

Components and Coupling in Enzyme-Catalyzed Reactions

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Many enzyme-catalyzed reactions involve coupling of two or more reactions that could otherwise be catalyzed separately. When biochemical reactions are coupled, the equilibrium composition is very different from that when the reactions are not coupled. The number of components in a chemical reaction is equal to the number of independent conservation equations for atoms of elements, but the number of components in an enzyme-catalyzed reaction that is coupled is larger than the number of independent conservation equations for atoms of elements. The investigation of these additional conservation equations by use of linear algebra is complicated by the fact that in dilute aqueous solutions, the activity of water is taken to be unity. This causes an incompatibility of conservation matrices and stoichiometric number matrices that can be avoided by use of the further transformed Gibbs energy G'' that provides the criterion for spontaneous change and equilibrium when the standard transformed Gibbs energy of water is constant. In the most striking example discussed, the enzyme mechanism of a ligase reaction introduces three constraints in addition to conservation of atoms of elements. This is completely unheard of in chemical reaction thermodynamics.

The specificity and speed of enzyme-catalyzed reactions are remarkable, but another remarkable aspect of enzyme catalysis is that an enzyme can couple two or more reactions that could otherwise be catalyzed separately. When this occurs, the reactions that are coupled do not occur, but the sum of reactions is catalyzed. Because chemical reactions and biochemical reactions are matrix equations, linear algebra provides insight into components and coupling. Because the thermodynamics of enzyme-catalyzed reactions is usually studied at a specified pH, the pH introduces a constraint beyond the usual constraints of temperature and pressure of chemical thermodynamics. When the pH is specified, a Legendre transform is used to define a transformed Gibbs energy G' that provides the criterion for spontaneous change and equilibrium.¹ When H_2O is a reactant in a biochemical reaction, its activity is not significantly affected by the extent of reaction in dilute solutions and $[\text{H}_2\text{O}]$ is not included in the expression for the apparent equilibrium constant K' . Therefore, it is useful to define a further transformed Gibbs energy G'' that provides the criterion for spontaneous change and equilibrium^{2,3} when the activity of water is considered to be constant.

This paper emphasizes the choice of components,⁴ which are the things that are conserved in a reaction system. In chemical thermodynamics, components are usually taken to be atoms of different elements. In biochemical thermodynamics, components can be taken to be atoms of different elements and conservation equations that tie coupled reactions together. When G' is used, hydrogen ions are not conserved, and when G'' is used, oxygen atoms are also not conserved. An element that provides a redundant conservation equation is not a component. Components can also be chosen from the list of reactants, but not all possible choices are allowed.

When an enzyme-catalyzed reaction does not involve H_2O , oxygen atoms are conserved by the reactants, but when H_2O appears in the reaction equation, there is the complication that $[\text{H}_2\text{O}]$ does not appear in the expression for the apparent equilibrium constant K' . This causes a serious problem in calculating equilibrium compositions in systems of biochemical reactions because computer programs^{2,5,6} for carrying out the

Newton–Raphson method use linear algebra with conservation matrices and stoichiometric number matrices for the reaction system. Because $[\text{H}_2\text{O}]$ is omitted in the expression for the apparent equilibrium constant, the stoichiometric number matrix for an enzyme-catalyzed reaction cannot include a stoichiometric number for H_2O . The equations of linear algebra⁷ that apply to reaction systems are described by Smith and Missen.⁸ Applications of linear algebra to systems of reactions are also discussed in *Thermodynamics of Biochemical Reactions*.⁹

In treating enzyme-catalyzed reactions hydrogen atoms are not conserved because the pH is specified, and in principle hydrogen ions are added to the system or are withdrawn to hold the pH constant. The conservation matrix \mathbf{A}' at specified pH has a row for each element, other than hydrogen, and a row for each conservation equation introduced by the enzyme mechanism. \mathbf{A}' has a column for each reactant (sum of species). Redundant rows and columns are omitted. \mathbf{A}' contains the coefficients in the conservation equations for a reaction system. The number N' of reactants in a system of enzyme-catalyzed reactions is equal to the number C' of components⁴ plus the number R' of independent biochemical reactions; that is, $N' = C' + R'$ and \mathbf{A}' is C' by N' . There is a corresponding stoichiometric number matrix ν' with a row for each reactant and a column for each independent reaction; that is, ν' is N' by R' . The matrix product of these two matrices is a zero matrix:

$$\mathbf{A}'\nu = \mathbf{0} \quad (1)$$

The zero matrix $\mathbf{0}$ is C' by R' . ν' is referred to as the null space of \mathbf{A}' , but because of the nature of matrices, only a basis for ν' can be calculated from \mathbf{A}' . However, if two matrices have the same row-reduced form, they contain the same information. Equation 1 can be rearranged to

$$(\nu')^T(\mathbf{A}')^T = \mathbf{0} \quad (2)$$

where T indicates the transpose. This equation shows that a basis for \mathbf{A}' can be calculated from ν' . In discussing coupling, it is useful to go back and forth between conservation matrices

and stoichiometric number matrices. If two matrices have the same row-reduced form, they contain the same information.

There is a problem in using eqs 1 and 2 when H_2O is a reactant, because $[\text{H}_2\text{O}]$ does not appear in the expression for the apparent equilibrium constant K' , but the standard transformed Gibbs energy of formation of water $\Delta_f G'^{\circ}(\text{H}_2\text{O})$ is needed in the calculation of the apparent equilibrium constant K' . The solution of this problem^{2,3} in calculating equilibrium concentrations utilizes the further transformed Gibbs energy G'' defined by the Legendre transform

$$G'' = G' - n_c(\text{O}) \mu'^{\circ}(\text{H}_2\text{O}) \quad (3)$$

where $n_c(\text{O})$ is the amount of the oxygen component in the system and $\mu'^{\circ}(\text{H}_2\text{O})$ is the standard transformed chemical potential of H_2O at the specified pH and ionic strength. The standard further transformed Gibbs energy of formation $\Delta_f G''^{\circ}$ of a reactant is calculated using

$$\Delta_f G''^{\circ} = \Delta_f G'^{\circ} - N(\text{O}) \Delta_f G'^{\circ}(\text{H}_2\text{O}) \quad (4)$$

where $N(\text{O})$ is the number of oxygen atoms in the reactant and $\Delta_f G'^{\circ}$ is the standard transformed Gibbs energy of H_2O at the specified pH and ionic strength. When standard further transformed Gibbs energies of formation $\Delta_f G''^{\circ}$ are used, the row for oxygen and the column for H_2O are omitted in the conservation matrix \mathbf{A}'' . The corresponding stoichiometric number matrix ν'' does not have a row for H_2O . The matrix product of these two matrices is a zero matrix.

$$\mathbf{A}''\nu'' = \mathbf{0} \quad (5)$$

The \mathbf{A}'' matrix is C'' by N'' , and the ν'' matrix is N'' by R'' , where R'' is the number of independent reactions. The zero matrix $\mathbf{0}$ is C'' by R'' . ν'' is referred to as the null space of \mathbf{A}'' , but because of the nature of matrices, only a basis for ν'' can be calculated from \mathbf{A}'' . Equation 5 can be rearranged to

$$(\nu'')^T (\mathbf{A}'')^T = \mathbf{0} \quad (6)$$

Thus a basis for \mathbf{A}'' can be calculated from ν'' . Mathematica provides a convenient way to make these matrix calculations.¹⁰

The current situation with respect to databases for thermodynamic properties of reactant is that $\Delta_f G'^{\circ}$ is used because the pH is treated as an independent variable.¹¹ From a physical chemical viewpoint, it might appear more logical to use the further transformed Gibbs energy G'' and tabulate $\Delta_f G''^{\circ}$ of biochemical reactants as functions of pH and ionic strength. However, biochemists balance biochemical reactions with H_2O when it is a reactant. A further disadvantage of a table of values of $\Delta_f G''^{\circ}$ for reactants is that reactants that differ in atomic composition only in the number of oxygen atoms and hydrogen atoms become pseudoisomers and should be aggregated. For example, the reactants citrate, isocitrate, and *cis*-aconitate would be aggregated in such a table. Therefore, G'' is sometimes needed, but G' is generally more useful to biochemists.

It is of interest to consider coupling in the context of the six classes of enzyme-catalyzed reactions, as defined by IUBMB.¹² Oxidoreductases (class 1) all involve coupling because these reactions can be divided into two or more half reactions. Transferases (class 2) all involve coupling. Hydrolases (class 3) do not involve coupling. Lyases (class 4) generally do not involve coupling. Isomerases (class 5) do not involve coupling. Ligases (class 6) all involve coupling by definition.

The identification of coupling is useful in making thermodynamic calculations because when a biochemical reaction is

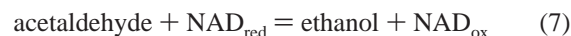
TABLE 1: Conservation Matrix \mathbf{A}' and Its Row-Reduced Form for the Alcohol Dehydrogenase Reaction

component	acetaldehyde	NAD_{red}	ethanol	NAD_{ox}
C	2	0	2	0
NAD	0	1	0	1
eq 10	1	0	0	1
acetaldehyde	1	0	0	1
NAD_{red}	0	1	0	1
ethanol	0	0	1	-1

the result of coupling two or more biochemical reactions, the standard transformed thermodynamic properties of the coupled reaction can be calculated by simply adding the standard transformed thermodynamic properties of the reactions that are coupled. For example, it may be easier to determine K' for two hydrolase reactions than for the coupled reaction.

Components in Oxidoreductase Reactions

An example of an oxidoreductase reaction is the alcohol dehydrogenase reaction that is represented by



The conservation matrix for this reaction with conservation equations for carbon and NAD shows that there are two independent reactions. An additional conservation equation, referred to here as con1 is needed to couple the following two half-reactions:



One way to ensure coupling is to include the conservation equation

$$n'(\text{acetaldehyde}) + n'(\text{NAD}_{\text{red}}) = \text{const} \quad (10)$$

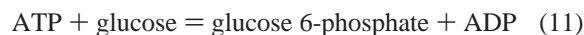
in \mathbf{A}' . The amount of a reactant is represented by n' . This equation requires that when an acetaldehyde molecule disappears, an NAD_{red} molecule appears. The conservation matrix and the row-reduced conservation matrix for reaction 7 are given in Table 1.

The components can also be considered to be acetaldehyde, NAD_{red} , and ethanol. The last column in the row-reduced matrix shows how NAD_{ox} is made up of components: $\text{NAD}_{\text{ox}} = \text{acetaldehyde} + \text{NAD}_{\text{red}} - \text{ethanol}$. This is, of course, simply reaction 7. NullSpace in Mathematica can be used to calculate a basis for the stoichiometric matrix ν' .

Components in Transferase Reactions

Transferase reactions all involve the coupling of two reactions, and so it is of interest to see how this shows up in the conservation matrix for the reaction system. There are two types of transferase reactions, those that do not involve H_2O as a reactant and those that do. Three enzyme-catalyzed reactions are discussed in this section, but only the third reaction involves H_2O as a reactant.

First consider the hexokinase reaction:

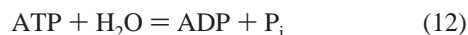


Atoms of carbon, oxygen, and phosphorus are conserved in this reaction. Conservation of nitrogen atoms is redundant. The conservation matrix \mathbf{A}' can be used to obtain a basis for the stoichiometric matrix that is correct. This makes it look like

TABLE 2: Conservation Matrix A' for Reaction 11 and Its Row-Reduced Form

component	ATP	glucose	glucose 6-phosphate	H ₂ O	ADP
C	10	6	6	0	10
O	13	6	9	1	10
P	3	0	1	0	2
eq 14	1	0	1	0	0
ATP	1	0	0	0	1
glucose	0	1	0	0	1
glucose 6-phosphate	0	0	1	0	-1
H ₂ O	0	0	0	1	0

the coupling does not involve an additional conservation equation, but because H₂O is not included in the conservation matrix this is a false impression. It is necessary to consider coupled reactions that do not involve H₂O in a broader context; for example, if this reaction is considered in a system of reactions in which some reactions do involve H₂O, then H₂O will be in the conservation matrix for the system. When H₂O is included in the conservation matrix A' , the conservation matrix indicates that the system involves two reactions, which can be taken to be



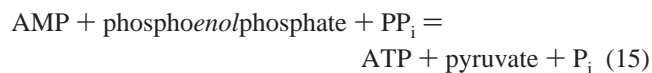
To couple these two reactions, an additional conservation equation has to be added to A' . Coupling requires that every time an ATP molecule disappears a glucose 6-phosphate molecule appears. This yields the following conservation equation:

$$n'(\text{ATP}) + n'(\text{glucose 6-phosphate}) = \text{const} \quad (14)$$

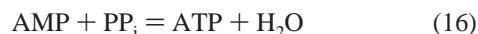
This constraint can be expressed in other ways with the same results. Adding this conservation equation yields the conservation matrix in Table 2.

This shows that there is now a single biochemical reaction, specifically reaction 11. This is evident from the last column, which shows that ADP is made up of three components: $\text{ADP} = \text{ATP} + \text{glucose} - \text{glucose 6-phosphate}$. The constraint represented by eq 14 is provided by the mechanism of action of hexokinase. An example of the way this is accomplished is discussed later.

Now consider a second transferase reaction that does not involve H₂O.



This reaction can be considered to result from the coupling of two hydrolase reactions.



Nitrogen atoms provide an independent constraint, even though they did not provide an independent constraint in the reaction $\text{ATP} + \text{glucose} = \text{glucose 6-phosphate} + \text{ADP}$. Consider reaction 15 in a system of reactions including some reactions where H₂O is a reactant. Because this system involves seven reactants, six conservation equations are required, but there are only four conservation equations for elements. Therefore, two

TABLE 3: Conservation Matrix A' for Reaction 13 and Its Row-Reduced Form

component	AMP	PEP ^a	PP _i	ATP	pyruvate	H ₂ O	P _i
C	10	3	0	10	3	0	0
O	7	6	7	13	3	1	4
P	1	1	2	3	0	0	1
N	5	0	0	5	0	0	0
eq 18	0	1	0	1	0	0	0
eq 19	0	0	1	0	0	1	0
AMP	1		0	0	0	0	1
PEP	0	1	0	0	0	0	1
PP _i	0	0	1	0	0	0	1
ATP	0	0	0	1	0	0	-1
pyruvate	0	0	0	0	1	0	-1
H ₂ O	0	0	0	0	0	1	0

^a PEP is phosphoenolphosphate.

TABLE 4: Conservation Matrix A'' for Reaction 20

components	AMP	PEP	P _i	ATP	pyruvate
C	10	3	0	10	3
P	1	1	1	3	0
N	5	0	0	5	0
eq 18	0	1	0	1	0
AMP	1	0	0	0	1
PEP	0	1	0	0	1
P _i	0	0	1	0	1
ATP	0	0	0	1	-1

conservation equations have to be added to tie the two hydrolase reactions together:

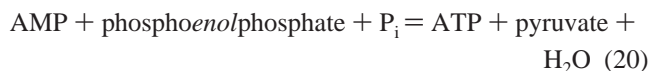
$$n'(\text{ATP}) + n'(\text{phosphoenolpyruvate}) = \text{const} \quad (18)$$

$$n'(\text{P}_i) + n'(\text{PP}_i) = \text{const}. \quad (19)$$

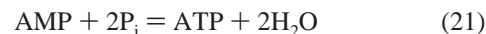
Adding these constraints yields the A' conservation that is shown in Table 3.

This conservation matrix yields a stoichiometric number matrix that shows there is a single reaction (as can be confirmed by the use of NullSpace in Mathematica).

Now consider a third transferase reaction that involves H₂O and is catalyzed by pyruvate, water dikinase.



This reaction can be viewed as the result of coupling the following two reactions:



Because this reaction is so much like the preceding reaction, we will start out with the conservation matrix involving C, O, P, N, and eq 18. The row-reduced A' conservation matrix shows that there is a single reaction, but it has the disadvantage that the use of NullSpace yields a stoichiometric matrix with a stoichiometric number for H₂O. The correct stoichiometric number matrix can be obtained by use of the point of view of the further transformed Gibbs energy G'' . This involves deleting the oxygen row and the H₂O column in A' to obtain A'' because $\mu^\circ(\text{H}_2\text{O})$ is constant. Table 4 shows the conservation matrix at specified $\mu^\circ(\text{H}_2\text{O})$.

This shows that there is a single reaction.

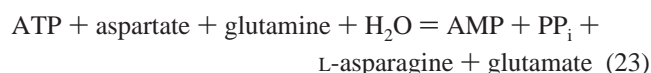
Components in Ligase Reactions

Ligase reactions necessarily involve coupling because they are defined¹² as “joining together of two molecules coupled with

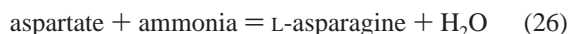
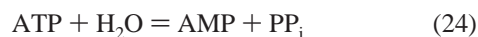
TABLE 5: Conservation Matrix A'' for Reaction 23 and Its Row-Reduced Form

components	ATP	aspartate	glutamine	AMP	PP _i	asparagine	glutamate
C	10	4	5	10	0	4	5
N	5	1	2	5	0	2	1
P	3	0	0	1	2	0	0
eq 27	1	0	0	0	0	1	0
eq 28	1	0	0	0	0	0	1
eq 29	0	1	0	0	1	0	0
ATP	1	0	0	0	0	0	1
aspartate	0	1	0	0	0	0	1
glutamine	0	0	1	0	0	0	1
AMP	0	0	0	1	0	0	-1
PP _i	0	0	0	0	1	0	-1
asparagine	0	0	0	0	0	1	-1

the hydrolysis of a pyrophosphate bond in ATP of a similar triphosphate." The asparagine synthase (glutamine hydrolyzing) reaction has eight reactants:



(Note that there is an error in ref 12 where the H₂O is missing.) This enzyme couples three hydrolase reactions:



The conservation matrix A' for the asparagine synthase (glutamine hydrolyzing) reaction assuming that C, O, N, and P atoms are conserved indicates that there are four independent reactions. To obtain reaction 23, it is necessary to put in three additional conservation equations that couple the three reactions. There are a number of ways to do this, but the three conservation equations used here are

$$n'(\text{ATP}) + n'(\text{L-asparagine}) = \text{const} \quad (27)$$

$$n'(\text{ATP}) + n'(\text{glutamate}) = \text{const} \quad (28)$$

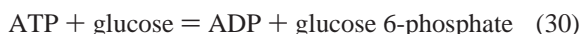
$$n'(\text{aspartate}) + n'(\text{PP}_i) = \text{const} \quad (29)$$

When these four conservation equations are included, conservation matrix A' indicates that there is a single biochemical reaction, but the use of NullSpace on this conservation matrix yields a stoichiometric number for H₂O, which is unsatisfactory. Therefore, reaction 23 is considered from the point of view of the further transformed Gibbs energy G''. The oxygen row and H₂O column are deleted to obtain the conservation matrix A'' given in Table 5 with its row-reduced form.

The last column in the row-reduced form yields reaction 23.

Coupling in Mechanisms of Enzyme-Catalyzed Reactions

To see how enzyme mechanisms introduce conservation equations beyond those required by atom balances, consider the hexokinase reaction:



Assume the following hypothetical two-step mechanism for the enzymatic catalysis:

**TABLE 6: Conservation Matrix A' for Reactions 31 and 32 and the Row-Reduced Form**

components	ATP	glucose	ADP	E	EP	glucose 6-phosphate
E	0	0	0	1	1	0
C	10	6	10	0	0	6
O	13	6	10	0	3	9
P	3	0	2	0	1	1
ATP	1	0	0	0	1	1
glucose	0	1	0	0	0	1
ADP	0	0	1	0	-1	-1
E	0	0	0	1	1	0

This is the way the enzyme ensures that a molecule of glucose 6-phosphate is formed for each molecule of ATP that disappears.

In treating the thermodynamics of reactions 31 and 32, components can be taken to be E, C, O, and P. Table 6 gives the conservation matrix for the mechanism.

This indicates that there are two reactions in the mechanism. If we want the mechanism to yield only net reaction 30, we have to add the conservation equation

$$n'(\text{ATP}) + n'(\text{glucose 6-phosphate}) = \text{const} \quad (33)$$

Row reducing this larger matrix yields reaction 30.

Calculation of Equilibrium Concentrations in a Two-Reaction System

Consider a reaction system containing ATP, ADP, P_i, glucose, and glucose 6-phosphate, all initially at 1 mM concentrations at 298.15 K, pH 7, and 0.25 M ionic strength. This section describes calculations of the equilibrium concentrations when the reactions



are catalyzed separately. The next section deals with the equilibrium concentrations when reactions 34 and 35 are coupled so that the only reaction is the hexokinase reaction:



There are two ways to make equilibrium calculations on biochemical reaction systems: the Mathematica program equalcc uses the conservation matrix as, and the Mathematica program equalcrx uses the stoichiometric number matrix nt (2,5,6,9). The first program uses the Newton–Raphson method, and the second program calls on the first program.

The program equalcc cannot be used when H₂O is a reactant because when H₂O is in the conservation matrix, the program tries to calculate [H₂O], just like it calculates the equilibrium concentrations of the other reactants. When H₂O is a reactant, the program equalcrx has to be used because it is based on the stoichiometric number matrix, which treats H₂O correctly; that is, it leaves it out. This program uses the stoichiometric number matrix nt and the vector lnkr of the natural logarithms of the apparent equilibrium constants to calculate the input as and lnk needed for equalcc. Then it calls on equalcc to calculate the equilibrium composition using the Newton–Raphson method.

The details of the calculation of the equilibrium composition when reactions 34 and 35 are catalyzed are given in the Supporting Information, and the equilibrium concentrations are given in Table 7 for a solution initially containing 0.001 M of each of the five reactants.

TABLE 7: Equilibrium Molar Concentrations for the Two-Reaction System and Equilibrium Molar Concentrations When the Two Reactions Are Coupled at 298.15 K, pH 7, and 0.25 M Ionic Strength

	ATP	ADP	P _i	glucose	glucose 6-phosphate
reactions 26 and 27	2.92×10^{-12}	0.00200	0.00300	0.00200	5.54×10^{-8}
reaction 28	1.44×10^{-5}	0.00199	0.00100	1.44×10^{-5}	0.00199

TABLE 8: Five Coupled Biochemical Reactions and Identification of Conservation Equations Required To Obtain Correct Conservation Matrices, Stoichiometric Number Matrices, and Equilibrium Concentrations^a

reactions	components
ethanol + 2e = acetaldehyde (8) NAD _{ox} + 2e = NAD _{red} (9) acetaldehyde + NAD _{red} = ethanol + NAD _{red} (7)	C, NAD, eq 10
ATP + H ₂ O = ADP + P _i (12) glucose 6-phosphate + H ₂ O = glucose + P _i (13) ATP + glucose = glucose 6-phosphate + ADP (11)	C, O, P, eq 14
AMP + PP _i = ATP + H ₂ O (16) phosphoenolphosphate + H ₂ O = pyruvate + P _i (17) AMP + phosphoenolphosphate + PP _i = ATP + pyruvate + P _i (15)	C, O, P, N, eq 18, eq 19
AMP + 2P _i = ATP + 2H ₂ O (21) phosphoenolphosphate + H ₂ O = pyruvate + P _i (22) AMP + phosphoenolphosphate + P _i = ATP + pyruvate + H ₂ O (20)	C, P, N, eq 20
ATP + H ₂ O = AMP + PP _i (24) glutamine + H ₂ O = glutamate + ammonia (25) L-asparagine + H ₂ O = aspartate + ammonia (26) ATP + aspartate + glutamine + H ₂ O = AMP + PP _i + L-asparagine + glutamate (23)	C, N, P, eq 27, eq 28, eq 29

^a The reactions that are coupled are listed above the coupled reaction.

When reactions 30 and 35 are catalyzed, ATP and glucose 6-phosphate are essentially completely hydrolyzed. When only reaction 36 is catalyzed, the reaction goes very far to the right so that ATP and glucose are essentially used up. The result of coupling is to produce a lot of glucose 6-phosphate.

Discussion

One of the problems encountered in discussing components and coupling in enzyme-catalyzed reactions is that when H₂O is a reactant, oxygen atoms are not conserved. The convention in chemical thermodynamics of aqueous solutions is that the solvent is treated on the mole fraction scale, rather than the molal or molar concentration scales used for the other reactants. In the dilute solutions studied in biochemical thermodynamics, the activity of H₂O is taken as unity in the expression for the apparent equilibrium constant, although $\Delta_f G'^{\circ}$ is included in the calculation of $\Delta_r G''^{\circ}$. Because the activity of H₂O is taken to be independent of the extent of a biochemical reaction, n' -(H₂O) in the constraint equation for oxygen atoms is effectively infinite, and so oxygen atoms are not conserved. In this article we have seen how this problem is solved with the further transformed Gibbs energy G'' . As mentioned after eq 4, when G'' is used, the oxygen row and the H₂O column in the conservation matrix are omitted.

Five enzyme-catalyzed reactions that are coupled have been discussed, and Table 8 summarizes the additional conservation equations that are introduced by the coupling provided by the enzyme mechanism.

The last reaction is a particularly dramatic example of a fundamental difference between coupled enzyme-catalyzed reactions and chemical reactions. Chemical reactions involve only balances of atoms of elements so that it is quite exceptional to find chemical reactions involving more than four species or three components.

Coupling can also be discussed in terms of the addition of constraints on a system. For example, the addition of hexokinase to an aqueous solution containing ATP, glucose, ADP, and

glucose 6-phosphate introduces the conservation equation n' -(atp) + n' -(glucose6phos) = constant in addition to balances of atoms of elements.

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Glossary

- A': conservation matrix at a specified pH
- A'': conservation matrix at a specified pH and specified μ'° -(H₂O)
- C': number of components at a specified pH
- C'': number of components at a specified pH and specified μ'° -(H₂O)
- G': transformed Gibbs energy defined by $G' = G - n_c(\text{H})\mu_{\text{H}^+}$
- G'': further transformed Gibbs energy defined by $G'' = G' - n_c(\text{O})\mu'^{\circ}(\text{H}_2\text{O})$
- $\Delta_f G'^{\circ}$: standard transformed Gibbs energy of formation of a reactant at a specified pH
- $\Delta_f G''^{\circ}$: standard further transformed Gibbs energy of formation of a reactant at a specified pH and specified μ'° -(H₂O)
- n' : amount of a reactant (sum of species) in a system
- $n_c(\text{O})$: amount of the oxygen component in a system
- N': number of different reactants (sum of species) in a reaction system at specified pH
- N'': number of different reactants (sum of species) in a reaction system at specified pH and specified μ'° -(H₂O)
- N(O): number of oxygen atoms in a reactant
- R': number of independent reactions in a reaction system at specified pH
- R'': number of independent reactions in a reaction system at specified pH and specified μ'° -(H₂O)
- ν' : stoichiometric number matrix at a specified pH
- ν'' : stoichiometric number matrix at a specified pH and specified μ'° -(H₂O)

$\mu'^{\circ}(\text{H}_2\text{O})$: standard transformed chemical potential of H_2O at a specified pH

Supporting Information Available: A *Mathematica* notebook containing the calculations described in this article. This notebook shows all the calculations described in this article and more calculations to clarify these results. Programs for calculating equilibrium compositions are also given. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

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