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Changes in binding of hydrogen ions in enzyme-catalyzed reactions

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Abstract

Most enzyme-catalyzed reactions produce or consume hydrogen ions, and this is expressed by the change in the binding of hydrogen ions in the biochemical reaction, as written in terms of reactants (sums of species). This property of a biochemical reaction is important because it determines the change in the apparent equilibrium constant K' with pH. This property is also important because it is the number of moles of hydrogen ions that can be produced by a biochemical reaction for passage through a membrane, or can be accepted from a transfer through a membrane. There are two ways to calculate the change in binding of hydrogen ions for an enzyme-catalyzed reaction. The first, which has been used for a long time, involves calculating the partial derivative of the standard transformed Gibbs energy of reaction with respect to pH. The second involves calculating the average numbers of hydrogen ions in each reactant and adding and subtracting these average numbers. The changes in binding of hydrogen ions calculated by the second method at pHs 5, 6, 7, 8, and 9 are given for 23 enzyme-catalyzed reactions. Values are given for 206 more reactions on the web. This database can be extended to include more reactions for which p*K*s of reactants are known or can be estimated.

Keywords: Binding hydrogen ions; Enzyme-catalyzed reactions; Transformed thermodynamic properties; Standard transformed Gibbs energy of reaction; Binding polynomial

1. Introduction

Most enzyme-catalyzed reactions produce or consume hydrogen ions, and this is expressed by the change in the binding of hydrogen ions $\Delta_r N_H$ in the reaction, as written in terms of reactants (sums of species) [1]. It is necessary to write biochemical reactions in terms of sums of species because pH is taken as an independent variable like T and P. Since pH is specified, it is assumed that the pH is held constant while the reaction occurs. The change in binding of hydrogen ions $\Delta_r N_H$ can be measured directly at a specified pH by use of a pHstat. Therefore, biochemical equations at specified pH do not balance hydrogen atoms or electric charge. $\Delta_r N_H$ is important because it determines how the apparent equilibrium constant K' depends on pH. If hydrogen ions are produced ($\Delta_r N_H < 0$), raising the pH causes K' to increase, and if hydrogen ions are consumed ($\Delta_r N_H > 0$), raising the pH causes K' to decrease. This is an example of Le Chatelier's principle that if the conditions are changed on a system at equilibrium, the system will shift in the direction to oppose the change. In Biochemical Thermodynamics: Applications of Mathematica [2] $\Delta_r N_H$ is calculated at 298.15 K, 0.25 M ionic strength, and five pHs for 229 enzyme-catalyzed reactions using

$$\Delta_r \mathrm{N}_H = \frac{1}{RT\ln(10)} \frac{\partial \Delta_r G'^\circ}{\partial \mathrm{pH}} \tag{1}$$

where $\Delta_r G'^\circ$ is the standard transformed Gibbs energy of reaction [3–5] that is given by

$$\Delta_r G'^\circ = -RT\ln K' \tag{2}$$

These two equations show that $\Delta_r N_H$ can also be calculated using the following partial derivative:

$$\Delta_r \mathrm{N}_H = -\frac{\partial \log K'}{\partial \mathrm{pH}} \tag{3}$$

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Thus when $\Delta_r N_H = -1$ in the range pH 5 to pH 9, K' increases by a factor of 10 for $\Delta_{pH} = 1$.

The standard transformed Gibbs energy of reaction $\Delta_r G'^\circ$ plays the same role in biochemical thermodynamics that the standard Gibbs energy of reaction $\Delta_r G^\circ$ plays in chemical thermodynamics. The connection between chemical thermodynamics and biochemical thermodynamics is that when the standard Gibbs energies of formation $\Delta_f G^\circ$ of all the species are known, $\Delta_r G'^\circ$ can be calculated at desired pHs and ionic strengths. The calculations here are restricted to 298.15 K.

However, there is a simpler way to obtain $\Delta_r N_H$ that does not depend on knowing the standard Gibbs energies of formation of all the species in the biochemical reaction. This calculation is a two-step process. First, the average number of hydrogen ions $\bar{N}_{Hi}$ in the various reactants (sums of species) are calculated using

$$\overline{N}_{Hi} = \sum r_j \mathrm{N}_{Hj} \tag{4}$$

where r_j is the equilibrium mole fraction of species j in reactant i at the specified pH and N_{Hj} is the number of hydrogen atoms in species j. Second, these average values are added and subtracted to obtain $\Delta_r N_H$ for the biochemical reaction using

$$\Delta_r \mathrm{N}_H = \sum \nu_i' \overline{N}_{Hi} \tag{5}$$

The prime on the stoichiometric number ν_i' for reactant i indicates that the stoichiometric numbers are for biochemical reactions, rather than the underlying chemical reactions. This type of equation also applies to $\Delta_r G'^\circ$, $\Delta_r H'^\circ$, and $\Delta_r S'^\circ$. Therefore, the correct stoichiometry for a biochemical reaction is very important. If H_2O is included in a biochemical reaction to balance oxygen atoms, it contributes a term in Eq. (5), even though the term for H_2O is omitted in the expression for the apparent equilibrium constant since it is the solvent.

2. Calculation of the average binding of hydrogen ions by a biochemical reactant

Calculations of equilibrium mole fractions r_i of the species in a reactant can be illustrated with ATP [2]. Since ATP is made up of three species in the pH range 5 to 9, its concentration is given by

$$[\mathrm{ATP}] = [\mathrm{ATP}^{4-}] + [\mathrm{HATP}^{3-}] + [\mathrm{H_2ATP}^{2-}] \tag{6}$$

When the acid dissociations are at equilibrium, substituting the expressions for the two acid dissociation constants yields

$$\begin{aligned}[\mathrm{ATP}] &= [\mathrm{ATP}^{-4}](1 + 10^{pK1\mathrm{ATP}-\mathrm{pH}} \\ &\quad + 10^{pK1\mathrm{ATP}+pK2\mathrm{ATP}-2pH}) \\ &= [\mathrm{ATP}^{-4}]p \end{aligned} \tag{7}$$

where $\mathrm{p}K_{1\mathrm{ATP}} = -\log K_{1\mathrm{ATP}}$ is the highest pK that has to be considered in the range pH 5 to pH 9. The factor multiplying $[\mathrm{ATP}^{-4}]$ is referred to as a binding polynomial [6] and is represented here by p. Eq. (7) shows that the equilibrium mole fraction r_j for ATP^{4-} is given by

$$r_1 = 1/p \tag{8}$$

The other two mole fractions are given by

$$r_2 = 10^{pK1\mathrm{ATP}-\mathrm{pH}}/p \tag{9}$$

$$r_3 = 10^{pK1\mathrm{ATP}+pK2\mathrm{ATP}-2\mathrm{pH}}/p \tag{10}$$

The pKs are at the desired temperature and ionic strength. Substituting Eqs. (8)–(10) and $N_H(\mathrm{ATP}^{4-}) = 12$, $N_H(\mathrm{HATP}^{3-}] = 13$, and $N_H(\mathrm{H_2ATP}^{2-}) = 14$ in Eq. (4) yields $\bar{N}_H(\mathrm{ATP})$ as a function of pH. Mathematica® [7] is especially useful for these calculations because of its symbolic and calculus capabilities. A short Mathematica program calcavnoH (see Appendix) was written to derive the mathematical function for $\bar{N}_{Hi}$ by typing in pK_1, pK_2, and the number of hydrogen atoms in the species that dominates at the highest pH. This program expresses $\bar{N}_{Hi}$ as a function of pH at the temperature and ionic strength for which the pKs are given. The functions of pH for $\bar{N}_{Hi}$ at 298.15 K and 0.25 M ionic strength have been calculated for all 199 reactants in BasicBiochemData3 [8], and they are available in the Mathematica notebook changeHbind.nb [9] by typing atpnhfun, for example. Reading MathSource requires MathReader, which is free from Wolfram Research [7]. In order to use a Mathematica program it is necessary to have the Mathematica application. Titration curves at desired temperatures and ionic strengths can be obtained by plotting $\bar{N}_{Hi}$ versus pH for these 199 reactants.

There is another source of functions for the calculation of $\bar{N}_{Hi}$ in the Mathematica package BasicBiochemData3 [8] that were calculated using Eq. (1). There are two types of functions there: (1) Functions of pH and ionic strength at 298.15 K for 199 biochemical reactants; the functions are named atpNH, for example. (2) Functions of temperature, pH, and ionic strength for 94 reactants; the functions are named atpNHT, for example. These functions were all derived using Eq. (1).

3. Calculation of changes in the binding of hydrogen ions in enzyme-catalyzed reactions using Eq. (5)

The objective of this article is to show that $\Delta_r N_H$ for an enzyme-catalyzed reaction can be calculated with less information than is required by Eq. (1), namely pKs at the desired temperature and ionic strength and the number of hydrogen atoms in the most basic form of the reactant.

The changes in binding of hydrogen ions in enzyme-catalyzed reactions can be calculated by adding and subtracting the functions described in the preceding section according to the stoichiometric numbers in the biochemical reaction and using ReplaceAll in Mathematica to specify the temperature, pH and ionic strength. A more convenient way to make these calculations is to write a computer program in which the desired reaction is typed in. The Mathematica program chgNHbindSummary has

been written to calculate the change in binding of hydrogen ions $\Delta_r N_H$ for a biochemical reaction under the desired conditions. The output has a line with the EC number and the name of the enzyme, a line showing the reaction, and a line showing a list of $\Delta_r N_H$ for specified pHs. This program is given in the Appendix. The program round, which controls the number of digits in the output, is also given in the Appendix. These programs are also available in the notebook changeHbind.nb [9].

Calculations of $\Delta_r G'^\circ$ and $\Delta_r N_H$ for 229 biochemical reactions at 298.15 K and five pHs are given in Biochemical Thermodynamics: Applications of Mathematica [2], but only about 10% of these tables are given in Table 1. The names and EC numbers of the enzyme-catalyzed reactions are from Enzyme Nomenclature [10]. These reactions are all written in the direction they are spontaneous at 298.15 K, pH 7, and 0.25 M ionic strength. The fact that the same $\Delta_r N_H$ are obtained by these two methods validates both approaches.

4. Verification of $\Delta_r N_H$ at high pH by use of chemical half reactions

The reactions in Table 1 that produce the most hydrogen ions at pH 7 are EC 1.2.1.3 ($\Delta_r N_H = -2.00$) and EC 1.2.2.4 ($\Delta_r N_H = -2.89$). Reaction EC 1.2.1.3 is

$$\text{Acetaldehyde} + \text{NAD}_{\text{red}} + \text{H}_2\text{O} = \text{acetate} + \text{NAD}_{\text{red}} \quad (11)$$

This reaction produces 2 mol of hydrogen ions for every mole of reaction at higher pHs and a little less at pH 6 because of the binding of hydrogen ions by acetate. The values of $\Delta_r N_H$ for EC 1.2.1.3 at high pH can be verified by writing the chemical half reactions and adding them:

$$C_{21}H_{26}N_7O_{14}P_2^- + H^+ + 2e^- = C_{21}H_{27}N_7O_{14}P_2^{-2} \quad (12)$$

$$CH_3CHO + H_2O = CH_3CO_2^- + 3H^+ + 2e^- \quad (13)$$

$$C_{21}H_{26}N_7O_{14}P_2^- + CH_3CHO + H_2O = C_{21}H_{27}N_7O_{14}P_2^{-2} + CH_3CO_2^- + 2H^+ \quad (14)$$

Reaction EC 1.2.2.4 is

$$\text{Carbonmonoxide} + 2H_2O + 2\text{ferricytochrome b-561} = CO_2\text{tot} + 2\text{ferrocytochrome b-561} \quad (15)$$

where CO_2tot is the sum of CO_2(aq), H_2CO_3, HCO_3^-, and CO_3^{2-}. The chemical half reactions for EC 1.2.2.4 and their sum at pH 8 are (ferredoxin is represented by Fe)

$$2Fe^{3+} + 2e^- = 2Fe^{2+} \quad (16)$$

$$CO + 2H_2O = HCO_3^- + 2e^- + 3H^+ \quad (17)$$

$$CO + 2H_2O + 2Fe^{3+} = HCO_3^- + 2Fe^{2+} + 3H^+ \quad (18)$$

Table 1
Changes in the binding of hydrogen ions $\Delta_r N_H$ at 298.15 K, 0.25 M ionic strength and five pHs

pH 5	pH 6	pH 7	pH 8	pH 9
EC 1.1.1.1 Alcohol dehydrogenase Acetaldehyde + NAD_{red} = ethanol + NAD_{ox}				
1.00	1.00	1.00	1.00	1.00
EC 1.1.1.94 Glycerol-3-phosphate dehydrogenase (NAD(P)) Dihydoxyacetonephosphate + NAD_{red} = glycerol-3-phosphate + $NADP_{ox}$				
1.35	1.44	1.1	1.01	1.00
EC 1.2.1.2 Formate dehydrogenase Formate + NAD_{ox} + H_2O = CO_2tot + NAD_{red}				
−0.08	−0.45	−0.89	−1.01	−1.15
EC 1.2.1.3 Aldehyde dehydrogenase Acetaldehyde + NAD_{ox} + H_2O = acetate + NAD_{red}				
−1.77	−1.97	−2.00	−2.00	−2.00
EC 1.2.1.9 Glyceraldehyde-3-phosphate + $NADP_{ox}$ + H_2O = 3-phospho-D-glycerate + $NADP_{red}$ D-glyceraldehyde 3-phosphate + $NADP_{ox}$ + H_2O = 3-phospho-D-glycerate + $NADP_{red}$				
−1.59	−1.29	−1.69	−1.96	−2.00
EC 1.2.2.4 Carbon-monoxide dehydrogenase (cytochrome *b*-561) CO + 2H_2O + 2ferricytochrome *b*-561 = CO_2tot + 2ferrocytochrome *b*-561				
−2.08	−2.45	−2.89	−3.01	−3.15
EC 1.7.1.4 Nitrite reductase ($NAD(P)_{red}$) Nitrite + 3$NADP_{red}$ = ammonia + 3$NADP_{ox}$ + 2H_2O				
4.99	5.00	4.99	4.95	4.64
EC 1.7.2.2 Nitrite reductase (cytochrome; ammonia-forming) Nitrite + 6ferrocytochrome *c* = ammonia + 2H_2O + 6ferricytochrome *c*				
7.99	8.00	7.99	7.95	7.64
EC 1.8.1.2 Sulfite reductase ($NADP_{red}$) Sulfite + 3$NADP_{red}$ = hydrogen sulfide + 3$NADP_{ox}$				
4.00	4.02	4.03	4.01	4.00
EC 1.8.7.1 Sulfite reductase (ferredoxin) Sulfite + 6$ferredoxin_{red}$ = hydrogen sulfide + 6$ferredoxin_{red}$ + 3H_2O				
7.00	7.02	7.03	7.01	7.00
EC 1.18.6.1 Nitrogenase 8$ferredoxin_{red}$ + N_2 + 16ATP + 16 H_2O = 8$ferredoxin_{ox}$ + H_2 + 2ammonia + 16ADP + 16P_I				
9.37	6.01	−1.90	−5.55	−6.66
EC 2.3.1.8 Phosphate acetyltransferase CoA + acetyl phosphate = acetyl-CoA + Pi				
−0.42	−0.23	−0.55	−0.88	0.78
EC 2.3.3.1 Citrate (*Si*)-synthase Acetyl-CoA + H_2O + oxaloacetate = citrate + CoA				
−0.09	−0.74	−1.04	−1.44	−1.89
EC 2.4.2.8 Hypoxanthine phosphoribosyltransferase Hypoxanthine + 5-phospho-a-D-ribose 1-diphosphate = IMP + PP_I				
0.10	0.46	0.08	−0.45	−1.45
EC 2.7.1.80 Diphosphate-serine phosphotransferase PP_i + L-serine = P_i + *O*-phospho-L-serine				
−0.02	−0.18	−0.65	−0.64	−0.17
EC 2.7.9.1 Pyruvate, phosphate dikinase AMP + phosphoenolpyruvate + PP_i = ATP + pyruvate + P_I				
0.21	0.97	1.26	1.36	1.83
EC 3.1.2.1 Acetyl-CoA hydrolase Acetyl-CoA + H_2O = CoA + acetate				
−0.77	−0.98	−1.07	−1.44	−1.89
EC 3.5.1.5 Urease Urea + 2H_2O = CO_2tot + 2ammonia				
1.92	1.54	1.09	0.89	0.13
EC 3.5.4.4 Adenosine deaminase Adenosine + H_2O = inosine + ammonia				
0.97	0.99	0.97	0.77	−0.04
EC 3.6.1.3 Adenosinetriphosphatase ATP + H_2O = ADP + P_i				
−0.04	−0.25	−0.74	−0.96	−1.00
EC 4.1.3.1 Isocitrate lyase Succinate + glyoxylate = isocitrate				
0.30	0.16	0.02	0	0
EC 5.3.1.9 Glucose-6-phosphate isomerase D-fructose 6-phosphate = D-glucose 6-phosphate				
0.04	0.08	0.02	0	0
EC 6.4.1.6 Acetone carboxylase Acetone + CO_2tot + ATP + H_2O = acetoacetate + AMP + 2P_I				
−1.01	−1.07	−1.59	−1.92	−1.85

The biggest consumers of hydrogen ions at pH 7 are EC 1.7.1.4 (4.99), EC 1.7.2.2 (7.99), and EC 1.8.7.1 (7.03). Reaction EC 1.7.1.4 is

$$\text{Nitrite} + 3\text{NADP}_{\text{red}} = \text{ammonia} + 3\text{NADP}_{\text{ox}} + 2\text{H}_2\text{O} \quad (19)$$

The two chemical half reactions and sum for EC 1.7.1.4 at pH 7 are

$$3\text{C}_{21}\text{H}_{26}\text{N}_7\text{O}_{14}\text{P}_2^{-4} = 3\text{C}_{21}\text{H}_{25}\text{N}_7\text{O}_7\text{P}_3^{-3} + 3\text{H}^+ + 6\text{e}^- \quad (20)$$

$$\text{NO}_2^- + 8\text{H}^+ + 6\text{e}^- = \text{NH}_4^+ + 2\text{H}_2\text{O} \quad (21)$$

$$3\text{C}_{21}\text{H}_{26}\text{N}_7\text{O}_{14}\text{P}_2^{-4} + \text{NO}_2^- + 5\text{H}^+ = 3\text{C}_{21}\text{H}_{25}\text{N}_7\text{O}_7\text{P}_3^{-3} + \text{NH}_4^+ + 2\text{H}_2\text{O} \quad (22)$$

At pH 9 fewer hydrogen ions are consumed by this reaction because some NH_3 is formed.

Reaction EC 1.7.2.2 is

$$\text{nitrite} + 6\text{ferrocytochromec} = \text{ammonia} + 2\text{H}_2\text{O} + 6\text{ferricytochromec} \quad (23)$$

The two chemical half reactions and their sum for EC 1.7.2.2 at pH 7 are

$$\text{NO}_2^- + 8\text{H}^+ + 6\text{e}^- = \text{NH}_4^+ + 2\text{H}_2\text{O} \quad (24)$$

$$6\text{Fe}^{2+} = 6\text{Fe}^{3+} + 6\text{e}^- \quad (25)$$

$$\text{NO}_2^- + 6\text{Fe}^{2+} + 8\text{H}^+ = \text{NH}_4^+ + 2\text{H}_2\text{O} + 6\text{Fe}^{3+} \quad (26)$$

Reaction EC 1.8.7.1 is

$$\text{Sulfite} + 3\text{ferredoxin}_{\text{red}} = \text{hydrogen sulfide} + 3\text{ferredoxin}_{\text{ox}} + 3\text{H}_2\text{O} \quad (27)$$

The two chemical half reactions and their sum for EC 1.8.7.1 at pH 9 are

$$\text{SO}_3^{2-} + 7\text{H}^+ + 6\text{e}^- = \text{HS}^- + 3\text{H}_2\text{O} \quad (28)$$

$$6\text{Fe}^{2+} = 6\text{Fe}^{3+} + 6\text{e}^- \quad (29)$$

$$\text{SO}_3^{2-} + 7\text{H}^+ + 6\text{Fe}^{2+} = \text{HS}^- + 6\text{Fe}^{3+} + 3\text{H}_2\text{O} \quad (30)$$

The most interesting reaction in Table 1 is EC1.18.6.1 Nitrogenase. This reaction is

$$\text{N}_2 + 8\text{ferredoxin}_{\text{red}} + 16\text{ATP} + 16\text{H}_2\text{O} = 2\text{ammonia} + \text{H}_2 + 8\text{ferredoxin}_{\text{ox}} + 16\text{ADP} + 16\text{P}_i \quad (31)$$

This reaction is made up of three chemical half reactions at about pH 8.5:

$$\text{N}_2 + 10\text{H}^+ + 8\text{e}^- = 2\text{NH}_4^+ + \text{H}_2 \quad (32)$$

$$8\text{Fe}^{2+} = 8\text{Fe}^{3+} + 8\text{e}^- \quad (33)$$

$$16\text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_{13}\text{P}_3^{4-} + 16\text{H}_2\text{O} = 16\text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_{13}\text{P}_2^{3-} + 16\text{HPO}_4^{2-} + 16\text{H}^+ \quad (34)$$

The sum of these reactions is

$$\text{N}_2 + 8\text{Fe}^{2+} + 16\text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_{13}\text{P}_3^{4-} + 16\text{H}_2\text{O} = 2\text{NH}_4^+ + \text{H}_2 + 8\text{Fe}^{3+} + 16\text{C}_{10}\text{H}_{12}\text{N}_5\text{O}_{13}\text{P}_2^{3-} + 16\text{HPO}_4^{2-} + 6\text{H}^+ \quad (35)$$

This represents the reaction at pH 8.5, but at pH 9 more hydrogen ions are produced because NH_3 is formed, rather than NH_4^+. At pH 7 there is a low production of hydrogen ions because the hydrogen atoms required to make N_2 and H_2 are supplied by the hydrolysis of ATP. In 1994 it was pointed out that "The function of the hydrolysis of ATP may be to provide the $10H^+$ required per mole of N_2 consumed" in reaction 25 [11]. Note the very great change of hydrogen ion production with pH: $\Delta_r N_H(\text{pH } 9) = -6.66$ and $\Delta_r N_H(\text{pH } 5) = 9.37$.

5. Effect of temperature on the production of hydrogen ions in a biochemical reaction

For an enzyme-catalyzed reaction where each reactant is a single species there is no effect of temperature on $\Delta_r N_H$. If one or more reactants have pKs that have to be taken into account in the range 5 to 9, and these pKs are functions of temperature, there will be an effect when the temperature is changed because of the changing proportions of protonated species of reactants. For the 23 reactions in Table 1, eight have reactants with pKs for which temperature dependencies are known. The $\Delta_r N_H$ for these eight reactions at 273.15 K and 0.25 M ionic strength have been calculated and are given in Table 2. There are some changes from Table 1, but the changes are relatively small.

Table 2
Changes in the binding of hydrogen ions $\Delta_r N_H$ at 273.15 K, 0.25 M ionic strength and five pHs

EC	pH 5	pH 6	pH 7	pH 8	pH 9
1.2.1.2	−0.06	−0.38	−0.86	−0.99	−1.09
1.2.1.3	−1.77	−1.97	−2.00	−2.00	−2.00
1.7.1.4	4.98	5.00	5.00	4.99	4.92
1.8.1.2	4.02	4.15	4.28	4.07	4.01
3.5.1.5	1.94	1.62	1.15	0.99	0.76
3.5.4.4	0.95	0.99	0.99	0.92	0.49
3.6.1.3	−0.03	−0.23	−0.70	−0.96	−1.00
5.3.1.9	0.10	0.16	0.03	0.00	0.00

6. Changes in the binding of magnesium ions in biochemical reactions

When species of reactants bind magnesium ions there are effects of pMg = − log[Mg^{2+}] on the changes in binding of both magnesium ions and hydrogen ions. Since standard transformed thermodynamic properties of reactants are functions of pMg, the value of pMg has to be specified in connection with the value of an apparent equilibrium constant, and it is assumed that pMg is held constant during a reaction. Since pMg is an independent variable, a transformed Gibbs energy G' provides the criterion for spontaneous change and equilibrium. This transformed Gibbs energy is defined by a Legendre transform that includes both pH and pMg.

$$G' = G - n_c(\mathrm{H})\mu(\mathrm{H}^+) - n_c(\mathrm{Mg})\mu(\mathrm{Mg}^{2+}) \tag{36}$$

n_c(H) is the amount of the hydrogen component (total amount of hydrogen atoms), n_c(Mg) is the amount of the magnesium component (total amount of magnesium atoms), $\mu(H^+)$ is the chemical potential of hydrogen ions (related to pH), and $\mu(Mg^{2+})$ is the chemical potential of magnesium ions (related to pMg). Since H^+ and Mg^{2+} compete for binding on species of reactants, it is more complicated to summarize the change in binding of magnesium ions because $\Delta_r N_{Mg}$ depends on both pH and pMg and so does $\Delta_r N_H$. The reciprocal nature of these effects for a reactant is shown by [6]

$$\frac{\partial \overline{N}_H}{\partial \mathrm{pMg}} = \frac{\partial \overline{N}_{Mg}}{\partial \mathrm{pH}} \tag{37}$$

and for an enzyme-catalyzed reaction is shown by

$$\frac{\partial \Delta_r \mathrm{N_H}}{\partial \mathrm{pMg}} = \frac{\partial \Delta_r \mathrm{N_{Mg}}}{\partial \mathrm{pH}} \tag{38}$$

Calculations of these effects have been made for the ATP series [2], but more dissociation constants need to be measured for magnesium complex ions of other reactants.

7. Discussion

The change in the binding of hydrogen ions in an enzyme-catalyzed reaction is very important because this determines the effect of pH on the thermodynamics of the reaction that is catalyzed. The standard transformed Gibbs energy of reaction $\Delta_r G'^\circ$, standard transformed enthalpy of reaction $\Delta_r H'^\circ$, standard transformed entropy of reactions $\Delta_r S'^\circ$, and the apparent equilibrium constant K' each change with the pH, and in each case the effect is determined by $\Delta_r N_H$. These quantitative relationships are discussed in a number of places in the literature [2]. In the past, $\Delta_r N_H$ has been calculated as a function of temperature, pH, and ionic strength for reactions where standard chemical thermodynamic properties are known for all the species. But this article shows that $\Delta_r N_H$ can be calculated when the pKs of the reactants are known or can be estimated. Thus $\Delta_r N_H$ can be calculated for most of the reactions in Enzyme Nomenclature [10] by estimating pKs. The pKs of some 60 biochemical reactants are available at 298.15 K [12]. Knowledge of $\Delta_r N_H$ and $\Delta_r N_{Mg}$ is important in considering the transfer of hydrogen ions and magnesium ions through membranes. For there to be membrane transport, reactions to produce these ions are needed and other reactions are needed to accept these ions on the other side of the membrane.

Some of the $\Delta_r N_H$ in Table 1 indicate that the apparent equilibrium constants are very sensitive to the experimental pH. If $\Delta_r N_H$ is independent of pH over a range in pH, Eq. (3) can be integrated to obtain

$$\frac{K'(\mathrm{pH})}{K'(\mathrm{pH}\ 7)} = 10^{-\Delta_r N_H(\mathrm{pH}-7)} \tag{39}$$

The largest $\Delta_r N_H$ in Table 1 is 8.00 for nitrite reductase. This means that if the pH is raised by 0.10, the apparent equilibrium constant will be decreased by a factor of 0.16.

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Appendix

The calculation of the average number of hydrogen ions in a reactant is too laborious to do by hand when a personal computer is available. The calculations here have been made with Mathematica [7], but other mathematical applications could be used. The first program yields the function of pH that gives $\overline{N}_{Hi}$ of a reactant at the temperature and ionic strength for which pKs are known.

```
calcavnoH[pK1_, pK2_, nHbasicform_] :=Module[{p, r1, r2, r3},(*This program derives the function of pH that gives the average number of hydrogen atoms in a reactant with three species. The pKs are for the desired temperature and ionic strength. nHbasicform is the number of hydrogen atoms in the species that dominates at the highest pH. If there is a single pK, pK2 can be set equal to zero. When there is a single species in the range pH 5 to 9, both pK1 and pK2 are set equal to zero.*)
p=1+10^(pK1−pH)+10^(pK1+pK2−2*pH);
r1=1/p; r2=10^(pK1−pH)/p; r3=10^(pK1+pK2−2*pH)/p;
nHbasicform*r1+(nHbasicform+1)*r2+(nHbasicform+2)*r3];
```

The Mathematica application carries out mathematical calculations to about 10 digits, but pKs are not known to this accuracy, and so the program round is used in the next program to round output to 0.01.

```
round[vec_, params_:{6, 2}] :=(*When a list of numbers has more digits to the right of the decimal point than you want, say 6, you can request 2 by using round[vec,{6,2}],*) Flatten [Map[NumberForm[#1, params] &, {vec}, {2}]]
```

For a given enzyme-catalyzed reaction, the next program makes a small table summarizing the EC number, name of the enzyme-catalyzed reaction, and $\Delta_r N_H$ at pHs 5, 6, 7, 8, and 9. The temperature and ionic strength are the temperature and

ionic strength for the p*K*s. Tables 1 and 2 were prepared in this way.

chgNHbindSummary[eq_,title_,reaction_,pHlist_]:=Module[{ functiom,vectorNH},(*When this program is given the equation for a*biochemical reaction in the form acetaldehydenhfun+nadrednhfun+de==ethanolnhfun+nadoxnhfun, it calculates the change in binding of hydrogen ions in the reaction. The temperature and ionic strength are the temperature and ionic strength for the pKs. title_ is in the form "EC 1.1.1.1 Alcohol dehydrogenase". reaction_ is in the form
"acetaldehyde+nadred=ethanol+nadox".*)
function=Solve[eq,de];
vectorNH=round[function[[1,1,2]]/.pH->pHlist,{4,2}];
Print[title];Print[reaction];Print[vectorNH]]

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Glossary

$\Delta_r G'^\circ$: Standard transformed Gibbs energy of reaction
$\Delta_r H'^\circ$: Standard transformed enthalpy of reaction
K': Apparent equilibrium constant
$n_c(H)$: Amount of the hydrogen component (amount of hydrogen atoms)
$n_c(Mg)$: Amount of the magnesium component (amount of magnesium atoms)
N_{Hj}: Number of hydrogen atoms in species j
$\bar{N}_{Hi}$: Average number of hydrogen atoms in reactant i
$\Delta_r N_H$: Change in the binding of hydrogen ions in a biochemical reaction
$\Delta_r N_{Mg}$: Change in the binding of magnesium ions in a biochemical reaction
p: Binding polynomial
pH: $-\log[H^+]$
pMg: $-\log[Mg^{2+}]$
pK: $-$ log(acid dissociation constant)
r_j: Equilibrium mole fraction of species j in a reactant
$\Delta_r S'^\circ$: Standard transformed entropy of reaction
ν_i': Stoichiometric number of reactant i in a biochemical reaction
$\mu(H^+)$: Chemical potential of hydrogen ions (related to pH)
$\mu(Mg^{2+})$: Chemical potential of magnesium ions (related to pMg)