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Constraints in biochemical reactions

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Abstract

Chemical reactions satisfy element and charge conservation equations, but generally do not satisfy additional independent conservation equations. Biochemical reactions at a specified pH satisfy conservation equations for elements in addition to hydrogen, but they often satisfy additional independent conservation equations because of the coupling of reactions through the enzymatic mechanism. The enzyme may couple together two or more biochemical reactions so that only the sum of the coupled reactions is catalyzed. The biochemical reactions that are coupled together may, or may not, share reactants, but the type of coupling discussed here is stoichiometric. Since chemical equations and biochemical equations are mathematical equations, linear algebra provides the means for determining the number and types of constraints involved in the enzymatic mechanism. Constraints in addition to element balances indicate missing reactions. The identification of conservation equations is essential for calculations of equilibrium compositions using computer programs that minimize the transformed Gibbs energy at a specified pH subject to the conservation equations that apply.

Keywords: Coupling; Constraints; Conservation equations; Components; Biochemical equations

1. Introduction

Some biochemical equations are quite remarkable to a chemist because of the large number of different reactants and products. The reason for this is that an enzymatic mechanism can couple two or more biochemical reactions stoichiometrically. When this occurs, something more than elements is conserved, and that is discussed here. Chemical reactions conserve elements and charge, and a chemical equation is simply another way of expressing the conservation equations [1]. The coefficients in the conservation equations for a system described in terms of species form the conservation matrix **A**, and the stoichiometric numbers in a set of independent chemical equations for the system form the stoichiometric number matrix **v**. The matrix multiplication of **v** by **A** by yields the null matrix **0**.

$$\mathbf{A}\mathbf{v} = \mathbf{0}. \tag{1}$$

The **v** matrix is the null space [2] of the **A** matrix, and it can be calculated from **A** by use of a computer program such as *Mathematica* [3]. Conversely, the **v** matrix can be used to calculate the conservation matrix **A** by use of

$$\mathbf{v}^{\mathrm{T}}\mathbf{A}^{\mathrm{T}} = \mathbf{0} \tag{2}$$

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where T indicates the transpose. Thus $\mathbf{A}^T$ is the null space of $\mathbf{v}^T$. Neither the **A** matrix nor the **v** matrix is unique for a reaction system because the conservation equations can be written in different ways and different sets of independent reactions can be used to describe the changes in composition that can occur in the system. However, the row reduced forms of these matrices are unique for a given order of the columns (species). The row reduced form of a matrix, often referred to as the row-echelon form, is obtained by multiplying and dividing rows by integers and adding and subtracting them to obtain a unit matrix on the left of the matrix. A unit matrix is a square matrix with ones on the diagonal and zeros everywhere else. The rank of the **A** matrix is equal to the number C of components required to describe the equilibrium composition of the system; various choices can be made for the C components, but these components must contain all of the elements in the system. The rank of the **v** matrix is equal to the number R of independent reactions; this number is independent of the way the chemical equations are written. The number N of species in the system under consideration is given by

$$N = C + R. \tag{3}$$

Thus we can say that the species in a system can be divided into C components and R non-components. For systems involving chemical reactions, the number of components is generally equal to the number of elements involved. There are two types of exceptions: (1) When two or more elements appear in a certain ratio in all species where they are present, they constitute a pseudoelement and the redundant rows in the **A** matrix can be deleted. In this case, C is less than the number of elements. (2) In a few special situations one or more additional constraints in the **A** matrix arise from the mechanism of chemical change, so that C is greater than the number of independent elements. Schott [4] showed how the necessary constraints for the restricted equilibrium can be used in a general equilibrium program. Bjornbom [5] has discussed examples of restricted equilibrium, and Krambeck [6], Smith and Missen [1], and Alberty [7] have shown how to handle additional constraints that arise in mechanisms by adding a row (or rows) to the **A** matrix. The number N_{con} of constraints due to the mechanism of chemical change is given by

$$N_{con} = C - N_e, \tag{4}$$

where N_e is the number of independent elements. The number of independent elements in a system can be obtained by writing the **A** matrix for the system with a row for each element and row reducing it; note that this is not really the **A** matrix because it lacks non-element constraints. If there are constraints in the mechanism, **A** can be obtained from the **v** matrix for the system using eq. (2).

Biochemical reactions are generally studied at a specified pH, and this means that the conservation equation for hydrogen is lost [8–10] and the equilibrium data are interpreted using the apparent equilibrium constant K', which corresponds with a biochemical equation written in terms of sums of species. To indicate these changes, primes are placed on symbols in the preceding equations. The biochemical equations are written using words or abbreviations for reactants to show that hydrogen is not conserved and that the reactants are actually sums of species. Biochemical equations express the conservation of elements other than hydrogen. The coefficients in the conservation equations for a biochemical reaction form the apparent conservation matrix **A**′, and the stoichiometric numbers in the biochemical equations that are catalyzed by enzymes form the apparent stoichiometric number matrix **v**′. Thus, the biochemical equations to be used in an equilibrium calculation are determined by the enzymes present. The **A**′ matrix contains the coefficients in the conservation equations for the independent elements and may contain rows for additional constraints introduced by the enzyme mechanism. The matrix multiplication of **v**′ by **A**′ yields the null matrix **0**.

$$\mathbf{A}'\mathbf{v}' = \mathbf{0}. \tag{5}$$

The $\mathbf{v}'$ matrix is the null space of the $\mathbf{A}'$ matrix, and it can be calculated from $\mathbf{A}'$ by use of a computer program. Conversely, we can can start with the $\mathbf{v}'$ matrix and calculate the conservation matrix $\mathbf{A}'$ by use of

$$(\mathbf{v}')^{\mathrm{T}}(\mathbf{A}')^{\mathrm{T}} = \mathbf{0} \tag{6}$$

where T indicates the transpose. Thus $(\mathbf{A}')^{\mathrm{T}}$ is the null space of $(\mathbf{v}')^{\mathrm{T}}$. Neither the $\mathbf{A}'$ matrix nor the $\mathbf{v}'$ matrix is unique for a biochemical reaction system because the conservation equations can be written in different ways and the reactants can be listed in a different order. However, the row reduced forms of these matrices are unique for a given order of the columns (reactants). The rank of the $\mathbf{A}'$ matrix is equal to the number C' of apparent components required to describe the equilibrium composition of the system. The rank of the $\mathbf{v}'$ matrix is equal to the number R' of independent biochemical reactions. The number N' of reactants (sums of species) is given by

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

Thus the reactants in a biochemical reaction system can be divided into C' apparent components and R' apparent non-components. The reason for this article is that for biochemical reactions, the number C' of apparent components is often greater than the number of independent elements. When this is the case, the number N'_{con} of constraints due to the enzymatic mechanism is given by

$$N'_{\mathrm{con}} = C' - N'_{\mathrm{e}}, \tag{8}$$

where N'_{e} is the number of independent elements other than hydrogen.

In this paper all considerations start with a single biochemical equations (in other words, $\mathbf{v}'$ consists of a single column), and eq. (6) is used to calculate the apparent conservation matrix $\mathbf{A}'$. For example, for the NAD synthase (glutamine-hydrolyzing) reaction (EC 6.3.5.1) [11]

$$\begin{aligned}&\mathrm{ATP} + \text{deamido-NAD}_{\mathrm{ox}} + \text{L-glutamine} + \mathrm{H_2O}\\ &\quad = \mathrm{AMP} + \mathrm{PP_i} + \mathrm{NAD_{ox}} + \text{L-glutamate}\ ^{1}\end{aligned} \tag{9}$$

the transpose of the apparent stoichiometric matrix is

$$(\mathbf{v}')^{\mathrm{T}} = [-1\ -1\ -1\ -1\ 1\ 1\ 1\ 1\ 1], \tag{10}$$

where the reactants are in the order of eq. (9). Eq. (6) indicates that the null space [2] of eq. (10) yields the apparent conservation matrix for the NAD synthase (glutamate hydrolyzing) reaction. The following $\mathbf{A}'$ is obtained with the function NullSpace in *Mathematica* [3].

$$\mathbf{A}' = \begin{pmatrix} -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ -1 & 0 & 1 & 0 & 0 & 0 & 0 & 0 \\ -1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \end{pmatrix}. \tag{11}$$

[1] Abbreviations used in this paper are: ammonia for the sum of NH_3 and NH_4^+ at a specified pH; carbonate for the sum of $CO_2(aq)$, H_2CO_3, HCO_3^-, CO_3^{2-}; P_i for orthophosphate; PP_i for inorganic pyrophosphate; NAD_{ox} for the oxidized form of nicotinamide adenine dinucleotide at a specified pH; NAD_{red} for the reduced form of nicotinamide adenine dinucleotide at a specified pH; G3P for D-glyceraldehyde 3-phosphate; and 3PGP for 3-phospho-D-glycerol phosphate.

Since $C' = \text{rank } \mathbf{A}'$, seven components are conserved. The question is what these are because there are only four independent element (C, O, N, P) balance equations. Since the apparent conservation equations are not unique, we have to figure out a set of conservation equations that is equivalent to eq. (11). The process to find the constraints used here is as follows: The part of the $\mathbf{A}'$ matrix due to elements other than hydrogen was row reduced to be sure that the element conservation equations are independent. The row reduced form of this partial $\mathbf{A}'$ matrix indicates the number of biochemical reactions required to represent the changes in composition that can take place in the system as limited by the element balances. If this number is greater than one, a row is added to represent a possible additional constraint, and the $\mathbf{A}'$ matrix is row reduced to see whether the constraint is independent of the element constraints and whether more than one biochemical reaction is still required to represent the changes in composition that can occur in the system. This process is continued until the $\mathbf{A}'$ matrix is in the row-echelon form with a unit matrix making up all but the last column. The interpretation of matrix (11) is given below in eq. (53). The constraints in addition to element balances indicate missing biochemical reactions. The form of the constraints is not unique, but the number of constraints is. The dimensions of the $\mathbf{A}'$ matrix are $C' \times N'$, which is $(N_e' + N_{con}') \times N'$. The interpretation of the $\mathbf{A}'$ matrices will be developed by considering examples.

2. Adenosinetriphosphatase

The adenosinetriphosphatase reaction (EC 3.6.1.3) is

$$\text{ATP} + \text{H}_2\text{O} = \text{ADP} + \text{P}_i. \tag{12}$$

The transpose of the apparent stoichiometric number matrix is

$$(\mathbf{v}')^T = [-1\ -1\ 1\ 1], \tag{13}$$

where the reactants are in the order of the biochemical equation. The null space of $(\mathbf{v}')^T$ calculated with *Mathematica* using eq. (6) yields

$$\mathbf{A}' = \begin{array}{c} \begin{matrix} \text{ATP} & \text{H}_2\text{O} & \text{ADP} & \text{P}_i \end{matrix} \\ \begin{pmatrix} -1 & 1 & 0 & 0 \\ 1 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 \end{pmatrix} \end{array}. \tag{14}$$

Thus $C' = 3$, $N' = 4$, $R' = 1$. (Note that H_2O is always included as one of the substances in an aqueous system, whether it is involved in the reaction or not.) There are three independent elements, and eq. (8) indicates that $N_{con}' = 0$ so that there are no constraints in addition to element balances. Thus the biochemical reaction for ATPase, like most chemical reactions, does not involve any additional constraints. Eq. (14) can be compared with the apparent conservation matrix obtained from the conservation equations for C, O, and P, which is

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{P} \end{array} \begin{array}{c} \begin{matrix} \text{ATP} & \text{H}_2\text{O} & \text{ADP} & \text{P}_i \end{matrix} \\ \begin{pmatrix} 10 & 0 & 10 & 0 \\ 13 & 1 & 10 & 4 \\ 3 & 0 & 2 & 1 \end{pmatrix} \end{array} \tag{15}$$

(The row for nitrogen is omitted because it is redundant with the row for C.) Matrices (14) and (15) look different, but they are actually equivalent, as can be shown by calculating the row reduced form, which is

$$\mathbf{A}' = \begin{array}{c} \\ \text{ATP} \\ \text{H}_2\text{O} \\ \text{ADP} \end{array} \begin{array}{c} \begin{array}{cccc} \text{ATP} & \text{H}_2\text{O} & \text{ADP} & \text{P}_\text{i} \end{array} \\ \begin{pmatrix} 1 & 0 & 0 & 1 \\ 0 & 1 & 0 & 1 \\ 0 & 0 & 1 & -1 \end{pmatrix} \end{array} \quad (16)$$

for both. An example of a row-echelon form is matrix (16). When there is a single column on the right of the unit matrix, this indicates that all possible changes in composition permitted can be reached with reaction indicated by the stoichiometric numbers in the last column. In matrix (16), P_i (a non-component) can be considered to be made up of ATP + H_2O − ADP; this statement is equivalent to biochemical eq. (12). The rows have been relabelled after the row reduction to the row-echelon form. When there is a single reaction, the stoichiometric coefficients can be obtained from the last column of the row-echelon form [1].

The remaining $\mathbf{A}'$ matrices in this article are like eq. (15) in the sense that they show the apparent conservation matrix that yields the desired biochemical equation.

3. Glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)

The glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) reaction (EC 1.2.1.12) is

$$\text{D-glyceraldehyde 3-phosphate} + \text{P}_\text{i} + \text{NAD}_\text{ox} = \text{3-phospho-D-glycerol phosphate} + \text{NAD}_\text{red} \quad (17)$$

The transpose of the apparent stoichiometric number matrix is

$$(\mathbf{v}')^\text{T} = [-1\ -1\ -1\ 1\ 0\ 1], \quad (18)$$

where H_2O is after 3-phospho-D-glycerol phosphate. For reaction (17), $N' = 6$, $R' = 1$, and $C' = 5$. There are four independent elements (C, O, N, and P), and so there is one constraint. The use of eq. (18) in eq. (6) yields $\mathbf{A}'$, which is equivalent to the following $\mathbf{A}'$:

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{con1} \end{array} \begin{array}{c} \begin{array}{cccccc} \text{G3P} & \text{P}_\text{i} & \text{NAD}_\text{ox} & \text{3PGP} & \text{H}_2\text{O} & \text{NAD}_\text{red} \end{array} \\ \begin{pmatrix} 3 & 0 & 21 & 3 & 0 & 21 \\ 6 & 4 & 14 & 10 & 1 & 14 \\ 0 & 0 & 7 & 0 & 0 & 7 \\ 1 & 1 & 2 & 2 & 0 & 2 \\ 0 & 1 & 0 & 0 & 0 & 1 \end{pmatrix} \end{array} \quad (19)$$

Eq. (19) involves element balances for C, O, N, P, and the following constraint:

$$[\text{P}_\text{i}] + [\text{NAD}_\text{red}] = \text{const}. \quad (20)$$

This constraint is not unique, but it is one of the constraints that will ensure that only reaction (17) can occur.

4. ATP citrate (*pro-S*)-lyase reaction

The ATP citrate (*pro-S*)-lyase reaction (EC 4.1.3.8) is

$$\text{ATP} + \text{citrate} + \text{CoA} = \text{ADP} + \text{P}_i + \text{acetyl-CoA} + \text{oxaloacetate}. \tag{21}$$

The transpose of the apparent stoichiometric number matrix is

$$(\mathbf{v}')^T = [-1\ -1\ -1\ 0\ 1\ 1\ 1\ 1], \tag{22}$$

where the reactants are in the order in eq. (21), except that H_2O is inserted after CoA. For reaction (21), $N' = 8$, $R' = 1$, and so $C' = 7$. There are five independent elements (C, O, N, P, and S), and so there are two constraints since eq. (8) gives $N'_{con} = 7 - 5 = 2$. The use of eq. (22) in eq. (6) yields $\mathbf{A}'$, which is equivalent to the following $\mathbf{A}'$:

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{CoA} \\ \text{con1} \\ \text{con2} \end{array} \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{citrate} & \text{CoA} & H_2O & \text{ADP} & P_i & \text{acetyl-CoA} & \text{oxalo acetate} \end{array} \\ \begin{pmatrix} 10 & 6 & 0 & 0 & 10 & 0 & 2 & 4 \\ 13 & 7 & 0 & 1 & 10 & 4 & 1 & 5 \\ 5 & 0 & 0 & 0 & 5 & 0 & 0 & 0 \\ 3 & 0 & 0 & 0 & 2 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 0 & 0 & 1 & 0 & 0 \end{pmatrix} \end{array} \tag{23}$$

This matrix is equivalent to that obtained from eq. (21).

Eq. (23) was obtained from element conservation and the following two constraints:

$$[\text{ATP}] + [\text{acetyl-CoA}] = \text{const.}, \tag{24}$$

$$[\text{citrate}] + [\text{P}_i] = \text{const.} \tag{25}$$

Note that in writing the apparent conservation matrix, CoA has been treated as an element rather than using sulfur as an element and counting all the atoms in CoA.

Reaction (21) is the sum of the following two reactions:

$$\text{ATP} + H_2O = \text{ADP} + \text{P}_i, \tag{26}$$

$$\text{citrate} + \text{CoA} + \text{acetyl-CoA} + \text{oxaloacetate} + H_2O. \tag{27}$$

These reactions share a single reactant. (Ref. [11] states that this enzyme can be dissociated into 4.1.3.34 and 6.2.1.18, which share (3*S*)-citryl-CoA as reactants. If (3*S*)-citryl-CoA is included in the system, an additional constraint must be added to matrix (23)).

5. Carbamoyl-phosphate synthase (ammonia) reaction

The biochemical reaction carbamoyl-phosphate synthase (ammonia) (EC 6.3.4.16) is

$$2\text{ATP} + \text{ammonia} + \text{carbonate} = 2\text{ADP} + \text{P}_i + \text{carbamoyl phosphate}, \tag{28}$$

The transpose of the apparent stoichiometric number matrix is

$$(\mathbf{v}')^T = [-2\ -1\ -1\ 0\ 2\ 1\ 1], \tag{29}$$

where the reactants are in the order of eq. (28) except that H_2O is inserted after carbonate. For reaction (28), $N' = 7$, $R' = 1$, and $C' = 6$ (C, O, N, P, con1, and con2). The use of eq. (6) yields

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{con1} \\ \text{con2} \end{array} \begin{array}{c} \begin{array}{ccccccc} \text{ATP} & \text{ammonia} & \text{carbonate} & \text{H}_2\text{O} & \text{ADP} & \text{P}_\text{i} & \text{carbamoylP} \end{array} \\ \begin{pmatrix} 10 & 0 & 1 & 0 & 10 & 0 & 1 \\ 13 & 0 & 3 & 1 & 10 & 4 & 5 \\ 5 & 1 & 0 & 0 & 5 & 0 & 1 \\ 3 & 0 & 0 & 0 & 2 & 1 & 1 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 \end{pmatrix} \end{array} \tag{30}$$

These constraints are taken to be

$$[\text{carbonate}] + [\text{P}_\text{i}] = \text{const.}, \tag{31}$$

$$[\text{ATP}] + [\text{ADP}] = \text{const.} \tag{32}$$

These constraints are not unique; for example, the second constraint can be replaced by

$$[\text{ATP}] + 2[\text{carbamoyl phosphate}] = \text{const.}, \tag{33}$$

Reaction (28) can be considered to be made up of the following two reactions:

$$2\text{ATP} + 2\text{H}_2\text{O} = 2\text{ADP} + 2\text{P}_\text{i}, \tag{34}$$

$$\text{ammonia} + \text{carbonate} + \text{P}_\text{i} = \text{carbamoyl phosphate} + 2\text{H}_2\text{O}, \tag{35}$$

which share two reactants. Reaction (35) does not involve constraints in addition to element balance; $N' = 5$, $R' = 1$, and $C' = 4$ (C, O, N, and P).

6. Carbamoyl-phosphate synthetase (glutamine hydrolyzing)

The biochemical reaction carbamoyl-phosphate synthetase (glutamine hydrolyzing) (EC 6.3.5.5) is

$$2\text{ATP} + \text{L-glutamine} + \text{carbonate} + \text{H}_2\text{O} = 2\text{ADP} + \text{P}_\text{i} + \text{L-glutamate} + \text{carbamoyl phosphate}. \tag{36}$$

The transpose of the apparent stoichiometric number matrix is

$$(\mathbf{v}')^\text{T} = [-2\ -1\ -1\ -1\ 2\ 1\ 1\ 1]. \tag{37}$$

For reaction (36), $N' = 8$, $R' = 1$, and $C' = 7$ (C, O, N, P, con1, con2, and con3). Use of eq. (6) yields

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{con1} \\ \text{con2} \\ \text{con3} \end{array} \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{glutamine} & \text{carbonate} & \text{H}_2\text{O} & \text{ADP} & \text{P}_\text{i} & \text{glutamate} & \text{carbamoylP} \end{array} \\ \begin{pmatrix} 10 & 5 & 1 & 0 & 10 & 0 & 5 & 1 \\ 13 & 3 & 3 & 1 & 10 & 4 & 4 & 5 \\ 5 & 1 & 0 & 0 & 5 & 0 & 0 & 1 \\ 3 & 0 & 0 & 0 & 2 & 1 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 & 2 & 0 \end{pmatrix} \end{array} \tag{38}$$

These constraints are taken to be

[L-glutamine] + [carbamoyl phosphate] = const., (39)

[carbonate] + [P_i] = const., (40)

[ATP] + 2[L-glutamate] = const. (41)

Reaction (36) can be considered to be made up of the following two reactions:

$2ATP + 2H_2O = 2ADP + 2P_i$, (42)

L-glutamate + carbonate + P_i = L-glutamate + carbamoyl phosphate + H_2O. (43)

Reaction (43) does involve a constraint; $N' = 6$, $R' = 1$, $C' = 5$ (C, O, N, P, con1). This constraint can be taken to be

[glutamine] + [carbamoyl phosphate] = const., (44)

which is passed on to reaction (36).

7. NAD synthase reaction

The biochemical reaction catalyzed by the enzyme NAD synthase (EC 6.3.1.5) is

$\text{ATP} + \text{deamido-NAD}_{ox} + \text{ammonia} = \text{AMP} + \text{PP}_i + \text{NAD}_{ox}$. (45)

The transpose of the apparent stoichiometric number matrix is

$$(\nu')^T = [-1\ -1\ -1\ 0\ 1\ 1\ 1], \tag{46}$$

where the order in eq. (45) is used, but H_2O is inserted after ammonia. For reaction (45), $N' = 7$, $R' = 1$, and $C' = 6$ (C, O, N, P, con1, and con2). The **A**′ matrix calculated using eq. (6) is equivalent to

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{con1} \\ \text{con2} \end{array} \begin{array}{c} \begin{array}{ccccccc} \text{ATP} & \text{dNAD}_{ox} & \text{amm.} & \text{H}_2\text{O} & \text{AMP} & \text{PP}_i & \text{NAD}_{ox} \end{array} \\ \begin{pmatrix} 10 & 21 & 0 & 0 & 10 & 0 & 21 \\ 13 & 15 & 0 & 1 & 7 & 7 & 14 \\ 5 & 6 & 1 & 0 & 5 & 0 & 7 \\ 3 & 2 & 0 & 0 & 1 & 2 & 2 \\ 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 \end{pmatrix} \end{array} \tag{47}$$

The two constraints are taken to be

$[\text{ATP}] + [\text{NAD}_{ox}] = \text{const.}$, (48)

[ATP] + [AMP] = const. (49)

Reaction (49) is the sum of the reactions

$ATP + H_2O = AMP + PP_i$, (50)

$\text{deamido-NAD}_{ox} + \text{ammonia} = \text{NAD}_{ox}$, (51)

which do not have a reactant in common. Neither reaction (50) nor (51) involves a constraint in addition to element balance; phosphorous is redundant in reaction (51).

8. NAD synthase (glutamine-hydrolyzing) reaction

The biochemical reaction NAD synthase (glutamine-hydrolyzing) (EC 6.3.5.1) is

$$\text{ATP} + \text{deamido-NAD}_{ox} + \text{L-glutamine} + H_2O = \text{AMP} + \text{PP} + \text{NAD}_{ox} + \text{L-glutamate}. \quad (52)$$

The transpose of the apparent stoichiometric number matrix and the apparent conservation matrix calculated from it using eq. (6) are given earlier in eqs. (10) and (11). For reaction (52), $N' = 8$, $R' = 1$, and $C' = 7$. Since four elements are involved, there are three constraints. The apparent conservation matrix involving the four elements and three additional constraints is

$$\mathbf{A}' = \begin{array}{c} \\ \text{C} \\ \text{O} \\ \text{N} \\ \text{P} \\ \text{con1} \\ \text{con2} \\ \text{con3} \end{array} \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{dNAD} & \text{glutam} & H_2O & \text{AMP} & \text{PP}_i & \text{NAD} & \text{glut} \end{array} \\ \begin{pmatrix} 10 & 21 & 5 & 0 & 10 & 0 & 21 & 5 \\ 13 & 15 & 3 & 1 & 7 & 7 & 14 & 4 \\ 5 & 6 & 1 & 0 & 5 & 0 & 7 & 0 \\ 3 & 2 & 0 & 0 & 1 & 2 & 2 & 0 \\ 1 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix} \end{array} \quad (53)$$

The constraints are taken to be

$$[\text{ATP}] + [\text{AMP}] = \text{const.}, \quad (54)$$
$$[\text{L-glutamine}] + [\text{L-glutamate}] = \textit{const.}, \quad (55)$$
$$[\text{ATP}] + [\text{L-glutamate}] = \text{const.} \quad (56)$$

These constraints are not unique; for example, constraint (56) can be replaced with

$$[\text{deamido-NAD}_{ox}] + [\text{NAD}_{ox}] = \text{const.} \quad (57)$$

Reaction (53) can be considered to be made up of the following two reactions:

$$\text{ATP} + H_2O = \text{AMP} + \text{PP}, \quad (58)$$
$$\text{deamido-NAD}_{ox} + \text{L-glutamine} = \text{NAD}_{ox} + \text{L-glutamate}. \quad (59)$$

Neither of these subreactions involve constraints in addition to element balances.

The row reduced form of eq. (53) shows that the first seven reactants can be taken to be the components,

$$\mathbf{A}' = \begin{array}{c} \\ \text{ATP} \\ \text{dNAD} \\ \text{glutam} \\ H_2O \\ \text{AMP} \\ \text{PP}_i \\ \text{NAD} \end{array} \begin{array}{c} \begin{array}{cccccccc} \text{ATP} & \text{dNAD} & \text{glutam} & H_2O & \text{AMP} & \text{PP}_i & \text{NAD} & \text{glut} \end{array} \\ \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 \end{pmatrix} \end{array}. \quad (60)$$

9. Discussion

When two or more biochemical reactions are coupled by an enzyme, the reactions that are coupled are not at equilibrium when the system is at equilibrium. The two reactions that are coupled are missing from the system and are replaced by the coupled reaction. The missing reactions are missing because there is not an enzyme in the system to catalyze them, and they do not occur spontaneously at a significant rate in the absence of an enzyme. In this way energy is channeled into desired pathways in the living cell.

Six of the seven biochemical reactions discussed involve constraints in addition to element balances. These constraints come from the enzyme mechanism. According to eqs. (7) and (8), the number of the number N'_{con} of constraints in addition to element balances (excluding hydrogen) for a single reaction is given by

$$N'_{con} = N' - 1 - N'_{e}. \tag{61}$$

Table 1 summarizes this calculation for the seven cases discussed here. Possible sets of constraints are identified, but they are not unique.

There are at least two ways to divide a biochemical reaction into two or more biochemical reactions: (1) Two or more biochemical reactions that add to give the desired reaction without completely canceling any reactant except for H_2O. (2) Two or more biochemical reactions that cancel a common reactant in addition to water when they are added. This article is concerned with subreactions of the first type. Subreactions of the second type introduce additional reactants and additional constraints. The second type is important because some enzymes can be dissociated into two enzymes that catalyze the separate reactions. ATP citrate (*pro-S*)-lyase (section 4) can be dissociated into enzymes, two of which are identical with citryl-CoA-lyase (EC 4.1.3.34) and citrate-CoA ligase (EC 6.2.1.18). In the cell, even larger numbers of enzymes can be associated in such a way that more reactions are coupled stoichiometrically.

If the equilibrium composition of a system in which a number of biochemical reactions occur is calculated by minimizing the transformed Gibbs energy subject to element balances other than hydrogen, the wrong answer will be obtained if there are constraints in addition to the element balances. The correct answer will be obtained if the constraints in addition to element balances are included or if the simultaneous biochemical equilibrium expressions are solved simultaneously with the applicable conservation equations. These conservation equations can be obtained from the **A'** matrix because it tells us what is conserved.

Any enzymatic mechanism that adds up to give the correct net reaction that is catalyzed by the enzyme involves the constraints calculated here. This is in agreement with the finding for glucokinase [12].

Table 1

Numbers N'_{con} of constraints in the enzyme catalysis of the seven biochemical reactions considered

Enzyme	N'	N'_e	$N'_{con} = N' - N'_e - 1$
adenosinetriphosphatase	4	3	0
glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)	6	4	1
ATP citrate (*pro-S*)-lyase	8	5	2
carbamoyl-phosphate synthase (ammonia)	7	4	2
carbamoyl-phosphate synthase (glutamine-hydrolyzing)	8	4	3
NAD synthase	7	4	2
NAD synthase (glutamine-hydrolyzing)	8	4	3

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Nomenclature

$\mathbf{A}$ conservation matrix ($C \times N$) (dimensionless),
$\mathbf{A}'$ apparent conservation matrix ($C' \times N'$) (dimensionless),
C number of components in a system (dimensionless),
C' number of apparent components in a system (dimensionless),
N number of species in a system (dimensionless),
N' number of reactants (sums of species) in a system (dimensionless),
N_e number of independent elements in a system (dimensionless),
N_e' number of independent elements other than hydrogen (dimensionless),
N_{con} number of constraints in addition to element balances and charge balance (dimensionless),
N_{con}' number of constraints in addition to element balances (excluding hydrogen) (dimensionless),
R number of independent chemical reactions in a system (dimensionless),
R' number of independent biochemical reactions in a system (dimensionless),
$\mathbf{v}$ stoichiometric number matrix for a system of chemical equations ($R \times N$) (dimensionless),
$\mathbf{v}'$ apparent stoichiometric number matrix for a system of biochemical equations at specified pH ($R' \times N'$) (dimensionless).

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