Constraints and Missing Reactions in the Urea Cycle

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ABSTRACT The stoichiometric relations in a series of biochemical reactions are summarized by a stoichiometric number matrix (with a column for each reaction) and a conservation matrix (with a row for each constraint). These two matrices for a series or cycle of biochemical reactions are related because the columns of the stoichiometric number matrix are in the null space of the conservation matrix, and the rows of the transpose of the conservation matrix are in the null space of the transpose of the stoichiometric number matrix. The conservation matrix for a system of biochemical reactions is of interest because it shows the nature of the constraints in addition to the conservation of atoms and groups. Constraints beyond those for the conservation of atoms and groups indicate "missing reactions" that do not occur because the enzymes involved couple reactions that could occur and still conserve atoms and groups. The interpretation of conservation matrices and stoichiometric matrices for a reaction system is complicated by the fact that they are not unique. However, their row-reduced forms are unique, as are their dimensions, which represent the number of reactants and number of independent reactions. Two matrices that look different contain the same information if they have the same row-reduced form. The urea cycle, which involves five enzyme-catalyzed reactions, and its net reaction are discussed in terms of the linear constraints produced by enzyme catalysis. A procedure to obtain a set of conservation equations that will yield the correct net reaction is described.

INTRODUCTION

Chemical equations are mathematical equations (Smith and Missen, 1982), and so are biochemical equations written in terms of sums of species at a specified pH (Alberty, 1992b). In utilizing the mathematical aspects of these equations, the stoichiometric numbers of products are taken as positive and those of reactants are taken as negative. For example, the biochemical equation $-\text{ATP} - \text{H}_2\text{O} + \text{ADP} + \text{P}_\text{i} = 0$ at specified pH can be written as

$$-1\begin{bmatrix}9\\3\\1\end{bmatrix} - 1\begin{bmatrix}1\\0\\0\end{bmatrix} + 1\begin{bmatrix}6\\2\\1\end{bmatrix} + 1\begin{bmatrix}4\\1\\0\end{bmatrix} = \begin{bmatrix}0\\0\\0\end{bmatrix}, \quad (1)$$

where the four reactants are represented by column vectors. The top number in the column vector for a reactant gives the number of oxygen atoms, the middle number gives the number of phosphorus atoms, and the bottom number gives the number of adenosine groups (including the oxygen atom that is phosphorylated). The abbreviations ATP, H_2O, ADP, and P_i represent sums of species, and because the pH is specified, hydrogen atoms are not conserved (Alberty, 1994b). Equation 1 can be rearranged into the following form:

$$\begin{bmatrix}9&1&6&4\\3&0&2&1\\1&0&1&0\end{bmatrix}\begin{bmatrix}-1\\-1\\1\\1\end{bmatrix} = \begin{bmatrix}0\\0\\0\end{bmatrix}. \quad (2)$$

Thus a biochemical reaction is represented by the product of a conservation matrix and a stoichiometric number matrix, which yields a zero matrix.

This approach can be extended to series or cycles of biochemical reactions. For example, two steps in the hydrolysis of ATP can be written

$$-\text{ATP} - \text{H}_2\text{O} + \text{ADP} + \text{P}_\text{i} = 0 \quad (3)$$

$$-\text{ADP} - \text{H}_2\text{O} + \text{AMP} + \text{P}_\text{i} = 0, \quad (4)$$

for which

$$\begin{bmatrix}9&1&6&4&3\\3&0&2&1&1\\1&0&1&0&1\end{bmatrix}\begin{bmatrix}-1&0\\-1&-1\\1&-1\\1&1\\0&1\end{bmatrix} = \begin{bmatrix}0&0\\0&0\\0&0\end{bmatrix}. \quad (5)$$

The order of the columns in this conservation matrix is ATP, H_2O, ADP, P_i, and AMP. This is also the order of the rows in the stoichiometric number matrix. Reactions 3 and 4 are like chemical reactions (i.e., reactions written in terms of species that balance atoms and charges) in that they do not involve constraints in addition to atom and group balances. Coupling of biochemical reactions introduces constraints in addition to atom and group conservations. When two biochemical reactions are coupled by an enzyme, only the equilibrium constant expression for the coupled reaction is satisfied at equilibrium, not the equilibrium constant expressions for the two reactions that are coupled. The reason for discussing the urea cycle in this article is to show that the enzymes involved bring in constraints in addition to atom and group balances. These constraints affect the composition that is obtained at equilibrium. The constraints in enzyme-catalyzed reactions are passed on to various net reactions for the system.

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These two examples show that a biochemical reaction system is represented by the matrix product of a conservation matrix **A** and a stoichiometric number matrix $\boldsymbol{\nu}$ to give a zero matrix.

$$\mathbf{A}\boldsymbol{\nu} = \mathbf{0}. \tag{6}$$

The rows in the conservation matrix give the coefficients in the conservation equations for atoms, groups, or other quantities that are conserved. The quantities that are conserved are referred to as components, and the number of components is represented by C. There is a row for each of the C components, and in Eqs. 2 and 5, $C = 3$. The number N of columns in the conservation matrix is equal to the number of reactants, and so the **A** matrix is $C \times N$. Any stoichiometric number matrix is $N \times R$, where R is the number of independent reactions. These matrices include only nonredundant information. The zero matrix in Eq. 6 is $C \times R$. The number of components is unique, but the choice of components is arbitrary. Different sets of components are related by an invertible set of linear equations, and so may be thought of as different bases of the "component space." Different bases of the same space can be put into a canonical form (e.g., row reduction to reduced row echelon form).

Conservation matrices and stoichiometric matrices are also used in discussing the kinetics of complex reaction systems (Clark, 1981; Schuster and Hilgetag, 1995), but when the kinetics of systems is discussed, all of the steps in the mechanism must be included. In this paper, the objective is the stoichiometric basis for equilibrium calculations on the biochemical reactants, but not the enzymes and enzyme-substrate complexes.

In simple chemical systems, there is usually a row for each element in the conservation matrix, and the components are elements. The number of rows is less than the number of elements involved if two elements always occur in the same ratio; in this case, the combination is treated like an element. In unusual chemical cases, the number of rows is greater than the number of elements because some combination of the elements is conserved, even though they do not always occur in this combination (Schott, 1964; Aris, 1965). For example, when benzene rings are conserved, they may be taken as components if this gives an independent conservation equation. Electric charge and oxidation number are conserved, but these conservation equations are not necessarily independent of those for the elements.

The number N of reactants is equal to the number C of components plus the number R of independent reactions:

$$N = C + R. \tag{7}$$

This involves the assumptions of independence and completeness. In previous papers on biochemical reactions (Alberty, 1992b, 1994a), primes have been put on C, N, and R to indicate that the pH is specified, but because this paper is concerned only with reactions at specified pH, this distinction is not needed here. It is incorrect stoichiometrically to write ATP + H_2O = ADP + P_i + H^+, as is done in so many introductory biochemistry textbooks, because the amount of H^+ produced per mole of ATP depends on the temperature, pressure, pH, pMg, and ionic strength. The amount of H^+ produced can be calculated from the derivative of the apparent equilibrium constant K' with respect to pH. In the presence of magnesium ions, Mg^{2+} is produced or consumed, and its amount per ATP hydrolyzed can be calculated from the derivative of the apparent equilibrium constant K' with respect to pMg.

Equation 6 shows that $\boldsymbol{\nu}$ is in the null space of the **A** matrix (Strang, 1988). The null space $\boldsymbol{\nu}$ of a conservation matrix **A** is a list of basis vectors whose linear combinations satisfy Eq. 6. The null space can be calculated by hand for a small matrix, but a computer is needed for larger matrices. Like the conservation matrix, the stoichiometric number matrix is not unique; in other words, the stoichiometric relations in a reaction system can be represented by different sets of independent reactions. For example, the stoichiometric number matrix for reactions 3 and 4 can be represented by any two reactions obtained by adding and subtracting reactions 3 and 4 or any multiples of them.

Equation 6 can also be written (Smith and Missen, 1982)

$$\boldsymbol{\nu}^{\mathrm{T}}\mathbf{A}^{\mathrm{T}} = 0, \tag{8}$$

where the superscript T indicates the transpose. Thus $\mathbf{A}^{\mathrm{T}}$ is in the null space of $\boldsymbol{\nu}^{\mathrm{T}}$. The important idea here is that a set of independent reactions that can represent the change in composition of a system can be calculated from a conservation matrix **A** using Eq. 6, or a conservation matrix for a system of reactions can be calculated from the transpose of a stoichiometric number matrix by use of Eq. 8. The $\boldsymbol{\nu}$ matrix calculated from **A** using Eq. 6 is not unique, but it can be compared with a $\boldsymbol{\nu}$ matrix obtained in a different way by row reduction of both matrices. Similarly, the **A** matrix calculated from a $\boldsymbol{\nu}$ matrix using Eq. 8 is not unique, but it can be compared with a conservation matrix obtained in a different way by row reduction of both matrices.

Another application of the mathematical nature of biochemical equations is the calculation of net reactions or pathways (Alberty, 1996). The dot product of the stoichiometric number matrix $\boldsymbol{\nu}$ and the column vector for the pathway is the column vector (nunet) for the net reaction.

	CO_2	ATP	H_2O	am	asp	AMP	urea	ADP	P_i	PP_i	fum
A =	1	0	0	0	0	0	0	0	0	0	1
	1	0	0	0	0	0	0	0	0	1	0
	2	0	0	0	0	0	0	0	1	0	0
	2	0	0	0	0	0	0	1	0	0	0
	1	0	0	0	0	0	1	0	0	0	0
	1	0	0	0	0	1	0	0	0	0	0
	-1	0	0	0	1	0	0	0	0	0	0
	-1	0	0	1	0	0	0	0	0	0	0
	-2	0	1	0	0	0	0	0	0	0	0
	-3	1	0	0	0	0	0	0	0	0	0

FIGURE 1 Conservation matrix for the net reaction calculated from the stoichiometric number matrix using Eq. 8 (10 × 11). am, ammonia; asp, aspartate; fum, fumarate.

A =	CO_2	ATP	H_2O	am	asp	AMP	urea	ADP	P_i	PP_i	fum
C	1	0	0	0	0	0	1	0	0	0	0
O	2	9	1	0	0	3	1	6	4	7	0
N	0	0	0	1	1	0	2	0	0	0	0
P	0	3	0	0	0	1	0	2	1	2	0
aden	0	1	0	0	0	1	0	1	0	0	0
fum	0	0	0	0	1	0	0	0	0	0	1
con/am	1	0	0	-1	0	0	0	0	0	0	0
con/H_2O	2	0	-1	0	0	0	0	0	0	0	0
con/PP_i	1	0	0	0	0	0	0	0	0	1	0
con/ATP	3	-1	0	0	0	0	0	0	0	0	0

FIGURE 2 Conservation matrix for the net reaction based on conservation equations (10 × 11). aden, adenosine.

The pathway, which is $R \times 1$, gives the number of times that each step has to occur to give the net reaction that is represented by the $N \times 1$ column vector nunet.

$$\nu \cdot \text{path} = \text{nunet}. \tag{9}$$

For the two steps in reactions 3 and 4, the net reaction is $-\text{ATP} - 2H_2O + 2P_i + \text{AMP} = 0$, as indicated by

$$\begin{bmatrix} -1 & 0 \\ -1 & -1 \\ 1 & -1 \\ 1 & 1 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 1 \\ 1 \end{bmatrix} = \begin{bmatrix} -1 \\ -2 \\ 0 \\ 2 \\ 1 \end{bmatrix}. \tag{10}$$

Conversely, the pathway for a particular net reaction nunet can be calculated from ν by use of a computer program that can solve a system of simultaneous linear equations. In *Mathematica* (Wolfram, 1991) the pathway is calculated using Linear Solve:

$$\text{pathway} = \text{LinearSolve}[\nu, \text{nunet}]. \tag{11}$$

Equation 10 is an overdetermined set of linear equations.

The primary objective of this paper is to discuss the stoichiometric relationships that provide the basis for the calculation of the equilibrium composition, excluding the enzymes and enzyme-substrate complexes. The correct equilibrium composition can be calculated using a correct

A =	CO_2	ATP	H_2O	am	asp	AMP	urea	ADP	P_i	PP_i	fum
CO_2	1	0	0	0	0	0	0	0	0	0	1
ATP	0	1	0	0	0	0	0	0	0	0	3
H_2O	0	0	1	0	0	0	0	0	0	0	2
am	0	0	0	1	0	0	0	0	0	0	1
asp	0	0	0	0	1	0	0	0	0	0	1
AMP	0	0	0	0	0	1	0	0	0	0	-1
urea	0	0	0	0	0	0	1	0	0	0	-1
ADP	0	0	0	0	0	0	0	1	0	0	-2
P_i	0	0	0	0	0	0	0	0	1	0	-2
PP_i	0	0	0	0	0	0	0	0	0	1	-1

FIGURE 3 Row-reduced form of the matrices in Figs. 1 and 2 (10 × 11).

A =	CO_2	ATP	H_2O	am	asp	AMP	urea	ADP	P_i	PP_i	fum
C	1	0	0	0	0	0	1	0	0	0	0
O	2	9	1	0	0	3	1	6	4	7	0
N	0	0	0	1	1	0	2	0	0	0	0
P	0	3	0	0	0	1	0	2	1	2	0
aden	0	1	0	0	0	1	0	1	0	0	0
fum	0	0	0	0	1	0	0	0	0	0	1
con/ADP	2	0	0	0	0	0	0	1	0	0	0

FIGURE 4 Conservation matrix for a system involving 11 reactants and 7 constraints (7 × 11).

stoichiometric number matrix or a correct conservation matrix. Neither matrix is unique, but all correct matrices yield the same equilibrium composition.

The conservation matrix **A** and the stoichiometric matrix $\boldsymbol{\nu}$ are also useful in writing the fundamental equation of thermodynamics for a biochemical reaction system at specified pH (Alberty, 1992b). The conservation matrix **A** is used as a transformation matrix to convert amounts of reactants to amounts of components, and the stoichiometric number matrix is used to write the fundamental equation in terms of the column matrix for the extents of the R reactions. The calculation of the equilibrium composition for a biochemical reaction system is conveniently carried out by using either the conservation matrix **A** or the stoichiometric number matrix $\boldsymbol{\nu}$ (Cheluget et al., 1987; Alberty and Krambeck, manuscript submitted for publication).

When biochemical reactions are coupled by a common intermediate involving the enzyme, there is a decrease in the number of independent reactions in the system. This decrease in the number of biochemical reactions is accompanied by an increase in the number of linear constraints in the **A** matrix—in other words, an increase in the number C of components. When there are added constraints of this type, we can speak of "missing reactions," which are stoichiometrically correct but cannot be obtained by adding and subtracting the reactions catalyzed by the enzymes that are present. For example, glycolysis involves ATP, ADP, and P_i, but the reactions of glycolysis cannot be combined with each other to cause the hydrolysis of ATP. Thus in glycolysis, $\text{ATP} + H_2O = \text{ADP} + P_i$ can be referred to as a "missing reaction" (Alberty, 1992a). It is fortunate that ATP is not hydrolyzed by the 10 reactions of glycolysis because energy is stored in ATP. The importance of the conserved moiety structure of reaction systems has also been emphasized by Schuster and Hilgetag (1985).

ν^T =	CO_2	ATP	H_2O	am	asp	AMP	urea	ADP	P_i	PP_i	fum
rx 22	0	0	0	1	-1	0	0	0	0	0	1
rx 23	0	-1	-1	0	0	1	0	0	0	1	0
rx 24	0	-1	-2	0	0	1	0	0	2	0	0
rx 25	-1	-1	1	-2	0	-1	1	2	0	0	0

FIGURE 5 Transpose of the stoichiometric number matrix obtained by calculating the null space of the conservation matrix in Fig. 4 (4 × 11).

FIGURE 6 Transpose of the stoichiometric number matrix for reactions 26–30 (5 × 16). orn, ornithine; arsucc, argininosuccinate; carb, carbamoyl phosphate; cit, citrulline; arg, arginine.

		CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
ν^T =	rx 26	-1	-2	-1	0	0	-1	0	1	0	0	0	0	2	1	0	0
	rx 27	0	0	0	-1	0	0	0	-1	1	0	0	0	0	1	0	0
	rx 28	0	-1	0	0	1	0	-1	0	-1	0	1	0	0	0	1	0
	rx 29	0	0	0	0	-1	0	0	0	0	1	0	0	0	0	0	1
	rx 30	0	0	-1	1	0	0	0	0	0	-1	0	1	0	0	0	0

Matrix equations 6 and 8 are applied here to the net reaction for the urea cycle and then to the five steps of the urea cycle.

CONSERVATION MATRIX FOR THE NET REACTION OF THE UREA CYCLE

The net reaction of the urea cycle is

$$CO_2 + 3ATP + 2H_2O + \text{ammonia} + \text{aspartate} = AMP + \text{urea} + 2ADP + 2P_i + PP_i + \text{fumarate}. \quad (12)$$

The transpose of the stoichiometric number matrix for reaction 12 is

$$\nu^T = [-1\ -3\ -2\ -1\ -1\ 1\ 1\ 2\ 2\ 1\ 1], \quad (13)$$

where the reactants are in the same order as in reaction 12. The corresponding conservation matrix **A** is calculated using Eq. 8. This calculation was carried out with NullSpace in *Mathematica*. This yields the 10 × 11 conservation matrix **A** shown in Fig. 1. There are four conservation equations for the elements C, O, N, and P, but there are also independent conservation equations for the adenosine group and the fumarate group because these groups are not broken up in forming urea. Thus the number of rows in Fig. 1 is quite striking, because we might have expected 6 rows rather than 10.

Now we want to account for the conservation matrix of Fig. 1 by constructing an equivalent conservation matrix from coefficients in the conservation equations for elements, groups, and other linear constraints. The first six rows in the matrix in Fig. 2 show the coefficients in the conservation equations for C, O, N, P, adenosine, and fumarate. Because we must account for 10 conservation equations, we must add four additional constraints. The four additional rows indicate missing reactions due to coupling. The four additional constraints can be taken to be the coefficients in the following constraint equations:

$$\text{con/am is } n(CO_2) - n(\text{ammonia}) = \text{constant} \quad (14)$$

$$\text{con/}H_2O \text{ is } 2n(CO_2) - n(H_2O) = \text{constant} \quad (15)$$

$$\text{con/}PP_i \text{ is } n(CO_2) + n(PP_i) = \text{constant} \quad (16)$$

$$\text{con/ATP is } 3n(CO_2) - n(ATP) = \text{constant}. \quad (17)$$

Note that when the amount of CO_2 decreases in the net reaction, the amount of ammonia decreases by a corresponding amount, so that the difference in amounts of these two reactants does not change, as indicated in Eq. 14. In constraint 16, when the amount of CO_2 decreases, the amount of PP_i increases by a corresponding amount. These constraints are independent, as they must be to be included in the **A** matrix, but they are not unique. Many other choices could have been made. The easiest way to test the independence of a set of constraints is make a row reduction of the conservation matrix; if there is one zero row (or more), the constraints are not independent. The conservation matrix constructed with the element and group conservations and Eqs. 14–17 is given in Fig. 2. The matrices in Fig. 1 and Fig. 2 look different, but when they are row reduced they both yield Fig. 3, which shows that they contain the same information. Note that the stoichiometric number matrix for the net reaction (Eq. 13) can be obtained from the last column of Fig. 3 by changing the signs and adding a 1 at the end. In Fig. 1 the components are unspecified, but in Fig. 2 the components are elements, groups, and the linear relations given in Eqs. 14–17. In Fig. 3 the components are reactants, except for fumarate. Different choices of components can be obtained by multiplying the conservation matrix times the inverse of the transformation matrix between the coordinate systems. The transformation matrix gives the

FIGURE 7 Conservation matrix calculated from the ν matrix in Fig. 6 using Eq. 8 (11 × 16).

	CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
A =	-2	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1
	-2	1	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	1	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	-1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0
	-2	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	-1	1	-1	0	1	0	0	0	0	1	0	0	0	0	0	0
	1	-1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
	2	0	-1	-1	0	0	0	1	0	0	0	0	0	0	0	0
	2	-1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	-1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

A =		CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
	C	1	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0
	O	2	9	1	0	0	0	0	5	1	0	3	1	6	4	7	0
	N	0	0	0	0	2	1	1	1	1	2	0	2	0	0	0	0
	P	0	3	0	0	0	0	0	1	0	0	1	0	2	1	2	0
	aden	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
	fum	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
	orn	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0
	con/am	1	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0
	con/H_2O	2	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
	con/asp	1	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0
	con/ATP	3	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

FIGURE 8 Conservation matrix for reactions 26–30 constructed from conservation equations for atoms and for adenosine, fumarate, and ornithine plus four additional constraints (11 × 16).

composition of the new components (columns) in terms of the old components (rows) (Alberty, 1993).

The four constraints in addition to atom and group balance (i.e., the constraints in Eqs. 14–17) indicate missing reactions. To obtain some indication about these missing reactions, the last four rows of the conservation matrix in Fig. 2 can be deleted, and this smaller matrix can be used to obtain a set of biochemical reactions for the system by calculation of the null space of **A**. When this is done, the biochemical equations look unfamiliar and are rather complicated, even though they balance atoms and groups. The calculation of the null space yields one of an infinite number of possible sets of biochemical reactions that can be used to represent changes in the composition of the reaction system. A better way to proceed is to write down an interesting set of uncoupled reactions and check that the set is consistent with the conservation matrix. Consider the following four possible missing reactions, which give the correct net reaction when they are coupled together:

$$CO_2 + 2\text{ammonia} = \text{urea} + H_2O \tag{18}$$

$$\text{aspartate} = \text{fumarate} + \text{ammonia} \tag{19}$$

$$2ATP + 2H_2O = 2ADP + 2P_i \tag{20}$$

$$ATP + H_2O = AMP + PP_i. \tag{21}$$

The stoichiometric number matrix for this set of independent reactions is 11 × 4, and when the null space of the transformed stoichiometric number matrix is calculated, it yields a 7 × 11 conservation number matrix. This indicates that one more constraint must be added to the conservation matrix in Fig. 2 with the last four rows deleted. The constraint $2n(CO_2) + n(\text{ADP}) = \text{constant}$ is added to provide the seventh row in Fig. 4. The calculation of the null space of this conservation matrix yields the stoichiometric number matrix in Fig. 5, which gives the following four reactions:

$$\text{aspartate} = \text{ammonia} + \text{fumarate} \tag{22}$$

$$ATP + H_2O = AMP + PP_i \tag{23}$$

$$ATP + 2H_2O = AMP + 2P_i \tag{24}$$

$$2\text{ ammonia} + AMP + ATP + CO_2 = 2ADP + H_2O + \text{urea}. \tag{25}$$

These reactions are not the same as reactions 18–21, but they do add up to give the correct net reaction. They satisfy another test that is useful for determining whether a given net reaction can be obtained by adding and subtracting reactions in a set of reactions. That involves the use of LinearSolve, as shown in Eq. 11. When LinearSolve is applied to the stoichiometric number matrix in Fig. 5 and the net reaction (Eq. 12), the pathway [1 1 1 1] is obtained, which means that each of these four reactions is taken once to obtain the desired net reaction. In considering biochemical reactions calculated by matrix operations, it is convenient to use a one-line program in *Mathematica* that actually prints out the biochemical equation in its familiar form (Alberty, 1996). Thomas Colthurst has written a second line for this program so that it will print out any number of reactions. The program is mkeq[c_List, s_List]: = Map[Max[#,0]&,-c].s = = Map[Max[#,0]&,c].s nameMatrix[m_List, s_List]: = Map[mkeq[#,s] &, m];
m_List is a transposed stoichiometric number matrix. s_List is a vector of names of reactants, each enclosed by parentheses.

A =		CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
	CO_2	1	0	0	0	0	0	0	0	0	0	0	0	1/2	0	0	0
	ATP	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0
	H_2O	0	0	1	0	0	0	0	0	0	0	0	1	1/2	0	0	0
	orn	0	0	0	1	0	0	0	0	0	0	0	-1	-1/2	1	0	0
	arsucc	0	0	0	0	1	0	0	0	0	0	0	0	0	0	-1	1
	am	0	0	0	0	0	1	0	0	0	0	0	0	1/2	0	0	0
	asp	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
	carb	0	0	0	0	0	0	0	1	0	0	0	0	-1	1	0	0
	cit	0	0	0	0	0	0	0	0	1	0	0	0	1/2	-1	1	0
	arg	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	-1
	AMP	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-1	0

FIGURE 9 Row-reduced conservation matrix of Fig. 7 (11 × 16).

A =		CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
	CO_2	1	0	0	0	0	0	0	0	0	0	0	-1/2	1/2	0	1/2	0
	ATP	0	1	0	0	0	0	0	0	0	0	0	-3/2	3/2	0	3/2	0
	H_2O	0	0	1	0	0	0	0	0	0	0	0	-1	1	0	1	0
	orn	0	0	0	1	0	0	0	0	0	0	0	3/2	-3/2	1	-1/2	0
	arsucc	0	0	0	0	1	0	0	0	0	0	0	1/2	-1/2	0	-1/2	1
	am	0	0	0	0	0	1	0	0	0	0	0	-1/2	1/2	0	1/2	0
	asp	0	0	0	0	0	0	1	0	0	0	0	-1/2	1/2	0	1/2	0
	carb	0	0	0	0	0	0	0	1	0	0	0	3	-2	1	-1	0
	cit	0	0	0	0	0	0	0	0	1	0	0	-3	2	-1	1	0
	arg	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	-1
	AMP	0	0	0	0	0	0	0	0	0	0	1	3/2	-1/2	0	-3/2	0

FIGURE 10 Row-reduced conservation matrix of Fig. 8 (11 × 16).

CONSERVATION MATRIX FOR 16 REACTANTS OF THE UREA CYCLE

The five steps of the urea cycle are

$$CO_2 + \text{ammonia} + 2\ \text{ATP} + H_2O = \text{carbamoyl phosphate} + 2\ \text{ADP} + P_i \quad (26)$$

$$\text{ornithine} + \text{carbamoyl phosphate} = \text{citrulline} + P_i \quad (27)$$

$$\text{citrulline} + \text{aspartate} + \text{ATP} = \text{AMP} + PP_i + \text{argininosuccinate} \quad (28)$$

$$\text{argininosuccinate} = \text{arginine} + \text{fumarate} \quad (29)$$

$$\text{arginine} + H_2O = \text{urea} + \text{ornithine}. \quad (30)$$

The transpose of the stoichiometric number matrix for reactions 26–30 is given in Fig. 6. The conservation matrix calculated by use of Eq. 8 is given in Fig. 7. Because $\boldsymbol{\nu}$ is 16 × 5, the conservation matrix **A** calculated from it by using Eq. 8 is 11 × 16. Now we want to account for this conservation matrix in terms of the conservation of atoms and groups, and other constraints. The constraints of the preceding section (that is C, O, N, P, adenosine, and fumarate, and the constraints of Eqs. 14–17) can be applied to the 11 reactants of the net reaction.

In constructing a conservation matrix for the 16-reactant system, it is convenient to add a constraint (component) for ornithine, because that combination of atoms exists in ornithine, argininosuccinate, citrullene, and arginine. When that is done and the constraints in Eqs. 14, 15, and 17 are included along with $n(CO_2) - n(\text{aspartate}) = \text{constant}$, the conservation matrix in Fig. 8 is obtained. Thus the number of constraints other than atom and group constraints is exactly the same as for the net reaction, namely four. This conservation matrix is 11 × 16, like that in Fig. 7, which was calculated from reactions 26–30 by using Eq. 8. We might hope that the row-reduced form of the matrix in Fig. 8 would be the same as for the preceding conservation matrix (Fig. 7) calculated from the stoichiometric number matrix for reactions 26–30. However, Figs. 9 and 10, which give the row-reduced forms of Figs. 7 and 8, show that they are different. The fact that they are different indicates that the constraints in the last four rows of the matrix in Fig. 8 are not unique, even though they are independent. Figs. 9 and 10 show that these conservation matrices each indicate five independent reactions. In both cases the five independent reactions can be added up after multiplying each of the reactions by an integer to obtain the net reaction for the urea cycle. These pathways can be calculated using LinearSolve, as shown in Eq. 11. It is not necessary to write these reactions out here, because they can be obtained from Figs. 9 and 10 by changing the signs in the last five columns and appending a 5 × 5 unit matrix below to obtain the stoichiometric number matrices. The pathways calculated in this way are {1, 1, 2, 2, 1} for Fig. 9 and {1, 1/2, 2, 1, 1/2} for Fig. 10. The 11 constraints indicate that five independent reactions are required to describe the stoichiometry of the

A =		CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
	C	1	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0
	O	2	9	1	0	0	0	0	5	1	0	3	1	6	4	7	0
	N	0	0	0	0	2	1	1	1	1	2	0	2	0	0	0	0
	P	0	3	0	0	0	0	0	1	0	0	1	0	2	1	2	0
	aden	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0
	fum	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1
	orn	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0	0
	con/am	1	0	0	0	0	-1	0	0	0	0	0	0	0	0	0	0
	con/H_2O	2	0	-1	0	0	0	0	0	0	0	0	0	0	0	0	0
	con/asp	1	0	0	0	0	0	-1	0	0	0	0	0	0	0	0	0
	con/ATP	3	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	con/urea	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
	con/fum	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	con/ADP	2	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	con/P_i	2	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0

FIGURE 11 Conservation matrix constructed from atom and group conservations and addtional constraints (15 × 16).

		CO_2	ATP	H_2O	orn	arsucc	am	asp	carb	cit	arg	AMP	urea	ADP	P_i	PP_i	fum
A =	CO_2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	ATP	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	3
	H_2O	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	2
	orn	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	arsucc	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	am	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
	asp	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
	carb	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	cit	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	arg	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	AMP	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	-1
	urea	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	-1
	ADP	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-2
	P_i	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	-2
	PP_i	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1

FIGURE 12 Row-reduced form of the conservation matrix of Fig. 9 (15 × 16).

system, but do not identify the actual reactions (see the discussion of Eqs. 18–21 and Eqs. 22–25).

Because the correct net reaction was obtained when the 11-reactant system was considered, it is important to show that the correct net reaction can also be obtained from an extension of the conservation matrix based on conservation of C, O, N, P, adenosine, fumarate, and ornithine and the four constraint equations used in Fig. 8. This can be done by adding four more constraints that are independent of the 11 constraints in the matrix in Fig. 8. These four constraints are

$$n(CO_2) + n(\text{urea}) = \text{constant} \tag{31}$$

$$n(CO_2) + n(\text{fumarate}) = \text{constant} \tag{32}$$

$$2n(CO_2) + n(\text{ADP}) = \text{constant} \tag{33}$$

$$2n(CO_2) + n(P_i) = \text{constant}. \tag{34}$$

When this is done, the conservation matrix in Fig. 11 is obtained. The row-reduced form of this matrix is given in Fig. 12. It is evident from the last column that the correct net reaction is obtained. The stoichiometric number matrix for the net reaction can be obtained by calculating the null space of the conservation matrix in Fig. 11.

The numbers of reactants, components, and independent biochemical reactions for the systems discussed are summarized in Table 1.

TABLE 1 Number (*N*) of reactants, number (*C*) of components, and number (*R*) of independent reactions for the urea cycle

System	*N*	*C*	*R*	Description of components
Net reaction	11	10	1	C, O, N, P, aden, fum, and four additional constraints (Figs. 1–3)
Five reactions	16	11	5	Above plus ornithine (Figs. 4–8)
Net reaction for system	16	15	1	Add four constraints for products in the net reaction (Figs. 9 and 10)

DISCUSSION

Enzyme-catalyzed reactions may involve coupling of two or more biochemical reactions that might otherwise occur separately. These couplings reduce the number of relations to be used in an equilibrium calculation and, correspondingly, increase the number of constraints beyond the conservation of atoms and groups. As an illustration of the coupling in biochemical reaction systems that is different from the effects in systems of chemical reactions, consider the glucokinase reaction

$$\text{ATP} + \text{glucose} = \text{ADP} + \text{glucose-6-phosphate} \qquad \Delta_r G'^\circ = -22 \text{ kJ mol}^{-1}, \tag{35}$$

with the two biochemical reactions that are coupled by the enzyme

$$\text{ATP} + H_2O = \text{ADP} + P_i \quad \Delta_r G'^\circ = -33 \text{ kJ mol}^{-1} \tag{36}$$

$$\text{Glucose} + P_i = \text{glucose-6-phosphate} + H_2O \qquad \Delta_r G'^\circ = +11 \text{ kJ mol}^{-1}, \tag{37}$$

where the standard transposed Gibbs energies of reaction are for pH 7. If ATP and glucose are added to solution containing ATPase and glucose-6-phosphatase, the ATP will be nearly completely hydrolyzed, and the phosphate produced will push reaction 37 just a little to the right. This is the sort of thing that occurs in series of chemical reactions. However, if ATP and glucose are added to a solution containing glucokinase, each ATP molecule that reacts produces a molecule of glucose-6-phosphate, and we speak of the energy in ATP as being conserved. This is accomplished by glucokinase by forming an enzyme-substrate complex, which then reacts with glucose to produce glucose-6-phosphate. Thus the stoichiometry of enzyme-catalyzed reactions is often very different from the stoichiometry of chemical reactions. When two reactions are coupled through a common intermediate involving the enzyme, there is a

"missing reaction" because two reactions are replaced by one. When a biochemical reaction system involves a series of reactions (like glycolysis) or a cycle (like the citric acid cycle), some of the reactions may involve coupling and others not. The effects of coupling are actually passed on to the net reaction.

Biochemical reactions involving coupling are very different from chemical reactions in that their constraint matrix **A** involves constraints in addition to atom and group balances. The coupling of biochemical reactions can be investigated by considering these linear constraints. The conservation matrix is not unique because the conservation equations can be written in different ways; in other words, different sets of components can be used. The conservation matrix that can be produced by considering constraints can be compared with the corresponding conservation matrix calculated from the stoichiometric number matrix by use of $\boldsymbol{\nu}^{\mathrm{T}}\mathbf{A}^{\mathrm{T}} = 0$. The two matrices may look different, but they are equivalent if they yield the same row-reduced form. The important point is that the rows, in addition to element and group conservations, indicate missing reactions. As indicated above, there are many possible answers to the question, What are the missing reactions? Although the methods of linear algebra tell something about the number of missing reactions and couplings, it alone cannot provide specific reactions, because there are often many possible sets of missing reactions that balance atoms and groups.

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