Thermodynamic Properties of Nucleotide Reductase Reactions†

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ABSTRACT: Recent thermodynamic measurements have made it possible to calculate the apparent equilibrium constants of the ribonucleoside diphosphate reductase reaction and the ribonucleoside triphosphate reductase reaction with various reducing agents. Third law heat capacity measurements on crystals of D-ribose and other calorimetric measurements make it possible to calculate $\Delta_f G°$ for D-ribose and two species of D-ribose 5-phosphate. The experimental value of the apparent equilibrium constant K' for the deoxyribose-phosphate aldolase reaction makes it possible to calculate the standard Gibbs energies of formation $\Delta_f G°$ for two protonation states of 2′-deoxy-D-ribose 5-phosphate. This shows that $\Delta_f G°$(2′-deoxy-D-ribose 5-phosphate^{2-}) − $\Delta_f G°$(D-ribose 5-phosphate^{2-}) = 147.86 kJ mol^{-1} at 298.15 K and zero ionic strength in dilute aqueous solutions. This difference between reduced and oxidized forms is expected to apply to D-ribose, D-ribose 1-phosphate, ribonucleosides, and ribonucleotides in general. This expectation is supported by two other enzyme-catalyzed reactions for which apparent equilibrium constants have been determined. The availability of $\Delta_f G°$ values for the species of 2′-deoxy-D-ribose and its derivatives makes it possible to calculate standard transformed Gibbs energies of formation of these reactants, apparent equilibrium constants for their reactions, changes in the binding of hydrogen ions in these reactions, and standard apparent reduction potentials of the half reactions involved as a function of pH and ionic strength at 298.15 K. The apparent equilibrium constant for ADP + thioredoxin$_{red}$ = 2′-deoxyADP + H_2O + thioredoxin$_{ox}$ is 1.4×10^{11} at 298.15 K, pH 7, and 0.25 M ionic strength.

The ribonucleoside-diphosphate reductase reaction

$$\text{EC 1.17.4.1 NDP + thioredoxin}_{red} = \text{dNDP + thioredoxin}_{ox} + H_2O \quad (1)$$

applies to ADP, CDP, UDP, and GDP, and the reducing agent can be NAD_{red},[1] $NADP_{red}$ FAD_{red}, or FMN_{red} in place of thioredoxin$_{red}$. In this paper, it will be convenient to use EC numbers (*1*) to identify reactions. The apparent equilibrium constants K' for reaction 1 and those with other reducing agents are expected to be independent of the base. These apparent equilibrium constants are too large to measure directly, but this article shows how they can be obtained indirectly. To make DNA, reaction 1 has to be followed by the nucleoside-diphosphate kinase reaction

$$\text{EC 2.7.4.6 dNDP + ATP = dNTP + ADP} \quad (2)$$

This reaction is expected to have an apparent equilibrium constant of essentially unity. Deoxyribonucleoside triphosphate can be produced directly by the reaction

$$\text{EC 1.17.4.2 NTP + thioredoxin}_{red} = \text{dNTP + thioredoxin}_{ox} + H_2O \quad (3)$$

The apparent equilibrium constant for this reaction is expected to be the same as for reaction 1. These apparent equilibrium constants are of interest because they determine the availability of dNTPs for the formation of DNA.

The recent determination of the standard molar entropy of crystals of D-ribose (*2*) by measuring heat capacities down to 10 K and additional thermodynamic measurements (*3, 4*) have made available the standard Gibbs energy of formation $\Delta_f G°$ and standard enthalpy of formation $\Delta_f H°$ for D-ribose in dilute aqueous solutions (*5*). Other data in the literature have made it possible to calculate $\Delta_f G°$ and $\Delta_f H°$ for the species of D-ribose 1-phosphate and D-ribose 5-phosphate (*6*). $\Delta_f G°$ and $\Delta_f H°$ are functions of temperature and ionic strength. These properties can be used to calculate apparent equilibrium constants and heats of chemical reactions written in terms of species, but when the pH is specified, the criterion for spontaneous change and equilibrium is provided by the transformed Gibbs energy G' (*7*−*9*). To calculate apparent equilibrium constants K' of enzyme-catalyzed reactions at specified pH and ionic strength, the standard transformed Gibbs energies of formation $\Delta_f G_i'°(j)$ of species j have to be calculated as functions of pH and ionic strength I using

$$\Delta_f G'°(j,\text{pH},I) = \Delta_f G°(j,I) - N_H(j)\{\Delta_f G°(H^+,I) - RT\ln(10)\text{pH}\} \quad (4)$$

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[1] Abbreviations: $NADP_{ox}$, β-nicotinamide adenine dinucleotide oxidized form; $NADP_{red}$, β-nicotinamide adenine dinucleotide phosphate reduced form; NDP, ribonucleoside diphosphate; NTP, ribonucleoside triphosphate; dNDP, 2′-deoxyribonucleoside diphosphate; dNTP, 2′-deoxyribonucleoside triphosphate.

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where $N_H(j)$ is the number of hydrogen atoms in species j and pH = $-\log[H^+]$. The extended Debye−Hückel equation is used to adjust $\Delta_f G°(j,I)$ and $\Delta_f G°(H^+,I)$ to the desired ionic strength (*10*). When a reactant consists of more than one species, the standard transformed Gibbs energy of formation of the reactant $\Delta_f G'°(i,\mathrm{pH},I)$ is calculated (*7*, *11*) using

$$\Delta_f G'°(i,\mathrm{pH},I) = -RT \ln \sum_{j=1}^{j=N_{sp}} \exp(-\Delta_f G'°(j,\mathrm{pH},I)/RT) \quad (5)$$

where N_{sp} is the number of species in reactant i. Temperature is not listed here as a variable here because all calculations in this paper are for 298.15 K. Substituting the extended Debye−Hückel equation in eq 1 and then substituting eq 1 in eq 2 yields a complicated function, but Mathematica® (*12*) makes it possible to write a program (calcdGmat) (*13*) that makes it to convenient to derive the mathematical function for $\Delta_f G'°(i,\mathrm{pH},I)$ and evaluate it at chosen pHs and ionic strengths.

The standard transformed Gibbs energies of formation $\Delta_f G'°(i)$ of reactants in a biochemical reaction can be used to calculate the standard transformed Gibbs energy of the reaction $\Delta_r G'°$ and the apparent equilibrium constant K':

$$\Delta_r G'° = \sum \nu'_i \Delta_f G'°(i) = -RT \ln K' \quad (6)$$

ν'_i is the stoichiometric number of reactant i in the biochemical reaction. Since K' for an enzyme-catalyzed reaction can be calculated from the $\Delta_f G°$ of species, the best way to store thermodynamic information is by means of tables of $\Delta_f G°$ and $\Delta_f H°$ for species (*14*).

The change in binding of hydrogen ions in a biochemical reaction can be calculated using

$$\Delta_r N_H = \frac{1}{RT \ln(10)} \left(\frac{\partial \Delta_r G'°}{\partial \mathrm{pH}} \right)_{T,P} \quad (7)$$

In discussing redox reactions, it is convenient to use half reactions. The standard apparent reduction potential $E'°$ for a half reaction, which is a function of pH and ionic strength, can be calculated using (*15*)

$$E'° = -\frac{\Sigma \nu'_i \Delta_f G'^{\circ}_i}{|\nu_e| F} \quad (8)$$

where $\Delta_f G'^{\circ}_i$ is the standard transformed Gibbs energy of formation of reactant i, ν'_i is the stoichiometric number of reactant i in the half reaction, $|\nu_e|$ is the number of electrons involved, and F is the Faraday constant (96 485 coulombs mol^{-1}). The apparent equilibrium constant for a redox reaction can be calculated by taking the difference between the apparent electromotive forces of half reactions. The apparent equilibrium constant for a redox reaction can be calculated from the difference in the standard apparent reduction potentials of the two half reactions.

$$K' = \exp\left[\frac{|\nu_e| F (E'^{\circ}_R - E'^{\circ}_L)}{RT} \right] \quad (9)$$

CALCULATION OF STANDARD GIBBS ENERGIES OF FORMATION OF DEOXYRIBOSE AND RELATED SPECIES

Equilibrium measurements have been made on the deoxyribose-phosphate aldolase reaction:

EC 4.1.2.4 2′-deoxy-D-ribose 5-phosphate = D-glyceraldehyde 3-phosphate + acetaldehyde (10)

The apparent equilibrium constant for this reaction was found to be $K' = 2.35 \times 10^{-4}$ at 310.15 K, pH 6.3, and 0.025 M ionic strength by Pricer and Horecker (*16*). Later Groth (*17*) obtained $K' = 2.5 \times 10^{-4}$ at 298.15 K, pH 7.5, and 0.005 M ionic strength. Since the $\Delta_f G°$ values are known for acetaldehyde (*18*) and the species of D-glyceraldehyde 3-phosphate are known (*14*) at 298.15 K, it is possible to calculate $\Delta_f G°$ for the species of 2′-deoxy-D-ribose 5-phosphate. The calculation of species properties at 298.15 K and zero ionic strength from an apparent equilibrium constant at a specified pH and ionic strength is rather complicated, and so the calculation has been made using the Mathematica program calcGef2sp (*13*). The pK of 2-deoxy-D-ribose 5-phosphate, which is needed in this calculation, can be assumed to be the same as for D-ribose 5-phosphate, that is, 6.69 at 298.15 K and zero ionic strength.

The calculation of species properties based on the more recent measurements (*17*) yields $\Delta_f G° = -1447.92$ kJ mol^{-1} for deoxy-D-ribose 5-phosphate^{2-} and -1486.11 kJ mol^{-1} for deoxy-D-ribose 5-phosphate^{-}, as shown in Table 1. The difference $\Delta_f G°$(2-deoxy-D-ribose 5-phosphate^{2-}) − $\Delta_f G°$(D-ribose 5-phosphate^{2-}) is $-1447.92 + 1595.78 = 147.86$ kJ mol^{-1}. The first question is whether this difference is reasonable. Since the $\Delta_f G°$ of methane, methanol, ethane, and ethanol are known in dilute aqueous solution at 298.15 K (*18*), the corresponding differences can be calculated for the two pairs: $\Delta_f G°$(methane,aq) − $\Delta_f G°$(methanol,aq) = $-34.33 + 175.31 = 140.98$ kJ mol^{-1}, and $\Delta_f G°$(ethane,aq) − $\Delta_f G°$(ethanol,aq) = $-17.01 + 181.64 = 164.63$ kJ mol^{-1}. These calculations indicate that 147.86 kJ mol^{-1} is reasonable as a measure of the effect of reduction of D-ribose 5-phosphate. It may seem surprising that five or six digits are used to represent these thermodynamic properties, in view of the fact that apparent equilibrium constants are at best uncertain by 1%. But since $RT \ln(1.01) = 0.02$ kJ mol^{-1} at 298.15 K, $\Delta_f G°$ values need to be given to two decimal places. The important information in these thermodynamic properties is in the differences between values in the table.

There are good reasons to assume that this same difference of 147.86 kJ mol^{-1} applies to D-ribose 1-phosphate, D-ribose, and ribonucleosides and ribonucleotides in general. This is based on the expectation that the replacement of C−OH at position 2′ in ribose with C−H of deoxyribose in position 2′ will not have a significant effect on the equilibrium constant of a reaction of a sugar residue with a base to form a nucleoside, the phosphorylation of a sugar residue, or the pK's of the phosphates. This expectation is confirmed by two reactions for which apparent equilibrium constants have been determined. The first is

EC 2.4.2.7 2′-deoxyinosine + adenine = 2′-deoxyadenosine + hypoxanthine (11)

Table 1: Standard Gibbs Energies of Formation of Species in Dilute Aqueous Solutions at 298.15 K and Zero Ionic Strength[a]

species	$\Delta_f G°$/kJ mol^{-1}	z	N_H	ref
ribose	−752.00	0	10	1
ribose 1-phosphate^{2-}	−1587.70	−2	9	5
Hribose 1-phosphate^{-}	−1625.88	−1	10	5
ribose 5-phosphate^{2-}	−1595.78	−2	9	5
Hribose 5-phosphate^{-}	−1633.96	−1	10	5
2′-deoxyribose	−604.14	0	10	this study[b]
2′-deoxyribose 1-phosphate^{2-}	−1439.84	−2	9	this study
2′-deoxyHribose 1-phosphate^{-}	−1478.02	−1	10	this study
2′-deoxyribose 5-phosphate^{2-}	−1447.92	−2	9	this study
2′-deoxyHribose 5-phosphate^{-}	−1486.11	−1	10	this study
2′-deoxadenosine^{0}	−46.64	0	13	this study
2′-deoxyHadenosine^{+}	−66.42	1	14	this study
2′-deoxyAMP^{2-}	−892.59	−2	12	this study
2′-deoxyHAMP^{-}	−931.00	−1	13	this study
2′-deoxyH_2AMP^{0}	−953.77	0	14	this study
2′-deoxyADP^{3-}	−1758.27	−3	12	this study
2′-deoxyHADP^{2-}	−1799.24	−2	13	this study
2′-deoxyH_2ADP^{-}	−1824.12	−1	14	this study
2′-deoxyATP^{4-}	−2620.24	−4	12	this study
2′-deoxyHATP^{3-}	−2663.62	−3	13	this study
2′-deoxyH_2ATP^{2-}	−2690.32	−2	14	this study
methanol	−175.31	0	4	17
methane	−34.33	0	4	17
ethanol	−181.64	0	6	17
ethane	−17.01	0	6	17
NAD^{-}	0	−1	26	13
NADH^{2-}	22.65	−2	27	13
NADP^{3-}	−835.18	−3	25	13
NADPH^{4-}	−809.19	−4	26	13
thioredoxin$_{ox}$	0	0	0	13
thioredoxin$_{red}^{2-}$	69.88	−2	0	13
thioredoxin$_{red}^{-}$	20.56	−1	11	13
thioredoxin$_{red}^{0}$	−25.37	0	2	13
FMN_{ox}^{2-}	0	−2	19	13
FMN_{red}^{2-}	−38.88	−2	21	13
FAD_{ox}^{2-}	0	−2	31	13
FAD_{red}^{2-}	−38.88	−2	33	13

[a] z is the charge number, and N_H is the number of hydrogen atoms. On the basis of the difference $\Delta_f G°$(2′-deoxy-D-ribose 5-phosphate^{2-}) − $\Delta_f G°$(D-ribose 5-phosphate^{2-}) = 147.86 kJ mol^{-1} at 298.15 K and zero ionic strength.

for which Danzin and Cardinaud (*19*) obtained $K' = 1.4$ at 313.15 K, pH 6, and 0.10 M ionic strength, as might be expected if the deoxy differences for adenosine and inosine are the same. The second example is provided by

EC 2.4.2.1 2′-deoxyinosine + P_i = hypoxanthine + 2′-deoxyribose 1-phosphate (12)

for which de Verier and Gould (*20*) obtained $K' = 30 \times 10^{-3}$ at 298.15 K and pH 7.0. This apparent equilibrium constant is not very different from the $K' = 16 \times 10^{-3}$ obtained for

EC 2.4.2.1 inosine + P_i = hypoxanthine + ribose 1-phosphate (13)

by Kim, King, Teague, Rufo, Veech, and Passonneau (*21*) at 311 K, pH 7.0, and ionic strength 0.25 M.

Therefore, $\Delta_f G°$ values for deoxy forms have been put in Table 1 for D-ribose, D-ribose 1-phosphate, adenosine, AMP, ADP, and ATP. It is well-known that the deoxy forms are more stable kinetically than the forms with C−OH in position 2′, but this does not mean that the deoxy adjustment of the Gibbs energy of formation should be different for various ribose derivatives.

Table 2: Standard Transformed Gibbs Energies of Formation in kJ Mol^{-1} of Reactants at 298.15 K, 0.25 M Ionic Strength, and Five pH Values[a]

	pH				
	5	6	7	8	9
ribose	−458.50	−401.42	−344.34	−287.26	−230.18
2′-deoxyribose	−310.64	−253.56	−196.48	−139.40	−82.32
ribose 1-phosphate	−1333.37	−1277.51	−1224.35	−1172.71	−1121.30
2′-deoxyibose 1-phosphate	−1185.51	−1129.65	−1076.49	−1024.85	−973.44
ribose 5-phosphate	−1341.45	−1285.59	−1232.43	−1180.79	−1129.38
2′-deoxyibose 5-phosphate	−1193.60	−1137.73	−1084.57	−1032.93	−981.52
2′-deoxy-adenosine	334.84	409.11	483.32	557.52	631.73
2′-deoxyAMP	−550.54	−477.36	−406.97	−338.18	−269.65
2′-deoxyADP	−1421.19	−1347.69	−1276.84	−1207.92	−1139.38
2′-deoxyATP	−2289.60	−2215.90	−2144.64	−2075.58	−2007.02
methanol	−57.91	−35.08	−12.25	10.59	33.42
methane	83.07	105.90	128.73	151.57	174.40
ethanol	−5.54	28.71	62.96	97.20	131.45
ethane	159.09	193.34	227.59	261.83	296.08
NAD_{ox}	762.29	910.70	1059.11	1207.51	1355.92
NAD_{red}	811.86	965.98	1120.09	1274.21	1428.33
$NADP_{ox}$	−108.72	33.98	176.68	319.38	462.08
$NADP_{red}$	−59.05	89.36	237.77	386.18	534.59
thioredoxin$_{ox}$	0	0	0	0	0
thioredoxin$_{red}$	33.33	44.70	55.74	64.03	66.35
FMN_{ox}	554.41	662.86	771.32	879.77	988.22
FMN_{red}	574.23	694.10	813.97	933.84	1053.70
FAD_{ox}	906.61	1083.56	1260.51	1437.46	1614.40
FAD_{red}	926.43	1114.79	1303.16	1491.52	1679.89
H_2O	−178.49	−167.07	−155.66	−144.24	−132.83

[a] The values in this table are intended for computational use, and the numbers of digits given do not signify accuracy in terms of significant figures. The differences are used computationally and are accurate to 2−3 significant figures.

CALCULATION OF STANDARD TRANSFORMED GIBBS ENERGIES OF FORMATION OF REACTANTS

The data in Table 1 can be used to calculate $\Delta_f G'°$ of each of these reactants at 298.15 K and any pH in the range 5−9 and any ionic strength in the range 0−0.35 M. This calculation involves using eq 4 with the extended Debye−Hückel equation inserted and eq 5. This calculation is conveniently carried out using the Mathematica program calcdGmat (*13*). The $\Delta_f G'°$ values given in Table 2 make it possible to calculate apparent equilibrium constants for the five reactions described in the next section, and many more. The values for adenosine, AMP, ADP, and ATP are not included because they have been published elsewhere (*9*, *14*).

CALCULATION OF APPARENT EQUILIBRIUM CONSTANTS FOR REDUCTASE REACTIONS

The data in Table 2 can be used to calculate apparent equilibrium constants using $K' = \exp(-\Delta_r G'°/RT)$. These calculations are conveniently carried out with the Mathematica program calckprime (*13*), by simply typing in the reaction. The apparent equilibrium constants for five reactions at 298.15 K, five pH values, and 0.25 M ionic strength

Table 3: Calculated Apparent Equilibrium Constants at Five pHs of Reactions at 298.15 K and 0.25 M Ionic Strength

	pH				
	5	6	7	8	9
1. ADP + $thioredoxin_{red}$ = 2′-deoxyADP + H_2O + $thioredoxin_{ox}$	1.6×10^{11}	1.6×10^{11}	1.4×10^{11}	3.8×10^{10}	9.8×10^{8}
2. ADP + $NADP_{red}$ = 2′-deoxyADP + H_2O + $NADP_{ox}$	1.1×10^{14}	1.1×10^{13}	1.1×10^{12}	1.2×10^{11}	1.2×10^{10}
3. ADP + FAD_{red} = 2′-deoxyADP + H_2O + FAD_{ox}	6.9×10^{8}	6.9×10^{8}	6.9×10^{8}	6.9×10^{8}	6.9×10^{8}
4. methanol + $thioredoxin_{red}$ = methane + H_2O + $thioredoxin_{ox}$	2.6×10^{12}	2.5×10^{12}	2.2×10^{12}	6.1×10^{11}	1.6×10^{10}
5. ethanol + $thioredoxin_{red}$ = ethane + H_2O + $thioredoxin_{ox}$	1.8×10^{8}	1.8×10^{8}	1.6×10^{8}	4.4×10^{7}	1.1×10^{6}

Table 4: Calculated Changes in Binding of Hydrogen Ions $\Delta_r N_H$ at Five pH Values of reactions at 298.15 K and 0.25 M ionic strength[a]

	pH				
	5	6	7	8	9
1. ADP + $thioredoxin_{red}$ = 2′-deoxyADP + H_2O + $thioredoxin_{ox}$	0.002	0.017	0.170	1.110	1.883
2. ADP + $NADP_{red}$ = 2′-deoxyADP + H_2O + $NADP_{ox}$	1.000	1.000	1.000	1.000	1.000
3. ADP + FAD_{red} = 2′-deoxyADP + H_2O + FAD_{ox}	0	0	0	0	0
4. methanol + $thioredoxin_{red}$ = methane + H_2O + $thioredoxin_{ox}$	0.002	0.017	0.170	1.110	1.883
5. ethanol + $thioredoxin_{red}$ = ethane + H_2O + $thioredoxin_{ox}$	0.002	0.017	0.170	1.110	1.883

[a] The values of $\Delta_r N_H$ are the number of moles of H^+ produced per mole of reaction, and so they are dimensionless.

are given in Table 3. The apparent equilibrium constants for reaction 1 are also expected to apply when ADP is replaced with ATP, CDP, CTP, UDP, UTP, GDP, and GTP.

The apparent equilibrium constants for reaction 2 in Table 3 apply when $NADP_{red}$ is replaced by NAD_{red}. The apparent equilibrium constants for reaction 3 apply when FAD_{red} is replaced by FMN_{red}. Reactions 4 and 5 are not enzyme-catalyzed reactions, but they are given to provide a comparison with the first reaction. The values for reaction 1 in Table 3 also apply to the reaction

$$\text{ATP} + \text{thioredoxin}_{red} = \text{2′-deoxyATP} + H_2O + \text{thioredoxin}_{ox} \quad (14)$$

A consequence of the assumption that the 147.86 kJ mol^{-1} difference applies to D-ribose 1-phosphate, D-ribose, and ribonucleosides and ribonucleotides in general is that the apparent equilibrium constant for the reaction

$$\text{2′-deoxyADP} + \text{ATP} = \text{ADP} + \text{2′-deoxyATP} \quad (15)$$

is equal to unity at all pH values and ionic strengths. Thus, the standard transformed Gibbs energy change for reaction 1 in Table 3 plus reaction 15 is the same as for reaction 14. Other reductants can be used in reaction 1 in Table 3 as shown. The values for the second reaction are also obtained with NAD_{red}. The values for the third reaction are also obtained with FMN_{red}.

CALCULATION OF CHANGES IN THE BINDING OF HYDROGEN IONS FOR REDUCTASE REACTIONS

The changes in the binding of hydrogen ions in the biochemical reactions in Table 3 cause the changes in the apparent equilibrium constants with pH. It is convenient to use the Mathematica program calcNHrx (*14*), which is based on eq 7. These calculations on the reactions in Table 3 yield Table 4. At high pH the predominant form of the first reaction is

$$ADP^{3-} + \text{thioredoxin}^{2-} + 2H^+ = \text{2′-deoxyADP}^{3-} + H_2O + \text{thioredoxin}^{0} \quad (16)$$

This causes the rather rapid decrease in K' for the first reaction at high pH. The pK's of ADP and 2′-deoxyADP are the same, and so their effects cancel. When ADP is replaced with ATP, CDP, CTP, UDP, UTP, GDP, or GTP the same results obtained in the first three reactions. In the fourth and fifth reactions methanol and methane have the same number of hydrogen atoms and so do ethanol and ethane. Therefore, the change in binding of hydrogen ions is the same as in the first reaction. In the second reaction the pK's in $NADP_{red}$ and $NADP_{ox}$ are the same, but $NADP^{3-} + H^+ + 2e^- = HNADP^{4-}$. Replacing $NADP_{red}$ with NAD_{red} does not affect this result. The reaction involving FAD_{red} does not involve a change in binding of hydrogen ions because $FAD^{2-} + 2e^- = H_2FAD^{4-}$.

CALCULATION OF STANDARD APPARENT REDUCTION POTENTIALS OF HALF REACTIONS

It is of interest to consider biochemical redox reactions in terms of standard apparent reduction potentials of half reactions because two half reactions determine the thermodynamics of the redox reaction and only formal electrons are exchanged between the half reactions. The best way to introduce half reactions at specified pH is to start with chemical reactions. The following chemical reaction represents the first reactions in Tables 3 and 4 at high pH, but as the pH is reduced additional species of ADP and 2′-deoxyADP have to be taken into account.

$$ADP^{3-} + \text{thioredoxin}_{red}^{2-} + 2H^+ = \text{2′-deoxyADP}^{3-} + H_2O + \text{thioredoxin}_{ox}^{0} \quad (17)$$

This chemical equation balances all atoms and charges, as it must. The half reaction involving thioredoxin at high pH is

$$\text{thioredoxin}_{red}^{2-} = \text{thioredoxin}_{ox}^{0} + 2e^- \quad (18)$$

Subtracting reaction 18 from reaction 17 yields the half reaction involving ADP^{3-} at high pH:

$$ADP^{3-} + 2H^+ + 2e^- = \text{2′-deoxyADP}^{3-} + H_2O \quad (19)$$

Table 5: Calculated Apparent Reduction Potentials at Five pH Values of Half Reactions at 298.15 K and 0.25 M Ionic Strength

	pH				
	5	6	7	8	9
1. ADP + $2e^-$ = 2′-deoxyADP + H_2O	0.1587	0.0996	0.0404	−0.0187	−0.0779
2. methanol + $2e^-$ = methane + H_2O	0.1944	0.1352	0.0761	0.0169	−0.0423
3. ethanol + $2e^-$ = ethane + H_2O	0.0718	0.0127	−0.0465	−0.1057	−0.1648
4. $thioredoxin_{ox}$ + $2e^-$ = $thioredoxin_{red}$	−0.1727	−0.2317	−0.2888	−0.3318	−0.3438
5. $NADP_{ox}$ + $2e^-$ = $NADP_{red}$	−0.2574	−0.2870	−0.3166	−0.3461	−0.3757
6. FAD_{ox} + $2e^-$ = FAD_{red}	−0.1027	−0.1619	−0.2210	−0.2802	−0.3393

This can be checked by adding reactions 18 and 19 to obtain reaction 17. To discuss reduction potentials, reaction 18 needs to be written as a reduction reaction:

$$\text{thioredoxin}_{ox}^{0} + 2e^- = \text{thioredoxin}_{red}^{2-} \qquad (20)$$

To discuss the thermodynamics of the ribonucleoside diphosphate reductase reaction at a specified pH, it is necessary to write the half reactions as

$$\text{ADP} + 2e^- = 2'\text{-deoxyADP} + H_2O \qquad (21)$$

$$\text{thioredoxin}_{ox} + 2e^- = \text{thioredoxin}_{red} \qquad (22)$$

where $thioredoxin_{ox}$ is a single species, $thioredoxin_{red}$ is made up of three species, and ADP and deoxyADP are each made up of three species. The difference between these half reactions is the first biochemical reaction in Tables 3 and 4. Since the thermodynamic properties of the first reaction in Table 3 and half reactions 21 and 22 depend on the pH, it is necessary to use standard transformed Gibbs energies of formation $\Delta_f G_i'^\circ$ of the reactants *i*. The standard apparent reduction potentials of half reactions 21, 22, and related reactions have been calculated using the Mathematica program calcappredpot (*14*). They are given in Table 5 as a function of pH at 298.15 K and 0.25 M ionic strength.

The corresponding chemical half reactions for methanol and ethanol are as follows:

$$CH_3OH + 2H^+ + 2e^- = CH_4 + H_2O \qquad (23)$$

$$CH_3CH_2OH + 2H^+ + 2e^- = CH_3CH_3 + H_2O \qquad (24)$$

where the species are in dilute aqueous solution. When the pH is specified these half reactions are written as

$$\text{methanol} + 2e^- = \text{methane} + H_2O \qquad (25)$$

$$\text{ethanol} + 2e^- = \text{ethane} + H_2O \qquad (26)$$

The standard apparent reduction potentials when ADP is replaced with ATP, CDP, CTP, UDP, UTP, GDP, or GTP are the same as for the first reaction in Table 5. Note that water is omitted in the expression of an apparent equilibrium constant, but $\Delta_f G_i'^\circ(H_2O)$ is included in the calculation of $\Delta_r G'^\circ$ and E'°. It is more difficult to reduce methanol than the OH in position 2′ in ribose, but it is easier to reduce ethanol. $NADP_{red}$ is a stronger reductant than $thioredoxin_{red}$, and FAD_{red} is a weaker reductant than $thioredoxin_{red}$.

DISCUSSION

Many enzyme-catalyzed reactions have such large or small apparent equilibrium constants that they cannot be measured directly because of the sensitivities of analytical methods. However, thermodynamics shows how these very large or small apparent equilibrium constants can be calculated by other paths. The recent determination of the molar entropy of D-ribose by use of the third law (*2*) and other thermodynamic measurements have made it possible to calculate the standard Gibbs energies of formation of the two species of D-ribose 5-phosphate (5). Since the standard Gibbs energies of formation of the two species of 2′-deoxy-D-ribose 5-phosphate can be calculated from measurements of the apparent equilibrium constant of the deoxyribose-phosphate aldolase reaction, it possible to determine $\Delta_f G^\circ$ of the two species of 2′-deoxy-D-ribose 5-phosphate. Since $\Delta_f G^\circ$ values of the two species of D-ribose 5-phosphate at 298.15 K and zero ionic strength have recently been calculated (*6*), the experimental value of the apparent equilibrium constant for reaction 10 has made it possible to show that $\Delta_f G^\circ$(2′-deoxy-D-ribose 5-$phosphate^{2-}$) − $\Delta_f G^\circ$(D-ribose 5-$phosphate^{2-}$) = 147.86 kJ mol^{-1}. There is experimental evidence (see reactions 11 and 12) that this difference is applicable to ribose, species of ribose 1-phosphate, and species of ribonucleosides and ribonucleotides. As a result many more species can be put in the table of basic biochemical data (*13*) and apparent equilibrium constants can be calculated for many reactions for which apparent equilibrium constants have been unknown. The apparent equilibrium constants for the formation of deoxynucleotides are important because these molecules provide the building blocks for DNA. Various reductants can be involved.

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