Thermodynamic Properties of Weak Acids Involved in Enzyme-Catalyzed Reactions

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Received: August 11, 2005; In Final Form: December 20, 2005

Measurements of apparent equilibrium constants and transformed enthalpies of enzyme-catalyzed reactions are making it possible to obtain $\Delta_fG°$ and $\Delta_fH°$ of species of biochemical reactants in dilute aqueous solution that could never have been determined classically. This article is concerned with the p*K*s that determine the pH dependencies of the standard transformed thermodynamic properties of biochemical reactants. The database BasicBiochemData3 makes it possible to calculate 82 p*K*s of 60 reactants as functions of ionic strength at 298.15 K. Standard enthalpies of formation of all the species are known for 27 of these reactants, and so their p*K*s can be calculated as functions of temperature and ionic strength. This article also presents calculations of $\Delta_rG°$, $\Delta_rH°$, and $\Delta_rS°$ at 298.15 K and three ionic strengths for the 42 p*K*s of these 27 reactants.

Introduction

Determinations of apparent equilibrium constants and standard transformed enthalpies of enzyme-catalyzed reactions are making it possible to calculate $\Delta_fG°$ and $\Delta_fH°$ of species of reactants at 298.15 K and zero ionic strength.[1,2] Because of the specificity and speed of the catalysis it is possible to determine $\Delta_fG°$ and $\Delta_fH°$ for species of large complicated molecules that could never have been obtained classically. It is convenient to store standard thermodynamic properties of species at 298.15 K and zero ionic strength in small matrices of the form[3]

$$\text{namesp} = \{\{\Delta_fG_1°, \Delta_fH_1°, z_1, N_{H1}\}, \{\Delta_fG_2°, \Delta_fH_2°, z_2, N_{H2}\},...\} \quad (1)$$

where z_j is the charge number for species j and N_{Hj} is the number of hydrogen atoms in species j. The first row is for the species with the fewest hydrogen atoms. The standard formation properties are functions of the ionic strength and even the buffer composition. Pitzer[4] has described the complicated equations that apply over wide ranges of ionic strength. However, because of the lack of parameters to use in these complicated equations and the fact that biochemical experiments are generally made at rather low ionic strengths, the calculations described here are based on the extended Debye−Hückel equation that yields the following equation[5,6] for the standard Gibbs energies of species in aqueous solution as a function of ionic strength I and temperature

$$\Delta_fG_j°(I) = \Delta_fG_j°(I=0) - RT\alpha z_j^2 I^{1/2}/(1 + BI^{1/2}) \quad (2)$$

α is the coefficient in the Debye equation ($\ln \gamma_j = -\alpha z_j^2 I^{1/2}$), and B is an empirical constant taken to be 1.6 $kg^{-1/2}$ $mol^{-1/2}$ independent of temperature. The temperature dependence of α is given by[7]

$$\alpha = 1.10708 - 1.54508 \times 10^{-3}T + 5.95584 \times 10^{-6}T^2 \quad (3)$$

Application of the Gibbs−Helmholtz equation ($H = -T^2\{\partial(G/T)/\partial T\}$) to eq 2 yields the following equation for the standard enthalpy of formation of a species[6]

$$\Delta_fH_j°(I) = \Delta_fH_j°(298.15\text{ K}, I=0) + RT^2(\partial\alpha/\partial T)z_j^2 I^{1/2}/(1 + 1.6I^{1/2}) \quad (4)$$

The p*K* for an acid dissociation can be calculated from the standard Gibbs energies of formation of the species involved using

$$pK = \Sigma\, \nu_j \Delta_fG_j°/RT\ln(10) \quad (5)$$

where the ν_j are the stoichiometric numbers of species.

When $\Delta_fG_j°(298.15\text{ K}, I=0)$ values for species in a reactant are known, p*K*s can be calculated as functions of ionic strength at 298.15 K, and when $\Delta_fH_j°(298.15\text{ K}, I=0)$ values are known as well, p*K*s can be calculated as functions of temperature and ionic strength.

Methods

1. Calculations of p*K*s of Biochemical Reactants at 298.15 K. BasicBiochemData3 gives species properties[3] for 60 reactants that have p*K*s in the range from pH 5 to 9. The substitution of eq 2 in eq 5 at 298.15 K leads to a very complicated function of ionic strength, but the symbolic capabilities of Mathematica can be used to derive these functions.[8] A program calcpK298is has been written in Mathematica to derive the functions of ionic strength that yield p*K*s for the acid groups in each reactant. This program is given in the Appendix. It has been applied to these 60 reactants in one step by the use of Map in Mathematica. The p*K*s obtained in this way are given in Table 1. Note that p*K*s either remain constant (see ammonia, adenine, and adenosine, where the numbers of charged groups are the same on the two sides of the dissociation equation) or decrease as the ionic strength is increased. The ionic strength effect is larger for more highly charged acid species. This table is useful in estimating p*K*s for acidic groups in similar reactants.

In using Table 1 it is important to understand that the acid dissociation constants calculated with eq 5 are of the form $K = [H^+][A^-]/[HA]$ so that the pH is defined by $-\log[H^+]$. To obtain p*K*s based on $-\log\{\gamma(H^+)[H^+]\}$, it is necessary[9] to add 0, 0.10, and 0.14 at ionic strengths 0, 0.1, and 0.25 M.

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10.1021/jp0545086 CCC: $33.50
Published on Web 02/21/2006

TABLE 1: p*K*s of Weak Acids at Three Ionic Strengths in Dilute Aqueous Solutions at 298.15 K

	$I = 0$ M	$I = 0.1$ M	$I = 0.25$ M		$I = 0$ M	$I = 0.1$ M	$I = 0.25$ M
acetate pK_1	4.75	4.54	4.47	glucose 1-phosphate pK_1	6.50	6.07	5.93
acetyl phosphate pK_1	8.69	8.26	8.12	glutathione$_{red}$ pK_1	8.34	7.91	7.77
acetyl phosphate pK_2	5.11	4.90	4.83	glyceraldehyde phosphate pK_1	5.70	5.27	5.13
adenine pK_1	4.20	4.20	4.20	glycerol 3-phosphate pK_1	6.67	6.24	6.10
adenosine pK_1	3.47	3.47	3.47	H_2S pK_1	12.92	12.48	12.35
ADP pK_1	7.18	6.53	6.33	H_2S pK_2	6.99	6.78	6.71
ADP pK_2	4.36	3.93	3.79	IDP pK_1	9.56	8.70	8.43
ammonia pK_1	9.25	9.25	9.25	IDP pK_2	7.18	6.54	6.33
AMP pK_1	6.73	6.30	6.16	IMP pK_1	9.63	8.99	8.78
AMP pK_2	3.99	3.77	3.71	IMP pK_2	6.73	6.30	6.16
arabinose 5-phosphate pK_1	6.69	6.26	6.12	inosine pK_1	8.96	8.74	8.67
ATP pK_1	7.60	6.74	6.47	ITP pK_1	10.09	9.02	8.67
ATP pK_2	4.68	4.03	3.83	ITP pK_2	7.60	6.74	6.47
bisphosphoglycerate pK_1	7.96	7.10	6.83	malate pK_1	5.26	4.83	4.69
citrate pK_1	6.39	5.75	5.54	malylCoA pK_1	4.21	4.00	3.93
citrate pK_2	4.76	4.33	4.19	mannitol 1-phosphate pK_1	6.50	6.07	5.93
isocitrate pK_1	6.40	5.76	5.55	mannose 1-phosphate pK_1	6.44	6.01	5.87
isocitrate pK_2	4.71	4.28	4.15	methyl maleate pK_1	6.27	5.84	5.70
CO_2tot pK_1	10.33	9.90	9.76	methylmalonylCoA pK_1	4.21	4.00	3.93
CO_2tot pK_2	6.37	6.15	6.08	NMN[a] pK_1	6.44	6.01	5.87
CoA pK_1	8.38	8.17	8.10	oxalate pK_1	4.28	3.85	3.71
cysteine pK_1	8.38	8.16	8.09	PEP[a] pK_1	7.00	6.36	6.15
deoxyribose 1-phosphate pK_1	6.69	6.26	6.12	2-phosphoglycerate pK_1	7.64	7.00	6.79
deoxyribose 5-phosphate pK_1	6.69	6.26	6.12	3-phosphoglycerate pK_1	7.53	6.89	6.68
deoxyadenosine pK_1	3.47	3.47	3.47	phosphoserine pK_1	6.44	6.01	5.87
deoxyAMP pK_1	6.73	6.30	6.16	phosphate pK_1	7.22	6.79	6.65
deoxyAMP pK_2	3.99	3.77	3.71	PRPP[a] pK_1	7.18	6.11	5.76
deoxyADP pK_1	7.18	6.53	6.33	PRPP[a] pK_2	6.69	5.83	5.56
deoxyADP pK_2	4.36	3.93	3.79	pyrophosphate pK_1	9.46	8.60	8.33
deoxyATP pK_1	7.60	6.74	6.47	pyrophosphate pK_2	6.72	6.08	5.87
deoxyATP pK_2	4.68	4.03	3.83	pyrophosphate pK_3	2.26	1.83	1.69
dihydroxyacetone phosphate pK_1	5.70	5.27	5.13	pyrophosphate pK_4	0.83	0.62	0.55
fructose 6-phosphate pK_1	6.27	5.84	5.70	ribose 1-phosphate pK_1	6.69	6.26	6.12
fructose 1,6-bisphosphate pK_1	6.65	5.79	5.52	ribose 5-phosphate pK_1	6.69	6.26	6.12
fructose 1,6-bisphosphate pK_2	6.05	5.41	5.20	ribulose 5-phosphate pK_2	6.69	6.26	6.12
fumarate pK_1	4.60	4.17	4.03	sorbitol 6-phosphate pK_1	6.42	5.99	5.85
fumarate pK_2	3.09	2.88	2.81	succinate pK_1	5.64	5.21	5.07
galactose 1-phosphate pK_1	6.15	5.72	5.58	succinate pK_2	4.21	3.99	3.92
galactose 6-phosphate pK_1	6.44	6.01	5.87	succinylCoA pK_1	4.21	3.99	3.92
gluconolactone 6-phosphate pK_1	6.42	5.99	5.85	thioredoxin$_{red}$ pK_1	8.64	8.21	8.07
glucose 6-phosphate pK_1	6.42	5.99	5.85	thioredoxin$_{red}$ pK_2	8.05	7.83	7.76

[a] The reactants NMN, PEP, and PRPP are nicotinamide mononucleotide, phosphoenolpyruvate, and 5-phosphoribosyl-α-pyrophosphate, respectively.

2. Calculations of p*K*s of Biochemical Reactants at Various Temperatures and Ionic Strengths. When the standard enthalpies of formation of species at zero ionic strength are independent of temperature, their standard Gibbs energies of formation are given by

$$\Delta_f G_j^\circ(T, I = 0) = \frac{T}{298.15}\Delta_f G_j^\circ(298.15\text{ K}, I = 0) + \left(1 - \frac{T}{298.15}\right)\Delta_f H_j^\circ(298.15\text{ K}, I = 0) \quad (6)$$

The coefficient $RT\alpha$ in eq 2 is given as a function of temperature by

$$RT\alpha = 9.20483 \times 10^{-3}T - 1.28467 \times 10^{-5}T^2 + 4.95199 \times 10^{-8}T^3 \quad (7)$$

The substitution of these relations in eq 2 and substitution of these expressions for $\Delta_f G_j^\circ(T, I)$ into eq 5 makes it possible to calculate p*K*s in the range from 273.15 K to about 313.15 K. This leads to a very complicated expression that would be impractical to write out, but Mathematica's symbolic capabilities make it possible to derive the expressions for the p*K*s of a reactant.

For 27 of the 60 reactants in Table 1, $\Delta_f H°(298.15\text{ K})$ is known for all of the species. A program calcpKTfn has been written to calculate functions of temperature and ionic strength that yield p*K*s of reactants. This program is given in the Appendix. It has been applied to these 27 reactants in one step by use of Map in Mathematica. The p*K*s obtained in this way are given in Table 2.

3. Calculations of Standard Thermodynamic Properties of Acid Dissociations at 298.15 K and Three Ionic Strengths. The standard Gibbs energy for an acid dissociation is determined by the standard enthalpy for the dissociation, the standard entropy for the dissociation, and the temperature. The values of these properties can be calculated at various temperatures and ionic strengths, but the changes in enthalpy and entropy are nearly independent of temperature because the calculations here are based on the assumption that $\Delta_r H°(I = 0)$ and $\Delta_r S°(I = 0)$ are independent of temperature. The program calcGHSdissfn has been written to derive the functions of temperature and ionic strength that yield $\Delta_r G°$, $\Delta_r H°$, and $\Delta_r S°$ for acid dissociations when $\Delta_f G°(298.15\text{ K}, I = 0)$ and $\Delta_f H°(298.15\text{ K}, I = 0)$ are known for all species. This program is given in the Appendix. Table 3 gives these properties at 298.15 K and three ionic strengths.

In the gas phase, dissociation reactions have positive $\Delta_r S°$ values, but for acid dissociations in aqueous solutions, the reactions discussed here all have negative values. This is attributed to the increase in order in the products due to the

TABLE 2: p*K*s of Weak Acids as Functions of Temperature and Ionic Strength

reactants	*I* (M)	273.15 K	298.15 K	313.15 K	reactants	*I* (M)	273.15 K	298.15 K	313.15 K
acetate pK_1	0	4.75	4.75	4.76	H_2S pK_1	0	13.73	12.92	12.49
	0.10	4.54	4.54	4.54		0.10	13.32	12.49	12.05
	0.25	4.48	4.47	4.47		0.25	13.18	12.35	11.91
adenine pK_1	0	4.52	4.20	4.03	H_2S pK_2	0	7.35	6.99	6.81
	0.10	4.52	4.20	4.03		0.10	7.14	6.78	6.59
	0.25	4.52	4.20	4.03		0.25	7.07	6.71	6.52
adenosine pK_1	0	3.73	3.47	3.33	IDP pK_1	0	10.02	9.56	9.32
	0.10	3.73	3.47	3.33		0.10	9.20	8.70	8.44
	0.25	3.73	3.47	3.33		0.25	8.93	8.43	8.15
ADP pK_1	0	7.09	7.18	7.22	IDP pK_2	0	7.09	7.18	7.23
	0.10	6.47	6.53	6.56		0.10	6.47	6.54	6.57
	0.25	6.27	6.33	6.35		0.25	6.27	6.33	6.35
ADP pK_2	0	4.64	4.36	4.21	IMP pK_1	0	10.20	9.63	9.34
	0.10	4.23	3.93	3.77		0.10	9.58	8.99	8.68
	0.25	4.10	3.79	3.63		0.25	9.38	8.78	8.47
ammonia pK_1	0	10.09	9.25	8.81	IMP pK_2	0	6.64	6.73	6.78
	0.10	10.09	9.25	8.81		0.10	6.23	6.30	6.34
	0.25	10.09	9.25	8.81		0.25	6.10	6.16	6.19
AMP pK_1	0	6.64	6.73	6.77	inosine pK_1	0	9.39	8.96	8.73
	0.10	6.23	6.30	6.33		0.10	9.19	8.74	8.51
	0.25	6.10	6.16	6.19		0.25	9.12	8.67	8.44
AMP pK_2	0	4.28	3.99	3.84	ITP pK_1	0	10.47	10.09	9.89
	0.10	4.07	3.77	3.62		0.10	9.44	9.02	8.79
	0.25	4.01	3.71	3.55		0.25	9.11	8.67	8.43
ATP pK_1	0	7.50	7.60	7.65	ITP pK_2	0	7.50	7.60	7.65
	0.10	6.67	6.74	6.77		0.10	6.67	6.74	6.77
	0.25	6.41	6.46	6.49		0.25	6.41	6.46	6.49
ATP pK_2	0	4.92	4.68	4.55	malate pK_1	0	5.26	5.26	5.26
	0.10	4.30	4.03	3.89		0.10	4.85	4.83	4.82
	0.25	4.10	3.83	3.68		0.25	4.72	4.69	4.68
citrate pK_1	0	6.45	6.39	6.36	mannose 6-phosphate pK_1	0	6.41	6.44	6.46
	0.10	5.83	5.75	5.70		0.10	6.00	6.01	6.01
	0.25	5.63	5.54	5.49		0.25	5.87	5.87	5.87
citrate pK_2	0	4.80	4.76	4.74	PEP pK_1	0	6.97	7.00	7.02
	0.10	4.38	4.33	4.30		0.10	6.35	6.36	6.36
	0.25	4.25	4.19	4.15		0.25	6.15	6.16	6.14
CO_2tot pK_1	0	10.57	10.33	10.20	phosphate pK_1	0	7.28	7.22	7.19
	0.10	10.16	9.90	9.76		0.10	6.86	6.79	6.75
	0.25	10.02	9.76	9.62		0.25	6.73	6.65	6.61
CO_2tot pK_2	0	6.49	6.37	6.20	pyrophosphate pK_1	0	9.48	9.46	9.45
	0.10	6.28	6.15	6.08		0.10	8.66	8.60	8.57
	0.25	6.22	6.08	6.01		0.25	8.39	8.33	8.28
fructose 1,6-phosphate pK_1	0	6.62	6.65	6.67	pyrophosphate pK_2	0	6.73	6.72	6.71
	0.10	5.80	5.79	5.78		0.10	6.11	6.08	6.05
	0.25	5.53	5.52	5.50		0.25	5.91	5.87	5.84
fructose 1,6-phosphate pK_2	0	6.02	6.05	6.06	pyrophosphate pK_3	0	2.18	2.26	2.30
	0.10	5.40	5.41	5.40		0.10	1.77	1.83	1.86
	0.25	5.20	5.20	5.19		0.25	1.63	1.69	1.72
fructose 6-phosphate pK_1	0	6.24	6.27	6.29	pyrophosphate pK_4	0	0.68	0.83	0.91
	0.10	5.83	5.84	5.85		0.10	0.48	0.62	0.69
	0.25	5.70	5.70	5.70		0.25	0.41	0.55	0.62
fumarate pK_1	0	4.56	4.60	4.63	ribose 1-phosphate pK_1	0	6.51	6.69	6.78
	0.10	4.14	4.17	4.19		0.10	6.10	6.26	6.34
	0.25	4.01	4.03	4.04		0.25	5.96	6.12	6.20
fumarate pK_2	0	3.10	3.09	3.09	ribose 5-phosphate pK_1	0	6.51	6.69	6.78
	0.10	2.89	2.88	2.87		0.10	6.10	6.26	6.34
	0.25	2.83	2.81	2.80		0.25	5.96	6.12	6.20
glucose 6-phosphate pK_1	0	6.39	6.42	6.44	succinate pK_1	0	5.64	5.64	5.64
	0.10	5.98	5.99	6.00		0.10	5.23	5.21	5.20
	0.25	5.85	5.85	5.85		0.25	5.10	5.07	5.05
glycerol 3-phosphate pK_1	0	6.64	6.67	6.69	succinate pK_2	0	4.26	4.21	4.18
	0.10	6.23	6.24	6.25		0.10	4.06	3.99	3.96
	0.25	6.10	6.10	6.10		0.25	3.99	3.92	3.89

hydration of the ions produced. For the dissociation of NH_4^+, $\Delta_r S°$ is -2.0 J K^{-1} mol^{-1} independent of the ionic strength because there is a single positive charge on each side of the reaction equation.

For an acid dissociation to be significant in the range of usual biochemical interest (pH 5−9), $\Delta_r G°$(298.15 K, $I = 0.25$ M) should be in the range from approximately 25 to 55 kJ mol^{-1}. Table 3 shows that in the absence of entropy effects only ammonia, H_2S, inosine, and inosine monophosphate would have p*K*s of biochemical interest. The entropy change for the dissociation of NH_4^+ is essentially zero, and the entropy changes for the other three are small (-58 to -77 J K^{-1} mol^{-1}). Thus for all the other acid dissociations discussed here, it is the negative entropy change that brings the p*K*s into the range of biochemical interest. The most negative entropy changes are found with ATP pK_1 (-156 J K^{-1} mol^{-1}), pyrophosphate (-165 J K^{-1} mol^{-1}), and bicarbonate (-142 J K^{-1} mol^{-1}).

4. Use of These Tables in the Estimation of Acid Dissociation Constants of Other Large Molecules. An interesting aspect of the acid dissociation constants of large molecules in

TABLE 3: $\Delta_r G°$, $\Delta_r H°$, and $\Delta_r S°$ for Acid Dissociations at 298.15 K and Three Ionic Strengths

reactant	I (M)	$\Delta_r G°$ (kJ mol^{-1})	$\Delta_r H°$ (kJ mol^{-1})	$\Delta_r S°$ (J K^{-1} mol^{-1})	reactant	I (M)	$\Delta_r G°$ (kJ mol^{-1})	$\Delta_r H°$ (kJ mol^{-1})	$\Delta_r S°$ (J K^{-1} mol^{-1})
acetate pK_1	0	27.14	−0.25	−91.9	H_2S pK_1	0	73.72	50.70	−77.2
	0.10	25.92	−0.87	−89.8		0.10	71.27	49.46	−73.2
	0.25	25.52	−1.07	−89.2		0.25	70.48	49.06	−71.9
adenine pK_1	0	23.97	20.10	−13.0	H_2S pK_2	0	39.91	22.10	−59.7
	0.10	23.97	20.10	−13.0		0.10	38.69	21.48	−57.7
	0.25	23.97	20.10	−13.0		0.25	38.29	21.28	−57.1
adenosine pK_1	0	19.78	16.40	−11.3	IDP pK_1	0	54.57	28.70	−86.8
	0.10	19.78	16.40	−11.3		0.10	49.67	26.22	−78.7
	0.25	19.78	16.40	−11.3		0.25	48.09	25.42	−76.1
ADP pK_1	0	40.97	−5.60	−156.2	IDP pK_2	0	40.98	−5.60	−156.2
	0.10	37.30	−7.46	−150.1		0.10	37.31	−7.46	−150.2
	0.25	36.11	−8.09	−148.2		0.25	36.12	−8.06	−148.2
ADP pK_2	0	24.88	17.60	−24.4	IMP pK_1	0	54.99	35.10	−66.7
	0.10	22.43	16.36	−20.4		0.10	51.32	33.24	−60.6
	0.25	21.64	15.96	−19.1		0.25	50.13	32.64	−58.7
ammonia pK_1	0	52.81	52.22	−2.0	IMP pK_2	0	38.42	−5.40	−147.0
	0.10	52.81	52.22	−2.0		0.10	35.97	−6.64	−142.9
	0.25	52.81	52.22	−2.0		0.25	35.18	−7.04	−141.6
AMP pK_1	0	38.41	−5.40	−146.9	inosine pK_1	0	51.13	27.10	−80.6
	0.10	35.96	−6.64	−142.9		0.10	49.91	26.48	−78.6
	0.25	35.17	−7.04	−141.6		0.25	49.51	26.28	−77.9
AMP pK_2	0	22.77	18.10	−15.7	ITP pK_1	0	57.59	23.95	−112.8
	0.10	21.55	17.48	−13.6		0.10	51.47	20.85	−102.7
	0.25	21.15	17.28	−13.0		0.25	49.49	19.85	−99.4
ATP pK_1	0	43.38	−6.30	−166.6	ITP pK_2	0	43.38	−6.35	−166.8
	0.10	38.48	−8.78	−158.5		0.10	38.48	−8.83	−158.7
	0.25	36.90	−9.58	−155.9		0.25	36.90	−9.63	−156.1
ATP pK_2	0	26.70	15.00	−39.2	malate pK_1	0	30.02	0.16	−100.2
	0.10	23.03	13.14	−33.2		0.10	27.57	−1.08	−96.1
	0.25	21.84	12.54	−31.2		0.25	26.78	−1.48	−94.8
citrate pK_1	0	36.49	3.35	−111.2	mannose 6-phosphate pK_1	0	36.76	−1.81	−129.4
	0.10	32.82	1.49	−105.1		0.10	34.31	−3.05	−125.3
	0.25	31.63	0.89	−103.1		0.25	33.52	−3.45	−124.0
citrate pK_2	0	27.15	2.42	−82.9	PEP pK_1	0	39.96	−1.80	−140.1
	0.10	24.70	1.18	−78.9		0.10	36.29	−3.66	−133.0
	0.25	23.91	0.78	−77.6		0.25	35.10	−4.26	−132.0
CO_2tot pK_1	0	58.96	14.85	−148.0	phosphate pK_1	0	41.20	3.60	−126.1
	0.10	56.51	13.61	−143.9		0.10	38.75	2.36	−122.1
	0.25	55.72	13.21	−142.6		0.25	37.96	1.96	−120.8
CO_2tot pK_2	0	36.34	7.64	−96.3	pyrophosphate pK_1	0	54.00	1.40	−176.4
	0.10	35.12	7.02	−94.2		0.10	49.10	−1.08	−168.3
	0.25	34.72	6.82	−93.6		0.25	47.52	−1.88	−165.7
fructose 1,6-phosphate pK_1	0	37.96	−1.80	−133.7	pyrophosphate pK_2	0	38.35	0.50	−127.0
	0.10	33.06	−4.28	−125.3		0.10	34.68	−1.36	−120.9
	0.25	31.48	−5.08	−122.6		0.25	33.49	−1.96	−118.9
fructose 1,6-phosphate pK_2	0	34.53	−1.80	−121.9	pyrophosphate pK_3	0	12.90	−5.00	−60.0
	0.10	30.86	−3.66	−115.8		0.10	10.45	−6.24	−56.0
	0.25	29.67	−4.26	−113.8		0.25	9.66	−6.64	−54.7
fructose 6-phosphate pK_1	0	35.80	−1.80	−126.1	pyrophosphate pK_4	0	4.74	−9.20	−46.8
	0.10	33.35	−3.04	−120.8		0.10	3.52	−9.82	−44.7
	0.25	32.56	−3.44	−120.8		0.25	3.12	−10.02	−44.1
fumarate pK_1	0	26.27	−2.93	−97.9	ribose 1-phosphate pK_1	0	38.18	−11.30	−166.0
	0.10	23.82	−4.17	−92.6		0.10	35.73	−12.54	−161.9
	0.25	23.03	−4.57	−92.6		0.25	34.94	−12.94	−160.6
fumarate pK_2	0	17.66	0.42	−57.8	ribose 5-phosphate pK_1	0	38.18	−11.30	−166.0
	0.10	16.44	−0.20	−55.8		0.10	35.73	−12.54	−161.9
	0.25	16.04	−0.40	−55.2		0.25	34.94	−12.94	−160.6
glucose 6-phosphate pK_1	0	36.65	−180	−129.0	succinate pK_1	0	32.18	0.16	−107.4
	0.10	34.20	−3.04	−124.9		0.10	29.73	−1.08	−103.4
	0.25	33.41	−3.44	−123.6		0.25	28.94	−1.48	−102.0
glycerol 3-phosphate pK_1	0	38.08	−1.80	−133.8	succinate pK_2	0	24.02	3.36	−69.30
	0.10	35.63	−3.04	−129.7		0.10	22.80	2.74	−67.3
	0.25	34.84	−3.44	−128.4		0.25	22.40	2.54	−66.6

aqueous solutions is that the acidic groups may be essentially independent of each other because they are far apart. An example of this is pK_1(ATP) and pK_2(ITP), which are both 7.60 at 298.15 K and zero ionic strength. In their article on the thermochemistry of inosine, Boeiro-Goates and co-workers[10] point out that "there are significant structural similarities between the inosine 5′-phosphate series and the adenosine 5′-phosphate series." They continue that "on the basis of structural similarity one would expect that the pK and $\Delta_r H°$ values for the H^+(aq) and Mg^{2+}(aq) binding reactions involving the corresponding phosphate groups in the adenosine 5′-phosphate series and inosine 5′-phosphate series to have essentially the same value." These comments also apply to pK_1(ADP) and pK_2(IDP) and to pK_1(AMP) and pK_2(IMP). This does not mean that the pH dependencies of the thermodynamic properties in the ATP series and ITP series are the same because the purine ring in ATP has pK(298.15 K, $I = 0$) = 4.68 and the purine ring in ITP has pK(298.15 K, $I = 0$) = 10.09. These pKs determine the

equilibriuum concentrations of the various species at a specified pH. There are other examples such as this in the tables presented here.

Discussion

The current database BasicBiochemData3 of species properties at 298.15 K can be extended a good deal from experimental data in the literature. Goldberg and Tewari[11−16] have compiled and critically evaluated thermodynamic data on enzyme-catalyzed reactions in the literature. However, a good deal of work is required to extract standard thermodynamic species properties from these measurements of apparent equilibrium constants and transformed enthalpies of reaction, even though programs have been written in Mathematica to make this easier.[1] Fortunately, with larger molecules of biochemical interest, acidic groups may be rather independent of each other and may have thermodynamic properties very much like those discussed here.

Acknowledgment. I am indebted to Robert N. Goldberg (National Institute of Standards and Technology) for helpful discussions and to the National Institutes of Health for support of this research by award 5-RO1-GM48358-10.

Appendix

```
calcpK298is[speciesmat_] := Module[{glist, hlist, zlist, nHlist,
glistis, ghydionis}
(*This program derives the functions of ionic strength that
yield the pKs at 298.15 K for weak acids. The first function of
ionic strength is for the acid with the fewest hydrogen atoms.
The program has a single argument so that it can be used with
Map. The functions can be evaluated by the use of calcpK298is-
[atpsp] /. is → {0, 0.1, 0.25}, for example.*)
{glist, hlist, zlist, nHlist} =Transpose[speciesmat];
glistis = Table[glist[[i]] − 2.91482*zlist[[i]]∧2*is∧0.5/(1 +
1.6*is∧0.5), {i, 1, Length[zlist]}];
ghydionis = −2.91482*is∧0.5/(1 + 1.6*is∧0.5);
Table[((glistis[[i − 1]] − glistis[[i]] + ghydionis)/
(8.31451*0.29815*Log[10])), {i, 2, Length[zlist]}]]
calcpKTfn[speciesmat_] := Module[{glist, hlist, zlist, nHlist,
coeff, speciesGT, spceiesGTis, hydionis}
(*This program derives the function of temperature, pH, and
ionic strength that gives the pKs for a weak acid. pKs are
numbered 1, 2, 3, ... from the highest pK to the lowest pK, but
the highest pK for a weak acid may be omitted if it is outside
of the range from 5 to 9. The first step is to calculate the standard
Gibbs energies of formation at zero ionic strength as a function
of temperature. The second step is to adjust these values to the
desired ionic strength. The output is a list of functions, with as
many functions as pKs. The third step is to make a table of the
pKs. For example, calcpKTfn[atpsp] /. t → {273.15, 298.15,
313.15} /. is → {0, 0.1, 0.25}.*)
{glist, hlist, zlist, nHlist} = Transpose[speciesmat];
(*Calculates functions of temperature for the Gibbs energies
of all species.*)
speciesGT = (t/298.15)*glist + (1 − t/298.15)*hlist;
(*Adjusts these functions of temperature to make them
functions of ionic
strength as well.*)
coeff = (9.20483*10∧−3)*t − (1.28467*10∧−5)*t∧2 +
(4.95199*10∧−8)*t∧3;
speciesGTis = speciesGT − coeff*zlist∧2*is∧0.5/(1 +
1.6*is∧0.5);
hydionis = −coeff*is∧0.5/(1 + 1.6*is∧0.5);
(*Makes a list of the Gibbs energies of dissociation for all
weak acids and convert them to pKs.*)
Table[((speciesGTis[[i − 1]] − speciesGTis[[i]] + hydionis)/
(8.31451*(t/1000)*Log[10])), {i, 2, Length[zlist]}]]
calcGHSdissfn[speciesmat_] := Module[{glist, hlist, zlist,
nHlist, glistis, ghydionis, gibbs, hlistis, hhydionis, enthalpy,
entropy}
(*This program derives the functions of ionic strength that
yield {G, H, S} functions of ionic strength at 298.15 K for weak
acids. The first function of ionic strength is for the acid with
the fewest hydrogen atoms. The program has a single argument
so that it can be used with Map. The functions can be evaluated
by use of calcGHSdissfn[atpsp] /. is → {0, 0.1, 0.25}, for
example.*)
{glist, hlist, zlist, nHlist} =Transpose[speciesmat];
glistis = Table[glist[[i]] − 2.91482*zlist[[i]]∧2*is∧0.5/(1 +
1.6*is∧0.5), {i, 1, Length[zlist]}];
ghydionis = −2.91482*is∧0.5/(1 + 1.6*is∧0.5);
gibbs = Table[((glistis[[i − 1]] − glistis[[i]] + ghydionis)),
{i, 2, Length[zlist]}];
hlistis = Table[hlist[[i]] − 1.4775*zlist[[i]]∧2*is∧0.5/(1 +
1.6*is∧0.5), {i, 1, Length[zlist]}];
hhydionis = −1.4775*is∧0.5/(1 + 1.6*is∧0.5);
enthalpy = Table[((hlistis[[i − 1]] − hlistis[[i]] + hhydionis)),
{i, 2, Length[zlist]}];
entropy = (enthalpy − gibbs)/0.29815;
Transpose[{gibbs, enthalpy, entropy}]]
```

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