ELSEVIER

Biophysical Chemistry 104 (2003) 327–334

Biophysical Chemistry

www.elsevier.com/locate/bpc

Standard transformed Gibbs energies of coenzyme A derivatives as functions of pH and ionic strength

Robert A. Alberty*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Received 1 November 2002; received in revised form 10 December 2002; accepted 10 December 2002

Abstract

The best way to store data on apparent equilibrium constants for enzyme-catalyzed reactions is to calculate the standard Gibbs energies of formation of the species involved at 298.15 K and zero ionic strength so that equilibrium constants can be calculated at the desired pH and ionic strength. These calculations are described for CoA, acetyl-CoA, oxalyl-CoA, succinyl-CoA, methylmalonyl-CoA, malyl-CoA and CoA-glutathione. The species properties are then used to calculate standard transformed Gibbs energies of formation for these reactants as functions of pH at ionic strength 0.25 M. The species data also make it possible to calculate apparent equilibrium constants of 23 enzyme-catalyzed reactions as a function of pH, including some that cannot be determined directly because they are so large.

Keywords: Coenzyme A; Standard Gibbs energies of formation; Apparent equilibrium constants; Standard transformed Gibbs energies; Inverse Legendre transform

1. Introduction

Apparent equilibrium constants K' have been determined for approximately 500 enzyme-catalyzed reactions involving approximately 1000 reactants (sums of species) [1–6]. Since these measurements have been made at various temperatures, pHs and ionic strengths, the apparent equilibrium constants cannot be compared directly with each other. When the same reaction has been studied by different investigators, different conditions have often been used. However, the experimental K' values can be used to calculate standard Gibbs energies of formation $\Delta_f G^\circ$ at zero ionic strength of the species involved. Then values of $\Delta_f G^\circ(I=0)$ for species calculated from different measurements of K' can be compared with each other. The calculation of $\Delta_f G^\circ(I=0)$ of species from measurements on different reactions not only checks the consistency of measurements of K', but makes it possible to calculate K' for other reactions that have not been studied and perhaps cannot be studied directly because K' is so large.

It has been known for some time that $\Delta_f G^\circ$ values of species can be obtained from K' values by calculating the value of K_{ref} for a reference

---

*Corresponding author. Tel.: +1-617-253-2456; fax: +1-617-253-7030.

E-mail address: alberty@mit.edu (R.A. Alberty).

0301-4622/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0301-4622(02)00390-3

reaction written in terms of species. When the values for $\Delta_f G^\circ(I=0)$ are known for all the species but one in the reference reaction, the value of $\Delta_f G^\circ(I=0)$ for that species can be calculated [7]. When $\Delta_f G^\circ(I=0)$ values are not known for two closely related species (like NAD^{-1} and $NADH^{-2}$), one of these species can be assigned $\Delta_f G^\circ(I=0)=0$ as a convention of the thermodynamic table. These calculations of species properties have been given new emphasis by the introduction of the transformed Gibbs energy G' that provides the criterion for spontaneous change and equilibrium at a specified pH and ionic strength [8,9]. The transformed Gibbs energy G' for a system is defined by the Legendre transform

$$\begin{aligned} G' &= G - n_c(\mathrm{H})\mu(\mathrm{H}+) \\ &= G - n_c(\mathrm{H})\{\Delta_f G^\circ(\mathrm{H}+) - RT\ln(10)\mathrm{pH}\} \end{aligned} \tag{1}$$

where G is the Gibbs energy of a reaction system, $n_c(\mathrm{H})$ is the amount of hydrogen atoms in the system, and $\mu(\mathrm{H}^+)$ is the specified chemical potential of hydrogen ions. This leads to a new path for calculating $\Delta_f G^\circ(I=0)$ values for species from K' (pH, I).

When the standard Gibbs energies of formation of the species involved in a reactant are known, the standard transformed Gibbs energy of formation $\Delta_f G_j'^\circ$ of the reactant at a specified pH and ionic strength can be calculated [10,11]. The standard transformed Gibbs energy of species j at 298.15 K and a specified pH and ionic strength I is calculated using

$$\begin{aligned} \Delta_f G_j'^\circ = \Delta_f G_j^\circ + N_\mathrm{H}(j)RT\ln(10)\mathrm{pH} - 2.91482 \\ \times\left[z_j^2 - N_\mathrm{H}(j)\right]I^{1/2}/(1+1.6\ I^{1/2}) \end{aligned} \tag{2}$$

where $\Delta_r G_j^\circ$ is the standard Gibbs energy of formation of the species, z_j is its electric charge, and $N_\mathrm{H}(j)$ is the number of hydrogen ions in the species. The coefficient of the third term has been calculated using the extended Debye–Hückel equation. In discussing the thermodynamics of enzyme-catalyzed reactions, the pH refers to $\mathrm{pH_c} = -\log[\mathrm{H}^+]$, rather than $\mathrm{pH_a} = -\log[\gamma(\mathrm{H}^+)]$ [11]. Thermodynamic properties in dilute aqueous solutions are taken to be functions of the ionic strength, so that expressions for equilibrium constants can be written in terms of concentrations of reactants. When a reactant consists of a single species, the standard transformed Gibbs energy of formation calculated using Eq. (2) is referred to as a property of a reactant.

When a reactant consists of several species, the standard transformed Gibbs energy of reactant i is calculated using

$$\Delta_f G_{i'_o} = -RT\ln\sum_{j=1}^{N_{\mathrm{iso}'}}\exp(-\Delta_f G_{j'_o}/RT) \tag{3}$$

where N_iso' is the number of species involved in the reactant (pseudoisomer group). The standard transformed reaction Gibbs energy for an enzyme-catalyzed reaction can be calculated using

$$\Delta_r G'^\circ = \Sigma \nu_i' \Delta_f G_i'^\circ = -RT\ln K' \tag{4}$$

where the stoichiometric numbers of the reactants have primes to distinguish them from the stoichiometric numbers of the underlying chemical reaction. This equation can be used to calculate $\Delta_f G_i'^\circ$ for reactant i from the experimental apparent equilibrium constant K' if $\Delta_f G_i'^\circ$ values are known for all the other reactants at the specified pH and ionic strength. When $\Delta_f G_i'^\circ$ has been determined for a reactant, the value $\Delta_f G_1'^\circ$ of the most basic species at that pH and ionic strength can be calculated using

$$\begin{aligned} \Delta_f G'^\circ = \Delta_f G_1'^\circ - RT\ln(1 + 10^{\mathrm{p}K_1 - \mathrm{pH}} \\ + 10^{\mathrm{p}K_1 +}\mathrm{p}K_2 - 2\mathrm{pH} + \ldots) \end{aligned} \tag{5}$$

where $\mathrm{p}K_1$ is the highest pK of the reactant in the pH range considered (usually 5–9), $\mathrm{p}K_2$ is the next highest, etc. The basic data on the species of a reactant are stored in a matrix of the form

$$\begin{aligned} \mathrm{namesp} = \{\{\Delta_f G_1^\circ(I=0), \Delta_f H_1^\circ(I=0), \\ z_1, N_\mathrm{H}1\}\{\Delta_f G_2^\circ(I=0), \\ \Delta_f H_2^\circ(I=0), z_2, N_{\mathrm{H}2}, \ldots\}\} \end{aligned} \tag{6}$$

with a row for each species, starting with the most basic.

Since the standard transformed Gibbs energies of formation of reactants can be calculated from $\Delta_f G_j^\circ$ of species, computer programs have been written in *Mathematica*® [12] to calculate the apparent equilibrium constant K' or $\Delta_r G'^\circ$ for a biochemical reaction at the desired pH and ionic strength by simply typing the reaction into the computer [13]. The data on approximately 200 species and 131 reactants at 298.15 K are in a *Mathematica* package that is freely available at MathSource [14]. More information on this data package is available in Ref. [15], which also provides a number of computer programs.

The calculation of $\Delta_f G^\circ(I=0)$ of the species of a reactant from an apparent equilibrium constant K' is more complicated than the calculation of K' from $\Delta_f G^\circ(I=0)$ values for species. Guidance for this process can be obtained from the inverse Legendre transform. Callen [16] discussed the Legendre transform to go from a function of (X,Y) to a function of (P,ϕ) and pointed out that 'the relationship between (X,Y) and (P,ϕ) is symmetrical with its inverse except for a change in sign in the equation for the Legendre transform.' The inverse Legendre transform is the definition of the Gibbs energy G in terms of the transformed Gibbs energy G'.

$$G = G' + n_c(\mathrm{H})\mu(\mathrm{H}^+) \tag{7}$$

The understanding of this inverse Legendre transform has led to writing computer programs in *Mathematica* that make it possible to type in an enzyme-catalyzed reaction with its apparent equilibrium constant at a specified pH and ionic strength and certain information about the reactant in question to obtain the data matrix [see Eq. (6)] for the reactant. Programs for doing this when the reactant consists of two or three species have been published [17], and the program calcGef1sp for doing this when the reactant consists of a single species is given in Appendix A. An advantage of using calcdGe1sp, calcGe2sp and calcdGe3sp is that the sensitivity of the calculation to the experimental values of K', pH and I can be quickly tested.

The compilations of Goldberg and Tewari [1–6] have been surveyed for data on reactions involving CoA and its derivatives in which the standard transformed Gibbs energies of formation can be calculated for all the other reactants using BasicBiochemData2 [14]. Enzyme Nomenclature [18] has also been surveyed for this type of reaction. This has yielded the 23 reactions listed in Table 1.

2. Standard Gibbs energies of formation of the species of CoA

At the present time, the $\Delta_f G^\circ(I=0)$ for species of CoA and related reactants cannot be connected with the elements, and so it is necessary to adopt the convention that $\Delta_f G^\circ(I=0)=0$ for the basic form of CoA. The value of $\Delta_f G^\circ(I=0)$ for the acidic form of CoA can be calculated from the pK of the sulfydryl group (pK = 8.38 at 298.15 K and zero ionic strength). In making these calculations, the electric charges and pKs of the groups at the other end of the CoA molecule are ignored because the reactivity of the sulfydryl group is expected to be quite independent of the acid dissociations there. Therefore, the data matrix for CoA is given by

$$\mathrm{coAsp} = [\{0,\_,-1,0\},\ \{-47.83,\_,0,1\}] \tag{8}$$

where the standard Gibbs energy of formation of the acid form of CoA is in kJ mol^{-1}. The number of hydrogen atoms in the basic form is arbitrarily taken to be zero. In *Mathematica*, the reactant CoA is referred to as coA because names have to start with lowercase letters.

3. Calculation of the standard Gibbs energy of formation of acetyl–CoA

CoA and acetyl–CoA are involved in six different reactions in Table 1: EC 1.1.1.37, 1.2.1.10, 2.3.1.54, 4.1.3.2, 4.1.3.7 and 6.2.1.1. Some of these apparent equilibrium constants are very large or very small and are not readily determined accurately, or have been calculated from kinetic parameters. Some of these six reactions were studied in the early 1950s when it was difficult to obtain pure reactants. Under these circumstances it seems best to select the best study to obtain the values

Table 1
Reactions of coenzyme A and experimental apparent equilibrium constants

EC	Reaction	K'	pH	I/M	Ref.
1.1.1.37	Malate + acetyl–CoA + NAD_{ox} + H_2O = citrate + CoA + NAD_{red}				
1.2.1.10	Acetaldehyde + CoA + NAD_{ox} = acetyl–CoA + NAD_{red}				
1.2.1.17	glyoxylate + CoA + $NADP_{ox}$ = oxalyl–CoA + $NADP_{red}$	73.4	6.90	0.05	21
1.2.1.51	Pyruvate + CoA + $NADP_{ox}$ = acetyl–CoA + CO_2tot + $NADP_{red}$				
1.2.1.52	Ketoglutarate + CoA + $NADP_{ox}$ = succinyl–CoA + CO_2tot + $NADP_{red}$				
1.2.7.1	Pyruvate + CoA + ferredoxin_{ox} = acetyl–CoA + CO_2tot + ferredoxin_{red}				
1.8.4.3	glutathione_{ox} + CoA = CoA–glutathione + glutathione_{red}	1.25	6.82	0.05	23
2.3.1.54	Acetyl–CoA + formate = CoA + pyruvate				
2.3.1.84	Acetyl–CoA + ethanol = CoA + ethylacetate				
3.1.2.1	Acetyl–CoA + H_2O = CoA + acetate				
3.1.2.3	Succinyl–CoA + H_2O = CoA + succinate				
3.1.2.18a	Oxalyl–CoA + H_2O = CoA + oxalate				
3.1.2.18b	Malyl–CoA + H_2O = CoA + malate				
4.1.3.2	Malate + CoA = acetyl–CoA + H_2O + glyoxalate				
4.1.3.7	Oxaloacetate + acetyl–CoA + H_2O = citrate + CoA				
4.1.4.8	ATP + citrate + CoA = ADP + P_i + acetyl–CoA + oxaloacetate				
4.1.3.24	malyl–CoA = acetyl–CoA + glyoxylate	0.0029	7.32	0.05	23
5.4.99.2	methylmalonyl–CoA = succinyl–CoA	18.6	7.42	0.05	22
6.2.1.1	ATP + acetate + CoA = AMP + PP_i + acetyl–CoA	11.1	6.86	0.25	19
6.2.1.4	GTP + succinate + CoA = succinyl–CoA + GDP + P_i	1.66	7.00	0.25	20
6.2.1.8	ATP + oxalate + CoA = AMP + PP_i + oxalyl–CoA				
6.2.1.9	ATP + malate + CoA = ADP + P_i + malyl–CoA				
6.2.1.13	ATP + acetate + CoA = ADP + P_i + acetyl–CoA				

Note that all EC classes of reactions are represented. Six of these reactions involve CoA and acetyl–CoA, but only one of these reactions is considered suitable for the calculation of $\Delta_f G^\circ$(acetyl–CoA). Another five reactions for which apparent equilibrium constants have been determined make it possible to calculate $\Delta_f G^\circ$ values for species of succinyl–CoA, oxalyl–CoA, methylmalonyl–CoA, malyl–CoA, and CoA–glutathione. These calculations make it possible to calculate apparent equilibrium constants for all 23 reactions at specified pHs in the range 5–9 and ionic strengths in the range 0–0.35 M.

for the species and use them. The study of EC 6.2.1.1 by Guynn, Webster and Veech [19] was very thorough. Their study shows that the reaction

$$\text{EC 6.2.1.1 ATP} + \text{acetate} + \text{CoA} = \text{AMP} + \text{PP}_i + \text{acetyl–CoA} \tag{9}$$

has an apparent equilibrium constant $K' = 11.1$ at 298.15 K, pH 6.86, and ionic strength 0.25 M. The program calcdGef1sp (see Appendix A) yields

$$\text{acetylcoAsp} = \{\{-180.36,\_,0,3\}\} \tag{10}$$

This type of calculation can be verified by using calcdGmat [14] to calculate the function of pH and ionic strength that represents $\Delta_f G_1^\circ$ at 298.15 K, and then this function can be used to calculate K' for the experimental conditions. This re-examination of the data on acetyl-CoA has yielded a different value from the one in BasicBiochemData2. In these calculations the effects of temperature and concentrations of divalent cations are ignored, except that the measurements at the lowest concentrations of divalent cations are used.

4. Calculation of standard Gibbs energies of formation for succinyl–CoA

The experimental apparent equilibrium constant has been measured for only one reaction involving succinyl–CoA for which the functions of pH and ionic strength are known for all the other reactants.

$$\text{6.2.1.4 GTP} + \text{succinate} + \text{CoA} = \text{succinyl–CoA} + \text{GDP} + \text{P}_i \tag{11}$$

This is the sixth reaction in the citric acid cycle. Lynn and Guynn [20] obtained $K' = 1.66$ at pH

7.00 and 0.25 M ionic strength. It is assumed that the p*K* for succinyl–CoA is the same as the lowest p*K* for succinic acid at 298.15 K and zero ionic strength, namely 4.21. Although the reaction was studied with GTP, the calculations can be made with ATP. Using calcGef2sp yields

$$\text{succinylcoAsp} = \{\{-509.72,\_,-1,4\}, \{-533.76,\_,0,5\}\} \quad (12)$$

This calculations can be verified by calculating the p*K* and *K*′ from the data matrix using the programs calcpK and calckprime.

5. Calculation of the standard Gibbs energy of formation for oxalyl–CoA

The standard Gibbs energy of formation of oxalyl–CoA can be calculated using the reaction

$$\text{EC 1.2.1.17 glyoxylate} + \text{CoA} + \text{NADP}_{\text{ox}} = \text{oxalyl–CoA} + \text{NADP}_{\text{red}} \quad (13)$$

Qua [21] obtained $K' = 73.4$ at pH 6.9 and ionic strength 0.05 M for this reaction. When the p*K*s of glyoxylate and oxalyl–CoA are both ignored because they are low, the following species matrix is obtained:

$$\text{oxalylcoAsp} = \{\{-509.96,\_,-1,0\}\} \quad (14)$$

6. Calculation of the standard Gibbs energies of formation for methylmalonyl–CoA

The standard Gibbs energies of formation of methylmalonyl–CoA can be calculated using the isomerization reaction

$$\text{EC 5.4.99.2 methylmalonyl–CoA} = \text{succinyl–CoA} \quad (15)$$

Kellermeyer, Allen, Stjernholm and Wood [22] obtained $K' = 18.6$ at pH 7.42 and 0.05 M ionic strength. Assuming the p*K* for methylmalonyl–CoA is 4.21, the same as for the lowest p*K* for of succinic acid, the following species matrix is obtained

$$\text{methylmalonylcoAsp} = \{\{-502.48,\_,-1,4\}, \{-526.51,\_,0,5\}\} \quad (16)$$

7. Calculation of the standard Gibbs energies of formation for malyl–CoA

The standard Gibbs energies of formation of malyl–CoA can be calculated using the reaction

$$\text{EC 4.1.3.24 malyl–CoA} = \text{acetyl–CoA} + \text{glyoxylate} \quad (17)$$

Hersh [23] obtained $K' = 0.0029$ at pH 7.32 and ionic strength 0.05 M. Assuming that the p*K* of malyl–CoA is the same as the first p*K* of succinic acid, the following species data are obtained:

$$\text{malylcoA} = \{\{-663.44,\_,-1,4\}, \{-687.47,\_,0,5\}\} \quad (18)$$

8. Calculations of standard Gibbs energy of formation for CoA–glutathione

The experimental apparent equilibrium constant has been measured for only one reaction involving CoA–glutathione for which the functions of pH and ionic strength are known for all the other reactants.

$$\text{1.8.4.3 glutathione}_{\text{ox}} + \text{CoA} = \text{CoA–glutathione} + \text{glutathione}_{\text{red}} \quad (19)$$

An apparent equilibrium constant of 1.25 has been obtained by Chang and Wilken [24] at pH 6.82 and 0.05 M ionic strength. The use of calcGef1sp yields -35.85 kJ mol^{-1}.

$$\text{coAglutathionesp} = \{\{-35.85,\_,-1,15\}\} \quad (20)$$

Note that the apparent equilibrium constants used in the calculation of the species of these six reactants have been in the range of 1–75, except for one.

The standard Gibbs energies of formation of species calculated from the studies of these six reactions are summarized in Table 2.

Table 2
Standard Gibbs energies of formation (in kJ mol^{-1}) of species at 298.15 K and zero ionic strength*

Species	$\Delta_f G_I^\circ$	z_i	$N_H(i)$
CoA^-	0	−1	0
CoA	−47.83	0	1
Acetyl–CoA	−180.36	0	3
Succinyl–CoA^-	−509.72	−1	4
Succinyl–CoA	−533.76	0	5
Oxalyl–CoA^-	−509.96	−1	0
Methylmalonyl–CoA^-	−502.48	−1	4
Methylmalonyl–CoA	−526.51	0	5
Malyl–CoA^-	−663.44	−1	4
Malyl–CoA	−687.47	0	5
CoA–$\mathrm{glutathione}^-$	−35.84	−1	15

* This table is based on the convention that $\Delta_f G^\circ(\mathrm{CoA}^-) = 0$ at zero ionic strength and $N_H(\mathrm{CoA}^-) = 0$.

9. Standard transformed Gibbs energies of formation of CoA and its derivatives as a function of pH

The program calcdGmat can be used to convert these matrices of species properties to functions of pH and ionic strength that give $\Delta_f G'^\circ$ for seven reactants. The values of this property are given in Table 3 for five pHs and ionic strength 0.25 M.

The data in Table 3 can be used with previously published tables [15] to calculate the apparent equilibrium constants of all the enzyme–catalyzed reactions in Table 1 at these pHs and ionic strength 0.25 M.

10. Calculation of apparent equilibrium constants of other reactions involving CoA and its derivatives

Table 4 gives K' at 298.15 K and 0.25 M ionic strength for all of the reactions in Table 1. Many of these reactions have apparent equilibrium constants that are so large that it would not be practical to determine them directly in the laboratory. The reactions used for the determinations of species data in Table 2 are included to show their pH dependencies. Table 4 gives values at five pH values and 0.25 M ionic strength, but the functions of pH and ionic strength that can be calculated using the values in Table 2 can be used to calculate K' and $\Delta_r N_H$ at any pH in the range 5–9 and any ionic strength in the range zero to 0.35 M.

Four of the reactions are hydrolysis reactions that would be expected to have approximately the same values of apparent equilibrium constants. Reaction 3.1.2.18a involving oxalyl–CoA is an exception because it does not have a $–CH_2–CO–$ group or $–CH(CH_3)–CO–$ group.

11. Discussion

A new way to calculate $\Delta_f G^\circ$ values of species from measurements of apparent equilibrium constants has been described and applied to CoA derivatives. Six reactions have been used to calculate $\Delta_f G^\circ$ values of species involved in six derivatives of CoA. These values have been used to calculate $\Delta_f G'^\circ$ values for these reactants at pHs 5, 6, 7, 8 and 9 and ionic strength 0.25 M. These

Table 3
Standard transformed Gibbs energies of formation (in kJ mol^{-1}) at 298.15 K and ionic strength 0.25 M*

Reactant	pH 5	pH 6	pH 7	pH 8	pH 9
CoA	−18.48	−12.79	−7.26	−2.82	−1.10
Acetyl–CoA	−92.31	−75.19	−58.06	−40.94	−23.81
Succinyl–CoA	−393.34	−370.32	−347.47	−324.64	−301.81
Oxalyl–CoA	−510.77	−510.77	−510.77	−510.77	−510.77
Methylmalonyl–CoA	−386.09	−363.08	−340.23	−317.39	−294.56
Malyl–CoA	−547.05	−524.04	−501.19	−478.40	−455.53
CoA–glutathione	403.59	489.21	574.84	660.46	746.08

* This table is based on the convention that $\Delta_f G^\circ(\mathrm{CoA}^-) = 0$ at zero ionic strength and $N_H(\mathrm{CoA}^-) = 0$.

Table 4
Calculations of apparent equilibrium constants at 298.15 K and ionic strength 0.25 M

EC	Reaction	pH 5	pH 6	pH 7	pH 8	pH 9
1.1.1.37	Malate dehydrogenase	0.19	7.2	6.2×10^{2}	1.0×10^{5}	5.0×10^{7}
1.2.1.10	Acetaldehyde dehydrogenase	2.9	29	2.7×10^{2}	1.6×10^{3}	3.2×10^{3}
1.2.1.17	Glyoxylate dehydrogenase	2.8	28	2.6×10^{2}	1.6×10^{3}	3.1×10^{3}
1.2.1.51	Pyruvate dehydrogenase	4.9×10^{35}	8.2×10^{33}	3.9×10^{32}	2.1×10^{31}	4.9×10^{39}
1.2.1.52	Ketoglutarate dehydrogenase	7.7×10^{36}	1.2×10^{35}	5.6×10^{33}	3.1×10^{32}	7.0×10^{30}
1.2.7.1	Pyruvate synthase	3.8×10^{37}	6.4×10^{36}	3.0×10^{36}	1.7×10^{35}	3.8×10^{35}
1.8.4.3	Glutathione–CoA -glutathione transhydrogenase	0.94	0.94	1.0	1.4	1.9
2.3.1.54	Formate C-acyltransferase	0.011	0.011	0.012	0.019	0.10
2.3.1.84	Alcohol O-acetyl transferase	1.3×10^{4}	1.3×10^{4}	1.4×10^{4}	2.3×10^{4}	1.2×10^{5}
3.1.2.1	Acetyl–CoA hydrolase	2.1×10^{5}	1.7×10^{6}	1.8×10^{7}	2.9×10^{8}	1.5×10^{10}
3.1.2.3	Succinyl–CoA hydrolase	2.4×10^{4}	1.3×10^{5}	1.2×10^{6}	2.0×10^{7}	1.0×10^{9}
3.1.2.18a	ADP-dependent short-chain acyl–CoA hydrolase	14	130	1.4×10^{3}	2.3×10^{4}	1.2×10^{6}
3.1.2.18b	ADP-dependent short-chain acyl–CoA hydrolase	8.5×10^{3}	6.5×10^{4}	6.7×10^{5}	1.1×10^{7}	5.6×10^{8}
4.1.3.2	Malate synthase	3.1×10^{-7}	4.4×10^{-8}	4.3×10^{-9}	2.6×10^{-10}	5.2×10^{-12}
4.1.3.7	Citrate synthase	3.1×10^{6}	8.5×10^{6}	7.0×10^{7}	1.1×10^{9}	5.6×10^{10}
4.1.4.8	ATP citrate lyase	0.16	0.078	0.029	0.014	0.0027
4.1.3.24	Malyl–CoA lyase	0.0027	0.0028	0.0029	0.0029	0.0029
5.4.99.2	Methylmalonyl–CoA mutase	19	19	19	19	19
6.2.1.1	Acetate–CoA ligase	40	13	11	9.8	7.7
6.2.1.4	Succinate–CoA ligase	21	5.2	1.7	0.77	0.15
6.2.1.8	Oxalate–CoA ligase	6.1×10^{5}	1.7×10^{5}	1.4×10^{5}	1.2×10^{5}	9.7×10^{4}
6.2.1.9	Malate–CoA ligase	60	10	3.1	1.4	0.27
6.2.1.13	Acetate–CoA ligase	2.4	0.39	0.12	0.053	0.010

In the six papers that were used here as a source of apparent equilibrium constants, none gave measurements over a sufficient range of pHs to test the pH dependencies shown in the above table; that is, a range of pH that caused a change in the apparent equilibrium constant that was significant in comparison with the experimental errors.

$\Delta_f G'^{\circ}$ values can be used to calculate $\Delta_r G'^{\circ}$ values for reactions involving these reactants at these pHs, but, more importantly, the species properties can be used to calculate $\Delta_r G'^{\circ}$ values at any desired pH in the range 5–9 and ionic strengths from zero to 0.35 M. As an example, K' has been calculated for all 23 reactions in Table 1 at pHs 5, 6, 7, 8 and 9 and 0.25 M ionic strength. The fact that the apparent equilibrium consents for the hydrolysis reactions (3.1.2.1, 3.1.2.3, 3.1.2.18a and 3.1.2.18b) are as close as they are indicates that hydrolysis constants can be estimated for a very large number of hydrolysis reactions not shown in this table.

Computer programs based on the inverse Legendre transform make it possible to calculate $\Delta_f G^{\circ}(I=0)$ values of species from K' in one step. The changes in binding of hydrogen ions in these reactions are also readily calculated.

It is important to recognize that large apparent equilibrium constants cannot be determined accurately because of experimental errors. However, large apparent equilibrium constants can be calculated by use of matrices of species data from other reactions.

Acknowledgments

I am indebted to Dr Robert N. Goldberg (NIST) for many helpful discussions. I am indebted to NIH for support of this research by award number 5-R01-GM48358-07.

Appendix A:

calcGef1sp[equat_, pHc_, ionstr_, z1_, nH1_]:=Module[{energy, trGereactant},

[*This program uses Σ\(Nu)i\(Capital-Delta)fGi′°=−RTlnK′ to calculate the standard Gibbs energy of formation of the species of a reactant that does not have a pK in the range 4–10. The equation is of the form pyruvate+atp-x-adp==−8.31451*.29815*Log(K′), where K′ is

```
the apparent equilibrium constant at 298.15 K,
pHc, and ionic strength is. The reactant has charge
number z1 and hydrogen atom number nH1. The
output is the species vector without the standard
enthalpy of formation.*]
energy=Solve(equat, x)/.pH -> pHc/. is ->
ionstr;
trGereactant=energy(1,1,2);
gef1=trGereactant-
nH1*8.31451*0.29815*Log[10]*pHc +
(2.91482*(z1^2-nH1)*ionstr^0.5)/
(1+1.6*ionstr^0.5);
{gef1, _, z1, nH1}]
```

References

[1] R.N. Goldberg, Y.B. Tewari, D. Bell, D.K. Fazio, E. Anderson, Thermodynamics of enzyme catalyzed reactions: part 1. oxidoreductases, J. Phys. Chem. Ref. Data 22 (1993) 515–582.

[2] R.N. Goldberg, Y.B. Tewari, Thermodynamics of enzyme catalyzed reactions: part 2. transferases, J. Phys. Chem. Ref. Data 23 (1994) 547–617.

[3] R.N. Goldberg, Y.B. Tewari, Thermodynamics of enzyme catalyzed reactions: part 3 hydrolases, J. Phys. Chem. Ref. Data 23 (1994) 1035–1103.

[4] R.N. Goldberg, Y.B. Tewari, Thermodynamics of enzyme catalyzed reactions: part 4 lyases, J. Phys. Chem. Ref. Data 24 (1995) 1669–1698.

[5] R.N. Goldberg, Y.B. Tewari, Thermodynamics of enzyme catalyzed reactions: part 5 isomerases and ligases, J. Phys. Chem. Ref. Data 24 (1995) 1765–1801.

[6] R.N. Goldberg, Thermodynamics of enzyme catalyzed reactions: part 6—update, J. Phys. Chem. Ref. Data 28 (1999) (1999) 931–965.

[7] R.A. Alberty, R.N. Goldberg, Calculation of thermodynamic formation properties for the ATP series at specified pH and pMg, Biochemistry 31 (1992) 10610–10615.

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

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

[10] R.A. Alberty, Calculation of standard transformed Gibbs energies and standard transformed enthalpies of biochemical reactants, Arch. Biochem. Biophys. 353 (1998) 116–130.

[11] R.A. Alberty, Effect of temperature on the standard transformed Gibbs energies of reactants at various pH and ionic strength and apparent equilibrium constants of biochemical reactions, J. Phys. Chem. B 105 (2001) 7865–7870.

[12] Wolfram Research, Inc., 100 World Trade Center, Champaign, IL 61820–7237.

[13] R.A. Alberty, Standard apparent reduction potentials for biochemical half reactions as a function of pH and ionic strength, Arch. Biochem. Biophys. 389 (2001) 94–109.

[14] R.A. Alberty, BasicBiochemData2 (2002), httpwwwmathsource.com/cgi-bin/msitem? 0211–622.

[15] R.A. Alberty, Thermodynamics of Biochemical Reactions, Wiley, Hoboken, NJ, in press.

[16] H.B. Callen, Thermodynamics and an Introduction to Thermostatics, Wiley, Hoboken, NJ, 1985.

[17] R.A. Alberty, Inverse Legendre transform in biochemical thermodynamics: Applied to the last five reactions of glycolysis, J. Phys. Chem. 106B (2002) 6594–6599.

[18] E.V. Webb, Enzyme Nomenclature, New York, Academic Press, 1992.

[19] R.W. Guynn, L.T. Webster Jr, R.L. Veech, Equilibrium constants of the reactions of acetyl-CoA synthetase: The hydrolysis of adenosine triphosphate to amp and inorganic pyrophosphate, J. Biol. Chem. 249 (1974) 3248.

[20] R. Lynn, R.W. Guynn, Equilibrium constants under physiological conditions for the reactions of succinyl coenzyme A synthetase and the hydrolysis of succinyl coenzyme A to coenzyme A and succinate, J. Biol. Chem. 253 (1978) 2546.

[21] J.R. Qua, Carbon assimilation by *Pseudomanus oxalaticus* (OK1): 6. Reactions of oxalyl-coenzyme A, Biochem. J. 87 (1963) 368.

[22] R.W. Kellermeyer, S.H.G. Allen, R. Stjernholm, H.G. Wood, Methylmalonyl isomerase: purification and properties of the enzyme from propionibacteria, J. Biol. Chem. 239 (1964) 2562.

[23] L.B. Hersh, Malate adenosine triphosphate lyase, J. Biol. Chem. 248 (1973) 7295.

[24] S.H. Chang, D.R. Wilken, Participation of the unsymmetrical disulfide of coA and glutathione in an enzymatic sulfhydryl disulfide interchange, J. Biol. Chem. 241 (1966) 425.