Thermodynamics and Kinetics of the Glyoxylate Cycle†

Robert A. Alberty*

Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139

Received September 1, 2006; Revised Manuscript Received October 18, 2006

ABSTRACT: Because the standard Gibbs energies of formation of all the species of reactants in the glyoxylate cycle are known at 298.15 K, it is possible to calculate the apparent equilibrium constants of the five reactions in the cycle in the pH range 5−9 and ionic strengths from 0 to ∼0.35 M. In making calculations on such a system, it is convenient to specify concentrations of coenzymes like NAD_{ox} and NAD_{red} because they are involved in many reactions and may be in steady states. Calculations are given for $[NAD_{ox}] = 1000[NAD_{red}]$ and $[NAD_{ox}] = 10[NAD_{red}]$. Equilibrium compositions are calculated using computer programs when all the reactants are present initially and when only glyoxylate and CoA are present initially. The kinetics of the reactions in the glyoxylate cycle at specified concentrations of NAD_{ox} and NAD_{red} are calculated by numerical solution of the steady-state rate equations for the case where the reactant concentrations are below their Michaelis constants and only glyoxylate and CoA are present initially.

To understand how living cells work, it is not enough to know the thermodynamics and kinetics of individual enzyme-catalyzed reactions because these reactions have to work together in a network of series and cycles of reactions. Thermodynamics can make major contributions to this understanding because it can do two things: (1) For a system involving many reactions, thermodynamics can tell whether each reaction will go to the right or left when the concentrations of all the reactants are known. (2) For such a system, thermodynamics can predict the composition that will be reached at equilibrium. To study the kinetics of the approach to equilibrium, steady-state rate equations for the enzyme-catalyzed reactions can be solved numerically. Both the thermodynamics and kinetics of enzyme-catalyzed reactions are based on the assumption that hydrogen ions are added to the solution or neutralized to hold the pH constant. The steady-state rate equations must include rate parameters for both the forward and reverse reactions. The equilibrium composition calculated by solving the rate equations must agree with the equilibrium composition calculated using thermodynamics. In the calculations presented here, it is assumed that the concentrations of NAD_{ox} and NAD_{red}[1] are constant because they are involved in many other reactions. Calculations are presented for oxidizing conditions ($[NAD_{ox}] = 1000[NAD_{red}]$) and less oxidizing conditions ($[NAD_{ox}] = 10[NAD_{red}]$).

The simplest example of a steady-state rate equation including both the forward and reverse reactions is the Michaelis−Menten rate equation for the reaction S = P, for which the rate v of reaction is given by

$$v = \frac{(V_S/K_S)[S] - (V_P/K_P)[P]}{1 + [S]/K_S + [P]/K_P} \quad (1)$$

where K_S and K_P are the Michaelis constants for the forward and reverse reactions and V_S and V_P are the limiting velocities of the forward and reverse reactions. At substrate concentrations significantly below the Michaelis constants for S and P, the steady-state rate equation reduces to

$$v = (V_S/K_S)[S] - (V_P/K_P)[P] \quad (2)$$

The apparent equilibrium constant K' for the catalyzed reaction at a specified pH is given by the Haldane relation

$$K' = [P]_{eq}/[S]_{eq} = V_S K_P/V_P K_S \quad (3)$$

This is quite remarkable because all of the kinetic parameters depend on the properties of the enzyme, but K' is completely independent of the properties of the enzyme. If V_S/K_S and K' are known, V_P/K_P can be calculated. At sufficiently low reactant concentrations, the rate of the forward reaction is proportional to [S] and the enzyme concentration, and the rate of the reverse reaction is proportional to [P] and the enzyme concentration. In this paper, eqs 2 and 3 are used in the form $v = k_f[S] - k_r[P]$ and $K' = k_f/k_r$. When the steady-state concentrations of NAD_{ox} and NAD_{red} are specified, K'' will be used instead of K'.

The steady-state rate equation for a rapid-equilibrium random-order ternary-complex mechanism for the reaction A + B = C + D is (*1*,*2*)

$$v = \frac{(V_{AB}/K_{AB})[A][B] - (V_{CD}/K_{CD})[C][D]}{1 + [A]/K_A + [B]/K_B + [C]/K_C + [D]/K_D + [A][B]/K_{AB} + [C][D]/K_{CD}} \quad (4)$$

where V_{AB} and V_{CD} are proportional to the enzyme concentration and the other constants will be referred to here as Michaelis constants. At substrate concentrations significantly below the Michaelis constants

† This research was supported by NIH grant 5-RO1-GM48358-10.

* Corresponding Author. E-mail: alberty@mit.edu. Telephone: 617-253-2456. Fax: 617-253-7030.

[1] Abbreviations: NAD_{ox}, nicotinamide adenine dinucleotide oxidized form; NAD_{red}, nicotinamide adenine dinucleotide reduced form; malate, L-malic acid.

10.1021/bi061829e CCC: $33.50
Published on Web 12/05/2006

$$\upsilon = (V_{AB}/K_{AB})[A][B] - (V_{CD}/K_{CD})[C][D] \quad (5)$$

The apparent equilibrium constant K' for the catalyzed reaction is given by the Haldane relation

$$K' = [C]_{eq}[D]_{eq}/[A]_{eq}[B]_{eq} = V_{AB}K_{CD}/V_{CD}K_{AB} \quad (6)$$

In the calculations discussed here, it is assumed that the rate equations at substrate concentrations significantly below the Michaelis constants are of the general form of eqs 2 and 5. Rate equations may be more complicated than eqs 1 and 4, but there is always a Haldane relation of the type shown here. Equations 5 and 6 will be used in the form $\upsilon = k_f[A][B] - k_r[C][D]$ and $K' = k_f/k_r$.

METHODS

1. Calculations of Equilibrium Constants in the Glyoxylate Cycle. The reactions in the glyoxylate cycle are (*3*)

EC 2.3.3.9 glyoxylate + acetyl-CoA + $H_2O \rightleftarrows$ malate + CoA (7)

EC 1.1.1.37 malate + $NAD_{ox} \rightleftarrows$ oxaloacetate + NAD_{red} (8)

EC 2.3.1.1 oxaloacetate + acetyl-CoA + $H_2O \rightleftarrows$ citrate + CoA (9)

EC 4.2.1.3 citrate $\rightleftarrows$ isocitrate (10)

EC 4.1.3.1 isocitrate $\rightleftarrows$ succinate + glyoxylate (11)

The net reaction is

2acetyl-CoA + NAD_{ox} + $2H_2O \rightleftarrows$ succinate + 2CoA + NAD_{red} (12)

Because the standard thermodynamic properties of the species of all these reactants are in BasicBiochemData3 (*4*), it is possible to use the program calckprime (*5*) in Mathematica (*6*) to calculate the apparent equilibrium constants K' of these five reactions at 298.15 K, in the pH range 5−9, and at 0.25 M ionic strength. Values of these apparent equilibrium constants using molar concentrations are shown in Table 1.

It is evident that reaction 8 does not go very far to the right. To accomplish the net reaction, it is pushed by reaction 7 and pulled by reaction 9. Note that

$$K_7'K_8'K_9'K_{10}'K_{11}' = K_{12}' \quad (13)$$

In calculating equilibrium concentrations and kinetics for systems of reactions, it is convenient to specify concentrations of coenzymes like NAD_{ox} and NAD_{red} because they are involved in many reactions and are usually in steady states. When $[NAD_{ox}] = 1000[NAD_{red}]$ (oxidizing conditions) or $[NAD_{ox}] = 10[NAD_{red}]$ (less oxidizing conditions), the five reactions in the glyoxylate cycle can be represented as follows:

EC 2.3.3.9 glyoxylate(1) + acetyl-CoA(2) $\rightleftarrows$ malate(3) + CoA(4) (14)

EC 1.1.1.37 malate(3) $\rightleftarrows$ oxaloacetate(5) (15)

EC 2.3.1.1 oxaloacetate(5) + acetyl-CoA(2) $\rightleftarrows$ citrate(6) + CoA(4) (16)

EC 4.2.1.3 citrate(6) $\rightleftarrows$ isocitrate(7) (17)

EC 4.1.3.1 isocitrate(7) $\rightleftarrows$ succinate(8) + glyoxylate(1) (18)

The net reaction is

2acetyl-CoA $\rightleftarrows$ succinate + 2CoA (19)

Note that H_2O has been omitted because its concentration is not in the expressions for the apparent equilibrium constants or the rate equations. The eight reactants (excluding $[NAD_{ox}]$ and $[NAD_{red}]$) have been numbered because they are referred to by integers in rate equations given later.

When steady-state concentrations are specified for some reactants, it is necessary to use the symbol K'' for the apparent equilibrium constants, rather than K', to indicate that some reactant concentrations are specified in addition to the pH. When $[NAD_{ox}] = 1000[NAD_{red}]$, $K_{15}'' = 8.87 \times 10^{-3}$ at 298.15 K, pH 7, and 0.25 M ionic strength, rather than 8.87×10^{-6} in Table 1, but $K'' = K'$ for the other reactions. When $[NAD_{ox}] = 10[NAD_{red}]$, $K_{15}'' = 8.87 \times 10^{-5}$. The apparent equilibrium constants K'' for the five reactions that are used in the calculation of equilibrium concentrations are given in Table 2.

These rate constants are discussed later where changes will be made in k_f and k_r for reactions 14 and 16. The apparent equilibrium constant for the net reaction is given by the following equation:

$$K_{14}''K_{15}''K_{16}''K_{17}''K_{18}'' = K_{19}'' \quad (20)$$

When $[NAD_{ox}] = 1000[NAD_{red}]$, $K_{19}'' = 8.67 \times 10^{12}$; when $[NAD_{ox}] = 10[NAD_{red}]$, $K_{19}'' = 8.67 \times 10^{10}$.

2. Calculation of Equilibrium Concentrations in the Glyoxylate Cycle When All the Reactants are Initially Present at 10^{-3} M. Equilibrium concentrations for systems of reactions cannot be calculated analytically, but they can be calculated using the Newton−Raphson iteration method. In 1978, Krambeck (*7*) wrote the computer program equcalc in APL to carry out this calculation for a reaction system of ideal gases at a specified pressure. Later, he modified it to apply to reactions in ideal solutions and named it equcalcc. This program requires the conservation matrix for the system. Krambeck adapted this program to Mathematica. However, the program equcalcc cannot be used for reactions involving H_2O (as in reactions 7 and 9) because $[H_2O]$ is not included the expression for the apparent equilibrium constant. This problem is solved by use of the program equcalcrx, which uses the stoichiometric number matrix for the reaction system (*8*). The program equcalcrx calculates an appropriate stoichiometric number matrix and calls on equcalcc to calculate the equilibrium composition. These programs are available in BasicBiochemData3 (*4*), Thermodynamics of Biochemical Reactions (*9*), and Biochemical Thermodynamics: Applications of Mathematica (*10*).

The transposed stoichiometric matrix for the glyoxylate cycle at specified NAD_{ox} and NAD_{red} is given in Table 3. Note that H_2O is omitted.

Table 1: Apparent Equilibrium Constants K' of Reactions 7−12 at 298.15 K, in the pH Range 5−9, and at 0.25 M Ionic Strength

reaction	pH 5	pH 6	pH 7	pH 8	pH 9
7	3.18×10^{6}	2.25×10^{7}	2.31×10^{8}	3.83×10^{9}	1.92×10^{11}
8	5.98×10^{-8}	8.50×10^{-7}	8.87×10^{-6}	8.91×10^{-5}	8.91×10^{-4}
9	3.13×10^{6}	8.51×10^{6}	6.97×10^{7}	1.13×10^{9}	5.63×10^{10}
10	0.0886	0.0687	0.0684	0.0684	0.0684
11	0.411	0.748	0.887	0.906	0.908
12	1.67×10^{4}	8.35×10^{6}	8.67×10^{9}	2.39×10^{13}	5.98×10^{17}

Table 2: Apparent Equilibrium Constants and Rate Constants at 298.15 K, pH 7, and Specified $[NAD_{ox}]/[NAD_{red}]$ That Are Used in Calculations of Equilibrium Concentrations

		$[NAD_{ox}] = 10^3[NAD_{red}]$			$[NAD_{ox}] = 10[NAD_{red}]$	
reaction	K''	k_f	k_r	K''	k_f	k_r
14	2.31×10^{8}	1	4.33×10^{-9}	2.31×10^{8}	1	4.33×10^{-9}
15	8.87×10^{-3}	1	113	8.87×10^{-5}	1	1.13×10^{4}
16	6.97×10^{7}	1	1.43×10^{-8}	6.97×10^{7}	1	1.43×10^{-8}
17	0.0684	1	14.6	0.0684	1	14.6
18	0.887	1	1.13	0.887	1	1.13

Table 3: Transposed Stoichiometric Number Matrix for Reactions 14−18

reaction	glyoxylate	acetyl-CoA	malate	CoA	oxaloacetate	citrate	isocitrate	succinate
14	−1	−1	1	1	0	0	0	0
15	0	0	−1	0	1	0	0	0
16	0	−1	0	1	−1	1	0	0
17	0	0	0	0	0	−1	1	0
18	1	0	0	0	0	0	−1	1

Table 4: Equilibrium Concentrations at 298.15 K, pH 7, 0.25 M Ionic Strength and when the Eight Reactants are Initially at 10^{-3} M

reactant	initial concn/M	concn/M $[NAD_{ox}] = 1000[NAD_{red}]$	concn/M $[NAD_{ox}] = 10[NAD_{red}]$
glyoxylate	10^{-3}	1.00×10^{-3}	1.36×10^{-4}
acetyl-CoA	10^{-3}	3.38×10^{-11}	3.09×10^{-10}
malate	10^{-3}	3.92×10^{-3}	4.86×10^{-3}
CoA	10^{-3}	2.00×10^{-3}	2.00×10^{-3}
oxaloacetate	10^{-3}	3.48×10^{-5}	4.31×10^{-7}
citrate	10^{-3}	4.09×10^{-5}	4.64×10^{-6}
isocitrate	10^{-3}	2.80×10^{-6}	3.17×10^{-7}
succinate	10^{-3}	2.48×10^{-3}	2.07×10^{-3}

Table 5: Equilibrium Concentrations at 298.15 K, pH 7, 0.25 M Ionic Strength and when Glyoxylate and Acetyl-CoA Are Initially Present at 10^{-3} M

reactant	initial concn/M	concn/M $[NAD_{ox}] = 1000[NAD_{red}]$	concn/M $[NAD_{ox}] = 10[NAD_{red}]$
glyoxylate	10^{-3}	4.55×10^{-4}	1.35×10^{-4}
acetyl-CoA	10^{-3}	5.12×10^{-12}	2.78×10^{-11}
malate	0	5.38×10^{-4}	8.65×10^{-4}
CoA	0	1.00×10^{-3}	1.00×10^{-3}
oxaloacetate	0	4.77×10^{-6}	7.67×10^{-8}
citrate	0	1.70×10^{-6}	1.49×10^{-7}
isocitrate	0	1.16×10^{-7}	1.02×10^{-8}
succinate	0	2.27×10^{-4}	6.72×10^{-5}

Many types of equilibrium calculations can be made for a system like the glyoxylate cycle. The initial concentrations can be taken to be the same for the eight reactants, only several reactants may be present initially, and $[NAD_{ox}]/[NAD_{red}]$ can be changed. Here, equilibrium concentrations are calculated first when the initial concentrations of the eight reactants are 10^{-3} M and second when only glyoxylate and acetyl-CoA are present initially at 10^{-3} M. Table 4 gives the calculated equilibrium concentrations with oxidizing conditions ($[NAD_{ox}] = 1000[NAD_{red}]$) and with less oxidizing conditions ($[NAD_{ox}] = 10[NAD_{red}]$).

Note that, under oxidizing conditions, there is more glyoxylate at equilibrium than under less oxidizing conditions. There are also big changes in the concentrations of oxaloacetate, citrate, and isocitrate in the expected directions.

These calculated equilibrium concentrations can be tested by using them to calculate the apparent equilibrium constants of the five reactions. The apparent equilibrium constants can be calculated by using the dot product of the vector lnc of natural logarithms of the equilibrium concentrations of the eight reactants and the stoichiometric number matrix ν''.

$$K'' = \exp[\ln c\nu''] \qquad (21)$$

The equilibrium concentrations in Table 4 satisfy this test.

3. Calculation of Equilibrium Concentrations in the Glyoxylate Cycle When Only Glyoxylate and Acetyl-CoA are Initially Present at 10^{-3} M. The equilibrium composition can also be calculated when only several reactants are present initially. Table 5 gives the equilibrium composition when only glyoxylate and acetyl-CoA are present initially and there are oxidizing conditions or less oxidizing conditions.

4. Calculation of Reactant Concentrations in the Glyoxylate Cycle as Functions of Time with Oxidizing Conditions. The built-in Mathematica object NDSolve provides numerical solutions to systems of differential equations. The eight steady-state rate equations for the glyoxylate cycle (reactions 14 to 18) are typed in, and the initial concentrations of the reactants are also typed in as shown in the Appendix. NDSolve yields interpolation functions for the concentrations of the eight reactants that can be plotted or tabulated.

These calculations are made for the case that glyoxylate and acetyl-CoA are present initially at 10^{-3} M in a buffer at 298.15 K, pH 7, and 0.25 M ionic strength. For Figures 1 and 2, $[NAD_{ox}] = 1000[NAD_{red}]$ is maintained during the reaction. The ratio of the rate constants for the forward and

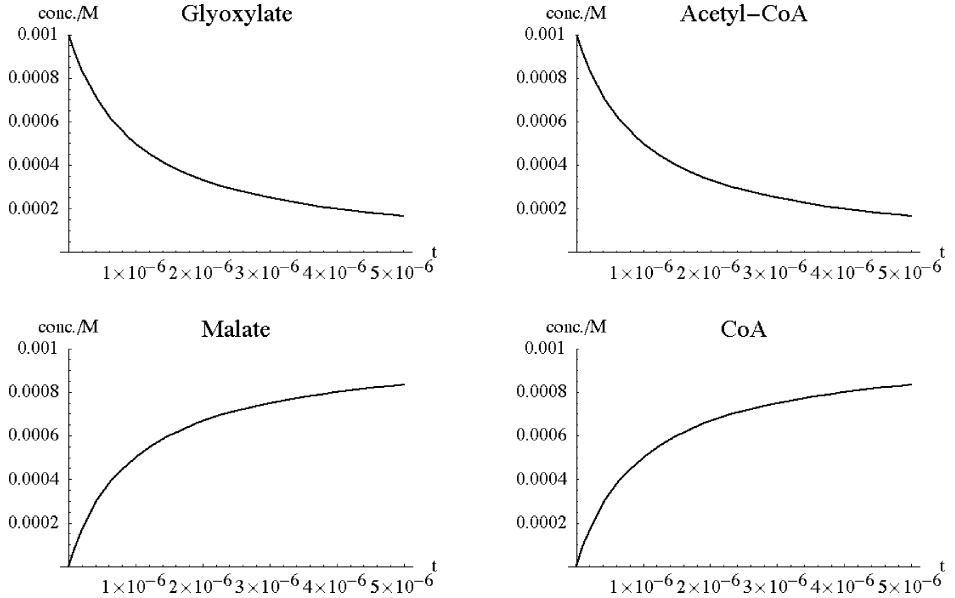

FIGURE 1: Concentrations of four reactants in the first stage of the glyoxylate cycle at 298.15 K, pH 7, and 0.25 M ionic strength under oxidizing conditions ($[NAD_{ox}] = 1000[NAD_{red}]$).

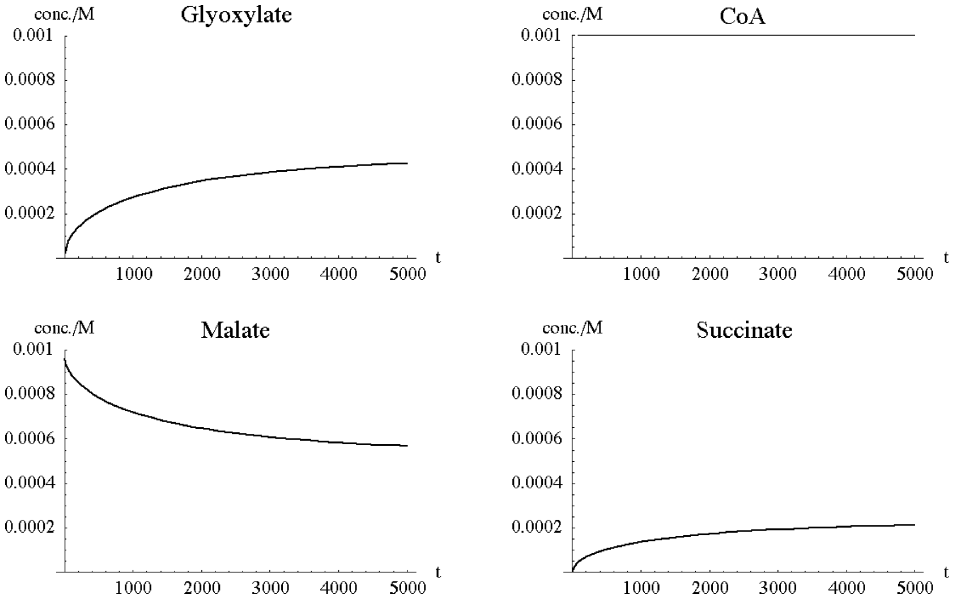

FIGURE 2: Concentrations of four reactants in the second stage of the glyoxylate cycle at 298.15 K, pH 7, and 0.25 M ionic strength under oxidizing conditions ($[NAD_{ox}] = 1000[NAD_{red}]$).

reverse reactions of each enzyme-catalyzed reaction must satisfy the following expression for the apparent equilibrium constant: $K'' = k_f/k_r$. This ensures that the correct equilibrium concentrations will be approached at long times. The k_f and k_r are proportional to enzyme concentrations; thus, enzyme concentrations can be selected so that equilibrium is reached in approximately 50 000 units of arbitrary time. In order to accomplish this, the forward and reverse rate constants for both reactions 14 and 16 are multiplied by 10^9 so that there will not be any rate constants in the system less than unity. If this is not done, it takes much longer to get close to equilibrium. For Figures 1 and 2, the forward rate constants k_f for reactions 14−18 are taken to be 10^9, 1, 10^9, 1, and 1. The reverse rate constants k_r are taken to be 4.33, 113, 14.3, 14.6, and 1.13. These rate constants satisfy $K'' = k_f/k_r$. This means that reaction 14 occurs very rapidly to produce stoichiometric amounts of malate and CoA. Thus, the system reaches equilibrium in two stages. In the first stage, glyoxylate and acetyl-CoA react to form malate and CoA. In the second stage, malate is essentially the initial reactant, and CoA remains at its equilibrium concentration because it is not involved in any forward reaction.

The concentrations of the reactants in the first stage are shown as functions of time up to 5×10^{-6} arbitrary units of time in Figure 1.

The concentrations of four reactants in the second stage of the reaction are shown in Figure 2. The concentrations of the other four reactants are too low to show in plots on this concentration scale, but they can be calculated from the solution of NDSolve and do satisfy the equilibrium expressions at long times. Figure 2 shows that glyoxylate is formed in reaction 18 because of the cyclic nature of this mechanism and builds up to 4.55×10^{-4} M at equilibrium. Malate is reduced to 5.38×10^{-4} M at equilibrium, and succinate is formed to the extent of 2.27×10^{-4} M at equilibrium. The solution of the rate equations shows that, at much longer

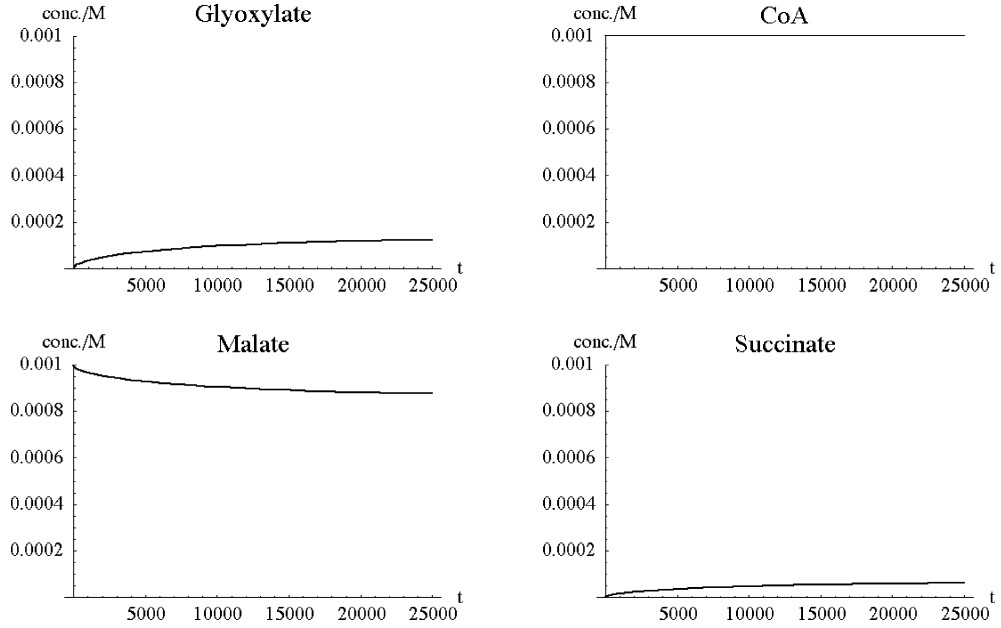

FIGURE 3: Concentrations of four reactants in the second stage of the glyoxylate cycle at 298.15 K, pH 7, and 0.25 M ionic strength under more reducing conditions ($[NAD_{ox}] = 10[NAD_{red}]$).

times (of the order of 50 000 units of arbitrary time), the equilibrium concentrations of all reactants are equal to those calculated using equcalcrx and are shown in Table 5. Net reaction 19 suggests that the amount of succinate at equilibrium should be half of the initial amount of acetyl-CoA, but it is significantly less. The initial amount of carbon in the system (excluding CoA) is distributed between glyoxylate, malate, and succinate at equilibrium. This is a kind of warning about the significance of net reactions for cycles.

Also note that eq 20 at specified concentrations of $[NAD_{ox}]$ and $[NAD_{red}]$ can be written as

$$\frac{k_{14f}}{k_{14r}}\frac{k_{15f}}{k_{15r}}\frac{k_{16f}}{k_{16r}}\frac{k_{17f}}{k_{17r}}\frac{k_{18f}}{k_{18r}} = K_{19}'' \qquad (22)$$

where $K_{19}'' = 8.67 \times 10^{12}$ when $[NAD_{ox}] = 1000[NAD_{red}]$.

5. Calculation of Reactant Concentrations in the Glyoxylate Cycle as Functions of Time with Less Oxidizing Conditions. As in the previous section, k_f and k_r for reactions 14 and 16 have been multiplied by 10^9 so that the rate constants used in the calculations are all greater than unity. Thus, the forward rate constants for the five reactions are 10^9, 1, 10^9, 1, and 1, the same as in the preceding calculation. The reverse rate constants are 4.33, 1.13×10^4, 14.3, 14.6, and 1.13, different from the preceding calculation. Therefore, the system reaches equilibrium in two stages. In the first stage, glyoxylate and acetyl-CoA react to form malate and CoA. In the second stage, malate is essentially the initial reactant, and CoA remains at its equilibrium concentration because it is not involved in any forward reaction. The kinetics for the first stage is the same as under oxidizing conditions; thus, it is not necessary to recalculate Figure 1.

The apparent equilibrium constant K'' for reaction 15 is now smaller by a factor of 100; thus, there will be much lower concentrations of oxaloacetate at equilibrium. This is a result of the less oxidizing conditions. The concentrations of four reactants in the second stage of the reaction are shown in Figure 3. The concentrations of the other four reactants are too low to show in plots on this concentration scale, but they can be calculated from the solution of NDSolve and do satisfy the equilibrium expressions at long times. Because oxaloacetate does not build up, more malate remains at equilibrium and less glyoxylate is formed at equilibrium. The initial amount of carbon in the system (excluding CoA) is distributed between glyoxylate, malate, and succinate at equilibrium. Note that, for this case, eq 22 yields 8.67×10^{10} for the net reaction, compared with 8.67×10^{12} for the oxidizing conditions.

DISCUSSION

The glyoxylate cycle is a good example for the calculation of the thermodynamics and kinetics of a biochemical reaction system because of the complications that it involves. It is a cyclic system; thus, the net reaction does not give a very useful description of the equilibrium concentrations. The glyoxylate cycle involves coenzymes so that oxidizing conditions can be compared with less oxidizing conditions. A wide range of apparent equilibrium constants is involved, and a wide range of rate constants is involved.

The kinetics of the approach to equilibrium in a system of reactions like the glyoxylate cycle depends on the concentrations of the five enzymes, the kinetic parameters for the forward and reverse reactions that they catalyze, and the initial composition. At low substrate concentrations, the rates of the forward and reverse reactions are proportional to the concentrations of reactants. When Michaelis constants and other kinetic parameters are known for a system of reactions, additional terms can be put into the steady-state rate equations. Note that the denominator terms in the rate law always reduce the rate and that this can be compensated for by raising the enzyme concentration. The limiting concentrations at long times have to yield the correct apparent equilibrium constants for the catalyzed reactions.

The thermodynamic treatment given here does assume that the cycle has reached equilibrium, but this shows where the system is headed. If reactants are being input and products are being withdrawn, equilibrium will not be reached and

reactants will have higher concentrations and products will have lower concentrations than the equilibrium concentrations. With information on inputs and outputs, steady-state concentrations can be calculated. If actual enzyme concentrations and limiting velocities for these enzymes are known, calculations can be made using this information, but the calculations given here shown the main features as to how this cyclic system approaches equilibrium.

Different initial concentrations of reactants can be studied. Effects of pH and ionic strength and concentrations of coenzymes can be studied. These effects are too complicated to be summarized, but with the rate equations in a computer, it is easy to change all these variables.

Frieden and co-workers (*11*, *12*) have made available the computer program KINSIM to calculate the concentrations of species in enzyme mechanisms as functions of time, given the rate constants.

ACKNOWLEDGMENT

I am indebted to Robert N. Goldberg (NIST) for many helpful suggestions.

APPENDIX

Typing the following input into Mathematica yields the concentrations of the eight reactants from $t = 10$ to $t = 5000$ when the eight reactants are initially at 10^{-3} M. The use of Plot yields the plots in Figure 2.

```
eqns3 = {c1′[t] == −(10^9)*c1[t]*c2[t] +
                4.33*c3[t]*c4[t] + c7[t] − 1.13*c8[t]*c1[t]
c2′[t] == −(10^9)*c1[t]*c2[t] + 4.33*c3[t]*c4[t] −
                (10^9)*c5[t]*c2[t] + 14.3*c4[t]*c6[t]
c3′[t] == (10^9)*c1[t]*c2[t] - 4.33*c3[t]*c4[t] −
                c3[t] + 113*c5[t]
c4′[t] == (10^9)*c2[t]*c5[t] − 14.3*c4[t]*c6[t] +
                (10^9)*c1[t]*c2[t] − 4.33*c3[t]*c4[t]
c5′[t] == c3[t] − 113*c5[t] − (10^9)*c5[t]*c2[t] +
                14.3*c4[t]*c6[t]
c6′[t] == (10^9)*c2[t]*c5[t] − 14.3*c4[t]*c6[t] −
                c6[t] + 14.6*c7[t]
c7′[t] == c6[t] − 15.6*c7[t] + 1.13*c1[t]*c8[t]
c8′[t] == c7[t] − 1.13*c1[t]*c8[t],
c1[0] == 1.*10^−3, c2[0] == 1.*10^−3, c3[0] ==
    0, c4[0] == 0, c5[0] == 0, c6[0] == 0, c7[0] == 0,
                c8[0] == 0}

vars = {c1[t], c2[t], c3[t], c4[t], c5[t], c5[t], c7[t], c8[t]}
solution15 = NDSolve[eqns3, vars, {t, 10, 5000}]
Plot[Evaluate[vars /. solution15],
        {t, 10, 5000}, AxesLabel -> {“t”, “conc./M”}]
```

REFERENCES

1. Roberts, D. V. (1977) *Enzyme Kinetics*, Cambridge University Press, Cambridge, U.K.
2. Cornish-Bowden, A. (2004) *Fundamentals of Enzyme Kinetics*, Portland Press Ltd, London.
3. Webb, E. C. (1992) *Enzyme Nomenclature 1992*, Academic Press, New York, http://www.chem.qmw.ac.uk/iubmb/enzyme.
4. Alberty, R. A. BasicBiochemData3, http://library.wolfram.com/infocenter/MathSource/797.
5. Alberty, R. A. (2002) Inverse Legendre Transform in Biochemical Thermodynamics: Applied to the Last Five Reactions of Glycolysis, *J. Phys. Chem. B 106*, 6594−6559.
6. Wolfram Research, 100 World Trade Center Drive, Champaign, IL.
7. Krambeck, F. J. (1978) Presented at the 71st Annual Meeting of the AIChE, Miami Beach, FL, Nov 16.
8. Alberty, R. A. (2001) Calculation of Equilibrium Compositions of Biochemical Reactions Systems Involving Water as a Reactant, *J. Phys. Chem. B 105*, 1109−1114.
9. Alberty, R. A. (2003) *Thermodynamics of Biochemical Reactions*, Wiley, Hoboken, NJ.
10. Alberty, R. A. (2006) *Biochemical Thermodynamics; Applications of Mathematica*, Wiley, Hoboken, NJ.
11. Barshop, B. A., Wenn, R. F., and Frieden, C. (1983) Analysis of Numerical Methods for Computer Simulation of Kinetic Processes: Development of KINSIM-A Flexible, Portable System, *Anal. Biochem. 130*, 134−145.
12. KINSIM can be downloaded from http://www.biochem.wustl.edu/cflab.

BI061829E