4 ± 7.2
T-ligation[a]	−7.68 ± .06	3.67 ± .39	11.35 ± .39	38.1 ± 1.3
R-ligation[a]	−9.70 ± .04	−10.42 ± .54	−.72 ± .54	−2.4 ± 1.8

[a] The thermodynamic parameters in these rows refer to the reaction of a molecule of CO with a single arbitrary site on a T- or R-conformation hemoglobin molecule.

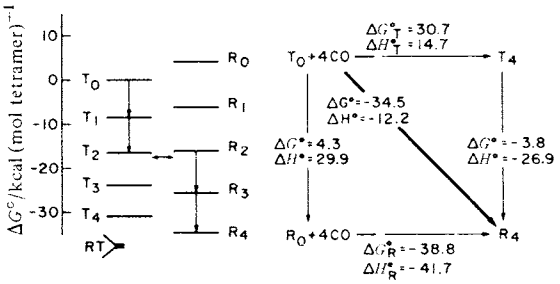

Fig. 8 (left). Free energy manifolds for CO binding by Trout Hb I according to the MWC model. The manifolds are equal in energy crossover at $i \simeq 2.1$ and are symmetrically disposed one to another.

Fig. 8 (right). Thermodynamic cycle for CO binding and associated allosteric transitions by hemoglobin trout I at 25°C. For visual clarity, entropy changes are omitted. The bold faced arrow indicates the main course of the ligation reaction as experimentally observed.

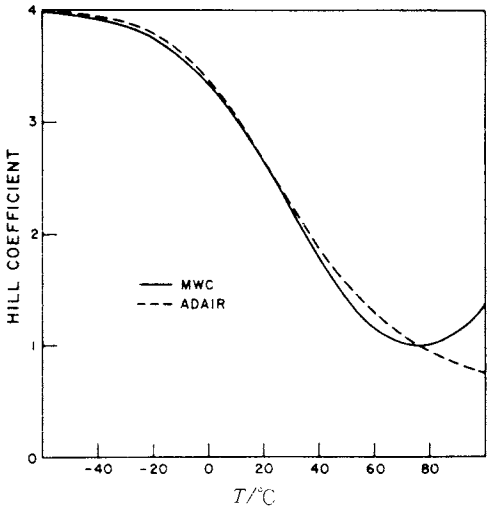

Fig. 9. Temperature dependence of the Hill coefficient for CO binding of hemoglobin trout I. The solid line is that predicted by the MWC model while the broken line is the prediction of the Adair stepwise reaction scheme. In both cases the thermodynamic parameters used are those determined from the experimental data and listed in Tables II and III.

than unity whereas the MWC case switches the role of the T and R forms at the minimum value of the Hill coefficient of unity. This later prediction is a general consequence of allosteric reaction properties[43]. Unfortunately the high temperature makes it impractical to explore this behaviour with the Trout I hemoglobin system.

The increased cooperativity as the temperature is lowered is highly suggestive as a factor conferring physiological advantage to the trout in its natural environment of low temperature water. The oxygen delivery capacity is increased for a given oxygen partial pressure change at low temperatures in order to overcome various kinetic effects in the fish. This hemoglobin component thus serves as a finely tuned temperature sensitive molecular transducer that adjusts oxygen transport efficiently to the low temperature natural surroundings. A second component, Trout Hb IV, shows extremely sensitive pH dependent properties that have formed the basis of a floatation control system[44]. Our understanding of such systems is clearly enhanced through the detailed knowledge of the thermodynamics of the binding reactions.

Conclusion:

The combination of various physico-chemical techniques such as microcalorimetric, pH, and binding curve titrations provide the fundamental data for exploring thermodynamic properties of allosteric proteins. Such properties determine the chemical operational characteristics of these inter-

esting molecular devices. Hemoglobin has been a particularly useful protein in these studies since it has highly desirable physical chemical properties and exists in a wide diversity of environments.

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