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Calculation of thermodynamic properties of species from binding of a ligand by a macromolecule

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Abstract

In the absence of experimental methods for determining concentrations of species in protein-ligand binding, it is not possible to determine the thermodynamic properties of species directly. However, this article on a simple reaction system shows that measurements of the average number of oxygen molecules bound at various T, pH and concentrations of molecular oxygen can be used to calculate thermodynamic properties of species. The simple system considered has some of the characteristics of the binding of oxygen by hemoglobin, but it has been simplified so that the method for obtaining thermodynamic information can be clarified. A table of standard thermodynamic properties of species is the most efficient way to store thermodynamic information on a reaction system. All the standard further transformed thermodynamic properties at specified T, pH and concentrations of molecular oxygen, all the standard transformed thermodynamic properties at specified T and pH, and all the standard thermodynamic properties of species at a specified temperature can be calculated. These calculations are based on the fact that the mathematical function for the standard further transformed Gibbs energy of the system contains all the thermodynamic information on the system. These properties are all interrelated by Maxwell equations.

Keywords: Ligand binding; Thermodynamic properties; Transformed thermodynamic properties; Hemoglobin; Gibbs energies; Entropies

1. Introduction

The previous article [1] dealt with the fundamental equations for the Gibbs energy G, transformed Gibbs energy G', and the further transformed Gibbs energy G'' for protein-ligand binding and emphasized that it is the Maxwell relations that provide the connection between observables and standard thermodynamic properties of reactants. In that article various thermodynamic properties of a simple protein-ligand system were calculated from pKs, a chemical equilibrium constant and corresponding standard enthalpies of reaction. It was pointed out that it is more difficult to calculate species properties from experimental values of standard further transformed properties and standard transformed properties than to calculate properties of pseudoisomer groups from species properties. The calculation of thermodynamic properties of a system at a specified pH and

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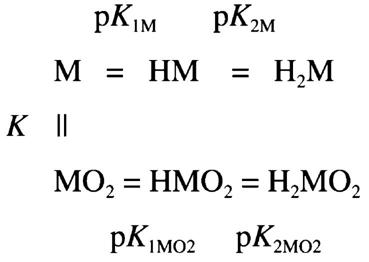
concentrations of molecular oxygen requires the use of two Legendre transforms. The current article is about calculations of species properties from experimental values of average numbers of oxygen molecules bound.

The simple reaction system considered is



$$\begin{array}{ccccc} & \mathrm{p}K_{1\mathrm{M}} & & \mathrm{p}K_{2\mathrm{M}} & \\ \mathrm{M} & = & \mathrm{HM} & = & \mathrm{H_2M} \\ K \;\| & & & & \\ \mathrm{MO_2} & = & \mathrm{HMO_2} & = & \mathrm{H_2MO_2} \\ & \mathrm{p}K_{1\mathrm{MO2}} & & \mathrm{p}K_{2\mathrm{MO2}} & \end{array}$$

where $K = [\mathrm{M}][\mathrm{O_2}]/[\mathrm{MO_2}]$. This reaction system has some of the characteristics of the hemoglobin–oxygen system, but it is not meant to be a model of that system that involves in addition the successive binding of four molecules of oxygen and the partial dissociation of the tetramer into dimers. This simple system is used to show how important thermodynamic information can be obtained. This simple reaction system was chosen because Antonini, Wyman, Brunori, Fronticelli, Bucci and Rossi-Fanelli [2] made a very thorough study of the equilibrium constants and enthalpies of reaction involved in human and horse hemoglobin. This system can be referred to as $\mathrm{H_{av}MO_{2av}}$, where the subscript av refers to the average numbers of hydrogen ions and molecules of oxygen bound by the macromolecule M. When the pH is specified, the criterion for spontaneous change and equilibrium is provided by the transformed Gibbs energy G' that is defined by a Legendre transform of the Gibbs energy [3,4]. When the equilibrium concentration of molecular oxygen is specified in addition, a second Legendre transform is used to define a further transformed Gibbs energy G'' [5]. When a system like this is studied at a series of temperatures, pHs and concentrations of molecular oxygen, all the thermodynamic information about reactants and species can be obtained from the standard further transformed Gibbs energy of formation $\Delta_f G''^\circ$ of $\mathrm{H_{av}MO_{2av}}$. Further information on transformed thermodynamic properties is given in [6].

2. Derivation of a convenient expression for the standard further transformed Gibbs energy of a system at specified temperature, pH and concentration of molecular oxygen

The standard further transformed Gibbs energy of formation of $\mathrm{H_{av}MO_{2av}}$ is calculated from the standard further transformed Gibbs energies of formation of $\mathrm{H_{av}M}$ and $\mathrm{H_{av}MO_2}$ by using

$$\Delta_f G''^\circ(\mathrm{H_{av}MO_{2av}}) = -RT\ln\{\exp(-\Delta_f G''^\circ(\mathrm{H_{av}M})/RT) + \exp(-\Delta_f G''^\circ(\mathrm{H_{av}MO_2})/RT)\} \quad (2)$$

The symbols used here are the same as in the previous paper [1], which includes a Glossary. It is convenient to write this equation in terms of the hydrogen ion binding polynomials (partition functions) for $\mathrm{H_{av}M}$ and $\mathrm{H_{av}MO_2}$ that are given by [7]

$$P_\mathrm{M} = 1 + 10^{-\mathrm{pH}+\mathrm{p}K_{1\mathrm{M}}} + 10^{-2\mathrm{pH}+\mathrm{p}K_{1\mathrm{M}}+\mathrm{p}K_{2\mathrm{M}}} \quad (3)$$

$$P_{\mathrm{MO_2}} = 1 + 10^{-\mathrm{pH}+\mathrm{p}K_{1\mathrm{MO_2}}} + 10^{-2\mathrm{pH}+\mathrm{p}K_{1\mathrm{MO2}}+\mathrm{p}K_{2\mathrm{MO_2}}} \quad (4)$$

The pKs are functions of ionic strength in addition to temperature, but the use of the extended Debye–Huckel theory is avoided here as a simplification.

The standard further transformed Gibbs energy of formation of $\mathrm{H_{av}M}$ is given by

$$\Delta_f G''^\circ(\mathrm{H_{av}M}) = \Delta_f G'^\circ(\mathrm{H_{av}M}) = \Delta_f G^\circ(\mathrm{M}) - RT\ln P_\mathrm{M} \quad (5)$$

where $\Delta_f G'^\circ(\mathrm{H_{av}M})$ is the standard transformed Gibbs energy of formation of the pseudoisomer group (M, HM and $\mathrm{H_2M}$) and $\Delta_f G^\circ(\mathrm{M})$ is the standard Gibbs energy of formation of the species M. The standard further transformed Gibbs energy of $\mathrm{H_{av}M}$ is equal to the standard transformed Gibbs energy of $\mathrm{H_{av}M}$ because this pseudoisomer group does not contain bound oxygen. Note that $\Delta_f G^\circ(\mathrm{M}) = 0$ at each temperature by convention.

The standard further transformed Gibbs energy of formation of $\mathrm{H_{av}MO_2}$ is given by

$$\Delta_f G''^\circ(\mathrm{H_{av}MO_2}) = \Delta_f G'^\circ(\mathrm{H_{av}MO_2}) - \Delta_f G(\mathrm{O_2,aq}) = \Delta_f G'^\circ(\mathrm{H_{av}MO_2}) - \Delta_f G^\circ(\mathrm{O_2,aq}) - RT\ln[\mathrm{O_2}] \quad (6)$$

The standard transformed Gibbs energy of formation of $H_{av}MO_2$ can be written in terms of its hydrogen ion binding polynomial as follows:

$$\Delta_f G'^\circ(H_{av}MO_2) = \Delta_f G^\circ(MO_2) - RT\ln P_{MO_2} \tag{7}$$

Substituting this equation into Eq. (6) yields

$$\Delta_f G''^\circ(H_{av}MO_2) = -RT\ln([O_2]P_{MO_2}/K) \tag{8}$$

since $-RT\ln K = \Delta_f G^\circ(O_2,aq) - \Delta_f G^\circ(MO_2)$. The expression for $\Delta_f G''^\circ(H_{av}MO_{2av})$ is obtained by substituting Eq. (5) and Eq. (8) in Eq. (2):

$$\begin{aligned}\Delta_f G''^\circ(H_{av}MO_{2av}) &= -RT\ln\left(P_M + \frac{[O_2]P_{MO_2}}{K}\right)\\ &= -RT\ln P_M - RT\ln\left(1 + \frac{[O_2]}{K'_{O_2}}\right)\end{aligned} \tag{9}$$

where the apparent equilibrium constant for the biochemical reaction

$$H_{av}MO_2 = H_{av}M + O_2 \tag{9b}$$

is given by

$$\begin{aligned}K'_{O_2} &= \frac{[H_{av}M][O_2]}{[H_{av}MO_2]} = K\frac{P_M}{P_{MO_2}}\\ &= K\frac{1 + 10^{pK_{1M} - pH} + 10^{pK_{1M} + pK_{2M} - 2pH}}{1 + 10^{pK_{1MO_2} - pH} + 10^{pK_{1MO_2} + pK_{2MO_2} - 2pH}}\end{aligned} \tag{10}$$

On the basis of this rather brief introduction to the thermodynamics of ligand binding, it is possible to state the main objective of this paper. If $\Delta_f G''^\circ(H_{av}MO_{2av})$ can be obtained as a function of T, pH and concentration of molecular oxygen, all of the standard further transformed thermodynamic properties, standard transformed properties, and standard properties of the species M, HM, H_2M, MO_2, HMO_2 and H_2MO_2 can be calculated. The standard transformed Gibbs energy of formation $\Delta_f G''^\circ(H_{av}MO_{2av})$ cannot be determined directly, but the average numbers of oxygen molecules bound at various T, pH, $[O_2]$ can be measured. In this paper, $\bar{N}_{O_2}$ will be calculated for system (1) at specified values of the three independent variables, and then this table will be treated as the only experimental data to be used to calculate all the thermodynamic properties and a number of relations between them.

3. Calculation of the average number of oxygen molecules bound at specified temperatures, pHs and concentrations of molecular oxygen

The values determined by Antonini and coworkers [2] on the basis of the assumption that the acid dissociations are independent were used to calculate pK_{1M} (293.15 K) = 7.85 ($\Delta_r H^\circ = 37.7$ kJ mol^{-1}), pK_{2M} (293.15 K) = 5.46 ($\Delta_r H^\circ = -6.3$ kJ mol^{-1}), pK_1MO_2 (293.15 K) = 6.67 ($\Delta_r H^\circ = 37.7$ kJ mol^{-1}), pK_2MO_2 (293.15 K) = 6.04 ($\Delta_r H^\circ = -6.3$ kJ mol^{-1}), and K (293.15 K) = 10^{-5} ($\Delta_r H^\circ = 60.7$ kJ mol^{-1}). It is assumed that these standard enthalpies of reaction are independent of temperature. When these functions of temperature are substituted in Eqs. (3), (4), (9) and (10) a very complicated function of T, pH and $[O_2]$ is obtained for $\Delta_f G''^\circ(H_{av}MO_{2av})$, but this equation can be derived with the *Mathematica*^{R} [8] program calcstdfrtrGe given in Appendix A (see *MathSource* [9]).

The average number of oxygen molecules bound can be calculated from $\Delta_f G''^\circ$ using the Maxwell relation [1]

$$\bar{N}_{O_2} = -\frac{[O_2]}{RT}\left(\frac{\partial \Delta_f G''^\circ}{\partial [O_2]}\right)_{T,P,pH} \tag{11}$$

and calculated values of $\bar{N}_{O_2}$ are given in Table 1 for two temperatures, nine pHs and six concentrations of molecular oxygen (in M). A very wide range of oxygen concentrations has been included so that limiting values at both high and low oxygen concentrations can be discussed.

Mathematica usually gives six numbers (not counting zeros), and so it is difficult to decide how many to round off. Here five places are kept

Table 1
Average numbers of oxygen molecules bound

	$[O_2]=10^{-7}$	$[O_2]=10^{-6}$	$[O_2]=10^{-5}$	$[O_2]=10^{-4}$	$[O_2]=10^{-3}$	$[O_2]=10^{-2}$
			293.15 K			
pH 5.0	0.00203	0.01997	0.16931	0.67085	0.95323	0.99512
pH 5.5	0.00156	0.01539	0.13520	0.60989	0.93988	0.99364
pH 6.0	0.00117	0.01158	0.10489	0.53956	0.92138	0.99154
pH 6.5	0.00118	0.01163	0.10527	0.54057	0.92167	0.99157
pH 7.0	0.00183	0.01801	0.15496	0.64711	0.94829	0.99458
pH 7.5	0.00353	0.03417	0.26133	0.77963	0.97251	0.99718
pH 8.0	0.00609	0.05771	0.37983	0.85964	0.98394	0.99837
pH 8.5	0.00822	0.07656	0.45327	0.89237	0.98808	0.99880
pH 9.0	0.00930	0.08578	0.48407	0.90368	0.98945	0.99894
			313.15 K			
pH 5.0	0.00043	0.00426	0.04101	0.29954	0.81048	0.97715
pH 5.5	0.00034	0.00339	0.03288	0.25374	0.77273	0.97143
pH 6.0	0.00028	0.00279	0.02726	0.21887	0.73698	0.96554
pH 6.5	0.00035	0.00353	0.03417	0.26131	0.77961	0.97251
pH 7.0	0.00065	0.00650	0.06144	0.39564	0.86749	0.98495
pH 7.5	0.00117	0.01156	0.10471	0.53908	0.92123	0.99152
pH 8.0	0.00164	0.01614	0.14091	0.62125	0.94254	0.99394
pH 8.5	0.00189	0.01856	0.15907	0.65417	0.94979	0.99474
pH 9.0	0.00199	0.01950	0.16592	0.66547	0.95214	0.99500

to the right of the decimal point. The sensitivity of these numbers to changes in the input is different in different parts of the table.

4. Calculation of K'_{O_2} for $H_{av}MO_2=H_{av}M+O_2$ from Table 1 treated as experimental data

Since a single molecule of molecular oxygen is bound by M, the rows in Table 1 can be interpreted in terms of

$$\bar{N}_{O_2}=\frac{[O_2]/K'_{O_2}}{1+[O_2]/K'_{O_2}} \tag{12}$$

where the definition of K'_{O_2} is given in Eq. (10). This apparent equilibrium constant can be calculated in a number of ways, of which a familiar one is the Lineweaver–Burk method of enzyme kinetics. The values of K'_{O_2} at the nine pH values and two temperatures in Table 1 are calculated by use of Fit in *Mathematica*, and one way to do it is to fit $1/\bar{N}_{O_2}$ linearly with respect to $1/[O_2]$. These 'experimental' values are given in Table 2.

There are two additional ways to obtain K'_{O_2} as a function of pH and temperature from Table 1. The other two ways are based on the observation that plots involving the dependence of functions of $\bar{N}_{O_2}$ vs. pH at a given temperature approach the same limiting form at both low $[O_2]$ and high $[O_2]$. These two additional ways to construct Table 2 from Table 1 are described in Appendix A.

5. Calculation of K and pKs from K'_{O_2}

Table 2 makes it possible to calculate K, pK_{1M},

Table 2
Values of $K'_{O_2}\times10^5$ Calculated from Table 1

pH	293.15 K	313.15 K
5	4.91	23.38
5.5	6.40	29.41
6	8.53	35.69
6.5	8.50	28.27
7	5.45	15.28
7.5	2.83	8.55
8	1.63	6.10
8.5	1.21	5.29
9	1.07	5.03

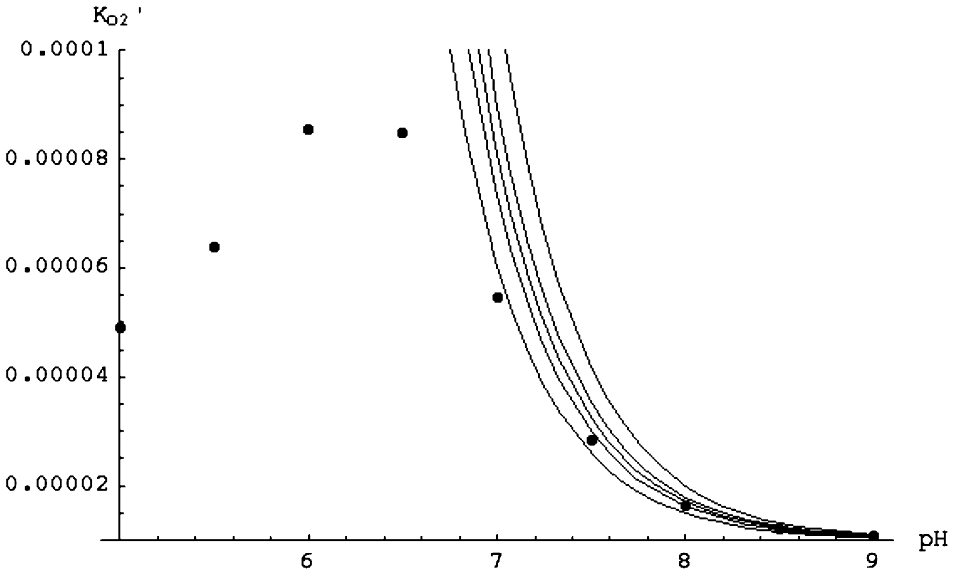


Fig. 1. Fit of plot of apparent equilibrium constants from Table 2 at 293.15 K for $K=1.00\times10^{-5}$ and $\mathrm{p}K_{1\mathrm{M}}=7.70$, 7.80, 7.85, 7.90 and 8.00 in Eq. (13).

$\mathrm{p}K_{2\mathrm{M}}$, $\mathrm{p}K_1\mathrm{MO}_2$ and $\mathrm{p}K_{2\mathrm{MO}_2}$ at 293.15 and 313.15 K. The form of the function K'_{O_2} (see Eq. (10)) makes it difficult to determine these five constants by non-linear curve fitting, but *Mathematica* can be used in a multi-step process starting at high pH where only K and $\mathrm{p}K_{1\mathrm{M}}$ are involved so that

$$K_{\mathrm{O}_2}\text{ ‘(high pH)}=K(1+10^{-\mathrm{pH}+\mathrm{p}K1\mathrm{M}}) \tag{13}$$

Various values of K and $\mathrm{p}K_{1\mathrm{M}}$ were tested by making plots, and the best fit of the apparent equilibrium constants in Table 2 for 293.15 K was obtained with $K=1.00\times10^{-5}$ and $\mathrm{p}K_{1\mathrm{M}}$ very close to 7.85. This is shown in Fig. 1.

This plot shows that K'_{O_2} is very sensitive to the value of $\mathrm{p}K_{1\mathrm{M}}$ at high pH, as expected since it is in the exponent. At pH 7.5 and lower, Eq. (13) gives too high a value because the exponential terms in the denominator are becoming important. It is not possible to determine $\mathrm{p}K_1\mathrm{MO}_2$ separately from $\mathrm{p}K_2\mathrm{MO}_2$ because these values are close and their sum is in the term involving the square of the hydrogen ion concentration. Fig. 2 shows calculations using

$$K'_{\mathrm{O}_2}(\text{intermediate pH})=K\frac{1+10^{\mathrm{p}K_{1\mathrm{M}}-\mathrm{pH}}}{1+10^{\mathrm{p}K_{1\mathrm{MO}_2}-\mathrm{pH}}+10^{\mathrm{p}K_{1\mathrm{MO}_2}+\mathrm{p}K_{2\mathrm{MO}_2}-2\mathrm{pH}}} \tag{14}$$

with $\mathrm{p}K_{1\mathrm{M}}=7.85$ and various values of the other two p*K*s.

Curve (b) gives the best fit on the right side, but the fit is poor on the left side because the squared term in the numerator has not been included. This plot shows that the first and second dissociations of MO_2 come very close to being cooperative; note there is cooperativity when the difference in two p*K*s is less than 0.60 [10,11]. Fig. 3 give plots for K'_{O_2} when $\mathrm{p}K_{2\mathrm{M}}$ in Eq. (10) is varied from 5.36 to 5.56.

In summary, this section has shown how K, $\mathrm{p}K_{1\mathrm{M}}$, $\mathrm{p}K_{2\mathrm{M}}$, $\mathrm{p}K_1\mathrm{MO}_2$ and $\mathrm{p}K_2\mathrm{MO}_2$ at 293.15 K can be calculated from Table 1 treated as experimental data. These calculations can be repeated

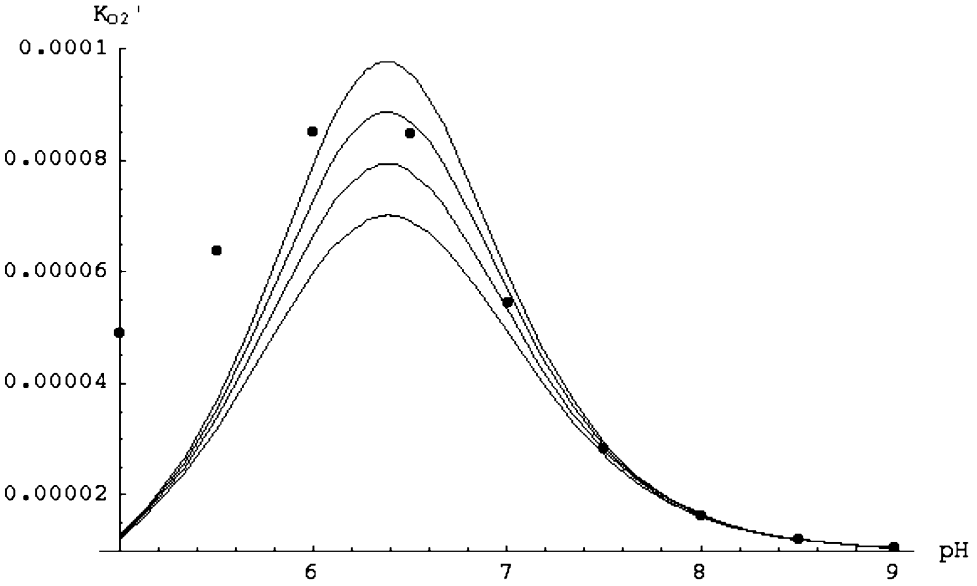


Fig. 2. Plot of K'_{O_2} at 293.15 K using Eq. (14) with (a) 6.77 and 5.94, (b) 6.67 and 6.04, (c) 6.57 and 6.14 and (d) 6.47 and 6.24. The lowest curve is (a) and the highest curve is (d).

with the values of K'_{O_2} in Table 2 at 313.15 K, and $\Delta_r H^o$ can be calculated for each of the five reactions involving species.

6. Calculation of standard further transformed Gibbs energies of formation of $H_{av}MO_{2av}$

There are two ways to calculate $\Delta_f G''^o(H_{av}MO_{2av})$ as a function of T, pH and $[O_2]$ from measurements of $\bar{N}_{O_2}$. One way is to integrate Maxwell relation (11). The other way is to simply use Eq. (9). To use the first way, the expression for $\bar{N}_{O_2}$ in Eq. (12) is substituted in Maxwell relation (11) and the integration is carried out.

$$\Delta_f G''^o(H_{av}MO_{2av}) + \text{const} = -RT\int \frac{1}{1+x}\mathrm{d}x = -RT\ln(1+x) = -RT\ln\left(1+\frac{[O_2]}{K'_{O_2}}\right) \qquad (15)$$

where $x = [O_2]/K'_{O_2}$. This yields the second term in Eq. 9, and so it is clear that the integration constant is $-RT\ln P_M$, which is a function of T and pH, but not $[O_2]$. Thus, $\Delta_f G''^o(H_{av}MO_{2av})$ as a function of temperature, pH and $[O_2]$ can be calculated from measurements of $\bar{N}_{O_2}$ at a series of temperatures, pHs and concentration of molecular oxygen.

The second way is to simply use Eq. (9). The program calcstdfrtrGe for doing this in *Mathematica* is given in Appendix A. This program has been used to calculate Table 3.

The function of T, pH and $[O_2]$ that yields $\Delta_f G''^o$ is very important because it can be used to calculate all of the other thermodynamic properties of the system.

7. Calculation of other further transformed thermodynamic properties of $H_{av}MO_{2av}$

Now the following Maxwell relations involving $\Delta_f G''^o$ can be used to calculate $\bar{N}_H$, $\Delta_f H''^o$, and $\Delta_f S''^o$.

$$\bar{N}_H = \frac{1}{RT\ln(10)}\left(\frac{\partial \Delta_f G''^o}{\partial \mathrm{pH}}\right)_{T,P,[O_2]} \qquad (16)$$

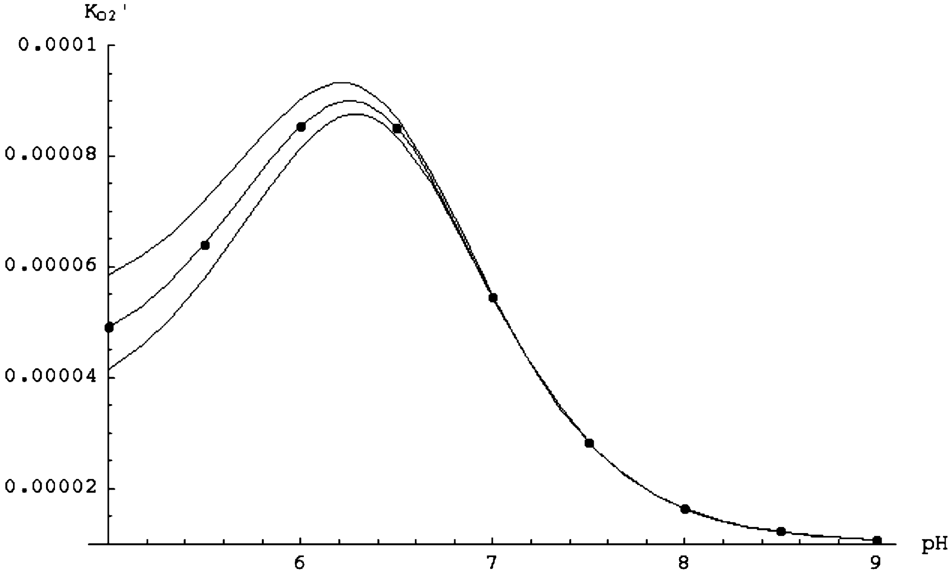


Fig. 3. Plot of K'_{O_2} at 293.15 K using Eq. (10) with $pK_{2M} = 5.36$, 5.46 and 5.56.

$$\Delta_f H''^{\circ} = -T^2 \left(\frac{\partial(\Delta_f G''^{\circ}/T)}{\partial T} \right)_{P,\text{pH},[O_2]} \tag{17}$$

$$\Delta_f S''^{\circ} = -\left(\frac{\partial \Delta_f G''^{\circ}}{\partial T} \right)_{P,\text{pH},[O_2]} \tag{18}$$

Tables of these properties at the two temperatures are given in Tables 4–6.

Note that although $\Delta_f H^{\circ}$ of species are assumed to be independent of temperature, standard further transformed enthalpies do depend on temperature because the composition of pseudoisomer groups depends on temperature.

Note that although $\Delta_f S^{\circ}$ of species are assumed to be independent of temperature, standard further transformed entropies do depend on temperature because the composition of pseudoisomer groups depends on temperature. The previous article [1] called attention to Maxwell relations that show that the dependence of $\Delta_f H''^{\circ}$ and $\Delta_f S''^{\circ}$ on pH and $[O_2]$ are determined by the dependencies of $\bar{N}_H$ and $\bar{N}_{O_2}$ on temperature.

8. Calculation of standard transformed thermodynamic properties of the biochemical reaction $H_{av}MO_2 = H_{av}M + O_2$

Since K'_{O_2} is known as a function of T and pH, $\Delta_r G'^{\circ}$ for the biochemical reaction $H_{av}MO_2 = H_{av}M + O_2$ can be calculated as a function of T and pH by using $\Delta_r G'^{\circ} = -RT \ln K'_{O_2}$. All the other standard transformed reaction properties of this reaction can be calculated by taking partial derivatives of $\Delta_r G'^{\circ}$. Table 7 gives the thermodynamic properties at five pH values and two temperatures.

Note that $\Delta_r H'^{\circ}$ and $\Delta_r S'^{\circ}$ do depend on temperature because $H_{av}M$ and $H_{av}MO_2$ are pseudoisomer groups that change in composition with temperature. The change in binding of hydrogen ions is positive at pH 7 and pH 8. Thus, when oxygen is dissociated, there is an uptake of hydrogen ions at the binding site. This Bohr effect is important because it means that when oxygen is taken up by hemoglobin in the lungs, H^+ ions are dissociated. This serves the function of converting HCO_3^- to H_2CO_3, which dissociates to H_2O and CO_2 under the action of carbonic anhydrase.

Table 3
Standard further transformed Gibbs energies of formation of $H_{av}MO_{2av}$ at two temperatures

	$[O_2]=10^{-7}$	$[O_2]=10^{-6}$	$[O_2]=10^{-5}$	$[O_2]=10^{-4}$	$[O_2]=10^{-3}$	$[O_2]=10^{-2}$
293.15 K						
pH 5.0	−19308	−19352	−19755	−22012	−26768	−32275
pH 5.5	−14778	−14812	−15129	−17069	−21627	−27103
pH 6.0	−11030	−11055	−11297	−12917	−17226	−22659
pH 6.5	−7890	−7916	−8158	−9783	−14095	−19529
pH 7.0	−5158	−5198	−5564	−7692	−12373	−17869
pH 7.5	−2888	−2964	−3618	−6566	−11640	−17191
pH 8.0	−1323	−1453	−2472	−6094	−11377	−16954
pH 8.5	−512	−687	−1965	−5926	−11290	−16876
pH 9.0	−190	−385	−1780	−5871	−11262	−16851
313.15 K						
pH 5.0	−18376	−18386	−18484	−19302	−22706	−28214
pH 5.5	−13434	−13442	−13520	−14195	−17291	−22690
pH 6.0	−9355	−9361	−9426	−9997	−12831	−18123
pH 6.5	−6056	−6064	−6146	−6844	−9993	−15413
pH 7.0	−3426	−3442	−3590	−4736	−8687	−14351
pH 7.5	−1594	−1622	−1879	−3608	−8208	−14012
pH 8.0	−615	−653	−1007	−3139	−8049	−13906
pH 8.5	−214	−258	−660	−2973	−7998	−13873
pH 9.0	−73	−119	−540	−2919	−7981	−13862

The energies are in J mol^{-1}, and the oxygen concentrations are molar.

Table 4
Average binding of hydrogen ions by $H_{av}MO_{2av}$ at two temperatures

	$[O_2]=10^{-7}$	$[O_2]=10^{-6}$	$[O_2]=10^{-5}$	$[O_2]=10^{-4}$	$[O_2]=10^{-3}$	$[O_2]=10^{-2}$
293.15 K						
pH 5.0	1.7423	1.7453	1.7709	1.8567	1.9050	1.9122
pH 5.5	1.4740	1.4778	1.5109	1.6420	1.7331	1.7479
pH 6.0	1.2108	1.2126	1.2286	1.3031	1.3686	1.3806
pH 6.5	1.0407	1.0386	1.0195	0.9308	0.8531	0.8389
pH 7.0	0.9030	0.8944	0.8220	0.5619	0.4028	0.3783
pH 7.5	0.6975	0.6802	0.5525	0.2611	0.1526	0.1388
pH 8.0	0.4141	0.3950	0.2756	0.0977	0.0516	0.0462
pH 8.5	0.1818	0.1703	0.1068	0.0328	0.0167	0.0149
pH 9.0	0.0656	0.0609	0.0364	0.0106	0.0053	0.0047
313.15 K						
pH 5.0	1.7714	1.7719	1.7774	1.8159	1.8921	1.9169
pH 5.5	1.5096	1.5103	1.5171	1.5684	1.6887	1.7348
pH 6.0	1.2193	1.2194	1.2202	1.2262	1.2425	1.2497
pH 6.5	0.9898	0.9884	0.9754	0.8786	0.6577	0.5755
pH 7.0	0.7553	0.7520	0.7205	0.5292	0.2591	0.1918
pH 7.5	0.4617	0.4575	0.4197	0.2434	0.0883	0.0598
pH 8.0	0.2096	0.2068	0.1828	0.0904	0.0286	0.0187
pH 8.5	0.0770	0.0758	0.0657	0.0303	0.0091	0.0059
pH 9.0	0.0257	0.0252	0.0217	0.0098	0.0029	0.0019

Oxygen concentrations are molar.

Table 5
Standard further transformed enthalpies of formation of $H_{av}MO_{2av}$ at two temperatures

	$[O_2]=10^{-7}$	$[O_2]=10^{-6}$	$[O_2]=10^{-5}$	$[O_2]=10^{-4}$	$[O_2]=10^{-3}$	$[O_2]=10^{-2}$
293.15 K						
pH 5.0	−33131	−34199	−43094	−72965	−89784	−92278
pH 5.5	−34705	−35513	−42511	−70236	−89510	−92650
pH 6.0	−35962	−36545	−41773	−66125	−87516	−91447
pH 6.5	−35769	−36282	−40871	−62204	−80881	−84307
pH 7.0	−33068	−33721	−39250	−59121	−71281	−73150
pH 7.5	−26233	−27446	−36437	−56953	−64588	−65564
pH 8.0	−15930	−18344	−33404	−55837	−61648	−62323
pH 8.5	−7347	−11061	−31536	−55401	−60603	−61186
pH 9.0	−3035	−7501	−30754	−55252	−60260	−60814
313.15 K						
pH 5.0	−32829	−33057	−35248	−50661	−81122	−91059
pH 5.5	−34257	−34434	−36144	−48948	−79038	−90557
pH 6.0	−35122	−35255	−36549	−46686	−74095	−86187
pH 6.5	−33479	−33615	−34930	−44677	−66917	−75194
pH 7.0	−27456	−27686	−29848	−42998	−61564	−66186
pH 7.5	−17262	−17736	−21976	−41749	−59145	−62345
pH 8.0	−7969	−8745	−15417	−41100	−58280	−61028
pH 8.5	−3013	−3980	−12130	−40848	−57995	−60602
pH 9.0	−1087	−2134	−10890	−40762	−57904	−60467

The enthalpies are in J mol^{-1}. The oxygen concentrations are molar.

Table 6
Standard further transformed entropies of formation of $H_{av}MO_{2av}$ at two temperatures

	$[O_2]=10^{-7}$	$[O_2]=10^{-6}$	$[O_2]=10^{-5}$	$[O_2]=10^{-4}$	$[O_2]=10^{-3}$	$[O_2]=10^{-2}$
293.15 K						
pH 5.0	−47.15	−50.65	−79.61	−173.82	−214.96	−204.69
pH 5.5	−67.98	−70.62	−93.41	−181.37	−231.56	−223.59
pH 6.0	−85.05	−86.95	−103.96	−181.50	−239.78	−234.65
pH 6.5	−95.10	−96.76	−111.59	−178.82	−227.82	−220.97
pH 7.0	−95.21	−97. 30	−114.91	−175.44	−200.95	−188.57
pH 7.5	−79.63	−83.51	−111.95	−171.88	−180.62	−165.01
pH 8.0	−49.83	−57.62	−105.52	−169.69	−171.49	−154.77
pH 8.5	−23.31	−35.39	−100.88	−168.77	−168.22	−151.15
pH 9.0	−9.71	−24.27	−98.84	−168.45	−167.14	−149.97
313.15 K						
pH 5.0	−46.15	−46.85	−53.53	−100.14	−186.55	−200.69
pH 5.5	−66.50	−67.03	−72.24	−110.98	−197.18	−216.72
pH 6.0	−82.28	−82.69	−86.61	−117.16	−195.64	−217.35
pH 6.5	−87.57	−87.98	−91.92	−120.81	−181.78	−190.91
pH 7.0	−76.73	−77.42	−83.85	−122.18	−168.86	−165.53
pH 7.5	−50.03	−51.46	−64.18	−121.80	−162.66	−154.35
pH 8.0	−23.48	−25.84	−46.02	−121.22	−160.41	−150.48
pH 8.5	−8.94	−11.89	−36.63	−120.95	−159.66	−149.23
pH 9.0	−3.24	−6.44	−33.05	−120.85	−159.42	−148.83

The entropies are in J K^{-1} mol^{-1}. The oxygen concentrations are molar.

Table 7
Thermodynamic properties of the biochemical reaction $H_{av}MO_2 = H_{av}M + O_2$

	pH 5	pH 6	pH 7	pH 8	pH 9
293.15 K					
$K'_{O_2} \times 10^5$	4.91	8.53	5.45	1.63	1.07
$\log K'_{O_2}$	−4.31	−4.07	−4.26	−4.79	−4.97
$\Delta_r G'^{\circ}$	24184	22835	23927	26867	27906
$\Delta_r N_H$	−0.1711	−0.1715	0.5285	0.3708	0.0615
$\Delta_r H'^{\circ}$	59559	56024	40375	46754	58383
$\Delta_r S'^{\circ}$	120.67	113.22	56.11	67.84	103.96
313.15 K					
$K'_{O_2} \times 10^5$	23.38	35.69	15.28	6.10	5.03
$\log K'_{O_2}$	−3.63	−3.45	−3.82	−4.21	−4.30
$\Delta_r G'^{\circ}$	21769	20668	22877	25269	25771
$\Delta_r N_H$	−0.1490	−0.0315	0.5725	0.1923	0.0240
$\Delta_r H'^{\circ}$	59618	52902	39348	53470	59797
$\Delta_r S'^{\circ}$	120.87	102.94	52.60	90.06	108.66

The energies are given in J mol^{-1} and the entropies are given in J K^{-1} mol^{-1}.

The fact that $\Delta_r S'^{\circ}$ is always positive means that it is easier for O_2 to dissociate. The dependencies of $\Delta_r S'^{\circ}$ and $\Delta_r H'^{\circ}$ on pH are a consequence of the effects of temperature on $\Delta_r N_H$ as indicated by the Maxwell relations discussed in the previous paper [1].

9. Calculation of standard thermodynamic properties of the chemical reaction $MO_2 = M + O_2$ and the acid dissociations

The equations that give the temperature dependencies of K and the four pKs are in the *Mathematica* program in Appendix A. These equations contain all the thermodynamic information on the five reactions involving species. These standard reaction properties can be calculated at any temperature in the range approximately 273.15–313.15 K, but here they are calculated at 298.15 K because that is the usual temperature for chemical thermodynamic tables. The calculations in this paper are based on the assumption that $\Delta_r S'^{\circ}$ and $\Delta_r H'^{\circ}$ are independent of temperature for these reactions. For reactions in ideal gases, standard entropies of dissociation are positive because the products are more random than the reactants. The acid dissociations are exceptions to this rule because there is a reorganization of H_2O around

Table 8
Standard reaction properties at 298.15 K of the five dissociation reactions in terms of species

	K or pK	$\Delta_r G^{\circ}$	$\Delta_r H^{\circ}$	$\Delta_r S^{\circ}$
$K(MO_2 = M + O_2)$	0.0000152	27.50	60.70	111.34
$pK_{1M}(HM = H + M)$	7.74	44.17	37.70	−21.68
$pK_{2M}(H_2M = H + HM)$	5.48	31.27	−6.30	−126.02
$pK_1MO_2(HMO_2 = H + MO_2)$	6.56	37.43	37.70	0.91
$pK_2MO_2(H_2MO_2 = H + HMO_2)$	6.06	34.58	−6.30	−137.13

The energies are in kJ mol^{-1}, and the entropies are in J K^{-1} mol^{-1}.

Table 9
Standard thermodynamic properties of species at 298.15 K

	$\Delta_f G^{\circ}$/kJ mol^{-1}	$\Delta_f H^{\circ}$/kJ mol^{-1}	$\Delta_f S^{\circ}$/J K^{-1} mol^{-1}
M	0	0	0
HM	−44.17	−37.7	21.68
H_2M	75.44	44.00	147.70
MO_2	−11.11	−72.40	−205.59
HMO_2	−48.54	−110.10	−206.50
H_2MO_2	−83.12	−103.80	−169.37
H^+	0	0	0
O_2	16.40	−11.70	−94.25

the products that decreases the entropies of the products.

10. Calculation of standard thermodynamic properties of species

Eight species are involved in these five chemical reactions, and the standard thermodynamic properties of two of them [H^+ and $O_2(aq)$] are available in the NBS Tables [12]. Since the standard thermodynamic properties of the remaining six species cannot be calculated from the properties of the five reactions, the properties of M are assigned values of zero at each temperature. This is the same as the convention of the usual chemical thermodynamic tables for H^+. The standard formation properties of species calculated from $\Delta_r G^o$, $\Delta_r H^o$ and $\Delta_r S^o$ in Table 8 are given in Table 9.

The entropy of formation of $O_2(aq)$ at 298.15 K, which is not given in the NBS Tables is $(\Delta_f H^o - \Delta_f G^o)/298.15 = -94.25$ J K^{-1} mol^{-1}.

This is the most efficient way to store thermodynamic information because Table 9 can be used to calculate all the tables discussed in this paper. Calculations can also be made on other reactions that involve these species. These values are compatible with the NBS tables except for the lack of treatment of ionic strength effects. The standard Gibbs energies of formation of these species can be calculated at temperatures not too far away from 298.15 K by use of the following equation:

$$\Delta_f G^\circ(T) = (T/298.15)\Delta_f G^\circ(298.15) + (1 - T/298.15)\Delta_f H^\circ(298.15) \quad (19)$$

11. Discussion

This paper shows how measurements of $\bar{N}_{O_2}$ at specified temperatures, pHs and concentrations of molecular oxygen for a simple binding system can be used to calculate the standard further transformed properties, standard transformed properties, and standard properties of species. This simple system was not chosen as a model of hemoglobin–oxygen binding, but is used to show how important thermodynamic information on species involved in protein–ligand binding can be obtained by use of transformed thermodynamic properties. The first step in this process is to calculate K'_{O_2} for the reaction $H_{av}MO_2 = H_{av}M + O_2$ at each pH and temperature. There are three different ways this can be done: one in the text and two in Appendix A. The second step is to calculate K for the reaction $MO_2 = M + O_2$ and the four pKs at each temperature by the use of curve fitting. When measurements are made at two temperatures, the standard enthalpies of reaction can be calculated for each chemical reaction on the assumption that $\Delta_f H_i^o$ is independent of temperature. The third step is to calculate $\Delta_f G''^o$ of $H_{av}MO_{2av}$ as a function of T, pH and $[O_2]$ using Eq. (9). This thermodynamic property is especially important because it contains all the thermodynamic information about the reaction system. Tables are presented for $\Delta_f G''^o$, $\bar{N}_H$, $\Delta_f H''^o$ and $\Delta_f S''^o$, all calculated from Table 1 of $\bar{N}_{O_2}$ values. These thermodynamic properties are related to each other by Maxwell relations, as described in the previous paper. In addition $\Delta_r G'^o$, $\log K'$, $\Delta_r H'^o$, $\Delta_r S'^o$ and $\Delta_r N_H$ for $H_{av}MO_2 = H_{av}M + O_2$ are calculated. These properties are interrelated by a different set of Maxwell relations. Finally, the reaction $MO_2 = M + O_2$ is considered, and the standard thermodynamic properties of species are calculated. The values of $\Delta_f G^o$ and $\Delta_f H^o$ for eight species at one temperature can be used to calculate all the properties discussed in this paper.

The expressions for $\Delta_r G'^o$ and $\Delta_f G''^o$ are quite complicated, but their various partial derivatives can be calculated using *Mathematica*. Programs used here are available free in digital form from *MathSource* [9].

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Appendix A:

calcstdfrtrGe:=Module[{pK1M, pK2M, pK1MO2, pK2MO2, k, pM, pMO2, kprimeO2},

```
(*This program derives the function of temperature, pH, and concentration of molecular oxygen that yields the standard further transformed Gibbs energy of formation of

HavMO2av . Energies are in J mol-1. *)

pK1M = 7.85 - (37.7*10^3/(8.3145*Log[10])) *(1/293.15 - 1/t);

pK2M = 5.46 - (-6.3*10^3/(8.3145*Log[10])) *(1/293.15 - 1/t);

pK1MO2 = 6.67 - (37.7*10^3/(8.3145*Log[10]))*(1/293.15 - 1/t);

pK2MO2 = 6.04 - (-6.3*10^3/(8.3145*Log[10])) *(1/293.15 - 1/t);

k = 10^-5*Exp[((60.7*10^3)/8.3145)*(1/293.15 - 1/t)];

pM = (1 + 10^(-pH + pK1M) + 10^(-2*pH + pK1M + pK2M));

pMO2 = (1 + 10^ (-pH + pK1MO2) + 10^ (-2*pH + pK1MO2 + pK2MO2)); kprimeO2 =

k*pM/pMO2;

-8.3145*t*Log[pM] - 8.3145*t*Log[1 + co2/kprimeO2]]
```

Two alternate ways to obtain K'_{O_2} as a function of T and pH:

As $[O_2]$ becomes much smaller than K'_{O_2}, Eq. (12) approaches $[O_2]/K'_{O_2}$, so that in the limit of low $[O_2]$, $K'_{O_2}=[O_2]/\bar{N}_{O_2}$. This plot is illustrated in Fig. 4. The pairs of points at the two lowest oxygen concentrations are almost superimposed.

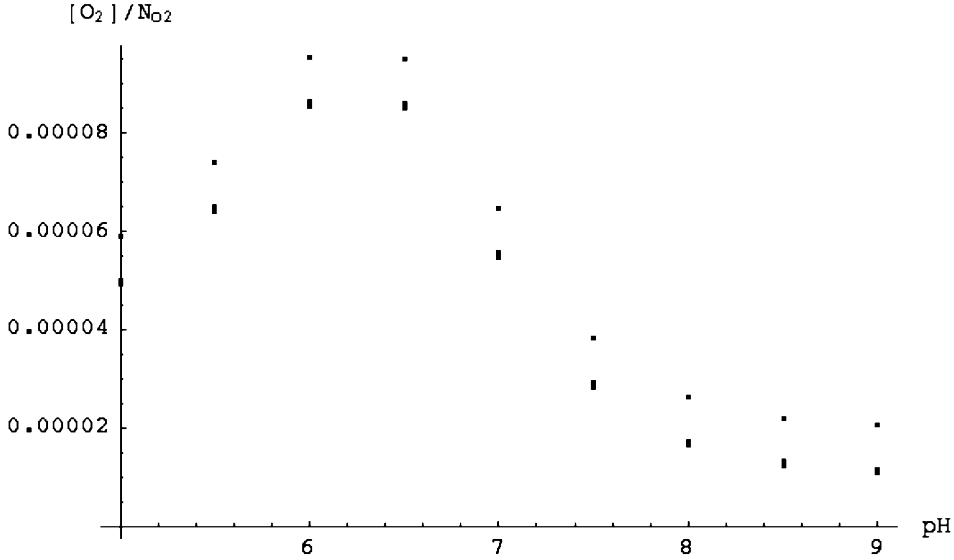

Fig. 4. Plot of $[O_2]/\bar{N}_{O_2}$ from Table 1 vs. pH at 293.15 K for $[O_2]=10^{-7}$ M (lowest points), 10^{-6} M, and 10^{-5} M (highest points), which shows that this function converges to a plot of K'_{O_2} vs. pH as $[O_2]$ is reduced.

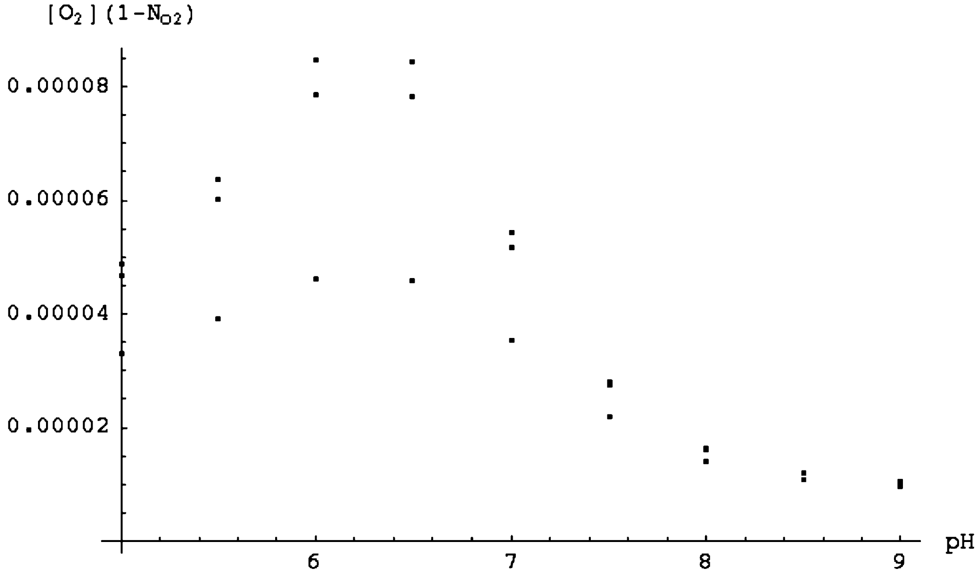

Fig. 5. Plot of $[O_2](1-\bar{N}_{O_2})$ from Table 1 vs. pH at 293.15 K for $[O_2]=10^{-2}$ M (highest points), 10^{-3} M and 10^{-4} M (lowest points), which shows that this function converges to a plot of K'_{O_2} as $[O_2]$ is raised.

Eq. (12) can also be written as

$$\bar{N}_{O_2}=\frac{1}{1+K'_{O_2}/[O_2]} \quad \text{(A1)}$$

As $[O_2]$ becomes much larger than K'_{O_2}, this equation can be written as $\bar{N}_{O_2}=1-K'_{O_2}/[O_2]$ by taking the first two terms of a series expansion. Thus, as $[O_2]$ increases, the limiting form of Eq. (12) can be written as

$$[O_2](1-\bar{N}_{O_2})=K'_{O_2} \quad \text{(A2)}$$

This is illustrated in Fig. 5.

These two methods are not used here, but are described to show what can be done when $\bar{N}_{O_2}$ can be determined more accurately at high or low oxygen concentrations. The limiting plots of K'_{O_2} can be calculated from measurements at high and low $[O_2]$ by use of linear extrapolations described for determining equilibrium constants for dimer-tetramer equilibria [13].

References

[1] R.A. Alberty, Fundamental equation of thermodynamics for protein-ligand binding, Biophys. Chem. 107 (2003) 3631–3635.

[2] E. Antonini, J. Wyman, M. Brunori, C. Fronticelli, E. Bicci, A. Rossi-Fanelli, Studies of the relations between molecular and functional properties of hemoglobin, J. Biol. Chem. 240 (1965) 1090–1103.

[3] R.A. Alberty, Equilibrium calculations on systems of biochemical reactions, Biophys. Chem. 42 (1992) 117–131.

[4] R.A. Alberty, Calculation of transformed thermodynamic properties of biochemical reactants at specified pH and pMg, Biophys. Chem. 43 (1992) 239–254.

[5] R.A. Alberty, Thermodynamics of the binding of ligands by macromolecules, Biophys. Chem. 62 (1996) 141–159.

[6] R.A. Alberty, Thermodynamics of Biochemical Reactions, Wiley, Hoboken, NJ, 2003.

[7] J. Wyman, S.J. Gill, Binding and Linkage, University Science Books, Mill Valley, CA, 1990.

[8] Wolfram Research, Inc., 100 Trade Center Drive, Champaign, IL 61820–7237.

[9] R.A. Alberty, ProteinLigandProg, http://librarywolfram.com/database/MathSource/4808/.

[10] I.M. Klotz, Ligand-Receptor Energetics, Wiley, Hoboken, NJ, 1997.

[11] R.A. Alberty, Effect of pH on protein-ligand equilibria, J. Phys. Chem. Part B 104 (2000) 9929–9934.

[12] D.D. Wagman, W.H. Evans, V.B. Parker, R.H. Schumm, I. Halow, S.M. Bailey, K.L. Churney, R.L. Nutall, The NBS tables of chemical thermodynamic properties, J. Phys. Chem. Ref. Data, 11, Suppl. 2 (1982).

[13] R.A. Alberty, Determination of the seven apparent equilibrium constants for the binding of oxygen by hemoglobin from measured fractional saturations, Biophys. Chem. 63 (1997) 119–132.