Calculation of Biochemical Net Reactions and Pathways by Using Matrix Operations

Robert A. Alberty
Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 USA

ABSTRACT Pathways for net biochemical reactions can be calculated by using a computer program that solves systems of linear equations. The coefficients in the linear equations are the stoichiometric numbers in the biochemical equations for the system. The solution of the system of linear equations is a vector of the stoichiometric numbers of the reactions in the pathway for the net reaction; this is referred to as the pathway vector. The pathway vector gives the number of times the various reactions have to occur to produce the desired net reaction. Net reactions may involve unknown numbers of ATP, ADP, and P_i molecules. The numbers of ATP, ADP, and P_i in a desired net reaction can be calculated in a two-step process. In the first step, the pathway is calculated by solving the system of linear equations for an abbreviated stoichiometric number matrix without ATP, ADP, P_i, NAD_{red}, and NAD_{ox}. In the second step, the stoichiometric numbers in the desired net reaction, which includes ATP, ADP, P_i, NAD_{red}, and NAD_{ox}, are obtained by multiplying the full stoichiometric number matrix by the calculated pathway vector.

INTRODUCTION

Inasmuch as the stoichiometry of biochemical reactions can be complicated, there are advantages in using generalized methods that give an overview of the stoichiometry. The first step in providing an overview is to use biochemical reactions written in terms of sums of species, such as ATP, which represents the sum of six species, rather than writing all the chemical reactions in terms of ionic species (Alberty, 1991b). The justification for this is that when a biochemical reaction is studied at a specific pH, H^+ is not conserved and should not appear in the biochemical equation (Alberty, 1992b, 1994). Many biochemical textbooks write some of the 21 biochemical reactions discussed here incorrectly, in the sense that H^+ is included. For example, when the hydrolysis of ATP is written ATP + H_2O = ADP + P_i + H^+, suggesting that a mole of H^+ is produced for each mole of ATP hydrolyzed, this is incorrect. At 25°C, pH 7, pMg, and 0.25-M ionic strength, calculations show that 0.62 mol of H^+ is produced (Alberty and Goldberg, 1993). Biochemical reactions balance atoms of carbon, oxygen, nitrogen, sulfur, and perhaps other elements but do not balance hydrogen atoms or atoms of metal ions at specified free concentrations.

The second step in providing an overview of a system of biochemical reactions is to add up series of reactions, such as those in glycolysis or the citric acid cycle, and obtain the net reaction, which shows what the series of reactions has accomplished. In this calculation, intermediates are eliminated because they are produced and used in equal amounts in the series of reactions. The reactions that add up to give a net reaction are referred to as a pathway, and an important feature of a pathway is the number of times each reaction has to occur to produce the net reaction.

The calculation of net reactions by adding biochemical equations by hand is laborious and really becomes difficult when as many as a dozen biochemical equations have to be added. Some of the biochemical reactions have to be multiplied by an integer or be written in the reverse direction so that intermediates will cancel. Fortunately, chemical equations are matrix equations (Alberty, 1991a), and, because chemical equations can be converted into biochemical equations, biochemical equations are also matrix equations (Alberty, 1992a). The mathematics of systems of reactions is explored in the next section. Because of the mathematical character of the stoichiometry of biochemical equations, mathematical operations on them can be carried out with personal computers using mathematical programs such as Mathematica (Wolfram Research, Inc., Champaign, IL), MATLAB (MathWorks, Inc., Natick, MA), and Maple (Waterloo Maple, Waterloo, Ontario, Canada). By use of computer programs for linear algebra, it is convenient to calculate net reactions and pathways even for large systems of biochemical reactions.

Net reactions and pathways are important in their own right, but the calculations described here also provide the stoichiometric framework for thermodynamic and kinetic calculations.

THE MATHEMATICS OF PATHWAYS

The one thing we know for sure about a chemical reaction is that it conserves atoms and electric charges. These conservation relationships for a system of chemical reactions can be represented in two ways that are mathematically equivalent: 1) a conservation matrix giving the number of atoms of different types and charges for all the species and 2) a stoichiometric number matrix that gives the stoichio-

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Address reprint requests to Dr. Robert Arnold Alberty, Department of Chemistry, Room 6–215, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139. Tel: 617-253-2456; Fax: 617-253-7030; E-mail: alberty@mit.edu.

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metric numbers for the various species in an independent set of reactions (Smith and Missen, 1982).

The calculation of a biochemical pathway is based on the stoichiometric number matrix for the biochemical reactions that can occur in the system. A stoichiometric number matrix has a column for each reaction and a row for each reactant. Reactants on the left have stoichiometric numbers with negative signs, and reactants on the right have stoichiometric numbers with positive signs. In dealing with biochemical reactions it is useful to differentiate among three types of stoichiometric matrix according to the level of thermodynamic treatment (Alberty, 1993). The discussion of thermodynamics in terms of species is referred to as Level 1; the stoichiometric number matrix $\boldsymbol{\nu}$ includes acid dissociation reactions, complex ion dissociations, and reference reactions written in terms of species. The chemical equations used at Level 1 balance atoms and electric charges, and they lead to the expression for the equilibrium constant, which is represented by K. A matrix of this type has been given for the 12 reactions and 17 species involved in the dissociation of ATP to ADP and P_i in the presence of magnesium ion (Alberty, 1991b). The discussion of thermodynamics in terms of sums of species at specified pH (and perhaps at specified free concentrations of cations bound by reactants) is referred to as Level 2. The conversion of a system of chemical equations to biochemical equations has been described (Alberty, 1992a), and the stoichiometric number matrix is smaller at Level 2. Stoichiometric number matrices at Level 2 have been used in calculating equilibrium concentrations in glycolysis (Alberty, 1992b) and in the calculation of the number of degrees of freedom in a biochemical reaction system (Alberty, 1992c). When the pH is specified, the stoichiometric number matrix is represented by $\boldsymbol{\nu}'$ to distinguish it from $\boldsymbol{\nu}$ for the underlying chemical reactions. Biochemical reactions should be written by using abbreviations or words that represent sums of species and do not suggest that they refer to specific ionic species, as recommended in an IUBMB-IUPAC report (Alberty et al., 1994). The expressions for the apparent equilibrium constants K' of biochemical reactions correspond to the way they are written, except that the concentration of water is replaced by unity for the activity of H_2O in dilute solutions. The thermodynamics of a biochemical reaction system can also be discussed when the concentrations of reactants such as ATP, ADP, P_i, NAD_{ox}, and NAD_{red} are specified; this is referred to as Level 3 (Alberty, 1993). The stoichiometric number matrix is smaller at Level 3 and is represented by $\boldsymbol{\nu}''$ to distinguish it from $\boldsymbol{\nu}'$ for the underlying biochemical reactions. The reaction equation at Level 3 leads to the expression for the apparent equilibrium constant, which is represented by K''. The matrix methods discussed here can be used at all three levels. The equations for the thermodynamics of reactions at Levels 1–3 are based on Legendre transforms to define the new thermodynamic potentials that are minimized when certain concentration variables are specified, as described in Appendix A.

The relation between a stoichiometric number matrix $\boldsymbol{\nu}$ for a set of R reactions involving N species and the stoichiometric number matrix $\boldsymbol{\nu}_{net}$ for a particular net reaction is a system of linear equations that is represented by

$$\begin{bmatrix} \nu_{11} & \nu_{12} & \cdots & \nu_{1R} \\ \nu_{21} & \nu_{22} & \cdots & \nu_{2R} \\ \cdots & \cdots & \cdots & \cdots \\ \cdots & \cdots & \cdots & \cdots \\ \nu_{N1} & \nu_{N2} & \cdots & \nu_{NR} \end{bmatrix} \begin{bmatrix} s_1 \\ s_2 \\ \cdots \\ s_R \end{bmatrix} = \begin{bmatrix} \nu_{net1} \\ \nu_{net2} \\ \cdots \\ \cdots \\ \nu_{netN} \end{bmatrix} \quad (1)$$

where the column matrix $\mathbf{s}$ is the set of stoichiometric numbers for the R reactions. The column matrix $\mathbf{s}$ is referred to as the pathway vector. The nomenclature of stoichiometric numbers s_i for reactions comes from chemical kinetics, where it is also convenient to use net reactions. The pathway vector shows the number of times the various reactions in the pathway have to go from left to right to accomplish the given net reaction. Biochemical reactions that have to go in the reverse direction in a pathway are represented by negative numbers in the pathway vector. When the pathway vector is known, the net reactions for that pathway can be calculated by multiplying the pathway vector by a stoichiometric number matrix.

In Eq. 1, $N > R$, and so Eq. 1 is an overdetermined set of linear equations. The reason that $N > R$ is that these reaction equations can be used to discuss the thermodynamics of the system, and in thermodynamics the number C of components is given by $C = N - R$ (Smith and Missen, 1992), and a system has at least one component. For a given set of reactions, solutions for a given $\boldsymbol{\nu}_{net}$ may or may not exist. A solution will exist if the right-hand side of Eq.1 is a linear combination of the columns in the stoichiometric number matrix (Stewart, 1973). Equation 1 can be written in the form

$$s_1 \begin{bmatrix} \nu_{11} \\ \nu_{21} \\ \cdots \\ \nu_{N1} \end{bmatrix} + s_2 \begin{bmatrix} \nu_{12} \\ \nu_{22} \\ \cdots \\ \nu_{N2} \end{bmatrix} + \cdots + s_R \begin{bmatrix} \nu_{1R} \\ \nu_{2R} \\ \cdots \\ \nu_{NR} \end{bmatrix} = \begin{bmatrix} \nu_{net1} \\ \nu_{net2} \\ \cdots \\ \nu_{netN} \end{bmatrix}. \quad (2)$$

This shows that the solution $\mathbf{s}$ to the system of linear equations is made up of the numbers s_i that give the number of times the various reactions have to occur to accomplish the net reaction. Equations 1 and 2 are conveniently written in matrix notation as

$$\boldsymbol{\nu}\mathbf{s} = \boldsymbol{\nu}_{net}. \quad (3)$$

The stoichiometric number matrix $\boldsymbol{\nu}$ for the system is $N \times R$, the stoichiometric number matrix $\mathbf{s}$ (pathway vector) for the reactions is $R \times 1$, and the stoichiometric number matrix $\boldsymbol{\nu}_{net}$ for the net reaction is $N \times 1$. When the pH (and perhaps the free concentrations of cations that are bound) is specified, a prime is used on the symbols in Eq. 2 to distinguish the stoichiometric numbers of the biochemical reactions at Level 2 from those of the underlying chemical reactions. At Level 2, the apparent stoichiometric number matrix $\boldsymbol{\nu}'$ is $N' \times R'$. When, in addition, the concentrations of reactants

such as ATP are specified, a double prime is used to indicate that these additional concentrations have been specified. At Level 3, the apparent stoichiometric number matrix $\boldsymbol{\nu}''$ is $N'' \times R''$. In the systems discussed here, specification of the concentrations of ATP, etc. does not reduce the number of equations, and so $R'' = R'$.

Equation 3 shows that the net reaction matrix $\boldsymbol{\nu}_{net}$ for a system of reactions can be calculated by multiplying the stoichiometric number matrix $\boldsymbol{\nu}$ by the path vector **s**. It is a lot easier to do this with a personal computer than by hand. But Eq. 3 makes it possible to do something even more interesting, and that is to calculate the pathway vector for any desired net reaction by solving the system of linear equations by using a personal computer.

Example: As these equations look rather abstract, consider the following simplest meaningful example. The first two reactions in glycolysis are as follows:

$$\text{glucose} + \text{ATP} = \text{glucose 6-phosphate} + \text{ADP}$$

$$\text{glucose 6-phosphate} = \text{fructose 6-phosphate}$$

Equation 1 for this system of reactions is

$$\begin{array}{c} \\ \text{glc} \\ \text{ATP} \\ \text{G6P} \\ \text{ADP} \\ \text{F6P} \end{array}\!\!\begin{array}{c} \begin{array}{cc} \text{rx 1} & \text{rx 2} \end{array} \\ \begin{bmatrix} -1 & 0 \\ -1 & 0 \\ 1 & -1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \end{array} \begin{array}{c} \text{path} \\ \begin{bmatrix} 1 \\ 1 \end{bmatrix} \end{array} = \begin{array}{c} \text{net rx} \\ \begin{bmatrix} -1 \\ -1 \\ 0 \\ 1 \\ 1 \end{bmatrix} \end{array}.$$

If you know the path, the stoichiometric number matrix is multiplied by the path to yield the net reaction. Simple operations like this can be done by inspection, but larger matrices and longer paths can be multiplied by using a personal computer with a program that makes matrix multiplications. If you have the stoichiometric number matrix and want to know the path, Eq. 1 for this system is a set of of five linear equations with two unknowns. A personal computer with a program that solves linear equations can be used to calculate the path, which in this case is the vector {1,1}. Simple systems can be treated by hand, but the point is that with a computer large systems of reactions can be considered, and long paths can be obtained. Note that these calculations involve overdetermined sets of linear equations; in other words, there are more linear equations than elements in the path vector.

In the calculations reported here, *Mathematica* (Wolfram Research, Champaign, IL) has been used, but other programs for making matrix operations can be used. It is convenient to use some *Mathematica* notation here so that it is clear how this is done in at least one program; the notation for other programs is similar. In *Mathematica*, Eq. 3 involves the dot product

$$numat \cdot path = nunet, \tag{4}$$

where *numat* represents the stoichiometric number matrix $\boldsymbol{\nu}$, $\boldsymbol{\nu}'$, or $\boldsymbol{\nu}''$ for the system, *path* represents the matrix **s**, **s**′, or **s**″ of stoichiometric numbers, and *nunet* represents the desired net reaction $\boldsymbol{\nu}_{net}$, $\boldsymbol{\nu}'_{net}$, or $\boldsymbol{\nu}''_{net}$. The objective is to find the pathway that yields a particular net reaction. This calculation can be made by using one of a number of mathematical programs on personal computers. In *Mathematica* the pathway is calculated with LinearSolve:

$$path = \text{LinearSolve}[numat, nunet]. \tag{5}$$

Very large matrices can be handled. LinearSolve gives the vector of the stoichiometric numbers of the biochemical reactions in the pathway for the net reaction.

First, systems of biochemical equations at specified pH and free concentrations of any metal ions that are bound by reactants are considered. Second, the corresponding systems of equations without ATP, ADP, P_i, NAD_{ox}, and NAD_{red} are considered. These reaction equations look unusual because they do not appear to balance, but they do balance carbon, oxygen, and sulfur atoms. The reaction equations have the apparent equilibrium constant K'' at specified concentrations of ATP, ADP, P_i, NAD_{ox}, and NAD_{red}.

AN OVERVIEW OF THE ENZYMATIC OXIDATION OF GLUCOSE TO CARBON DIOXIDE AND WATER

As an introduction to the calculation of pathways, the oxidation of glucose to carbon dioxide and water can be considered to be accomplished by 1) the net reaction for glycolysis, 2) the net reaction catalyzed by the pyruvate dehydrogenase complex, 3) the net reaction for the citric acid cycle, and 4) the net reaction for oxidative phosphorylation:

$$\text{glucose} + 2\,P_i + 2\,\text{ADP} + 2\,NAD_{ox} = 2\,\text{pyruvate} + 2\,\text{ATP} + 2\,NAD_{red} + 2\,H_2O, \tag{6}$$

$$\text{pyruvate} + \text{CoA} + NAD_{ox} = \text{acetyl-CoA} + CO_2 + NAD_{red}, \tag{7}$$

$$\text{acetyl-CoA} + 4\,NAD_{ox} + \text{ADP} + P_i + 2\,H_2O = 2\,CO_2 + 4\,NAD_{red} + \text{ATP} + \text{CoA}, \tag{8}$$

$$NAD_{red} + \frac{1}{2}O_2 + 3\,P_i + 3\,\text{ADP} = NAD_{ox} + 4\,H_2O + 3\,\text{ATP}. \tag{9}$$

Reaction 9 represents the stoichiometry of oxidative phosphorylation (Lehninger, 1971; Mathews and van Holde, 1990). When these reactions were written, GTP and GDP were replaced by ATP and ADP because it has been assumed that the following net reaction can occur in various ways:

$$\text{GTP} + \text{ADP} = \text{GDP} + \text{ATP}. \tag{10}$$

It is also assumed that the net reaction

$$FAD_{red} + NAD_{ox} = FAD_{ox} + NAD_{red} \qquad (11)$$

occurs because of the glycerol 3-phosphate dehydrogenase shuttle (Appendix B). These assumptions are not necessary, but they provide a simplification.

The stoichiometric number matrix for the system of biochemical reactions 6–9 is

$$\nu_i' = \begin{array}{r} \\ \text{glucose} \\ P_i \\ \text{ADP} \\ NAD_{ox} \\ \text{pyruvate} \\ \text{ATP} \\ NAD_{red} \\ H_2O \\ \text{CoA} \\ \text{acetyl-CoA} \\ CO_2 \\ O_2 \end{array} \begin{array}{c} \begin{array}{cccc} \text{rx 6} & \text{rx 7} & \text{rx 8} & \text{rx 9} \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 0 \\ -2 & 0 & -1 & -3 \\ -2 & 0 & -1 & -3 \\ -2 & -1 & -4 & 1 \\ 2 & -1 & 0 & 0 \\ 2 & 0 & 1 & 3 \\ 2 & 1 & 4 & -1 \\ 2 & 0 & -2 & 4 \\ 0 & -1 & 1 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 1 & 2 & 0 \\ 0 & 0 & 0 & -1/2 \end{bmatrix} \end{array} \qquad (12)$$

The net reaction for the system of reactions 6–9 can be calculated by hand by multiplying the column vectors of these four reactions by 1, 2, 2, and 12, respectively, and adding. This yields

$$\text{glucose} + 6\,O_2 + 40\,\text{ADP} + 40\,P_i = 46\,H_2O + 6\,CO_2 + 40\,\text{ATP}. \qquad (13)$$

The same result is obtained with a computer by multiplying the stoichiometric number matrix ν_i' by the path vector {1, 2, 2, 12} to yield the stoichiometric coefficients in Eq. 13. The dot product used in Eq. 4 is

$$\nu_i' \,.\, \{1, 2, 2, 12\} = \{-1, -40, -40, 0, 0, 40, 0, 46, 0, 0, 6, -6\} = nunet1, \qquad (14)$$

where the right hand side is the stoichiometric number vector for net reaction 13.

The pathway for glucose to carbon dioxide is well known, but for other catabolic or anabolic net reactions of more complicated systems the pathways are not well known and are laborious to calculate. Thus it is useful to be able to calculate the pathway with LinearSolve. The pathway for the oxidation of glucose is calculated by using Eq. 5:

$$path1 = \text{LinearSolve}[numat1, nunet1] = \{1, 2, 2, 12\}, \qquad (15)$$

where $numat1 = \nu_i'$ is given by Eq. 12 and *nunet1* is given by Eq. 14. LinearSolve yields *path1* = {1, 2, 2, 12}. In this case we know that the net reaction is given by *nunet1* = {−1, −40, −40, 0, 0, 40, 0, 46, 0, 0, 6, −6}, but in other cases the stoichiometric numbers of ATP, ADP, and P_i will not be known; in fact, the objective of the calculation of the pathway may be to determine exactly that. The way this problem is solved is discussed in the following section.

ABBREVIATED SYSTEM OF FOUR NET REACTIONS FOR THE ENZYMATIC OXIDATION OF GLUCOSE TO CARBON DIOXIDE AND WATER

The section on the mathematics of pathways describes three levels of thermodynamic treatment. The previous section was at Level 2, and in this section Level 3 is used. The advantage of using Level 3 is that the number of reactants is reduced. In this case ATP, ADP, P_i, NAD_{ox}, and NAD_{red} are removed from the reaction equations because they are assumed to have specified concentrations at equilibrium. In biochemical thermodynamics, Level 3 is used when it is of interest to specify steady-state concentrations of reactants such as ATP, which are produced and consumed by many reactions, and calculate equilibrium compositions that can be reached by other reactants under these conditions. In discussing stoichiometry we want to take ATP, ADP, P_i, NAD_{ox}, and NAD_{red} temporarily out of consideration because we do not know a priori how much they are involved in a desired net reaction.

We take the terms for ATP, ADP, P_i, NAD_{ox}, and NAD_{red} out of Eqs. 6–9 by subtracting a multiple of ATP + H_2O = ADP + P_i and of NAD_{red} = NAD_{ox} from reactions 6–9. Note that to do that with Eq. 8 it is necessary to add H_2O to both sides. This leaves the reactions

$$\text{glucose} = 2\,\text{pyruvate}, \qquad (16)$$

$$\text{pyruvate} + \text{CoA} = \text{acetyl-CoA} + CO_2, \qquad (17)$$

$$\text{acetyl-CoA} + 3\,H_2O = 2\,CO_2 + \text{CoA}, \qquad (18)$$

$$\frac{1}{2}O_2 = H_2O. \qquad (19)$$

These reactions may look strange, but they do balance carbon, oxygen, and sulfur. They also correspond to the form of equilibrium constant K'' expressions obtained when the equilibrium concentrations of ATP, ADP, P_i, NAD_{ox}, and NAD_{red} are specified, as discussed above Eq. 1. The abbreviated stoichiometric number matrix for reactions 16–19 is

$$\nu_2'' = \begin{array}{r} \\ \text{glucose} \\ \text{pyruvate} \\ H_2O \\ \text{CoA} \\ \text{acetyl-CoA} \\ CO_2 \\ O_2 \end{array} \begin{array}{c} \begin{array}{cccc} \text{rx 16} & \text{rx 17} & \text{rx 18} & \text{rx 19} \end{array} \\ \begin{bmatrix} -1 & 0 & 0 & 0 \\ 2 & -1 & 0 & 0 \\ 0 & 0 & -3 & 1 \\ 0 & -1 & 1 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 1 & 2 & 0 \\ 0 & 0 & 0 & -1/2 \end{bmatrix} \end{array}. \qquad (20)$$

The net reaction for this system of reactions is

$$\text{glucose} + 6\,O_2 = 6\,CO_2 + 6\,H_2O, \qquad (21)$$

which corresponds to the reaction vector *nunet2* = {−1, 0, 6, 0, 0, 6, −6}. When this is used in LinearSolve, we obtain the same path as before in Eq. 15:

$$path2 = \text{LinearSolve}[numat2, nunet2] = \{1, 2, 2, 12\}, \quad (22)$$

where *numat2* is $\boldsymbol{\nu}_2''$. Thus one can calculate the pathway with an abbreviated stoichiometric number matrix without knowing what the coupling with ADP + P_i = ATP + H_2O and the reduction of NAD_{ox} may be.

The abbreviated net reaction *nunet2* = {−1, 0, 6, 0, 0, 6, −6} can be used with the abbreviated stoichiometric number matrix ($\boldsymbol{\nu}_2''$) to calculate the pathway {1, 2, 2, 12}. Once that has been done, the matrix multiplication of Eqs. 3 and 4 yields the desired complete net reaction involving ATP. In this case the dot product is

$$numat1 \,.\, \{1, 2, 2, 12\} \quad (23)$$

$$= \{-1, -40, -40, 0, 0, 40, 0, 46, 0, 0, 6, -6\},$$

which corresponds to Eq. 13.

Thus the stoichiometric number matrix of a biochemical reaction system can be represented in two ways: 1) a complete matrix involving all the reactants and 2) an abbreviated matrix omitting ATP, ADP, P_i, NAD_{ox}, and NAD_{red}, with some adjustments for H_2O described below. To calculate the pathway without knowing the involvement of ATP, the abbreviated net reaction is used in LinearSolve with the abbreviated stoichiometric number matrix to determine the path vector. Then the path vector is used in a matrix multiplication with the complete stoichiometric number matrix to calculate the complete net reaction. The next section discusses the two matrices for a system of 21 reactions and uses them to calculate pathways and complete net reactions.

A MORE DETAILED CONSIDERATION OF THE ENZYME-CATALYZED OXIDATION OF GLUCOSE TO CARBON DIOXIDE AND WATER

In reaction system 6–9, reaction 6 can be replaced by the 10 reactions of glycolysis, and reaction 8 can be replaced by the 9 reactions of the citric acid cycle. The complete set of biochemical reactions used is given here because these reactions are written in various ways in the literature:

1. glucose + ATP = glucose 6-phosphate + ADP,
2. glucose 6-phosphate = fructose 6-phosphate,
3. fructose 6-phosphate + ATP = fructose 1,6-bisphosphate + ADP,
4. fructose 1,6-bisphosphate = glycerone phosphate + glyceraldehyde 3-phosphate,
5. glycerone phosphate = glyceraldehyde 3-phosphate,
6. glyceraldehyde 3-phosphate + P_i + NAD_{ox} = 3-phospho-D-glyceroyl phosphate + NAD_{red},
7. 3-phospho-D-glyceroyl phosphate + ADP = 3-phospho-D-glycerate + ATP,
8. 3-phospho-D-glycerate = 2-phospho-D-glycerate,
9. 2-phosphoglycerate = phospho*enol*pyruvate + H_2O,
10. phospho*enol*pyruvate + ADP = pyruvate + ATP,
11. pyruvate + CoA + NAD_{ox} = acetyl-CoA + CO_2 + NAD_{red},
12. acetyl-CoA + oxaloacetate + H_2O = citrate + CoA,
13. citrate = *cis*-aconitate + H_2O,
14. *cis*-aconitate + H_2O = isocitrate,
15. isocitrate + NAD_{ox} = 2-oxoglutarate + CO_2 + NAD_{red},
16. 2-oxoglutarate + NAD_{ox} + CoA = succinyl-CoA + CO_2 + NAD_{red},
17. succinyl-CoA + P_i + ADp = succinate + ATP + CoA,
18. succinate + NAD_{ox} = fumarate + NAD_{red},
19. fumarate + H_2O = (*S*)-malate,
20. (*S*)-malate + NAD_{ox} = oxaloacetate + NAD_{red},
21. NAD_{red} + ½O_2 + 3 P_i + 3 ADP = NAD_{ox} + 4 H_2O + 3 ATP.

The 30 × 21 stoichiometric number matrix for this system of biochemical reactions is shown in Fig. 1. The 21 columns are for the above 21 reactions, and the 30 rows are for the 30 reactants in the order glucose, P_i, ADP, NAD_{ox}, pyruvate, ATP, NAD_{red}, H_2O, CoA, acetyl-CoA, CO_2, O_2, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate, glycerone phosphate, glyceraldehyde 3-phosphate, 3-phospho-D-glyceroyl phosphate, 3-phospho-D-glycerate, 2-phospho-D-glycerate, phospho*enol*pyruvate, oxaloacetate, citrate, *cis*-aconitate, isocitrate, 2-oxoglutarate, succinyl-CoA, succinate, fumarate, and (*S*)-malate.

The stoichiometric number matrix in Fig. 1 can be be used to calculate the ATP production when various reactants are oxidized to CO_2 and H_2O. The procedure is to write a vector for the pathway and use it to calculate the net reaction by use of the matrix multiplication in Eq. 1. The nine pathways considered here are summarized in Table 1. The enzyme-catalyzed reactions are numbered 1–21. The pathways that are discussed are labeled a–i. Note that the same amount of carbon is involved in each pathway so that the ATP productions can be compared. Some possible reactions starting with isomers have been omitted. The stoichiometric number for reaction 21 in each pathway has been adjusted so that no NAD_{red} is produced. The names of the enzymes are those recommended by the IUBMB Nomenclature Committee (Webb, 1992), and the EC numbers are given for biochemical reactions.

The net reactions in Table 2 were calculated from the pathways in Table 1 using Eq. 4, which is equivalent to Eq. 1, and were typed out by the computer using a *Mathematica* program given in Appendix C.

The calculation of Table 2 from Table 1 illustrates the calculation of net reactions by the use of matrix multiplication. Note that no reactions are included for the oxidation of intermediates such as citrate, . . . , oxalacetate in the citric acid cycle because the 21 enzyme-catalyzed reactions in Table 1 do not provide pathways for oxidation of these

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Out[129]//TableForm=

-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	-3
1	0	1	0	0	0	-1	0	0	-1	0	0	0	0	0	0	-1	0	0	0	-3
0	0	0	0	0	-1	0	0	0	0	-1	0	0	0	-1	-1	0	-1	0	-1	1
0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0
-1	0	-1	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	3
0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	0	1	0	1	-1
0	0	0	0	0	0	0	0	1	0	0	-1	1	-1	0	0	0	0	-1	0	4
0	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	-1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$-(\frac{1}{2})$
1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0

FIGURE 1 Stoichiometric number matrix for the system of 21 reactions with 30 reactants.

reactants to CO_2 and H_2O. For example, if reaction 12 is omitted, the remaining reactions convert citrate to oxaloacetate. But to oxidize oxaloacetate to CO_2 and H_2O, one must include reaction 12. The citric acid cycle does oxidize the acetyl group off acetylCoA. The stoichiometric number matrix for this system of 21 reactions can be used to yield pathways between intermediates in the citric acid cycle, but that is not discussed here. Note that the ATP production is greater than 40 in reactions b–d because they start with phosphorylated reactants. After that, the production of ATP

TABLE 1 Pathways used to calculate the nine net reactions given in Table 2

Enzyme	EC No.	a	b	c	d	e	f	g	h	i
1) Hexokinase	2.7.1.1	1	0	0	0	0	0	0	0	0
2) Glucose-6-phosphate isomerase	5.3.1.9	1	1	0	0	0	0	0	0	0
3) 1-Fructokinase	2.7.1.56	1	1	0	0	0	0	0	0	0
4) Fructose-bisphosphate aldolase	4.1.2.13	1	1	1	0	0	0	0	0	0
5) Triose-phosphatase isomerase	5.3.1.1	1	1	1	0	0	0	0	0	0
6) Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	2	2	2	2	0	0	0	0	0
7) Phosphoglycerate kinase	2.7.2.3	2	2	2	2	2	0	0	0	0
8) D-Phosphoglycerate 2,3-phosphomutase	5.4.2.1	2	2	2	2	2	0	0	0	0
9) Enolase	4.2.1.11	2	2	2	2	2	2	0	0	0
10) Pyruvate kinase	2.7.1.40	2	2	2	2	2	2	2	0	0
11) Pyruvate dehydrogenase	1.2.1.51	2	2	2	2	2	2	2	2	0
12) Citrate synthase	4.1.3.7	2	2	2	2	2	2	2	2	2
13) Aconitate hydratase	4.2.1.3	2	2	2	2	2	2	2	2	2
14) Aconitase	4.2.1.3	2	2	2	2	2	2	2	2	2
15) Isocitrate dehydrogenase (NAD)	1.1.1.41	2	2	2	2	2	2	2	2	2
16) Oxoglutarate dehydrogenase	1.2.1.52	2	2	2	2	2	2	2	2	2
17) Succinyl-CoA ligase (ADP-forming)	6.2.1.5	2	2	2	2	2	2	2	2	2
18) Succinate dehydrogenase	1.3.99.1	2	2	2	2	2	2	2	2	2
19) Fumarate hydratase	4.2.1.2	2	2	2	2	2	2	2	2	2
20) Malate dehydrogenase	1.1.1.37	2	2	2	2	2	2	2	2	2
21) Oxidative phosphorylation		12	12	12	12	10	10	10	10	8

TABLE 2 Net reactions calculated from the pathways of Table 1

a) 40 ADP + glucose + 6 O_2 + 40 P_i = 40 ATP + 6 CO_2 + 46 H_2O
b) 41 ADP + glucose 6-phosphate + 6 O_2 + 40 P_i = 41 ATP + 6 CO_2 + 46 H_2O
c) 42 ADP + fructose 1,6-bisphosphate + 6 O_2 + 40 P_i = 42 ATP + 6 CO_2 + 46 H_2O
d) 42 ADP + 2 glyceraldehyde 3-phosphate + 6 O_2 + 40 P_i = 42 ATP + 6 CO_2 + 46 H_2O
e) 36 ADP + 5 O_2 + 32 P_i + 2 3-phospho-D-glyceroyl phosphate = 36 ATP + 6 CO_2 + 38 H_2O
f) 34 ADP + 5 O_2 + 32 P_i + 2 2-phospho-D-glycerate = 34 ATP + 6 CO_2 + 38 H_2O
g) 34 ADP + 5 O_2 + 2 phospho*enol*pyruvate + 32 P_i = 34 ATP + 6 CO_2 + 36 H_2O
h) 32 ADP + 5 O_2 + 32 P_i + 2 pyruvate = 32 ATP + 6 CO_2 + 36 H_2O
i) 2 acetylCoA + 26 ADP + 4 O_2 + 26 P_i = 26 ATP + 2 CoA + 4 CO_2 + 28 H_2O

drops because the reactant that is oxidized has already been partially oxidized.

ABBREVIATED SYSTEM OF 21 REACTIONS FOR THE ENZYME-CATALYZED OXIDATION OF GLUCOSE TO CARBON DIOXIDE AND WATER

Net reactions are readily calculated by matrix multiplication of stoichiometric number matrices by path vectors, but the inverse problem of calculating the pathway for a desired net reaction is more difficult. The reason is that *numat* cannot be used to calculate a path with LinearSolve unless the complete net reaction is known for the system discussed here. This means that the stoichiometric numbers of ATP, ADP, P_i, and H_2O must be known. However, there is a way of avoiding that by using an abbreviated *numat* and using abbreviated net reactions. As shown in the overview of the oxidation of glucose, abbreviated biochemical reactions do not involve ATP, ADP, P_i, NAD_{red}, and NAD_{ox}. Table 3 gives abbreviated biochemical reactions for the nine reactions in Table 2. As discussed in connection with Eqs. 16–19, ATP + H_2O = ADP + P_i and NAD_{red} and NAD_{ox} are canceled in net equations a–i. However, a new problem is encountered in that in a sense ATP = ADP and H_2O = P_i are separated in some of these net reactions. For example, in reaction b one ATP can be canceled against one ADP, and then forty ATP + H_2O = ADP + P_i can be canceled. In some cases it may be necessary to add H_2O to both sides of an equation so that P_i can be canceled against H_2O. The cancellation of P_i versus H_2O accomplishes the conservation of oxygen.

TABLE 3 Abbreviated versions of the net reactions in Table 2

a) glucose + 6 O_2 = 6 CO_2 + 6 H_2O
b) glucose 6-phosphate + 6 O_2 = 6 CO_2 + 6 H_2O
c) fructose 1,6-bisphosphate + 6 O_2 = 6 CO_2 + 6 H_2O
d) 2 glyceraldehyde 3-phosphate + 6 O_2 = 6 CO_2 + 6 H_2O
e) 5 O_2 + 2 3-phospho-D-glyceroyl phosphate = 6 CO_2 + 6 H_2O
f) 5 O_2 + 2 2-phospho-D-glycerate = 6 CO_2 + 6 H_2O
g) 5 O_2 + 2 phospho*enol*pyruvate = 6 CO_2 + 4 H_2O
h) 5 O_2 + 2 pyruvate = 6 CO_2 + 4 H_2O
i) 2 acetylCoA + 4 O_2 = 2 CoA + 4 CO_2 + 2 H_2O

To use LinearSolve with the abbreviated net reactions in Table 3 to calculate pathways, it is necessary to construct the stoichiometric number matrix for the correspondingly abbreviated set of 21 reactions. The abbreviated stoichiometric number matrix contains the stoichiometric numbers for the 21 biochemical reactions in the system with ATP, ADP, P_i, NAD_{ox}, and NAD_{red} deleted in the way described, which involves the adjustment of the number of H_2O molecules in some reactions. Nine of the 21 reactions are unchanged. The 12 that are changed are as follows:

1. glucose = glucose 6-phosphate,
3. fructose 6-phosphate = fructose 1,6-bisphosphate,
6. glyceraldehyde 3-phosphate + H_2O= 3-phosphoglyceroyl phosphate,
7. 3-phosphoglyceroyl phosphate = 3-phosphoglycerate,
10. phospho*enol*pyruvate = pyruvate,
11. pyruvate + CoA = acetyl-CoA + CO_2,
15. isocitrate = 2-oxoglutarate + CO_2,
16. 2-oxoglutarate + CoA = succinyl-CoA + CO_2,
17. succinyl-CoA + H_2O = succinate + CoA,
18. succinate = fumarate,
20. (*S*)-malate = oxaloacetate,
21. ½O_2 = H_2O.

This yields the abbreviated stoichiometric number matrix in Fig. 2 that is 25 × 21. Note that the number of reactions has not changed, but the number of reactants has been reduced by five. The order of the reactions is the same as in Fig. 1, and the order of the reactants is the same except for the five deletions. When the net reactions a–i in Table 3 are used in LinearSolve with the abbreviated stoichiometric number matrix in Fig. 2, the pathways given in Table 1 are obtained. This shows that the abbreviated net reactions in Table 3 can be used to calculate pathways. Although these net reactions do not involve ATP, ADP, P_i, NAD_{red}, and NAD_{ox}, they do balance carbon, oxygen, and sulfur. Once the pathway for an abbreviated net reaction has been determined, that pathway can be used to calculate the complete net reaction by multiplication of the complete stoichiometric number matrix (Fig. 1) by the path vector. The complete net reaction shows the production of ATP.

DISCUSSION

Because chemical equations and biochemical equations are mathematical equations, they can be used in calculations based on linear algebra. The availability of mathematical programs for personal computers makes this convenient to do. A system of chemical reactions or biochemical reactions is represented by a stoichiometric number matrix that has as many rows as there are reactants and as many columns as

stoich.95-10-30 1

Out[131]//TableForm=

-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	-1	0	0	1	0	0	-1	1	-1	0	0	-1	0	-1	0	1
0	0	0	0	0	0	0	0	0	0	-1	1	0	0	0	-1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	$-(\frac{1}{2})$
1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	0

FIGURE 2 Abbreviated stoichiometric number matrix for the system of 21 reactions with 25 reactants.

there are reactions. Multiplication of this matrix times a pathway vector yields a net reaction vector, as shown in Eq. 1. The inverse problem of calculating the pathway for a desired net reaction vector requires a computer program for the solution of the system of linear equations. There is a solution only if the net reaction is a linear combination of the enzyme-catalyzed reactions.

In general catabolic net reactions produce ATP and anabolic net reactions consume ATP. One of the problems in the calculation of a pathway for reactions involving ATP is that the amount of ATP produced or consumed is unknown and may even be the objective of the calculation. The involvement of ATP can be calculated by using abbreviated net reactions that do not involve ATP, ADP, P_i, NAD_{red}, or NAD_{ox}, but these reactions must balance carbon, oxygen, and sulfur, as illustrated in the cases discussed here. An abbreviated net reaction can be used in LinearSolve (in *Mathematica*) with a corresponding abbreviated stoichiometric number matrix to calculate the pathway. This pathway can then be used in a matrix multiplication with the complete stoichiometric number matrix to yield the complete net reaction with ATP involved. In illustrating this process with a simplified system of biochemical reactions, it has been shown that the citric acid cycle does not provide a pathway for the complete oxidation of intermediates in the citric acid cycle, although the acetyl group of acetyl-CoA is oxidized to carbon dioxide and water.

This paper is a first step in the discussion of the thermodynamics of catabolic and anabolic net reactions. Synthetic reactions can be added to the stoichiometric number matrices used here to calculate pathways for net reactions involving oxidation of other reactants or their synthesis. A net reaction will go or not go to the right, depending on the equilibrium constants and the concentrations of intermediates. The requirement that a net reaction go in the indicated direction at a specified pH is that the change in the transformed Gibbs energy, $\Delta_r G'$, be less than zero for each reaction in the pathway. Some pathways can go to the right or the left, depending on the pH or the concentrations of intermediates, which do not appear in the net reaction for the pathway. If the concentrations of ATP, etc. are specified in addition, the condition that a net reaction will go to the right is that the change in the further transformed Gibbs energy, $\Delta_r G''$, be less than zero for each reaction in the pathway.

APPENDIX A

The Gibbs energy G is minimized at equilibrium at specified T and P, which is referred to here as Level 1. At Level 1, equilibrium constants are represented by K. When the concentration of a species is specified at equilibrium, G is no longer minimized at equilibrium, and it is necessary to use a Legendre-transformed Gibbs energy G' that is defined by (Alberty, 1994)

$$G' = G - n'_s\mu_s, \tag{A1}$$

where n'_s is the total amount of the specified species in the system, free and bound, and μ_s is the specified chemical potential of this species. n'_s is one of the independent variables for the system, and it is equal to the amount of the s component. When the concentration of a species is specified, G' is minimized at equilibrium, and this is referred to as Level 2. In most discussions of biochemical equilibria, pH is specified, and so H^+ is the

species with a specified chemical potential. Equation A1 leads to the following transformed chemical potentials of other species in the system:

$$\mu_i' = \mu_i - N_s(i)\mu_s, \qquad \text{(A2)}$$

where $N_s(i)$ is the number of s molecules in one molecule of species i. At Level 2 it is convenient to consider ATP, which is made up of six species when Mg^{2+} is present, to be a single reactant. At Level 2 equilibrium constants are represented by K'.

In considering systems of biochemical reactions, it is sometimes useful to consider that the concentrations of certain reactants such as ATP, ADP, and P_i are constant because they are produced and used by many reactions. When the concentration of such a reactant is specified, G' no longer provides the criterion for equilibrium, and it is necessary to use a further Legendre transformed Gibbs energy, G'', which is defined by

$$G'' = G' - n_s''\mu_s', \qquad \text{(A3)}$$

where n_s'' is the total amount of the specified reactant in the system, free and bound, and μ_s' is its transformed chemical potential. When the concentration of a reactant such as ATP is specified, G'' is minimized at equilibrium, and this is referred to as Level 3. This leads to the following further transformed chemical potential of other reactants in the system:

$$\mu_i'' = \mu_i' - N_s''(i)\mu_s', \qquad \text{(A4)}$$

where $n_s''(i)$ is the number of molecules of reactant s in one molecule of reactant i. At Level 3, equilibrium constants are represented by K'', and the concentrations of reactants with specified chemical potentials μ_s' do not appear in the equilibrium constant expression.

APPENDIX B

The net reaction

$$FAD_{red}(cyt) + NAD_{ox}(cyt) = FAD_{ox}(cyt) + NAD_{red}(cyt)$$

where cyt stands for cytosol, occurs because of the glycerol 3-phosphate shuttle, which consists of the following six steps:

$$\text{glycerone phosphate(mit)} + FAD_{red}(mit) = \text{glycerol 3-phosphate(mit)} + FAD_{ox}(mit)$$

$$FAD_{red}(cyt) = FAD_{red}(mit)$$

$$FAD_{ox}(mit) = FAD_{ox}(cyt)$$

$$\text{glycerone phosphate(cyt)} = \text{glycerone phosphate(mit)}$$

$$\text{glycerol 3-phosphate(mit)} = \text{glycerol 3-phosphate(cyt)}$$

$$\text{glycerol 3-phosphate(cyt)} + NAD_{ox}(cyt) = \text{glycerone phosphate(cyt)} + NAD_{red}(cyt)$$

where mit stands for mitochondria. The first reaction is catalyzed by glycerol-3-phosphate dehydrogenase (EC1.1.99.5), and the last reaction is catalyzed by glycerol-3-phosphate dehydrogenase (NAD) (EC1.1.1.8). The other four reactions are transfers from one phase to another. The net reaction for the glycerol 3-phosphate shuttle is obtained by adding these six reactions.

APPENDIX C

The following Mathematica program takes a vector *nunet* of stoichiometric coefficients of reactants, with negative values for reactants and positive values for products, and a vector *names* of the names of the reactants (each in quotation marks) such as in Table 2, which were actually typed out in this way:

```
mkeq[c_List,s_]:
    =Map[Max[#,0]&,-c].s==Map[Max[#,0]&,c].s
```

In other words, the biochemical equation is obtained by typing mkeq[*nunet,names*].

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