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Thermodynamic properties of enzyme-catalyzed reactions involving cytosine, uracil, thymine, and their nucleosides and nucleotides

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Abstract

The standard Gibbs energies of formation of species in the cytidine triphosphate series, uridine triphosphate series, and thymidine triphosphate series have been calculated on the basis of the convention that $\Delta_f G=0$ for the neutral form of cytidine in aqueous solution at 298.15 K at zero ionic strength. This makes it possible to calculate apparent equilibrium constants for a number of reactions for which apparent equilibrium constants have not been measured or cannot be measured because they are too large. This paper adds fifteen reactants to the database BasicBiochemData3 at MathSource that includes 199 reactants. The standard transformed Gibbs energies of formation of these fifteen reactants are used to calculate apparent equilibrium constants at 298.15 K, ionic strength 0.25 M, and pHs 5, 6, 7, 8, and 9 for thirty two reactions. The p*K*s, standard Gibbs energies of hydrolysis, and standard Gibbs energies of deamination are given for these fifteen reactants.

Keywords: Cytosine; Uracil; Thymine; Nucleotides; Thermodynamics; Apparent equilibrium constants

1. Introduction

This paper extends the calculations of the thermodynamic properties of enzyme-catalyzed reactions involving guanine, xanthine, and their nucleosides and nucleotides [1] to the cytidine triphosphate, uridine triphosphate, and thymidine triphosphate series [2]. These calculations are based on the convention that $\Delta_f G°$(cytidine,aq,298.15 K, I=0)=0. The calculated species properties are used to calculate apparent equilibrium constants for thirty two enzyme-catalyzed reactions at pHs 5 to 9. The requirement for these calculations is that moities of these reactants must appear on both sides of the catalyzed reactions. The calorimetric research by Boeiro–Goates and coworkers [3–5] has eliminated this requirement for reactions involving the ATP and IMP series. Species data for 199 biochemical reactants are stored in BasicBiochemData3 [6]. This has made it possible to calculate apparent equilibrium constants of many enzyme-catalyzed reactions [7].

The reactions that connect the reactants in the CTP, UTP, and TTP series are represented schematically in Eq. (1). The reactions in the vertical direction are

$$\begin{array}{ccccc} \text{CTP} & = & \text{UTP} & = & \text{TTP} \\ \| & & \| & & \| \\ \text{CDP} & = & \text{UDP} & = & \text{TDP} \\ \| & & \| & & \| \\ \text{CMP} & = & \text{UMP} & = & \text{TMP} \\ \| & & \| & & \| \\ \text{cytidine} & = & \text{uridine} & = & \text{thymidine} \\ \| & & \| & & \| \\ \text{cytosine} & = & \text{uracil} & = & \text{thymine} \end{array} \qquad (1)$$

hydrolysis reactions, but H_2O and other reactants are not shown. The first column of equal signs in the horizontal direction are deaminase reactions, but the H_2O and ammonia are not shown. The second column of arrows in the horizontal direction is discussed later in connection with the TTP series.

Abbreviations: CTP, cytidine triphosphate; CDP, cytidine diphosphate; CMP, cytidine monophosphate; UTP, uridine triphosphate; UDP, uridine diphosphate; UMP, uridine monophosphate; TTP, thymidine triphosphate; TDP, thymidine diphosphate; CMP, thymidine monophosphate.

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Calculations of the standard Gibbs energies of formation $\Delta_f G°$ of species of the cytidine triphosphate series, the uridine triphosphate series, and the thymidine triphosphate series are based on the convention that $\Delta_f G°$(cytidine,aq,298.15 K, I=0)=0. The apparent equilibrium constants for all the reactions that are indicated by equal signs and more can be calculated at 298.15 K, ionic strengths from zero to about 0.35 M, and pHs in the range 5 to 9. The apparent equilibrium constants are not presented for all of these possible reactions, but tables are given of reactions for the CTP series, the UTP series, and the TTP series with their EC numbers [8] to show that the apparent equilibrium constants can be calculated with the assistance of BasicBiochemData3 [6] for more reactions than those shown in Eq. (1).

The thermodynamic connection between the CTP series and the UTP series is provided by the assumption that the $\Delta_r G°$ for the deamination of cytidine is equal to the $\Delta_r G°$ for the deamination of adenosine [1]. The thermodynamic connection between the UTP series and the TTP series is obtained by use of the assumption that $\Delta_f G°$(uracil,aq,298.15 K, I=0)=$\Delta_f G°$(thymine, aq,298.15 K, I=0), based on related pairs of species.

2. Calculation of standard Gibbs energies of formation of the species of the CTP series

Since it is currently not possible to connect species in the CTP series with the elements in their reference states, $\Delta_f G°$(cytidine^0, 298.15 K, I=0)=0 is adopted as a convention of the thermodynamic tables. It is assumed that the CTP series is like the ATP series with respect to phosphate hydrolysis constants and deamination equilibrium constants so that the $\Delta_f G°$ for the ATP series can be used to calculate the $\Delta_f G°$ of species in the CTP. The $\Delta_f G°$ of the two most highly charged species of ATP, ADP, and AMP and the electrically neutral species of adenosine and adenine [6] are given in Table 1, where the electric charges of the species are shown.

Two things have to be done to Table 1 to make it apply to the CTP series. The first is to add 194.50 kJ mol^{-1} to each of the values in this table because $\Delta_f G°$(cytidine,aq,298.15 K, I=0)=0. This does not change the pKs of the phosphate groups or the equilibrium constants for the chemical reactions of hydrolysis of the most highly charged species. The second is to use the pKs of the pyrimidine rings in the CTP series to calculate the $\Delta_f G°$ of the species with a hydrogen ion bound by the pyrimidine ring. Since pK(adenine,298.15 K, I=0)=4.20 and pK(cytosine,298.15 K, I=0)=4.60 [9,10], the ring pKs in the CTP series are taken to be 0.40 larger than in the ATP series: namely, CTP(5.08), CDP (4.76), CMP(4.40), cytidine(3.87), and cytosine(4.60). As the pH is reduced to this range, the cytosine ring picks up a hydrogen ion.

Table 1
Some of the standard Gibbs energies of formation in the ATP series at 298.15 K and zero ionic strength [6][a,b]

Reactant	$\Delta_f G°$/kJ mol^{-1}	
ATP	−2768.10(−4)	−2811.48(−3)
ADP	−1906.13(−3)	−1947.10(−2)
AMP	−1040.45(−2)	−1078.86(−1)
Adenosine	−194.50(0)	
Adenine	313.40(0)	

[a] The charges of the species are shown in parentheses.

[b] The number of digits in this and subsequent tables does not signify the accuracy in terms of significant figures. The information in any thermodynamic table is in the differences between values. For example, in Table 2 the difference between the first two values is 43.38 kJ mol^{-1}. This can be used to calculate the pK of CTP: 43.38/(8.31451×0.29815×2.303)=7.60. Table 2 involves the assumption that the pK of CTP is the same as the pK of ATP because the phosphate groups are pretty far removed from the structural differences between ATP and CTP.

Table 2
Thermodynamic properties of the species in the CTP series at 298.15 K and zero ionic strength[a]

	$\Delta_f G°$/kJ mol^{-1}	z	N_H
CTP	−2573.60	−4	12
	−2616.98	−3	13
	−2645.98	−2	14
CDP	−1711.63	−3	12
	−1752.60	−2	13
	−1779.77	−1	14
CMP	−845.95	−2	12
	−884.36	−1	13
	−909.45	0	14
Cytidine	0	0	13
	−22.09	1	14
Cytosine	507.90	0	5
	481.64	1	6

[a] The charge number is represented by z, and the number of hydrogen atoms is represented by N_H.

This yields the species data in Table 2 that can be used to calculate standard transformed Gibbs energies of formation $\Delta_f G'°$ at 298.15 K, pHs in the range 5 to 9, and ionic strengths in the range zero to about 0.35 M. The charge on the species is z, and the number of hydrogen atoms it contains is N_H. The standard transformed Gibbs energies of formation of these reactants can be calculated at 298.15 K and desired pHs and ionic strengths using the Mathematica program calcdGmat [11].

3. Calculation of standard Gibbs energies of formation of species in the UTP series

The $\Delta_f G°$ for the species in the UTP series can be calculated from the $\Delta_f G°$ for some of the species in the CTP series in a three-step process. The first step is to select the $\Delta_f G°$ in the CTP series that contain information that applies to the UTP series.

Table 3
Some of the standard Gibbs energies of formation in the CTP series at 298.15 K and zero ionic strength that are used in calculating species properties in the UTP series[a]

Reactant	$\Delta_f G°$/kJ mol^{-1}	
CTP	−2573.06(−4)	−2616.98(−3)
CDP	−1711.63(−3)	−1752.60(−2)
CMP	−845.95(−2)	−884.36(−1)
Cytidine	0(0)	
Cytosine	507.90(0)	

[a] The charges of the species are shown in parentheses.

Table 4
Thermodynamic properties of the species in the UTP series at 298.15 K and zero ionic strength

	$\Delta_f G°$/kJ mol^{-1}	z	N_H
UTP	−2727.23	−5	10
	−2788.25	−4	11
	−2831.63	−3	12
UDP	−1868.29	−4	10
	−1926.28	−3	11
	−1967.25	−2	12
UMP	−1002.21	−3	10
	−1060.60	−2	11
	−1099.01	−1	12
Uridine	−160.08	−1	11
	−214.65	0	12
Uracil	347.48	−1	3
	293.25	0	4

Table 6
Thermodynamic properties of the species in the TTP series at 298.15 K and zero ionic strength

	$\Delta_f G°$/kJ mol^{-1}	z	N_H
TTP	−2724.87	−5	12
	−2788.25	−4	13
	−2831.63	−3	14
TDP	−1865.77	−4	12
	−1926.28	−3	13
	−1967.25	−2	14
TMP	−999.67	−3	12
	−1060.60	−2	13
	−1099.01	−1	14
Thymidine	−157.59	−1	13
	−214.65	0	14
Thymine	349.99	−1	5
	293.25	0	6

These values are the $\Delta_f G°$ of the two most highly-charged species of CTP, CDP, and CMP plus the electrically neutral species of cytidine and cytosine that are shown in Table 3.

The second step is to calculate $\Delta_f G°$(uridine^0,aq,298.15 K, $I=0$) following the convention that $\Delta_f G°$(cytidine^0,aq,298.15 K, $I=0$)=0. This is done on the assumption that the following two chemical reactions have the same equilibrium constant:

$$\text{Adenosine}^0 + H_2O = \text{inosine}^0 + NH_3 \quad (2)$$

$$\text{Citidine}^0 + H_2O = \text{uridine}^0 + NH_3 \quad (3)$$

Since $\Delta_r G°$(298.15 K, $I=0$) for reaction 2 is −3.96 kJ mol^{-1} and $\Delta_f G°$(cytidine^0,aq,298.15 K, $I=0$)=0, $\Delta_f G°$(uridine^0, aq,298.15 K, $I=0$)=−3.96 − $\Delta_f G°(NH_3)+\Delta_f G°(H_2O)$= −214.65 kJ mol^{-1}. Therefore, 214.65 kJ mol^{-1} is subtracted from each of the values in Table 3 so that $\Delta_f G°$(uridine^0, aq,298.15 K, $I=0$)=−214.65 kJ mol^{-1}.

The third step is to use the pKs of the uridine ring to calculate the $\Delta_f G°$ of the most negatively charged species. The pKs in the UTP series at 298.15 K and zero ionic strength are assumed to shift from uracil to UTP in the same way as in the ITP series where pK(hypoxanthine)=8.90, pK(inosine)=8.96, pK(IMP)= 9.63, pK(IDP)=9.56, and pK(ITP)=10.09. Since the pK of uracil is 9.5 [9,10], the highest pKs of the other reactants in the UTP series are taken to be pK(uridine)=9.56, pK(UMP)=10.23, pK(UDP)=10.16, and pK(UTP)=10.69. As the pH is raised to this range, the uracil ring loses a hydrogen ion. The species data for the UTP series obtained in this way are given in Table 4.

Table 5
Some of the standard Gibbs energies of formation in the UTP series at 298.15 K and zero ionic strength that are used in calculating species properties in the TTP series[a]

Reactant	$\Delta_f G°$/kJ mol^{-1}	
UTP	−2788.25(−4)	−2831.63(−3)
UDP	−1926.28(−3)	−1967.25(−2)
UMP	−1060.60(−2)	−1099.01(−1)
Uridine	−214.65(0)	
Uracil	293.25(0)	

[a] The charges of the species are shown in parentheses.

4. Calculation of standard Gibbs energies of formation for species in the TTP series

The replacement of a hydrogen atom in uracil with a methyl group to form thymine is not expected to change thermodynamic properties very much. The difference $\Delta_f G°$(thymine)−$\Delta_f G°$(-uracil) can be estimated by looking for similar situations. There are four examples in BasicBiochemData3 [7] of the effect of replacing a hydrogen atom by a methyl group in a solute in water: (1) going from methanol to ethanol decreases $\Delta_f G°$ by 6.33 kJ mol^{-1}, (2) going from ethanol to 1-propanol increases $\Delta_f G°$ by 5.83 kJ mol^{-1}, (3) going from glycine to alanine increases $\Delta_f G°$ by 8.91 kJ mol^{-1}, and (4) going from ethanol to 2-propanol decreases $\Delta_f G°$ by 3.59 kJ mol^{-1}. Thus it is reasonable to assume that $\Delta_f G°$(thymine^0,aq,298.15 K, $I=0$)=$\Delta_f G°$(uracil^0,

Table 7
pKs and $\Delta_r G°$ of chemical reactions at 298.15 K and zero ionic strength in the CTP, UTP, and TTP series calculated using Tables 2, 4, and 6)[a]

Reactant	pK_1(phosphate)	pK_2(base)	$\Delta_r G°$(hydrolysis)	$\Delta_r G°$(deamination)
CTP	7.60	5.08	−38.14	−3.96
CDP	7.18	4.76	−34.43	−3.96
CMP	6.73	4.40	−12.96	−3.96
Cytidine		3.87	−6.91	−3.96
Cytosine		4.60		−3.96

	pK_1(base)	pK_2(phosphate)	
UTP	10.69	7.60	−38.14
UDP	10.16	7.18	−34. 43
UMP	10.23	6.73	−12. 96
Uridine	9.56		−6.91
Uracil	9.50		
TTP	11.13	7.60	−38.14
TDP	10.60	7.18	−34.43
TMP	10.67	6.73	−12.96
Thymidine	10.00		−6.91
Thymine	9.94		

[a] The standard Gibbs energies of formation are given in kJ mol^{-1}.

Table 8
Standard transformed Gibbs energies of formation $\Delta_f G'^\circ$ of reactants in the CTP, UTP, and TTP series at 298.15 K, five pHs, and 0.25 M ionic strength

Reactant	pH 5	pH 6	pH 7	pH 8	pH 9
CTP	−2243.18	−2169.28	−2098.00	−2028.94	−1960.38
CDP	−1374.75	−1301.07	−1230.20	−1161.28	−1092.74
CMP	−504.07	−430.74	−360.33	−291.54	−223.01
Cytidine	381.37	455.74	529.96	604.16	678.37
Cytosine	653.82	683.09	711.72	740.27	768.81
UTP	−2486.80	−2418.95	−2353.42	−2290.19	−2228.27
UDP	−1618.40	−1550.75	−1485.64	−1422.62	−1361.22
UMP	−747.79	−680.42	−615.76	−552.76	−490.71
Uridine	137.55	206.04	274.53	342.91	410.48
Uracil	410.65	433.48	456.30	479.00	500.80
TTP	−2428.10	−2348.84	−2271.89	−2197.16	−2123.31
TDP	−1559.70	−1480.64	−1404.09	−1329.54	−1255.93
TMP	−689.09	−610.31	−534.22	−459.75	−385.83
Thymidine	196.25	276.16	356.07	435.94	515.46
Thymine	469.35	503.60	537.84	572.04	605.85

aq,298.15 K, I=0). This does not mean that the standard transformed Gibbs energies of formation $\Delta_f G'^\circ$ in the TTP series and the UTP series are the same because the pKs are not the same in the two series and thymine and uracil have different numbers of hydrogen atoms (thymine 6 and uracil 4). The pK [9,10] of thymine (9.94) is 0.44 greater than the pK of uracil (9.50), and so 0.44 is added to all the pKs in the UTP series to obtain the following pyrimidine ring pKs for the TTP series: TTP (11.13), TDP (10.60), TMP (10.67), and thymidine (10.00). These pKs are used to calculate $\Delta_f G^\circ$ for the most negative species of each reactant. Table 5 gives the properties of species in the UTP series that are used to make calculations of the properties of species in the TTP series. The use of the pKs yields the $\Delta_f G^\circ$ of the most negative species if each reactant. The thermodynamic properties of species in the TTP series are given in Table 6.

5. Checking the properties of species in the CTP, UTP, and TTP series

The species properties calculated in the preceding three sections can be checked by calculating three types of equilibrium constants and comparing them with previously published values of these properties in the ATP series: acid pKs, standard Gibbs energies of hydrolysis reactions, and standard Gibbs energies of deaminase reactions. The calculations of new species properties have been carried out to achieve certain properties that are assumed to be the same in different series. This is checked by using the data on species properties in Tables 2, 4, and 6 to calculate these properties. The pKs are calculated using the Mathematica program calcpK [11]. These calculated properties are given in Table 7. This table shows that these objectives have been met.

The chemical reactions for phosphate hydrolysis have been written as follows:

$$CTP^{4-} + H_2O = CDP^{3-} + H_2PO_4^- \quad (4)$$

$$CDP^{3-} + H_2O = CMP^{2-} + H_2PO_4^- \quad (5)$$

$$CMP^{2-} + H_2O = \text{cytidine}^0 + HPO_4^{2-} \quad (6)$$

$$\text{cytidine}^0 + H_2O = \text{cytosine}^0 + \text{ribose} \quad (7)$$

The same types of chemical reactions have been used for the UTP and TTP series.

The chemical reactions for deamination in the CTP series have been written as follows:

$$CTP^{4-} + H_2O = UTP^{4-} + NH_3 \quad (8)$$

The values in Table 7 can be compared with values for the GTP and XTP series [1].

The thermodynamic properties given in Tables 2, 4, and 6 can be used for the deoxy derivatives of the reactants in calculating apparent equilibrium constants where there are deoxy groups on both sides of the equation, since the deoxy adjustment cancels.

6. Calculations of standard transformed Gibbs energies of formation of reactants in the three series

The species matrices for the reactants in the three series have been used to calculate standard transformed Gibbs energies of formation $\Delta_f G'^\circ$ at 298.15 K, pHs 5 to 9, and 0.25 M ionic strength using calcdGmat [11]. These values can be added and subtracted to obtain $\Delta_r G'^\circ$ for enzyme-catalyzed reactions at these pHs provided there is one of these fifteen reactants on each side of the biochemical reaction. When the species matrices are put in a computer along with BasicBiochemData3, $\Delta_r G'^\circ$, K', and $\Delta_r N_H$ can be calculated. The following three sections give K'(298.15 K, pHs 5 to 9, 0.25 M ionic strength) for reactions involving reactants in these three series (Table 8).

It is possible to calculate species matrices for deoxy forms of all these reactants that involve ribose [2], but that is not done in

Table 9
Apparent equilibrium constants for enzyme-catalyzed reactions involving the CTP series at 298.15 K, five pHs and 0.25 M ionic strength

Biochemical reaction	pH 5	pH 6	pH 7	pH 8	pH 9
EC 2.4.2.2 Cytidine phosphorylase Cytosine+ribose 1-phosphate=cytidine+P_i	1.8×10^3	1.6×10^3	9.1×10^2	7.4×10^2	7.2×10^2
EC 2.7.1.40 Pyruvate kinase CDP+phosphoenolpyruvate=CTP+pyruvate	1.1×10^6	6.3×10^5	1.1×10^5	1.2×10^4	1.2×10^3
EC 2.7.1.86 NADH kinase CTP+NAD_{red}=CDP+$NADP_{red}$	3.3	37	430	4.6×10^3	4.6×10^4
EC 2.7.4.10 Nucleoside-triphosphate-adenylate kinase CTP+AMP=CDP+ADP	2.4	2.4	2.3	2.3	2.3
EC 2.7.4.14 Cytidylate kinase ATP+dCMP=ADP+dCDP	2.5	2.4	2.3	2.3	2.3
EC 3.2.2.10 Pyrimidine-5′-nucleotide nucleosidase CMP+H_2O=cytosine+ribose 5-phosphate	7.7	6.6	6.7	6.8	6.8
EC 3.6.1.12 deoxyCTP diphosphatase dCTP+H_2O=dCMP+diphosphate	8.2×10^6	2.1×10^7	2.0×10^8	2.9×10^9	1.1×10^{11}

Table 10
Apparent equilibrium constants for enzyme-catalyzed reactions involving the UTP series at 298.15 K, five pHs and 0.25 M ionic strength

Biochemical reaction	pH 5	pH 6	pH 7	pH 8	pH 9
EC 2.4.2.3 Uridine phosphorylase Uracil+ribose 1-phosphate=uridine+P_i	2.3×10^{3}	1.7×10^{3}	920	730	680
EC 2.4.2.9 Uracil phosphoribosyltransferase Uracil+PRPP=UMP+diphosphate	3.9×10^{6}	2.0×10^{6}	1.0×10^{6}	1.2×10^{6}	4.1×10^{6}
EC 2.7.1.30 Glycerol kinase UTP+glycerol=UDP+glycerol 3-phosphate	4.3×10^{5}	7.7×10^{5}	4.5×10^{6}	4.5×10^{7}	4.5×10^{8}
EC 2.7.1.40 Pyruvate kinase UDP+phosphoenolphosphate=UTP+pyruvate	1.1×10^{6}	6.3×10^{5}	1.1×10^{5}	1.2×10^{4}	950
EC 2.7.1.48 Uridine kinase ATP+uridine=ADP+UMP	920	1.6×10^{3}	8.8×10^{3}	8.1×10^{4}	7.5×10^{5}
EC 2.7.1.86 NAD_{red} kinase UTP+NAD_{red}=UDP+$NADP_{red}$	3.4	3.6	430	4.8×10^{3}	5.8×10^{4}
EC 2.7.4.14 Cytidylate kinase ATP+UMP=ADP+UDP	2.4	2.4	2.3	2.4	3.2
EC 3.2.2.3 Uridine nucleosidase Uridine+H_2O=ribose+uracil	16.2	16.2	16.3	16.4	17.1
EC 3.2.2.10 Pyrimidine-5′-nucleotide nucleosidase UMP+H_2O=ribose 5-phosphate+uracil	6.2	6.4	6.7	6.9	7.7
EC 3.5.4.1 Cytosine deaminase Cytosine+H_2O=uracil+ammonia	6.3×10^{4}	8.5×10^{3}	890	99	22
EC 3.5.4.5 Cytidine deaminase Cytidine+H_2O=uridine+ammonia	8.2×10^{4}	8.8×10^{3}	890	98	21
EC 3.5.4.12 dCMP deaminase dCMP+H_2O=dUMP+ammonia	7.9×10^{4}	8.7×10^{3}	8.9×10^{2}	97	20
EC 3.5.4.30 dCTP deaminase dCTP+2H_2O=dUMP+diphosphate+ammonia	6.5×10^{11}	1.9×10^{11}	1.8×10^{11}	2.8×10^{11}	2.2×10^{12}
EC 3.6.1.23 dUTP diphosphatase dUTP+H_2O=dUMP+diphosphate	8.5×10^{6}	2.1×10^{7}	2.0×10^{8}	2.9×10^{9}	1.0×10^{11}
EC 3.6.1.42 GDPase UDP+H_2O=UMP+P_i	2.1×10^{5}	2.8×10^{5}	8.9×10^{5}	6.4×10^{6}	4.8×10^{7}
EC 6.3.4.2 CTP synthase ATP+UTP+ammonia=ADP+P_i+CTP	6.7	75	2.3×10^{3}	1.6×10^{5}	7.2×10^{6}

this paper because there are so many reactions where there are deoxy moieties on both sides of the biochemical equation. In this case, the deoxy adjustment cancels, and the value of the apparent equilibrium constant is that calculated using the properties given here for the species in the three series.

7. Applications of standard transformed Gibbs energies of formation of reactants in the CTP series

The functions of pH and ionic strength that yield the standard transformed Gibbs energy of formation $\Delta_f G'^\circ$ at 298.15 K of the reactants in the CTP series have been calculated using calcdGmat [11]. These functions of pH and ionic strength are then used to calculate apparent equilibrium constants for a number of enzyme-catalyzed reactions using calcprime [11]. The apparent equilibrium constants are given in Table 9. This is not a complete list of reactions that can be calculated using the species properties given here. The cytosine moiety has to be on both sides of each reaction because the convention $\Delta_f G^\circ$(cytidine,aq,298.15 K, I=0)=0 is used.

8. Applications of standard transformed Gibbs energies of formation of reactants in the UTP series

The functions of pH and ionic strength for $\Delta_f G'^\circ$ at 298.15 K can be used to calculate apparent equilibrium constants for a number of enzyme-catalyzed reactions that involve the UTP and the CTP series. The list given in Table 10 is not complete. Apparent equilibrium constants have not been measured for any of these reactions; some can be measured, but others are too large.

9. Applications of standard transformed Gibbs energies of formation of reactants in the TTP series

The functions of pH and ionic strength for $\Delta_f G'^\circ$ at 298.15 K can be used to calculate apparent equilibrium constants for a number of enzyme-catalyzed reactions that involve the TTP, UTP, and the CTP series. The list given in Table 11 is not complete. Apparent equilibrium constants have not been measured for any of these reactions; some can be measured, but others are too large.

10. Discussion

These calculations of standard Gibbs energies of formation of species in the CTP, UTP, and TTP series are based on similarities in the thermodynamic properties of various pyrimidines, their nucleosides, and nucleotides. In large molecules the chemical properties of groups in the molecule may be nearly independent of other groups in the molecule. Therefore it is to be expected that the pKs of phosphate groups in the ATP series will be good predictors of pKs of phosphate groups in other ribonucleotide

Table 11
Apparent equilibrium constants for enzyme-catalyzed reactions involving the TTP series at 298.15 K, five pHs and 0.25 M ionic strength

Biochemical reaction	pH 5	pH 6	pH 7	pH 8	pH 9
EC 2.7.1.21 Thymidine kinase ATP+thymidine=ADP+thymidine 5′-phos	920	1.6×10^{3}	8.7×10^{3}	8.1×10^{4}	7.8×10^{5}
AMP+thymidine=adenosine+thymidine 5′-phosphate	1.0	1.0	1.0	1.0	1.0
EC 2.7.1.118 ADP-thymidine kinase ADP+thymidine=AMP+thymidine 5′-phos	375	671	3.8×10^{3}	3.5×10^{4}	3.4×10^{5}
EC 2.7.1.114 AMP-thymidine kinase AMP+thymidine=adenosine+TMP	1.0	1.0	1.0	1.0	1.0
EC 2.7.1.45 Deoxynucleoside kinase ATP+dT=ADP+dTMP	920	1.6×10^{3}	8.7×10^{3}	8.1×10^{4}	7.8×10^{5}
EC 2.7.4.9 dTMP kinase ATP+dTMP=ADP+dTDP	2.4	2.4	2.3	2.4	2.7
EC 3.1.3.35 Thymidylate 5′-phosphatase TMP+H_2O= thymidine+P_i	550	420	240	190	190
EC 3.6.1.39 thymidine-triphosphatase dTTP+H_2O= dTDP+P_i	5.1×10^{5}	6.6×10^{5}	2.1×10^{6}	1.6×10^{7}	1.7×10^{8}

series. In the ATP series, the p*K*s of the purine rings shift with the degree of phosphorylation, and so it is expected that these shifts in p*K*s will be found in the pyrimidine series. It is also to be expected that the chemical equilibrium constants for the deamination of species in the ATP series and the CTP series will be nearly the same. It is also expected that the chemical equilibrium constants for the hydrolysis of ribonucleosides and ribonucleotides will be the same. It would be better if there were more experimental data on the p*K*s and chemical reactions in these series, but, until there is, calculations like those presented here can provide information about the thermodynamics of enzyme-catalyzed reactions involving these reactants.

A more serious problem is that it is currently not possible to calculate apparent equilibrium constants for reactions of members of these three series that convert them to members of the purine series or to smaller molecules. This is because the standard Gibbs energies of formation of species in the CTP series, UTP series and TTP series cannot yet be connected with the elements in their reference states. There are two ways that these limitations can be removed: (1) Apparent equilibrium constants can be measured for a series of reactions connecting these species with the elements, or (2) The entropies of these species can be determined by use of the third law measurements, and enthalpies of formation can also be determined [3–5].

The usefulness of the species properties given here can be increased by recognizing that when the deoxy forms of these reactants are involved in reactions, the reactions have the same apparent equilibrium constants provided deoxy forms appear on both sides of the reaction.

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Glossary

$\Delta_f G°$	standard Gibbs energy of formation of a species (kJ mol^{-1})
$\Delta_f H°$	standard enthalpy of formation of a species (kJ mol^{-1})
$\Delta_f G'°$	standard transformed Gibbs energy of formation of a reactant (kJ mol^{-1})
$\Delta_r G'°$	standard transformed reaction Gibbs energy of reaction (kJ mol^{-1})
K	equilibrium constant of a chemical reaction (dimensionless)
K'	apparent equilibrium constant at a specified pH (dimensionless)
N_H	number of hydrogen atoms in a species (dimensionless)
$\Delta_r N_H$	change in the number of hydrogen ions bound in a reaction at a specified pH (dimensionless)
pK	$-\log K$
z	electric charge on an ion