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New Concepts in Biochemistry

Standard Free Energy Change for the Hydrolysis of the α,β-Phosphoanhydride Bridge in ATP[†]

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It appears to be common knowledge in biochemistry that the standard free energy change $\Delta G'^{\circ}$ for the hydrolysis of ATP to AMP and PP_i is −7.7 to −8.4 kcal mol^{-1}.[1] Most textbooks of biochemistry list values in this range (Zubay, 1993; Lehninger et al., 1992; Garrett & Grisham, 1994; Voet & Voet, 1990; Matthews & Van Holde, 1990). Moreover, many textbooks also list the standard free energy for the hydrolysis of PP_i as $\Delta G'^{\circ} = -7.9$ to 8.0 kcal mol^{-1}. However, the true value for pyrophosphate hydrolysis is significantly less negative (Flodgaard & Fleron, 1974). Therefore, the standard free energy change for the hydrolysis of ATP to AMP and PP_i must be more negative than 8 kcal mol^{-1}, as is explained in this paper.

Upon consulting the literature, we have found that the standard free energy change for the hydrolysis of ATP to AMP and PP_i is much more negative than the conventional value and much more negative than that for the hydrolysis of ATP to ADP and P_i. This fact has significant implications in metabolism and for the mechanisms of action of enzymes such as aminoacyl-tRNA synthetases, NAD$^+$ pyrophosphorylase, nucleotide sugar pyrophosphorylases, PRPP synthetase, DNA ligases, and RNA ligases, as well as DNA and RNA polymerases and a number of other ATP-dependent synthetases. These enzymes cleave ATP at the α,β-phosphoanhydride bridge and use the free energy released to drive biosynthetic reactions.

$\Delta G'^{\circ}$ for the Hydrolysis of ATP to AMP and PP_i. Literature data allow $\Delta G'^{\circ}$ for the hydrolysis of ATP to AMP and PP_i to be calculated as the sum of eqs 1 and 2 to give eq 3.

reaction	$\Delta G^{\circ\prime}$ (kcal mol^{-1})	
$ATP + 2H_2O = AMP + 2P_i$	−15.5	(1)
$2P_i = PP_i + H_2O$	+4.6	(2)
$ATP + H_2O = AMP + PP_i$	−10.9	(3)

The value of −15.5 kcal mol^{-1} as $\Delta G'^{\circ}$ for the hydrolysis of ATP to AMP and P_i in eq 1 is calculated from values for the standard free energies of formation of the reactants and products at pH 7.0 and 25 °C in the presence of 1 mM free Mg^{2+} (Alberty, 1994). The value of $\Delta G'^{\circ}$ for eq 2 is calculated from that for the hydrolysis of PP_i at pH 7.4 and 25 °C in the presence of 1 mM free Mg^{2+}, which is −4.37 ± 0.11 kcal mol^{-1} (Flodgaard & Fleron, 1974). Correction to pH 7.0 by taking account of the pK_a of P_i (Jencks & Regenstein, 1970) gives −4.6 ± 0.1 kcal mol^{-1} as $\Delta G'^{\circ}$ for the hydrolysis of PP_i and +4.6 ± 0.1 kcal mol^{-1} for its synthesis from 2 P_i. Differential complexation of Mg^{2+} by HPO_4^{2-} and $H_2PO_4^-$ has no significant effect on the free energy under these conditions. The stability constant for $MgHPO_4$ is 75.9 M^{-1} at 25 °C (Smith & Alberty, 1956), so

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[1] The recommendation of the International Union of Pure and Applied Chemistry is that $\Delta G'^{\circ}$ refers to total concentrations of the ionic species of each component of the reaction at pH 7.0, 25 °C, and 1 mM free Mg^{2+} (Alberty, 1994). In this paper, most standard free energy changes follow this recommendation. In cases in which free energy changes have been reported under other conditions, they will be so specified, e.g., $\Delta G^{\circ}_{\mathrm{pHx}}$, 37 °C, 5 mM Mg^{2+}. Literature values reported at 25 °C but at different pH and Mg^{2+} concentrations are corrected in this paper to pH 7.0 and 1 mM free Mg^{2+} by application of the relevant values of pK_a and Mg^{2+}-binding constants.

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that at pH 7.4 less than 2% of phosphate will be complexed by Mg^{2+}. At pH 7.0 a smaller percentage will be complexed because the stability constant for $MgH_2PO_4^+$ is even smaller than that for $MgHPO_4$. The algebraic sum of the standard free energies in eqs 1 and 2 is -10.9 kcal mol^{-1}, the standard free energy change for the hydrolysis of ATP to AMP and PP_i (eq 3). For the hydrolysis of ATP to ADP and P_i under the same conditions $\Delta G'^{\circ}$ is -7.7 kcal mol^{-1} (Alberty, 1994). Therefore, hydrolytic cleavage of the α,β-phosphoanhydride bridge at pH 7.0 releases 3.2 kcal mol^{-1} more free energy than cleavage of the β,γ-bridge.

The foregoing calculation is based on accurate experimental data and stands on its own. A similar value measured under different conditions was reported by Scheugraf et al. (1960) in their study of argininosuccinate synthetase. They estimated the standard free energy change for the hydrolysis of ATP to AMP and PP_i at pH 7.5, 37 °C, and 5 mM Mg^{2+} to be -10.3 kcal mol^{-1}. This value depended on the assumption that the free energies for the hydrolysis of ATP to ADP and of ADP to AMP are the almost the same, as they are, and it is comparable to the -10.9 kcal mol^{-1} reported here. The two values are difficult to compare directly because they refer to different conditions of pH and temperature. Another value of $\Delta G'^{\circ} = -7.7$ kcal mol^{-1} for ATP = AMP + PP_i was calculated from the free energy for the hydrolysis of acetyl-CoA ($\Delta G'^{\circ} = -7.3$ kcal mol^{-1}) and an equilibrium constant for the reaction ATP + CoA + acetate = acetyl-CoA + AMP + PP_i catalyzed by acetyl-CoA synthetase (Jencks & Gilchrist, 1964). The equilibrium constant adopted was representative of two variant values of 0.86 and 2.6 reported in the literature (Jones, 1953; Hele, 1954). In a third estimate, the value of $\Delta G'^{\circ} = -9.75$ kcal mol^{-1} was based on the assumption that it should be 1 kcal mol^{-1} more negative than that for the hydrolysis of ATP to ADP and P_i (Alberty, 1969).

Biosynthetic Significance of the α,β-Phosphoanhydride Bridge in ATP. The value of -10.9 kcal mol^{-1} offers a rationale for certain biosynthetic reactions being driven by cleavage of the α,β-phosphoanhydride bridge in ATP. PRPP biosynthesis proceeds according to eq 4, and the equilibrium

$$\text{ATP} + \text{ribose-5-P} = \text{AMP} + \text{PRPP} \qquad \Delta G^{\circ}_{\text{pH7.5}} = -2.0 \pm 0.5 \text{ kcal mol}^{-1} \quad (4)$$

constant at pH 7.5, 37 °C, and 5 mM Mg^{2+} indicates that the standard free energy change $\Delta G^{\circ}_{\text{pH7.5}}$ is -2.0 ± 0.5 kcal mol^{-1} (Switzer, 1969). Subtraction of eq 4 from eq 3 gives eq 5 for the hydrolysis of PRPP to ribse-5-P and PP_i. The

$$\text{PRPP} + H_2O = \text{ribose-5-P} + PP_i \qquad \Delta G^{\circ}_{\text{pH7.5}} = -8.3 \pm 0.5 \text{ kcal mol}^{-1} \quad (5)$$

standard free energy change for eq 3 under the same conditions as eq 4 was reported to be -10.3 kcal mol^{-1} (Sheugraf et al., 1960). Therefore, $\Delta G^{\circ}_{\text{pH7.5}}$ for the hydrolysis of PRPP is -8.3 kcal mol^{-1}, which is 3.3 kcal mol^{-1} more negative than the free energy change for the hydrolysis of glucose-1-P to glucose and P_i at pH 7.0 and 25 °C (Atkinson et al., 1961). The free energy for the hydrolysis of PRPP had previously been estimated at -7.0 kcal mol^{-1} based on the assumption that the free energy change for the hydrolysis of the α,β-phosphoanhydride bridge of ATP is the same as for the hydrolysis of the β,γ-bridge (Switzer, 1969). PRPP is the source of ribosyl groups for the biosynthesis of the *N*-ribosyl bonds in nucleotides, and its high 5-phosphoribosyl group transfer potential is important in driving these reactions forward. The formation of PRPP itself requires a high driving force, and this is provided by cleavage of the α,β-phosphoanhydride bridge of ATP.

The equilibrium constant for the formation of cAMP from ATP catalyzed by adenylyl cyclase according to eq 6 is

$$\text{ATP} = \text{cAMP} + PP_i \qquad \Delta G'^{\circ} = 1.6 \text{ kcal mol}^{-1} \quad (6)$$

reported to be 0.065 at pH 7.3, 25 °C, and 1 mM Mg^{2+} (Hayaishi et al., 1971). This corresponds to $\Delta G^{\circ}_{\text{pH7.3}} = 1.6$ kcal mol^{-1}. The correction to pH 7.0 should be negligible owing to the fact that the pK_a values for MgATP, cAMP, and $MgPP_i$ are all lower than 6, so that there are no significant differences in the ionic compositions of these compounds between pH 7.3 and 7.0. Subtraction of eq 6 from eq 3 gives eq 7 for the hydrolysis of cAMP to AMP.

$$\text{cAMP} + H_2O = \text{AMP} \qquad \Delta G'^{\circ} = -12.5 \text{ kcal mol}^{-1} \quad (7)$$

The calculated value of -12.5 kcal mol^{-1} for the standard free energy change should be compared with the calorimetrically measured value of the enthalpy change (-11.1 ± 0.2 kcal mol^{-1}) for this reaction at pH 7.3 (Gerlt et al., 1975). An earlier estimate of $\Delta G^{\circ}_{\text{pH7.3}} = -11.9$ kcal mol^{-1} for the hydrolysis of cAMP was based on the value of -9.75 kcal mol^{-1} as $\Delta G'^{\circ}$ for eq 3 corrected to -10.3 kcal mol^{-1} for pH 7.3 (Hayaishi et al., 1971). This value of $\Delta G^{\circ}_{\text{pH7.3}}$ for eq 7 can be recalculated as -12.8 kcal mol^{-1} after correcting the standard free energy change for eq 3 to pH 7.3. Because cAMP is a high energy compound, its formation from ATP depends upon the high negative free energy made available by cleaving the α,β-phosphoanhydride bridge.

The biosyntheses of coenzymes such as NAD^+ and FAD and of nucleotide sugars such as UDP-glucose are catalyzed by nucleotidyltransferases commonly known as nucleoside triphosphate dependent pyrophosphorylases. Two examples are NAD^+ pyrophosphorylase (eq 8) and UDP-glucose pyrophosphorylase (eq 9). Both enzymes transfer a 5′-nucleotidyl group from a nucleoside triphosphate to an acceptor phosphate to form new phosphoanhydride bridges in NAD^+ and UDP-glucose, and both reactions produce PP_i.

$$\text{ATP} + \text{nicotinamide mononucleotide} = NAD^+ + PP_i \quad (8)$$

$$\text{UTP} + \text{glucose 1-phosphate} = \text{UDP-glucose} + PP_i \quad (9)$$

These equilibria are fairly well balanced. For example, K_{eq} for eq 9 is 0.26 at pH 8.5 (Tsuboi et al., 1969). The value of K_{eq} should not be very pH-dependent between pH 7 and 8.5, so that the value of $\Delta G'^{\circ}$ may be about +0.8 kcal mol^{-1}. The free energy for the hydrolysis of UTP to UMP and PP_i should be the same as that for ATP, -10.9 kcal mol^{-1}. Subtraction of eq 9 from eq 3, substituting UTP for ATP in eq 3, gives the hydrolysis of UDP-glucose to UMP and glucose-1-P. The calculated value of $\Delta G'^{\circ}$ for this process is -10.1 kcal mol^{-1} based on a K_{eq} of 0.26 for the reaction of eq 9 at pH 7.0.

A similar value of -10.3 kcal mol^{-1} can be calculated independently for the hydrolysis of UDP-glucose to UMP and glucose-1-P (eq 14) at pH 7.0, 25 °C, and 1 mM free Mg^{2+}. This process is given by the sum of eqs 10−13. The

reaction	$\Delta G°$ (kcal mol^{-1})	
UDP-glucose + H_2O = UDP + Glc	-7.3	(10)
UDP + Mg^{2+} = MgUDP	-0.2	(11)
MgUDP + H_2O = UMP + P_i	-7.8	(12)
P_i + glucose = glucose-1-P	$+5.0$	(13)
UDP-glucose + H_2O = UMP + glucose-1-P	-10.3	(14)

value of $\Delta G°_{pH 7.4}$ for the hydrolysis of UDP-glucose to UDP and glucose at 25 °C (eq 10) was reported by Avigad (1964). Because neither glucose nor UDP-glucose undergoes a change in ionic state between pH 3 and 7.5, the standard free energy change is the same at pH 7.0 and 7.4. The value of $\Delta G'°$ for binding Mg^{2+} under the same conditions in the presence of 1.0 mM free Mg^{2+} (eq 11) is calculated as -0.2 kcal/mol from the binding constant reported by Taqui and Martell (1967). The value of $\Delta G'°$ for the hydrolysis of UDP under the same conditions in eq 12 is taken to be that for ADP, as calculated from free energies of formation (Alberty, 1994). The values for UDP and ADP should be the same because they refer to hydrolysis of the phosphoanhydride bridge, a process in which the nucleotide base plays no role. The value of $\Delta G'°$ for the hydrolysis of glucose-1-P under the same conditions is -5.0 kcal mol^{-1} (Atkinson et al., 1961) and for its formation in eq 13 it is + 5.0 kcal mol^{-1}. The sum of free energies in eqs 10−13 is -10.3 kcal mol^{-1}. Similar values can be expected for the hydrolysis of other P^1,P^2-dialkyl diphosphates such as NAD^+, FAD, other nucleoside diphosphate sugars, and CDP-diglyceride.

Cleavage of the α,β-phosphoanhydride bridge in nucleoside triphosphates and deoxynucleoside triphosphates drives RNA and DNA biosynthesis. A step in DNA biosynthesis may be represented by eq 15,which emphasizes the production of PP_i with the incorporation of each nucleotide. The production of PP_i takes place in many nucleoside triphosphate-dependent biosynthetic reactions, including most of those described in the preceding sections. The practical irreversibility of these processes in cells is often attributed to the presence of inorganic pyrophosphatase, which catalyzes the hydrolysis of PP_i and thereby drives reactions that produce it to completion. This effect depends upon the prevalence of equilibrium conditions. In some cases, for example in the DNA replication complex, equilibrium conditions are unlikely to exist. However, even in the absence of equilibrium conditions, the free energy available from the cleavage of the α,β-phosphoanhydride bridge in nucleoside triphosphates will drive reactions such as DNA and RNA polymerization practically to completion. Nucleoside 5′-diphosphates are also potential nucleotidyl group donors for nucleic acid biosynthesis. However, the standard free energy change for the hydrolysis of ADP (P_α/P_β-cleavage) is only -7.8 kcal mol^{-1}, so that phosphodiester bond formation from ADP would be less favorably by about 3.1 kcal mol^{-1} than from ATP as the adenylyl group donor.

$$\mathrm{poly(dNp)}_n + \mathrm{NTP} = \mathrm{poly(dNp)}_{n+1} + \mathrm{PP_i} \quad (15)$$

Significance of the α,β-Phosphoanhydride Bridge in Enzymatic Mechanisms. Certain ATP-dependent synthetases activate carboxylic acids as by formation of their adenylates with cleavage of the α,β-phosphoanhydride in ATP. In the second step, the enzyme-bound carboxylic acid adenylate reacts with an acyl group acceptor to form the product. The reaction of acetyl CoA synthetase is an example (eqs 16a−b).

$$\mathrm{H_3C{-}COO^-} + \mathrm{ATP} \rightleftharpoons \mathrm{H_3C{-}CO{-}AMP} + \mathrm{PP_i} \quad (16a)$$

$$\mathrm{H_3C{-}CO{-}AMP} + \mathrm{CoASH} \rightarrow \mathrm{H_3C{-}CO{-}SCoA} + \mathrm{AMP} \quad (16b)$$

The value of $\Delta G'°$ for the hydrolysis of simple carboxylic acid adenylates such as acetyl adenylate is -13.3 kcal mol^{-1} (Jencks, 1962, 1957; Rhodes & McElroy, 1958), just midway between the values of -21.8 kcal mol^{-1} for acetic anhydride (Jencks, 1976) and -4.6 kcal mol^{-1} for PP_i.[2] Therefore, the formation of acetyl adenylate in the reaction of ATP with acetate is unfavorable by 2.4 kcal mol^{-1} in free energy terms. One or two modest hydrogen bonding interactions, or a single electrostatic interaction, between the active site and acetyl adenylate would suffice to provide the necessary stabilization. It appears that much of the energy required to generate acetyl adenylate at the active site comes from the α,β-phosphoanhydride bridge of ATP.

A different situation exists in the case of tyrosyl tRNA synthetase. The value of K_{eq} for the reaction ATP + Tyr = Tyr−AMP + PP_i has been suggested to be 3.5×10^{-7} at pH 7.78 (Wells & Fersht, 1986). No correction need be made to pH 7.0 because the concentration of Mg^{2+} was above saturation and the pK_as of $MgPP_i$ and MgATP are below 7.0. The pK_as of tyrosyl adenylate are also well outside this range, so that the value of K_{eq} should be the same at pH 7.0. Therefore, $\Delta G'°$ for tyrosyl adenylate formation appears to be $+8.8$ kcal mol^{-1}. This value is extraordinarily positive, and even the high group transfer potential of the α,β-phosphoanhydride bridge could not drive tyrosyl adenylate production. Tyrosyl adenylate is postulated to be stabilized at the active site by multiple, individually weak hydrogen bonding interactions (Wells & Fersht, 1986; Fersht, 1987).

The standard free energy of $+8.8$ kcal mol^{-1} for tyrosyl adenylate production can be used with the -10.9 kcal mol^{-1} for the hydrolysis of (ATP + H_2O = AMP + PP_i) to calculate -19.7 kcal mol^{-1} as $\Delta G'°$ for the hydrolysis of tyrosyl adenylate (Tyr−AMP + H_2O = Tyr + AMP). This remarkable value is 6.4 kcal mol^{-1} more negative than that for the hydrolysis of acetyl adenylate. Based on $\Delta G'°$ for the hydrolyses of ethyl acetate and glycine ethyl ester (Jencks, 1976), about 3.7 kcal mol^{-1} of this difference can be attributed to the α-amino group in tyrosyl adenylate.

DNA ligase catalyzes the sealing of nicks in DNA by a mechanism that requires the adenylylation of the 6-amino group of a lysine residue at the active site through a reaction with ATP according to eq 17a. In the second step (eq 17b) the AMP moiety is transferred to a 5′-phosphate moiety in

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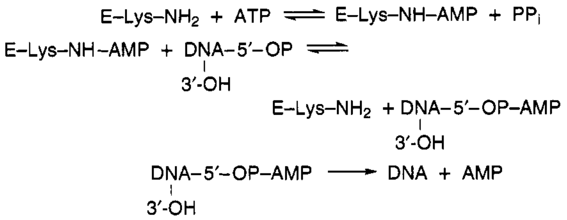
[2] A. Arabshahi and P. A. Frey, unpublished data from a manuscript in preparation.



$$\text{E-Lys-NH}_2 + \text{ATP} \rightleftharpoons \text{E-Lys-NH-AMP} + \text{PP}_i \qquad (17a)$$

$$\text{E-Lys-NH-AMP} + \underset{\substack{| \\ 3'\text{-OH}}}{\text{DNA}}\text{-5'-OP} \rightleftharpoons \text{E-Lys-NH}_2 + \underset{\substack{| \\ 3'\text{-OH}}}{\text{DNA}}\text{-5'-OP-AMP} \qquad (17b)$$

$$\underset{\substack{| \\ 3'\text{-OH}}}{\text{DNA}}\text{-5'-OP-AMP} \longrightarrow \text{DNA} + \text{AMP} \qquad (17c)$$

nicked DNA. In the third step (eq 17c), the 3′-hydroxyl group in the nick displaces AMP from the activated 5′-phosphate to seal the 3′,5′-phosphodiester linkage and release AMP. The second and third steps are thermodynamically spontaneous reactions. However, the adenosine-5′-phosphoramidate formed in the first step is a high energy intermediate. The standard free energy for the hydrolysis of a nucleoside-5′-phosphoramidate such as E−Lys−NH−AMP is not known; however, the value of $\Delta G'^\circ$ for the hydrolysis of UMP-imidazolate to UMP and imidazole is -14.7 kcal mol^{-1}.[2] It appears that the free energy available in the α,β-phosphoanhydride bridge of ATP is within about 4 kcal mol^{-1} of what is needed to form an AMP-phosphoramidate, so that modest enzymatic stabilization through noncovalent binding of the AMP moiety in E−NH−AMP can allow it to be formed as an intermediate.

Conclusion. Available data indicate that the standard free energy change for the hydrolysis of ATP to AMP and PP_i ($\Delta G'^\circ = -10.9$ kcal mol^{-1}) is much more negative than that for the hydrolysis of ATP to ADP and P_i ($\Delta G'^\circ = -7.8$ kcal mol^{-1}). Similarly, the value of $\Delta G'^\circ$ for the hydrolysis of UDP-glucose to UMP and glucose-1-P is -10.3 kcal mol^{-1}. These are significantly higher energy phosphoanhydride linkages than had previously been thought and are comparable in energy to the high energy bonds in acetyl phosphate and creatine phosphate. The free energy made available by the cleavage of α,β-phosphoanhydride bonds in nucleoside triphosphates and P^1,P^2-dialkyl diphosphates such as nucleoside diphosphate sugars appears to be put to effective use in certain metabolic reactions and enzymatic reaction mechanisms that require a large chemical driving force. Cleavage of the β,γ-phosphoanhydride bridge in ATP takes place in metabolic reactions in which a smaller driving force is required.

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