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Equilibrium concentrations for pyruvate dehydrogenase and the citric acid cycle at specified concentrations of certain coenzymes

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Abstract

It is of interest to calculate equilibrium compositions of systems of biochemical reactions at specified concentrations of coenzymes because these reactants tend to be in steady states. Thermodynamic calculations under these conditions require the definition of a further transformed Gibbs energy G'' by use of a Legendre transform. These calculations are applied to the pyruvate dehydrogenase reaction plus the citric acid cycle, but steady-state concentrations of CoA, acetyl-CoA and succinyl-CoA cannot be specified because they are involved in the conservation of carbon atoms. These calculations require the use of linear algebra to obtain further transformed Gibbs energies of formation of reactants and computer programs to calculate equilibrium compositions. At specified temperature, pH, ionic strength and specified concentrations of several coenzymes, the equilibrium composition depends on the specified concentrations of the coenzymes and the initial amounts of reactants.

Keywords: Citric acid cycle; Equilibrium compositions; Apparent equilibrium constants; Transformed thermodynamic properties; Legendre transforms

1. Theory

When the pH is specified, the criterion for spontaneous change and equilibrium is provided by the transformed Gibbs energy G' that is defined by a Legendre transform [1–3]. The definition of the transformed Gibbs energy leads to the calculation of standard transformed Gibbs energies of formation $\Delta_f G'^\circ$ of reactants at a specified temperature, pH and ionic strength. Knowledge of $\Delta_f G'^\circ$ for all the reactants in a biochemical reaction makes it possible to calculate the apparent equilibrium constant K' of the reaction. For a system of biochemical reactions like glycolysis at specified pH, the number of reactants N' is equal to the number of components C' plus the number R' of independent biochemical reactions; i.e. $N' = C' + R'$ [4]. Components are important because they are conserved in a reaction system; in other words, there is a conservation equation for each component. In considering the thermodynamics of chemical reactions, atoms of elements are conserved, but some of these conservation equations may be redundant. The number of components is the number of independent conservation equations. In considering biochemical reactions at specified pH,

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hydrogen atoms are not conserved, but atoms of other elements are. The superficial difference between chemical reactions and biochemical reactions is that chemical reactions are written in terms of species, including hydrogen ions, and biochemical reactions are written in terms of reactants (sums of species), hydrogen ions being omitted. But enzyme catalysts may introduce further conservation equations, and groups of atoms like adenine may be conserved and can be counted as components. The conservation matrix $\boldsymbol{A}'$ of a biochemical reaction system at a specified pH and its stoichiometric number matrix $\boldsymbol{\nu}'$ are related [4]:

$$\boldsymbol{A}'\boldsymbol{\nu}' = \mathbf{0} \text{ and } (\boldsymbol{\nu}')^{\mathrm{T}}(\boldsymbol{A}')^{\mathrm{T}} = \mathbf{0} \quad (1)$$

A matrix of zeros is represented by $\mathbf{0}$. The transpose of a matrix is indicated by a superscript T. The conservation matrix $\boldsymbol{A}'$ gives the coefficients in the conservation equations for components and has a row for each component and a column for each reactant; in other words, the conservation matrix is $C' \times N'$. The stoichiometric number matrix $\boldsymbol{\nu}'$ gives the coefficients in the reaction equations and has a row for each reactant and a column for each reaction; in other words, the stoichiometric number matrix is $N' \times R'$. Eq. (1) is important because it provides a way to go from conservation matrices to stoichiometric number matrices and vice versa. This article emphasizes the use of the second form of Eq. (1) because reaction equations are known for a system of biochemical reactions and the corresponding conservation matrix is needed for the calculations described here.

It is important to understand that neither $\boldsymbol{\nu}'$ nor $\boldsymbol{A}'$ is unique. In biochemistry it is clear that an enzyme catalyzes a certain reaction, but the equilibrium composition of a system of biochemical reactions can be calculated just as well when two of the reactions in the system are added and this new reaction is used to replace one of the original reactions. Similarly, the conservation matrix for a system of biochemical reactions is not unique because it can be row-reduced without changing the important information contained in it. When the conservation matrix $\boldsymbol{A}'$ is calculated from the stoichiometric number matrix $\boldsymbol{\nu}'$, the components become identified with the reactants listed first in the columns of the stoichiometric number matrix, provided these reactants are sufficiently different in terms of the atoms and groups they contain. Because of this lack of uniqueness, it is necessary to say that the null space of $\boldsymbol{A}'$ provides a basis for $\boldsymbol{\nu}'$ and the null space of $(\boldsymbol{\nu}')^{\mathrm{T}}$ provides a basis for $(\boldsymbol{A}')^{\mathrm{T}}$. Two matrices of the same size can be compared by row-reducing both of them. If the same row-reduced form is obtained from two sources, the matrices contain the same information.

If different sets of components can be used to express the conservation equations for a system of biochemical reactions, why is the choice of a particular set of components important? The choice of components is important because that determines the reactants that can have specified concentrations. The choice of independent intensive variables for a thermodynamic calculation on a system is an important first step in the calculation of an equilibrium composition. This is an example of the point made by Callen [5] that 'The choice of variables in terms of which a given system is formulated, while seemingly an innocuous step, is often the most crucial step in the solution'.

In order to specify the concentrations of several coenzymes, they must each be components. In a living cell the concentrations of coenzymes tend to be in steady states because they are involved in many other reactions. When several coenzymes are at specified concentrations, the criterion for spontaneous change and equilibrium is provided by a further transformed Gibbs energy G'' that is defined by the following Legendre transform:

$$G'' = G' - \sum n_{\mathrm{c}}(\mathrm{coenz}_i)\mu'(\mathrm{coenz}_i) \quad (2)$$

$n_{\mathrm{c}}(\mathrm{coenz}_i)$ is the amount of the component that corresponds with coenzyme i and $\mu'(\mathrm{coenz}_i)$ is the specified transformed chemical potential of coenzyme i. It is convenient to include a term for H_2O in this sum of products of conjugate variables because the thermodynamic convention that the activity of H_2O is taken as unity in dilute aqueous solutions makes the conservation matrix $\boldsymbol{A}'$ incompatible with $\boldsymbol{\nu}'$ for a system of biochemical reactions [6,7]. When a term $n_{\mathrm{c}}(\mathrm{H_2O})\mu'(\mathrm{H_2O})$ is included in the summation in Eq. (2), the conser-

vation matrix $\boldsymbol{A}''$ of a biochemical reaction system at specified concentrations of certain coenzymes and H_2O is consistent with its corresponding stoichiometric number matrix $\boldsymbol{\nu}''$, so that

$$\boldsymbol{A}''\boldsymbol{\nu}'' = \mathbf{0} \text{ and } (\boldsymbol{\nu}'')^{\mathrm{T}}(\boldsymbol{A}'')^{\mathrm{T}} = \mathbf{0} \tag{3}$$

The conservation matrix is $C'' \times N''$, the stoichiometric number matrix is $N'' \times R''$ and $N'' = C'' + R''$.

Specifying the concentrations of coenzymes makes it possible to obtain a more global view of a system of biochemical reactions. This is illustrated by the calculations that have been made for glycolysis [8,9]. When the concentrations of the coenzymes involved in glycolysis are specified, all the thermodynamic properties of the reaction system can be represented by a single reaction $C_6 = 2C_3 + 2H_2O$, where C_6 is the pseudoisomer group of reactants with six carbon atoms and C_3 is the pseudoisomer group of reactants with three carbon atoms. The equilibrium composition in terms of C_6 and C_3 is readily calculated, and it has been shown that the equilibrium concentrations of reactants within these two pseudoisomer groups can be calculated. The equilibrium concentrations of species can also be calculated. This approach provides more thermodynamic information than the net reaction.

Making the Legendre transform in Eq. (2) leads [4] to the following equation for the standard further transformed Gibbs energy of formation $\Delta_f G''^{\circ}$ of reactants when steady-state concentrations of certain coenzymes and the activity of H_2O are specified;

$$\Delta_f G''^{\circ}_i = \Delta_f G'^{\circ}_i - \sum N_{ik}(\mathrm{coenz}_k)\big(\Delta_f G'^{\circ}(\mathrm{coenz}_k) + RT\ \ln[\mathrm{coenz}_k]\big) - N_i(H_2O)\Delta_f G'^{\circ}(H_2O) \tag{4}$$

$N_{ik}(\mathrm{coenz}_i)$ is the number of molecules of coenzyme k required to make up a molecule of reactant i. The $N_{ik}(\mathrm{coenz}_i)$ and $N_i(H_2O)$ can be determined from the conservation matrix $\boldsymbol{A}'$ for the reaction system at the specified pH and ionic strength. In Section 3 we will see how that is done with matrix operations. The conservation matrix $\boldsymbol{A}''$ is obtained from $(\boldsymbol{\nu}'')^{\mathrm{T}}$ using the second form of Eq. (3).

2. The stoichiometric number matrix for the pyruvate dehydrogenase reaction and the citric acid cycle

The equilibrium compositions in the pyruvate dehydrogenase reaction and the citric acid cycle are discussed in terms of the following 10 reactions:

$$\mathrm{pyruvate} + \mathrm{CoA} + \mathrm{NAD_{ox}} + \mathrm{H_2O} = \mathrm{CO_2tot} + \mathrm{NAD_{red}} + \text{acetyl-CoA} \tag{5}$$

$$\text{acetyl-CoA} + \text{oxaloacetate} + \mathrm{H_2O} = \mathrm{CoA} + \text{citrate} \tag{6}$$

$$\text{citrate} = \textit{cis}\text{-aconitate} + \mathrm{H_2O} \tag{7}$$

$$\textit{cis}\text{-aconitate} + \mathrm{H_2O} = \text{iso-citrate} \tag{8}$$

$$\text{iso-citrate} + \mathrm{NAD_{ox}} + \mathrm{H_2O} = \text{ketoglutarate} + \mathrm{CO_2tot} + \mathrm{NAD_{red}} \tag{9}$$

$$\text{ketoglutarate} + \mathrm{NAD_{ox}} + \mathrm{CoA} + \mathrm{H_2O} = \text{succinyl-CoA} + \mathrm{CO_2tot} + \mathrm{NAD_{red}} \tag{10}$$

$$\text{succinyl-CoA} + \mathrm{P}_i + \mathrm{ADP} = \text{succinate} + \mathrm{ATP} + \mathrm{CoA} \tag{11}$$

$$\text{succinate} + \mathrm{FADenz_{ox}} = \text{fumarate} + \mathrm{FADenz_{red}} \tag{12}$$

$$\text{fumarate} + \mathrm{H_2O} = \text{malate} \tag{13}$$

$$\text{malate} + \mathrm{NAD_{ox}} = \text{oxaloacetate} + \mathrm{NAD_{red}} \tag{14}$$

The net reaction is

$$\mathrm{pyruvate} + 4\mathrm{NAD_{ox}} + \mathrm{FADenz_{ox}} + \mathrm{ADP} + \mathrm{P}_i + 5\mathrm{H_2O} = 3\mathrm{CO_2tot} + 4\mathrm{NAD_{red}} + \mathrm{FADenz_{red}} + \mathrm{ATP} \tag{15}$$

$\mathrm{FADenz_{ox}}$ and $\mathrm{FADenz_{red}}$ are used to indicate that this coenzyme is bound by a protein. Some of these reactions differ from those in textbooks because reactions involving carbon dioxide are

written in terms of CO_2tot to indicate that in making thermodynamic calculations it is more useful to deal with the total concentration of species of carbon dioxide (CO_2, H_2CO_3, HCO_3^- and CO_3^{2-}) in aqueous solution than with $P(CO_2)$. When CO_2(g) is changed to CO_2tot in a biochemical reaction, a H_2O has to be put on the other side [10–12]. The apparent equilibrium constants of these 11 reactions at 298.15 K, ionic strength 0.25 M and pHs 5, 6, 7, 8 and 9 have been published [4]. At a specified pH, this system of reactions involves 21 reactants and 10 independent reactions, and so there are $C' = N' - R' = 21 - 10 = 11$ components.

In the case of glycolysis, it was possible to specify the steady-state concentrations of all coenzymes, but this is not possible for the pyruvate dehydrogenase reaction and the citric acid cycle because CoA, succinyl-CoA and acetyl-CoA cannot all be components, as we will see from the conservation matrix in the next section. In calculating equilibrium compositions, the steady-state concentrations of CoA, succinyl-CoA and acetyl-CoA cannot be specified because they are involved in the conservation of carbon atoms. Therefore, the steady-state concentrations of only NAD_{ox}, NAD_{red}, $FADenz_{ox}$, $FADenz_{red}$, P_i, ADP and ATP will be specified in the calculations described here. The activity of water is also fixed in dilute aqueous solutions.

The stoichiometric number matrix $\boldsymbol{\nu}'$ for the system in Eqs. (5)–(14) is given in Table 1. The reactants can be put in any desired order, but the strategy of the calculations is to be able to specify concentrations of H_2O, NAD_{ox}, NAD_{red}, $FADenz_{ox}$, $FADenz_{red}$, P_i, ADP and ATP. Therefore, these eight reactants are listed first in the rows of the stoichiometric number matrix in that they can be treated as components.

This stoichiometric number matrix can be checked in three ways: (1) Adding each row should give the net reaction. (2) Since the reactions must be independent, transposing and row-reducing should not yield any zero rows. (3) Computer programs MKEQN [13] and NAMEMATRIX [14] can be used to print out reactions Eqs. (5)–(14) from the stoichiometric number matrix for proof reading.

3. Calculation of the conservation matrix for the pyruvate dehydrogenase reaction and the citric acid cycle

The stoichiometric number matrix $\boldsymbol{\nu}'$ for the 10 reactions of pyruvate dehydrogenase and the citric acid cycle in Table 1 can be used to calculate a basis for the corresponding conservation matrix using NullSpace in Mathematica® [15]. This is the way $(\boldsymbol{\nu}')^T(\boldsymbol{A}')^T = \mathbf{0}$ is used. The row-reduced form of this conservation matrix has the advantage that the C' rows can be labeled like the first C' reactants. The row-reduced form of the 11×21 conservation matrix is given in Table 2, which lists the components down the left side and reactants across the top.

This is a very important table because it divides the 21 reactants into 11 components (from H_2O to CO_2tot) and 10 non-components (acetyl-CoA to pyruvate). If the reactants are put in a different order, there will be different components and non-components, but the numbers of components and non-components will remain 11 and 10.

When the concentrations of NAD_{ox} to ATP are specified and H_2O is included in the Legendre transform, there are enough components left to represent the stoichiometric number matrix for the system at those specified concentrations. When H_2O to ATP are taken as components to be specified, these rows and columns in the conservation matrix are deleted. The remaining conservation matrix is 3×13, and it can be used to calculate a basis for the stoichiometric number matrix of this reaction system when concentrations of certain coenzymes are specified. Before discussing this calculation, note that there is other important information in Table 2.

The matrix in Table 2 can be divided into four sections: in the upper left there is an 8×8 unit matrix for the reactants for which steady-state concentrations are to be specified. In the lower left there is a 3×8 zero matrix for the components for which steady-state concentrations are not specified. The 11×13 matrix on the right side shows the 'contents' of the 11 components in the 13 reactants (CoA to pyruvate). As an example of the information in this section of the matrix, citrate is made up of $-2H_2O - 2NAD_{ox} + 2NAD_{red} - CoA +$ suc-

Table 1
The stoichiometric number matrix for reactions Eqs. (5)–(14) is 21×10

	Rx 5	Rx 6	Rx 7	Rx 8	Rx 9	Rx 10	Rx 11	Rx 12	Rx 13	Rx 14
H_2O	−1	−1	1	−1	−1	−1	0	0	−1	0
NAD_{ox}	−1	0	0	0	−1	−1	0	0	0	−1
NAD_{red}	1	0	0	0	1	1	0	0	0	1
$FADenz_{ox}$	0	0	0	0	0	0	0	−1	0	0
$FADenz_{red}$	0	0	0	0	0	0	0	1	0	0
P_i	0	0	0	0	0	0	−1	0	0	0
ADP	0	0	0	0	0	0	−1	0	0	0
ATP	0	0	0	0	0	0	1	0	0	0
CoA	−1	1	0	0	0	−1	1	0	0	0
Succinyl-CoA	0	0	0	0	0	1	−1	0	0	0
CO_2tot	1	0	0	0	1	1	0	0	0	0
Acetyl-CoA	1	−1	0	0	0	0	0	0	0	0
Citrate	0	1	−1	0	0	0	0	0	0	0
Cis-aconitate	0	0	1	−1	0	0	0	0	0	0
Iso-citrate	0	0	0	1	−1	0	0	0	0	0
Ketoglutarate	0	0	0	0	1	−1	0	0	0	0
Succinate	0	0	0	0	0	0	1	−1	0	0
Fumarate	0	0	0	0	0	0	0	1	−1	0
Malate	0	0	0	0	0	0	0	0	1	−1
Oxaloacetate	0	−1	0	0	0	0	0	0	0	1
Pyruvate	−1	0	0	0	0	0	0	0	0	0

cinyl-CoA + $2CO_2$tot. This accounts for the six carbon atoms of citrate. The column for pyruvate yields the net reaction for the citric acid cycle. The 8×13 upper part of this 11×13 matrix is referred to as the weighting matrix because it gives the numbers $N_{ik}(\text{coenz}_k)$ and $N_i(H_2O)$ of molecules of eight components with specified concentrations that are contained in 13 reactants. This information will make it possible to adjust $\Delta_f G'^\circ$ for the 13 reactants at specified temperature, pH and ionic strength to $\Delta_f G''^\circ$ at specified concentrations of seven coenzymes and H_2O (i.e. eight components). With these $\Delta_f G''^\circ$ values, the equilibrium concentrations of the 13 reactants can be calculated when the steady-state concentrations of certain coenzymes are specified. In the bottom right there is a 3×13 conservation matrix for the reaction system after the concentrations of certain

Table 2
The row-reduced conservation matrix $\boldsymbol{A}'$ for reactions Eqs. (5)–(14) is 11×21

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
H_2O	1	0	0	0	0	0	0	0	0	0	0	−4	−2	−3	−2	−1	0	0	1	1	−5
NAD_{ox}	0	1	0	0	0	0	0	0	0	0	0	−3	−2	−2	−2	−1	0	0	0	1	−4
NAD_{red}	0	0	1	0	0	0	0	0	0	0	0	3	2	2	2	1	0	0	0	−1	4
FAD_{ox}	0	0	0	1	0	0	0	0	0	0	0	−1	0	0	0	0	0	1	1	1	−1
FAD_{red}	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	−1	−1	−1	1
P_i	0	0	0	0	0	1	0	0	0	0	0	−1	0	0	0	0	1	1	1	1	−1
ADP	0	0	0	0	0	0	1	0	0	0	0	−1	0	0	0	0	1	1	1	1	−1
ATP	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	−1	−1	−1	−1	1
CoA	0	0	0	0	0	0	0	0	1	0	0	1	−1	−1	−1	−1	−1	−1	−1	−1	0
Succinyl-CoA	0	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1	0
CO_2tot	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2	1	0	0	0	0	3

The numbered columns are for (1) H_2O, (2) NAD_{ox}, (3) NAD_{red}, (4) $FADenz_{ox}$, (5) $FADenz_{red}$, (6) P_i, (7) ADP, (8) ATP, (9) CoA, (10) succinyl-CoA, (11) CO_2tot, (12) acetyl-CoA, (13) citrate, (14) *cis*-aconitate, (15) iso-citrate, (16) ketoglutarate, (17) succinate, (18) fumarate, (19) malate, (20) oxaloacetate and (21) pyruvate.

coenzymes and H_2O have been specified. This conservation matrix is obtained by deleting the rows and columns of H_2O to ATP, as mentioned in the preceding paragraph.

8×8 unit matrix	8×13 weighting matrix
3×8 zero matrix	3×13 conservation matrix

Before using the weighting matrix, it is of interest to consider the stoichiometric information in the 3×13 conservation matrix. Since there are 13 reactants and three components, this conservation matrix will lead to a stoichiometric number matrix with $R'' = N'' - C'' = 13 - 3 = 10$ independent reactions. The three components are CoA, succinyl-CoA and CO_2tot. The stoichiometric number matrix for the 10 enzyme-catalyzed reactions at specified concentrations of certain coenzymes and water is obtained by using Eq. (3). The row-reduced form of the stoichiometric number matrix obtained in this way is shown in Table 3.

The row-reduced form is used so that it can be compared with the row-reduced form of the stoichiometric number matrix given in the next section.

4. When steady-state concentrations of certain coenzymes are specified, a simpler stoichiometric matrix is obtained

When the steady-state concentrations of coenzymes other than CoA, acetyl-CoA and succinyl-CoA are specified (also H_2O is included in the Legendre transform) and those reactants in Eqs. (5)–(14) are omitted, the 10 reaction equations are simplified to

$$\text{pyruvate} + \text{CoA} = \text{CO}_2\text{tot} + \text{acetyl-CoA} \quad (16)$$

$$\text{acetyl-CoA} + \text{oxaloacetate} = \text{CoA} + \text{citrate} \quad (17)$$

$$\text{citrate} = \textit{cis}\text{-aconitate} \quad (18)$$

$$\textit{cis}\text{-aconitate} = \text{iso-citrate} \quad (19)$$

$$\text{iso-citrate} = \text{ketoglutarate} + \text{CO}_2\text{tot} \quad (20)$$

$$\text{ketoglutarate} + \text{CoA} = \text{succinyl-CoA} + \text{CO}_2\text{tot} \quad (21)$$

$$\text{succinyl-CoA} = \text{succinate} + \text{CoA} \quad (22)$$

$$\text{succinate} = \text{fumarate} \quad (23)$$

$$\text{fumarate} = \text{malate} \quad (24)$$

$$\text{malate} = \text{oxaloacetate} \quad (25)$$

The net reaction is

$$\text{pyruvate} = 3\text{CO}_2\text{tot} \quad (26)$$

These reactions will make it possible to calculate the equilibrium composition of the system when the steady-state concentrations of NAD_{ox}, NAD_{red}, $FADenz_{ox}$, $FADenz_{red}$, P_i, ADP and ATP are specified. Note that $N'' = C'' + R'' = 3 + 10 = 13$. The stoichiometric number matrix for reactions Eqs. (16)–(25) is given in Table 4.

When this stoichiometric number matrix is transposed, row-reduced and transposed back, it gives the same matrix as Table 3. This shows that the two matrices represent the same stoichiometric information.

5. Calculation of the adjustments to be subtracted from $\Delta_f G'^\circ$ for each reactant to obtain $\Delta_f G''^\circ$

Now we return to the use of the 8×13 weighting matrix to calculate the standard further transformed Gibbs energies of formation of the 13 reactants at specified concentrations of the coenzymes. The weighting matrix that is extracted from Table 2 is given in Table 5.

Note that CoA, succinyl-CoA and CO_2tot are not adjusted because they are components and are not made up of other components.

According to Eq. (4), the weighting matrix is multiplied by the list of $\Delta_f G'$ for the seven coenzymes at specified concentrations and $\Delta_f G^\circ$ for water to obtain the adjustments to be subtracted from the $\Delta_f G'^\circ$ values for the 13 reactants to obtain their $\Delta_f G''^\circ$ values. The $\Delta_f G''^\circ$ values are needed to calculate apparent equilibrium constants K'' for reactions Eqs. (16)–(25) at specified concentra-

Table 3
Row-reduced stoichiometric number matrix calculated from the 3×13 conservation matrix when steady-state concentrations of certain coenzymes have been specified

	Rx 1	Rx 2	Rx 3	Rx 4	Rx 5	Rx 6	Rx 7	Rx 8	Rx 9	Rx 10
CoA	1	0	0	0	0	0	0	0	0	0
Succinyl-CoA	0	1	0	0	0	0	0	0	0	0
CO_2tot	0	0	1	0	0	0	0	0	0	0
Acetyl-CoA	−1	−1	0	0	0	0	0	0	0	0
Citrate	0	0	0	1	0	0	0	0	0	0
Cis-aconitate	0	0	0	0	1	0	0	0	0	0
Iso-citrate	0	0	0	0	0	1	0	0	0	0
Ketoglutarate	0	0	0	0	0	0	1	0	0	0
Succinate	0	0	0	0	0	0	0	1	0	0
Fumarate	0	0	0	0	0	0	0	0	1	0
Malate	0	0	0	0	0	0	0	0	0	1
Oxaloacetate	0	−1	0	−1	−1	−1	−1	−1	−1	−1
Pyruvate	2/3	2/3	−1/3	−2/3	−2/3	−2/3	−1/3	0	0	0

These reactions have been numbered 1–10 because the form of these reactions is established in the next section.

Table 4
Stoichiometric number matrix $\boldsymbol{\nu}''$ for reactions Eqs. (16)–(25)

	Rx 16	Rx 17	Rx 18	Rx 19	Rx 20	Rx 21	Rx 22	Rx 23	Rx 24	Rx 25
CoA	−1	1	0	0	0	−1	1	0	0	0
Succinyl-CoA	0	0	0	0	0	1	−1	0	0	0
CO_2tot	1	0	0	0	1	1	0	0	0	0
Acetyl-CoA	1	−1	0	0	0	0	0	0	0	0
Citrate	0	1	−1	0	0	0	0	0	0	0
Cis-aconitate	0	0	1	−1	0	0	0	0	0	0
Iso-citrate	0	0	0	1	−1	0	0	0	0	0
Ketoglutarate	0	0	0	0	1	−1	0	0	0	0
Succinate	0	0	0	0	0	0	1	−1	0	0
Fumarate	0	0	0	0	0	0	0	1	−1	0
Malate	0	0	0	0	0	0	0	0	1	−1
Oxaloacetate	0	−1	0	0	0	0	0	0	0	1
Pyruvate	−1	0	0	0	0	0	0	0	0	0

Table 5
Weighting matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13
H_2O	0	0	0	−4	−2	−3	−2	−1	0	0	1	1	−5
NAD_{ox}	0	0	0	−3	−2	−2	−2	−1	0	0	0	1	−4
NAD_{red}	0	0	0	3	2	2	2	1	0	0	0	−1	4
FAD_{ox}	0	0	0	−1	0	0	0	0	0	1	1	1	−1
FAD_{red}	0	0	0	1	0	0	0	0	0	−1	−1	−1	1
P_i	0	0	0	−1	0	0	0	0	1	1	1	1	−1
ADP	0	0	0	−1	0	0	0	0	1	1	1	1	−1
ATP	0	0	0	1	0	0	0	0	−1	−1	−1	−1	1

The numbered columns are for (1) CoA, (2) succinyl-CoA, (3) CO_2tot, (4) acetyl-CoA, (5) citrate, (6) *cis*-aconitate, (7) iso-citrate, (8) ketoglutarate, (9) succinate, (10) fumarate, (11) malate, (12) oxaloacetate and (13) pyruvate.

Table 6
Calculation of $\Delta_f G''^\circ$ in kJ mol^{-1} of reactants at 298.15 K, pH 7, ionic strength 0.25 M and seven coenzymes at 0.001 M

	$\Delta_f G'^\circ$	$\Delta_f G'$(adjust)	$\Delta_f G''^\circ$
CoA	−7.26	0	−7.26
Succinyl-CoA	−347.47	0	−347.47
CO_2tot	−547.10	0	−547.10
Acetyl-CoA	−58.062	1007.34	−1065.40
Citrate	−966.23	433.29	−1399.52
Cis-aconitate	−802.12	588.95	−1391.07
Iso-citrate	−959.58	433.29	−1392.87
Ketoglutarate	−633.59	216.65	−850.23
Succinate	−530.64	−208.82	−321.83
Fumarate	−523.58	−201.75	−321.83
Malate	−682.85	−357.41	−325.44
Oxaloacetate	−715.00	−418.39	−296.60
Pyruvate	−350.78	1223.99	−1574.77

tions of certain coenzymes. This matrix multiplication is represented by

$$\{\Delta_f G'_1,\ \Delta_f G'_2,\ldots,\Delta_f G'_8\}\cdot\text{weighting matrix} = \Delta_f G'(\text{adjust}) \quad (27)$$

where $\Delta_f G_1'$, $\Delta_f G_2'$,…,$\Delta_f G_8'$ are $\Delta_f G'^\circ(H_2O)$ and the $\Delta_f G'$ at specified concentrations or the seven coenzymes. The $\Delta_f G'$(adjust) list is calculated using the program CALCTRGEF given in Appendix A. The result of the matrix multiplication in Eq. (27) is a list of adjustments $\Delta_f G'$(adjust) to be subtracted from the 13 $\Delta_f G'^\circ$ values for reactants to obtain their $\Delta_f G''^\circ$ values.

The first step is to calculate $\Delta_f G'^\circ$ for all reactants at 298.15 K, pH 7 and ionic strength 0.25 M. The basic thermodynamic data on the species of the 13 reactants and coenzymes are available in the package BASICBIOCHEMDATA2 that is available at MathSource [16]. The functions $\Delta_f G'^\circ$ of pH and ionic strength for the 13 reactants are used to calculate values at pH 7 and ionic strength 0.25 M.

Table 6 shows an example of the calculation of the further transformed Gibbs energies of formation of the 13 reactants at 298.15 K, pH 7 and ionic strength 0.25 M when NAD_{ox}, NAD_{red}, $FADenz_{ox}$, $FADenz_{red}$, P_i, ADP and ATP are all at 0.001 M. The transformed Gibbs energies of formation of these coenzymes at desired concentrations are calculated using the Mathematica program CALCTRGEF that is given in Appendix A. The first column in Table 6 gives $\Delta_f G'^\circ$ for reactants at 298.15 K, pH 7 and 0.25 M. The adjustments are given in the second column and the further transformed Gibbs energies of formation $\Delta_f G''^\circ$ are given in the third column.

The standard further transformed Gibbs energies of formation of the reactants at specified concentrations of certain coenzymes are used to calculate the standard further transformed Gibbs energies of the reactions Eqs. (16)–(25) by use of a matrix multiplication [4].

$$[\Delta_f G''_1{}^\circ,\ \Delta_f G''_2{}^\circ,\ldots,\Delta_f G''_N{}^\circ]\cdot\nu'' = [\Delta_r G''_1{}^\circ,\ \Delta_r G''_2{}^\circ,\ldots,\Delta_r G''_R{}^\circ] \quad (28)$$

(Note that $(1\times N'')(N''\times R'')=1\times R''$.) The use of Eq. (28) yields the following list of standard further transformed Gibbs energies of reactions Eqs. (16)–(25) at 298.15 K, pH 7, 0.25 M ionic strength and 0.001 M concentrations of certain coenzymes: −30.49, −44.77, 8.45, −1.80, −4.46, −37.09, 18.38, −0.01, −3.68 and 28.83 kJ mol^{-1}.

6. Calculation of equilibrium concentrations of 13 reactants when certain coenzymes are at 10^{-3} M

Krambeck [17] wrote EQUCALCC to calculate equilibrium concentrations in solution reactions when the standard Gibbs energies of formation of species and the conservation matrix are known for a system of chemical reactions. In biochemistry it is usually more convenient to use EQUCALCRX, which calls on EQUCALCC, but requires the stoichiometric number matrix and equilibrium constants. This short program calculates the input for EQUCALCC. For a given stoichiometric number matrix and set of apparent equilibrium constants, the equilibrium composition depends on the initial composition of the system. Two quite different starting compositions are considered here. The first calculation is for three initial reactants, and in the second calculation all 13 reactants are initially present at 10^{-3} M.

The first calculation of equilibrium concentra-

Table 7
Initial concentrations, equilibrium concentrations and changes in concentrations at 298.15 K, pH 7, 0.025 M when certain coenzyme concentrations are held at 10^{-3} M

	Initial concentration (M)	Equilibrium concentration (M)	Change in concentration (M)
CoA	0	0.0023	0.0023
Succinyl-CoA	0.01	0.0077	−0.0023
CO_2tot	0	0.03	0.03
Acetyl-CoA	0	1.0×10^{-12}	1.0×10^{-12}
Citrate	0	2.3×10^{-9}	2.3×10^{-9}
Cis-aconitate	0	7.5×10^{-11}	7.5×10^{-11}
Iso-citrate	0	1.5×10^{-10}	1.5×10^{-10}
Ketoglutarate	0	3.1×10^{-8}	3.1×10^{-8}
Succinate	0	0.00196	0.00196
Fumarate	0	0.00196	0.00196
Malate	0	0.00842	0.00842
Oxaloacetate	0.01	7.5×10^{-8}	−0.010
Pyruvate	0.01	6.0×10^{-17}	−0.010

tions is for [succinyl-CoA] = [oxaloacetate] = [pyruvate] = 0.01 M. This is starting with a minimum number of three reactants. Note that all the three components have to be represented in the initial composition. Table 7 shows the calculated equilibrium concentrations and the changes in reactant concentrations in going to equilibrium. Pyruvate is essentially completely converted to carbon dioxide, but the carbon atoms initially in oxaloacetate and part of the carbon atoms in succinyl-CoA end up at equilibrium in succinate, fumarate and especially malate. Note that the CoA moiety is conserved.

Table 8 gives the equilibrium concentrations when all the reactants are initially present at 10^{-3} M. Essentially, all the carbon atoms in acetyl-CoA, citrate, *cis*-aconitate, iso-citrate, ketoglutarate, oxaloacetate and pyruvate are converted to carbon dioxide and succinyl-CoA. Note that there are significant concentrations of succinate, fumarate and especially malate at equilibrium.

7. Calculation of equilibrium concentrations of 13 reactants when certain coenzymes are at 10^{-4} M and others are at 10^{-2} M

Since the reactions of pyruvate dehydrogenase and the citric acid cycle go pretty far to the right when the specified concentrations of NAD_{ox}, NAD_{red}, $FADenz_{ox}$, $FADenz_{red}$, P_i, ADP and ATP are all 0.001 M, the next two tables are calculated on the assumption that the concentrations of coenzymes on the left sides of reactions Eqs. (16)–(25) are 10^{-4} M and the concentrations of coenzymes on the right sides are 10^{-2} M. Using the same steps as above, the equilibrium concentrations are calculated first when succinyl-CoA, oxaloacetate and pyruvate are initially present at 0.01 M and second when the initial concentrations are 0.001 M for all the reactants. Table 9 shows the equilibrium concentrations and the changes in reactant concentrations in going to equilibrium.

In this case CoA, succinyl-CoA and acetyl-CoA are essentially in steady states. At equilibrium, there are significant concentrations of citrate, ketoglutarate and succinate.

Table 10 gives the equilibrium concentrations when all the reactants are initially at 10^{-3} M. The equilibrium concentrations in Table 10 are quite different from Table 8 in that there are significant concentrations of citrate, ketoglutarate and succinate at equilibrium.

There are at least two ways these calculations can be checked. The first uses EQUCALCC rather than EQUCALCRX; i.e. it uses the conservation matrix $\boldsymbol{A}''$ rather than the stoichiometric number matrix $\boldsymbol{\nu}''$. The same equilibrium concentrations are obtained when this is done. The second, which is really better, is to treat the equilibrium concentrations as experimental data and to calculate the $\Delta_r G''^\circ$ for the reactions at the specified temperature, pH and concentrations of certain coenzymes.

Table 8
Initial concentrations, equilibrium concentrations and changes in concentrations at 298.15 K, pH 7, 0.025 M when certain coenzyme concentrations are held at 10^{-3} M

	Initial concentration (M)	Equilibrium concentration (M)	Change in concentration (M)
CoA	0.001	0.00105	0.00005
Succinyl-CoA	0.001	0.00195	−0.00095
CO_2tot	0.001	0.013	0.012
Acetyl-CoA	0.001	8.6×10^{-14}	−0.001
Citrate	0.001	2.4×10^{-10}	−0.001
Cis-aconitate	0.001	8.0×10^{-12}	−0.001
Iso-citrate	0.001	1.7×10^{-11}	−0.001
Ketoglutarate	0.001	7.7×10^{-9}	−0.001
Succinate	0.001	0.0011	0.00012
Fumarate	0.001	0.0011	0.00012
Malate	0.001	0.0048	0.0038
Oxaloacetate	0.001	4.2×10^{-8}	−0.001
Pyruvate	0.001	4.9×10^{-18}	−0.001

This can be done in one step by use of the following matrix multiplication

$$-RT\{\ln[\text{reactant}_1],\ \ln[\text{reactant}_2],\ldots,\ \ln[\text{reactant}_{13}]\}\cdot\nu'' = \{\Delta_r G''_1{}^\circ,\ \Delta_r G''_2{}^\circ,\ldots,\Delta_r G''_{10}{}^\circ\} \quad (29)$$

The use of Eq. (29) yields the same values for $\Delta_r G_1''^\circ$ as the use of Eq. (28), as it must.

8. Discussion

This paper has shown how the thermodynamics of systems of biochemical reactions like pyruvate dehydrogenase and the citric acid cycle can be discussed at steady-state concentrations of certain coenzymes. This is more informative than the net reaction because equilibrium information on the other reactants is obtained. This approach is possible because Legendre transforms can be used to define further transformed Gibbs energies G'' of such systems. G'' provides the criteria for spontaneous change and equilibrium at specified temperature, pH, ionic strength and concentrations of certain coenzymes. Matrix operations are needed to divide reactants into a set of components and a set of non-components. For pyruvate dehydrogenase and the citric acid cycle, not all coenzymes

Table 9
Initial concentrations, equilibrium concentrations and changes in concentrations at 298.15 K, pH 7, 0.025 M when certain coenzyme concentrations are held at 10^{-4} M and other coenzyme concentrations are held at 10^{-2} M

	Initial concentration (M)	Equilibrium concentration (M)	Change in concentration (M)
CoA	0	3.1×10^{-6}	3.1×10^{-6}
Succinyl-CoA	0.01	0.010	-3.8×10^{-5}
CO_2tot	0	0.015	0.015
Acetyl-CoA	0	3.4×10^{-5}	3.5×10^{-5}
Citrate	0	0.058	0.058
Cis-aconitate	0	1.9×10^{-4}	1.9×10^{-4}
Iso-citrate	0	4.0×10^{-4}	4.0×10^{-4}
Ketoglutarate	0	0.0016	0.0016
Succinate	0	0.0019	0.0019
Fumarate	0	1.9×10^{-5}	1.9×10^{-5}
Malate	0	8.3×10^{-5}	8.3×10^{-5}
Oxaloacetate	0.01	7.4×10^{-12}	−0.010
Pyruvate	0.01	7.9×10^{-5}	−0.0099

Table 10
Initial concentrations, equilibrium concentrations and changes in concentrations at 298.15 K, pH 7, 0.025 M when certain coenzyme concentrations are held at 10^{-4} M and other coenzyme concentrations are held at 10^{-2} M

	Initial concentration (M)	Equilibrium concentration (M)	Change in concentration (M)
CoA	0.001	6.8×10^{-7}	−0.001
Succinyl-CoA	0.001	0.0030	0.0020
CO_2tot	0.001	0.0076	0.0066
Acetyl-CoA	0.001	1.9×10^{-6}	−0.001
Citrate	0.001	0.0020	0.00096
Cis-aconitate	0.001	6.5×10^{-5}	−0.00094
Iso-citrate	0.001	1.3×10^{-4}	−0.0087
Ketoglutarate	0.001	0.0011	6.4×10^{-5}
Succinate	0.001	0.0026	0.0016
Fumarate	0.001	2.6×10^{-5}	−0.00097
Malate	0.001	1.1×10^{-4}	−0.00089
Oxaloacetate	0.001	1.0×10^{-11}	−0.001
Pyruvate	0.001	9.7×10^{-6}	−0.001

can be at specified concentrations because enough components have to be left free to represent the stoichiometry of the system; in this case, three components are required. The components used here are CoA, succinyl-CoA and CO_2tot, but other sets of components could be used.

It is possible to calculate equilibrium compositions by substituting concentrations of certain coenzymes in expressions for apparent equilibrium constants, but a complete discussion of the thermodynamics of a system of biochemical reactions at specified concentrations of certain coenzymes requires the use of Legendre-transformed thermodynamic properties. The introduction of steady-state concentrations of certain coenzymes as independent variables increases the number of Maxwell relations. In the present case, these relations make it possible to calculate the change in the 'binding' of coenzymes, just like the introduction of the pH as an independent variable makes it possible to calculate the change in the binding of hydrogen ions at a specified pH. However, these calculations are not discussed here.

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Appendix A:

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CALCTRGEF[nadoxcon_, nadredcon_, fadenzoxcon_, fadenzredcon_, picon_, adpcon_, atpcon_]:= Module[{gh2o, gnadox, gnadred, gfadenzox, gfadenzred, gpi, gadp, gatp} (*This program calculates transformed Gibbs energies of formation of coenzymes at 298.15 K, pH 7 and ionic strength 0.25 M at specified concentrations of the coenzymes. The output is a list including water as the first member. This list has to be multiplied by the weighting matrix to obtain the adjustments to be subtracted from the list of standard transformed Gibbs energies of reactants to obtain the standard further transformed Gibbs energies of formation of the reactants at specified concentrations of coenzymes. Energies are in kJ mol^-1.*)

gh2o = -155.66;
gnadox = 1059.11 +
8.31451*0.29815*Log[nadoxcon];
gnadred = 1120.09 +
8.31451*0.29815*Log[nadredcon];
gfadenzox = 1260.51 +
8.31451*0.29815*Log[fadenzoxcon];
gfadenzred = 1253.44 +
8.31451*0.29815*Log[fadenzredcon];
gpi = -1059.49 + 8.31451*0.29815*Log[picon];
gadp = -1424.70 +
8.31451*0.29815*Log[adpcon];
```

gatp = −2292.50 +
8.31451*0.29815*Log[atpcon];
{gh2o, gnadox, gnadred, gfadenzox, gfadenzred, gpi, gadp, gatp}]

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