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Thermodynamics of the purine nucleotide cycle

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Abstract

Since the standard Gibbs energies of formation are known for all the species in the purine nucleotide cycle at 298.15 K, the functions of pH and ionic strength that yield the standard transformed Gibbs energies of formation of the ten reactants can be calculated. This makes it possible to calculate the standard transformed Gibbs energies of reaction, apparent equilibrium constants, and changes in the binding of hydrogen ions for the three reactions at desired pHs and ionic strengths. These calculations are also made for the net reaction and a reaction that is related to it. The equilibrium concentrations for the cycle are calculated when all the reactants are initially present or only some are present initially. Since the concentrations of GTP, GDP, and P_i may be in steady states, the equilibrium concentrations are also calculated for the system at specified steady-state concentrations.

Keywords: Adenylosuccinate; Purine nucleotide cycle; Equilibrium compositions; Steady state concentrations; Change in binding of hydrogen ions

1. Introduction

The purine nucleotide cycle consists of the following three enzyme-catalyzed reactions:
EC 6.3.4.4 Adenylosuccinate synthase

$$\text{aspartate} + \text{GTP} + \text{IMP} = \text{adenylosuccinate} + \text{GDP} + P_i \quad (1)$$

EC 4.3.2.2 Adenylosuccinate lyase

$$\text{adenylosuccinate} = \text{fumarate} + \text{AMP} \quad (2)$$

EC 3.5.4.6 AMP deaminase

$$\text{AMP} + H_2O = \text{IMP} + \text{ammonia} \quad (3)$$

Information about these reactions is available on the web [1]. The net reaction for the cycle is

$$\text{aspartate} + \text{GTP} + H_2O = \text{fumarate} + \text{GDP} + P_i + \text{ammonia} \quad (4)$$

A reaction that is similar to the net reaction is
EC 4.3.1.1 Aspartate ammonia–lyase

$$\text{Aspartate} = \text{fumarate} + \text{ammonia} \quad (5)$$

The purine nucleotide cycle plays an important role in skeletal muscle because it replenishes fumarate in the citric acid cycle. Also note that mutant human adenylosuccinate lyases (ASLs) have been associated with mental retardation, epilepsy, autism, and muscle wasting. The standard thermodynamic properties of species of aspartate, IMP, P_i, fumarate, AMP, H_2O, and ammonia are given in BasicBiochemData3 [2]. The properties of GTP and GDP, based on the convention that $\Delta_f G^\circ(\text{guanosine}) = 0$ at 298.25 K and zero ionic strength, are in press [3]. The standard Gibbs energies of formation $\Delta_f G^\circ$ of two species of adenylosuccinate are calculated from experimental data in the next section. These properties are used to derive the functions of pH and ionic strength that yield standard transformed Gibbs energies of formation of the reactants in the purine ribonucleotide cycle at 298.15 K, apparent equilibrium constants for reactions (1)–(5), and changes in the binding of hydrogen ions in these reactions. These apparent equilibrium constants are used to calculate equilibrium concentrations for the three-reaction cycle for various initial concentrations of the nine reactants.

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Table 1
Apparent equilibrium constants K' at 298.15 K, pHs 5 to 9, and 0.25 M ionic strength

Reaction	pH 5	pH 6	pH 7	pH 8	pH 9
1	6.1	142	7.0×10^{3}	5.4×10^{5}	3.0×10^{7}
2	9.7×10^{-3}	4.8×10^{-3}	2.94×10^{-3}	2.7×10^{-3}	2.7×10^{-3}
3	9.6×10^{4}	1.00×10^{4}	1.02×10^{3}	123	41
4	5.8×10^{3}	6.8×10^{3}	2.11×10^{4}	1.8×10^{5}	3.3×10^{6}
5	1.09×10^{-2}	9.9×10^{-3}	9.9×10^{-3}	1.04×10^{-2}	1.53×10^{-2}

Calculations of standard transformed Gibbs energies of formation $\Delta_fG°$ of biochemical reactants as functions of pH and ionic strength are sufficiently complicated that computer programs are required [4,5], and Mathematica [6] is a very convenient application for making these calculations because of its symbolic capabilities. The species data are stored in small matrices [2] in the form namesp={{$\Delta_fG_1°$, $\Delta_fH_1°$, z_1, N_{H1}}, {$\Delta_fG_2°$, $\Delta_fH_2°$, z_2, N_{H2}},...}}. $\Delta_fG_1°$ is the standard Gibbs energy of formation of species 1, $\Delta_fH_1°$ is the standard enthalpy of formation of species 1, z_1 is the electric charge on species 1, and N_{H1} is the number of hydrogen atoms in species 1. Programs can then be written in terms of matrix operations.

It is important to understand that although apparent equilibrium constants of enzyme-catalyzed reactions depend on the pH, it is the standard thermodynamic properties $\Delta_fG°$ and $\Delta_fH°$ that determine the equilibrium constants of the underlying chemical reactions, which are written in terms of species. The equilibrium constants of these chemical reactions do not depend on the pH. The best way to store information on the thermodynamics of enzyme-catalyzed reactions is to store $\Delta_fG°$ and $\Delta_fH°$ of the species that make up the reactants in the pH range of interest (usually pH 5 to 9). The literature data on apparent equilibrium constants can be used to calculate $\Delta_fG°$ and $\Delta_fH°$ for species that cannot be found in tables of chemical thermodynamic properties. These calculations are complicated, but computer programs have been written in Mathematica to make it convenient to carry out these calculations with a personal computer [4,5].

2. Calculation of standard Gibbs energies of formation of species of adenylosuccinate

The $\Delta_fG°$ of the species of adenylosuccinate can be calculated from the apparent equilibrium constant for reaction (2) determined by Carter and Cohen [7] that is quoted by Goldberg and Tewari [8]. Carter and Cohen obtained $K'=6.8\times10^{-3}$ at 308.15 K, pH 7.0 and $I=0.075$ M for reaction (2). Use of the Mathematica program calcdGf2sp [9] and data from BasicBiochemData3 [2] yields $\Delta_fG°$(adenylosuccinate^{4-}, 298.15 K, $I=0$)=−1650.53 kJ mol^{-1}, and this ion has 14 hydrogen atoms. The pK of adenylosuccinate is expected to be the same as for the phosphate group of AMP, namely 6.73 at 298.15 K and zero ionic strength. Therefore, $\Delta_fG°$(adenylosuccinate^{3-}, 298.15 K, $I=0$) is equal to −1688.95 kJ mol^{-1}. This ion has 15 hydrogen atoms. The succinate group does not add any pKs in the range pH 5–9. This many digits in $\Delta_fG°$ is required to preserve values of pKs.

The function of pH and ionic strength that gives the standard transformed Gibbs energy of formation $\Delta_fG'°$ of adenylosuccinate is derived by use of the Mathematica program calcdGmat [4]. This function of pH and ionic strength can be checked by calculating pK(adenylosuccinate) using the program calcpK and the apparent equilibrium constant under the experimental conditions of Carter and Cohen using the program calckprime [4]. The values of $\Delta_fG'°$(adenylosuccinate) at 298.15 K, pHs 5, 6, 7, 8, 9 and 0.25 M ionic strength are −1256.55, −1173.5, −1092.86, −1012.86, −932.94 kJ mol^{-1}.

The apparent equilibrium constants for reactions (1)–(5) at 298.15 K, pHs 5, 6, 7, 8, 9 and $I=0.25$ M are given in Table 1. The entries in this table were calculated using the program calckprime.

The changes in these apparent equilibrium constants with pH are caused by the production or consumption of hydrogen ions. The changes in binding Δ_rN_H can be calculated using

$$\Delta_rN_H = -\frac{1}{\ln(10)}\frac{\partial \ln K'}{\partial \mathrm{pH}} \tag{7}$$

The changes in binding of hydrogen ions are given as a function of temperature in Table 2.

The first and last reactions in the cycle push and pull the middle reaction so that the net reaction is spontaneous as shown by

$$K'_1K'_2K'_3 = K'_4 \tag{8}$$

Note that reaction (5) is not spontaneous. Its apparent equilibrium constant is given by

$$K'_5 = K'_4/K' \text{ (GTP hydrolysis)} \tag{9}$$

Bada and Miller [10] determined the apparent equilibrium constant for reaction (5). They obtained K'_5 (300.55 K, pH 7.0, and $I=0.10$ M)=0.0051. Use of BasicBiochemData3 yields K'_5 (298.15 K, pH 7.0, and $I=0.10$ M)=0.0072.

3. Calculation of equilibrium compositions for the purine nucleotide cycle

Thermodynamics can tell us two things about a system of reactions. First, it can tell us whether each reaction will go to the right or the left when the concentrations of all reactants are specified. Second, it can be used to calculate the equilibrium composition that will be reached for specified initial

Table 2
Changes in the binding of hydrogen ions Δ_rN_H at 298.15 K, pHs 5 to 9, and 0.25 M ionic strength

Reaction	pH 5	pH 6	pH 7	pH 8	pH 9
1	−1.17	−1.56	−1.82	−1.89	−1.52
2	0.28	0.32	0.09	0.01	0
3	0.95	1.00	0.98	0.81	0.02
4	0.07	−0.24	−0.75	−1.08	−1.50
5	0.10	0.01	0	−0.05	−0.36

Table 3
Transposed stoichiometric number matrix for the purine nucleotide cycle

Reaction	AMP	Asp	GTP	P_i	Amm	Fum	IMP	GDP	Adenylosucc
1	0	−1	−1	1	0	0	−1	1	1
2	1	0	0	0	0	1	0	0	−1
3	−1	0	0	0	1	0	1	0	0

concentrations of reactants. In calculating equilibrium compositions it is convenient to use matrix operations because a program using matrix operations can be used with a single reaction or a large system of reactions. Krambeck [11] wrote equcalc in the computer language APL to calculate the equilibrium compositions for gaseous reaction systems. Later he modified this program to calculate equilibrium compositions in solution reactions and named it equcalcc. He translated both of these programs into Mathematica. The program equcalcc requires the conservation matrix for the system; but, when H_2O is involved in a reaction in dilute solutions, the concentration of H_2O is not included in the expression for the apparent equilibrium constant. The problem caused by this omission is solved by using the Mathematica program equcalcrx, which requires the stoichiometric number matrix and the apparent equilibrium constants of a set of independent reactions to derive a suitable conservation matrix that includes the additional constraints caused by the enzyme mechanism as well. The program equcalcrx calls on equcalcc to calculate the equilibrium composition. These Mathematica programs have been published [12], and their uses have been described a number of times in the literature [4,5].

To calculate the equilibrium composition that will be reached in the purine nucleotide cycle, it is necessary to use its transposed stoichiometric number matrix. The stoichiometric number matrix nu for a system of reactions is a table of stoichiometric numbers for the reactions with a column for each reaction and a row for each reactant. The Mathematica program equcalcrx requires the transposed stoichiometric number matrix that is given in Table 3. Note that H_2O is omitted from the list of reactants because it does not appear in expressions for apparent equilibrium constants. To calculate the equilibrium composition it is necessary to specify the initial concentrations of all reactants. When all the reactants are initially present at 1 mM, the equilibrium concentrations at 298.15 K, pH 7, and ionic strength 0.25 M are obtained by typing the following into Mathematica:

```
equcalcrx[Transpose[nu], log[{7.00*10^3, 2.94*10^-3,
  1.02*10^3}], {10^-3, 10^-3, 10^-3, 10^-3, 10^-3,
  10^-3, 10^-3, 10^-3, 10^-3}]
```

In Mathematica * is the multiplication sign, and the ^ indicates that the next number is in the exponent. This input produces the list of the equilibrium concentrations that is shown in Table 4.

Under these conditions five reactants are produced in nearly stoichiometric amounts and four reactants are essentially used up. Note that the fumarate concentration is tripled. We can understand these results in the following way: Aspartate reacts nearly quantitatively with GTP and IMP to produce adenylosuccinate, GDP, and P_i. The reaction of adenylosuccinate to form fumarate and AMP is not favorable, but the concentration of AMP is reduced to a very low value by the third reaction. As a consequence, the concentrations of P_i, ammonia, fumarate, IMP, and GDP are significantly increased. Note that IMP is produced, even though it is not a product of the net reaction. These equilibrium concentrations can be checked by using them to calculate values of the three apparent equilibrium constants. This can be done by typing Exp[lnc.nu] into Mathematica, where lnc is the vector of natural logarithms of equilibrium concentrations and nu is the stoichiometric number matrix given in Table 3.

Equilibrium concentrations can also be calculated when fewer reactants are present initially. Table 5 gives the equilibrium concentrations when AMP, Aspartate, GTP are initially present at 1 mM.

Table 4
Equilibrium concentrations in the purine nucleotide cycle at 298.15 K, pH 7, I=0.25 M when all reactants are initially present at 1 mM

Reactant	Initial conc. (M)	Equilibrium conc. (M)
AMP	0.001	1.17×10^{-8}
Aspartate	0.001	4.78×10^{-8}
GTP	0.001	4.78×10^{-8}
P_i	0.001	0.002
Ammonia	0.001	0.004
Fumarate	0.001	0.003
IMP	0.001	0.003
GDP	0.001	0.002
Adenylosuccinate	0.001	1.20×10^{-8}

Table 5
Equilibrium concentrations in the purine nucleotide cycle at 298.15 K, pH 7, I=0.25 M when AMP, Aspartate, and GTP are initially present at 1 mM

Reactant	Initial conc. (M)	Equilibrium conc. (M)
AMP	0.001	1.96×10^{-9}
Aspartate	0.001	9.76×10^{-9}
GTP	0.001	9.76×10^{-9}
P_i	0	0.001
Ammonia	0	0.002
Fumarate	0	0.001
IMP	0	0.001
GDP	0	0.001
Adenylosuccinate	0	6.66×10^{-10}

Table 6
Equilibrium concentrations in the purine nucleotide cycle at 298.15 K, pH 7, I=0.25 M for steady-state concentrations [GTP]=[GDP]=[P_i]=10^{-3} M when the other reactants are initially at 1 mM

Reactant	Initial conc. (M)	Equilibrium conc. (M)
Aspartate	0.001	5.69×10^{-13}
IMP	0.001	0.003
Adenylosuccinate	0.001	1.19×10^{-8}
Fumarate	0.001	0.003
AMP	0.001	1.17×10^{-8}
Ammonia	0.001	0.004

Table 7
Equilibrium concentrations in the purine nucleotide cycle at 298.15 K, pH 7, I=0.25 M for steady-state concentrations [GTP]=[GDP]=[P_i]=10^{-3} M when aspartate and AMP are present initially at 1 mM

Reactant	Initial conc. (M)	Equilibrium conc. (M)
Aspartate	0.001	9.49×10^{-14}
IMP	0	0.001
Adenylosuccinate	0	6.64×10^{-10}
Fumarate	0	0.001
AMP	0.001	1.95×10^{-9}
Ammonia	0	0.002

The fact that the equilibrium composition can be calculated when only three of the nine reactants are present initially does not mean that any three reactants can be selected. The initial composition must be such that the other six reactants can be formed from them. This issue can be discussed in terms of components [12].

4. Calculation of equilibrium compositions at specified concentrations of GTP, GDP, and P_i

In calculating equilibrium concentrations for systems of enzyme-catalyzed reactions it is sometimes useful to specify steady-state concentrations of coenzymes because they are involved in so many different reactions. This provides a more global view of a multi-reaction system. When equilibrium calculations are to be made at steady-state concentrations of [GTP]=[GDP]=[P_i]=1 mM, it is necessary to change the symbols for the apparent equilibrium constants to K'' since one of them will be different from the values in Table 1.

The steady-state concentrations of GTP, GDT, and P_i are substituted in the expression for the apparent equilibrium constant of reaction (1), and their stoichiometric numbers are omitted from the stoichiometric number matrix for the system. This leads to K'' (aspartate+IMP=adenylosuccinate, 298.15 K, pH 7, and I=0.25 M)=7.0×10^6 when [GTP]=[GDP]=[P_i]=10^{-3} M. Table 6 gives the equilibrium concentrations when these three steady-state concentrations are specified and the other reactants are all at 1 mM initially.

Table 7 shows the equilibrium concentrations when [GTP]=[GDP]=[P_i]=1 mM and only aspartate and AMP are present initially.

This table can be compared with Table 5. AMP yields IMP in reaction (3), and then reaction (1) can occur. It is not possible to calculate equilibrium concentrations when reactants are arbitrarily set initially at zero. It must be possible to make all the reactants in the reaction system.

Since so many choices of pH, ionic strength, and initial concentrations are possible, it is obviously difficult to present an overview of the many possibilities. There is no substitute for a computer program that allows all of these variables to be specified.

5. Discussion

There is sufficient thermodynamic information on the species of the reactants in the purine nucleotide cycle to derive functions of pH and ionic strength that yield the standard transformed Gibbs energies of formation $\Delta_fG'^\circ$ of these nine reactants at 298.15 K in the range pH 5 to 9 and ionic strengths in the range zero to about 0.35 M. These functions have been used to calculate functions of pH and ionic strength that give the apparent equilibrium constants K' for five reactions under these conditions. The computer program equcalcrx has been used to calculate equilibrium concentrations of the nine reactants at 298.15 K, pH 7, and 0.25 M ionic strength for two sets of initial concentrations of reactants. Since the concentrations of GTP, GDP, and P_i may be in steady states, a more global view of the equilibria that are reached has been calculated at specified steady-state concentrations of GTP, GDP, and P_i using K'' values.

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