The Equilibrium Constants of the Adenosine Triphosphate Hydrolysis and the Adenosine Triphosphate-Citrate Lyase Reactions

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SUMMARY

The observed standard free energy change ($\Delta G^{\circ}_{\text{obs}}$) for the hydrolysis of the terminal pyrophosphate bond of ATP has been experimentally determined under physiological conditions using an entirely new set of reactions. The observed equilibrium constant ($K_{\text{obs}}$) for the combined reactions of acetate kinase (EC 2.7.2.1) and phosphate acetyltransferase (EC 2.3.1.8) has been determined at $38^\circ$, pH 7.0, ionic strength 0.25, and varying free [Mg$^{2+}$]. The $K_{\text{obs}}$ of these combined reactions reflects the difference between $\Delta G^{\circ}_{\text{obs}}$ for the hydrolysis of acetyl-CoA and the $\Delta G^{\circ}_{\text{obs}}$ for the hydrolysis of ATP. Using $\Sigma$ and square brackets to indicate total concentration,

$$K_{\text{obs}} = \frac{[\Sigma \text{ADP}] [\Sigma \text{Pi}]}{[\Sigma \text{ATP}] [\Sigma \text{H}_2\text{O}]} \times \frac{[\text{Acetyl-CoA}]}{[\text{Acetyl-CoA}]}$$

The observed value of this combined equilibrium constant varies with free [Mg$^{2+}$], being 0.984 ± 0.009 when [Mg$^{2+}$] = 0 and 0.218 ± 0.002 when free [Mg$^{2+}$] = $10^{-3}$ M. The $\Delta G^{\circ}_{\text{obs}}$ for the hydrolysis of acetyl-CoA is virtually unaffected by the free [Mg$^{2+}$] and has been previously determined to be -8.54 Cal per mole (-35.75 kJ per mole) under the same conditions of temperature, pH, and ionic strength. Therefore at pH 7.0, at ionic strength 0.25, at $38^\circ$, and taking the standard state of liquid water to have activity = unity ([H$_2$O] = 1) the $\Delta G^{\circ}_{\text{obs}}$ for the reaction

$$2\text{ATP}^+ + \text{H}_2\text{O} \rightleftharpoons 2\text{ADP}^+ + 2\text{Pi}$$
can be calculated to be -8.53 Cal per mole (-35.69 kJ per mole) at [Mg$^{2+}$] = 0 and -7.60 Cal per mole (-31.80 kJ per mole) at [Mg$^{2+}$] = $10^{-3}$ M. The corresponding values of $K_{\text{obs}}$ for the ATP hydrolysis reaction are 9.86 $\times$ 10$^5$ M (Mg$^{2+}$) = 0 and 2.19 $\times$ 10$^3$ M (Mg$^{2+}$) = $10^{-3}$ M. Equations have been developed for calculating from the experimental data the $\Delta G^{\circ}_{\text{obs}}$ of ATP hydrolysis at different free magnesium and hydrogen ion concentrations.

The $K_{\text{obs}}$ of ATP hydrolysis has been used in combination with the $K_{\text{obs}}$ of the citrate synthase reaction (EC 4.1.3.7) to calculate the $K_{\text{obs}}$ of the ATP-citrate lyase reaction (EC 4.1.3.8)

$$K_{\text{obs}} = \frac{[\Sigma \text{ADP}] [\Sigma \text{Pi}] [\Sigma \text{O}_{\text{Acacetyl}O}] [\Sigma \text{Citrate}]}{[\Sigma \text{ATP}] [\Sigma \text{CoA}] [\Sigma \text{Acetate}]}$$

Under the same near physiological conditions of $38^\circ$, pH 7.0, and ionic strength 0.25, the value of $K_{\text{obs}}$ for the ATP-citrate lyase reaction was found to be very sensitive to the free [Mg$^{2+}$], being 0.975 M at [Mg$^{2+}$] = 0 and 0.0985 M when free [Mg$^{2+}$] = $10^{-3}$ M.

The energy involved in the making or breaking of the terminal pyrophosphate bond of ATP is a common denominator of many cellular reactions including oxidative phosphorylation, membrane active transport systems, and certain steps in glycolysis, gluconeogenesis and lipogenesis. The fundamental reaction for the making and breaking of this bond, the hydrolysis reaction, may be written

$$\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{Pi} + \text{H}^+$$

The equilibrium position of this reaction lies very far to the right. The cell makes use of the energy of this reaction by coupling it to many other reactions, particularly synthetic ones, which have an unfavorable equilibrium position and are thus unable to proceed significantly toward completion otherwise. Knowledge of the free energy change of the ATP hydrolysis reaction expected under physiological conditions requires an accurate knowledge of the observed standard free energy change ($\Delta G^{\circ}_{\text{obs}}$) under the same conditions.$^{1}$ Experimentally, the most direct approach to the determination of this basic number under a given set of conditions would be through the determination of the observed equilibrium constant ($K_{\text{obs}}$) of Equation 1. However, the equilibrium of Reaction 1 lies far to the right and only traces of ATP would remain at equilibrium. Therefore, the equilibrium constant (and $\Delta G^{\circ}_{\text{obs}}$) cannot be determined directly but must be

$$\Delta G^{\circ}_{\text{obs}} = -RT\ln K_{\text{obs}}$$

where $K_{\text{obs}}$ is the experimentally determinable equilibrium constant of a reaction. $K_{\text{obs}}$ is defined as the equilibrium ratio of the total concentrations of the products and reactants of a reaction exclusive of $\text{H}^+$ or Mg$^{2+}$. $\Sigma$ and square brackets indicate total concentration. For the ATP hydrolysis reaction

$$K_{\text{obs}} = \frac{[\Sigma \text{ADP}] [\Sigma \text{Pi}]}{[\Sigma \text{ATP}] [\Sigma \text{H}_2\text{O}]}$$

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determined indirectly from two or more reactions, the sum of which is Equation 1. The most recent approaches to the expression of Reaction 1 which have included detailed consideration of the effect of magnesium have all been based upon the glutaminase (EC 3.5.1.2) and glutamine synthetase (EC 6.3.1.2) reactions. Benzinger et al. (1) determined the equilibrium constant of the glutaminase reaction and combined this data with the equilibrium constant of the glutamine synthetase reaction determined by Levintow and Meisler (2) to obtain values for the ATP hydrolysis reaction. Alberty (3) and Phillips et al. (4), using the same original data of Benzinger et al. (1) and Levintow and Meisler (2), have arrived at quite different values. Rosing and Slater (5) have redetermined the equilibrium constant of the glutamine synthetase reaction which they then combined with the equilibrium constant of the glutaminase reaction measured by Benzinger (1) to arrive at yet another set of values. The work based on the glutaminase and glutamine synthetase reactions has therefore yielded a considerable range of values for the $\Delta G_{\text{obs}}$ of Equation 1. For example at 37°C, pH 7.0, and free [Mg$^{2+}$] = 10^{-3} M, the calculated values of the $\Delta G_{\text{obs}}$ range from -6.79 Cal per mole (-28.4 kJ per mole) (5) to -8.74 Cal per mole (-36.57 kJ per mole) (4).

Because of the wide discrepancy in the values using the glutaminase and glutamine synthetase reactions, the experimental determination of the free energy of hydrolysis of ATP under physiological conditions has been approached using a completely different set of reactions. The combined equilibrium constant of acetate kinase (EC 2.7.2.1) (Equation 2) and phosphate acetyltransferase (EC 2.3.1.8) (Equation 3) is a measure of the difference between the free energies of hydrolysis of acetyl-CoA and ATP (Equations 4 and 5).

$$\text{Acetate} - \text{ATP}^{\gamma} \rightleftharpoons \text{Acetyl phosphate}^{\gamma} + \text{ADP} + \text{Pi}$$

$$K = \frac{[\text{Acetate}] [\text{ATP}] [\text{Pi}]}{[\text{Acetyl phosphate}^{\gamma}] [\text{ADP}]}$$

Since the $\Delta G_{\text{obs}}$ for the hydrolysis of acetyl-CoA under physiological conditions has been determined elsewhere, (6) the $\Delta G_{\text{obs}}$ for the hydrolysis of ATP can be calculated if appropriate values for Equation 5 are known.

In the present work the combined equilibrium constant (Equation 5) has been determined at 38°C, pH 7.0, and ionic strength 0.25. Variations of this constant with the free [Mg$^{2+}$] have been studied and from these results the value of $\Delta G_{\text{obs}}$ for the hydrolysis of ATP as a function of free [Mg$^{2+}$] has been determined.

**EXPERIMENTAL PROCEDURE**

**Enzymes**—Crystalline suspensions of acetate kinase (ATP:acetate phosphotransferase, EC 2.7.2.1) from *Escherichia coli* and phosphotase acetyltransferase (acyl-CoA:orthophosphate acetyltransferase, EC 2.3.1.8) from *Clavibacter kluyveri* were obtained from Boehringer Mannheim, N. Y. Immediately before use the enzymes were centrifuged at 45,000 $\times$ g for 20 min at 0°C, and the excess (NH$_4$)$_2$SO$_4$ was removed. The pellet of enzymes was resuspended in 0.15 M potassium phosphate buffer, pH 7.0. $\alpha$-Ketoglutarate dehydrogenase was prepared from pig heart by the method of Sanadi et al. (7). The other enzymes used for the metabolite assays were obtained from Boehringer Mannheim, N. Y.

**Substrates and Reagents**—Analytical grade potassium phosphate, potassium acetate, potassium chloride, and magnesium chloride and acetate were obtained from Mallinkrodt Chemical Co., St. Louis, Mo. Acetyl-CoA was obtained as the trillithium salt from P-L Biochemicals Inc., Milwaukee, Wisc. Dithiothreitol and Na-ADP-2H$_2$O were obtained from Calbiochem, La Jolla, Calif. Na$_2$-ATP-H$_2$O, cocarboxylic A (free acid), and potassium lithium acetyl phosphate were obtained from Boehringer Mannheim, N. Y.

**Instruments**—Metabolite assays were performed on a Zeiss PMQ II spectrophotometer. The total magnesium concentrations were analyzed with a Varian TECTRON atomic absorption spectrophotometer (type AA-5). pHe measurements were made at 38°C with a Radiometer Copenhagen microelectrode (type E5021a) and pH meter (type 27) using precision Radiometer buffers as standards.

**Metabolite Assays**—CoA was assayed using $\alpha$-ketoglutarate dehydrogenase (8). Stock solutions of CoA were also analyzed with phosphate acetyltransferase (9) with agreement of the two methods. Acetyl-CoA was assayed by the method of Wieland and Weiss (10) as modified by Pearson (11). Stock solutions of CoA were also measured by the method of Srere (12) with agreement of the two methods. ATP, ADP, AMP, and Pi were measured enzymatically as described previously (13, 14). Acetate was measured enzymatically (15). The values obtained for potassium acetate agreed well with that predicted from the weight of the dry salt. The values obtained for magnesium acetate agreed well with the magnesium content.

**Contaminations**—All reagents were assayed for possible contaminations of acetate, phosphate, and magnesium, and these contaminations have been taken into account. Total contaminations of phosphate and acetate were < 1% of the total added. Total magnesium contaminations were $\approx 0.02\%$ umole per ml in the final reaction mixture, coming mainly from the ATP and ADP.

**Procedure**—The details of the concentrations of the reaction mixtures are given in Table II. For each reaction mixture a total of 6.0 ml of final volume was prepared. The reaction was initiated at room temperature and allowed to stand for 10 min. Aliquots of 1.5 ml of the reaction mixture were then pipetted into each of three tubes (13 x 100 mm), and the tubes and reaction mixture remaining in the original tube were capped and placed in a 38°C shaking water bath. At 15, 30, and 45 min, 300 $\mu$l of 15% ice-cold perchloric acid were added to one of the tubes which was then shaken and chilled rapidly to 0°C. After all of the samples were taken, the pH was measured in the reaction mixture remaining in the original tube. After standing in ice for 20 min the tubes were centrifuged at 0°C at 700 $\times$ g for 15 min. A 1.3-ml portion of the clear supernatant was transferred to fresh tubes and 150 $\mu$l of 6 M K$_2$CO$_3$ was added slowly to the cold tube. The concentration of the K$_2$CO$_3$ was previously adjusted so that 150 $\mu$l would neutralize the extract to pH 5.5 to 6.0. After standing for 20 min in ice, the tubes were centrifuged at 700 $\times$ g at 0°C for 20 min and the clear supernatant was assayed for ATP, ADP, acetyl-CoA, and CoA. This extract contained no detectable activity of acetate kinase or phosphate acetyltransferase. AMP was also assayed in some tubes. The AMP found was no detectable activity of acetate kinase or phosphate acetyltransferase. The samples were taken, the pH was measured in the reaction mixture remaining in the original tube. After standing in ice for 20 min the tubes were centrifuged at 0°C at 700 $\times$ g for 15 min. A 1.3-ml portion of the clear supernatant was transferred to fresh tubes and 150 $\mu$l of 6 M K$_2$CO$_3$ was added slowly to the cold tube. The concentration of the K$_2$CO$_3$ was previously adjusted so that 150 $\mu$l would neutralize the extract to pH 5.5 to 6.0. After standing for 20 min in ice, the tubes were centrifuged at 700 $\times$ g at 0°C for 20 min and the clear supernatant was assayed for ATP, ADP, acetyl-CoA, and CoA. This extract contained no detectable activity of acetate kinase or phosphate acetyltransferase. AMP was also assayed in some tubes. The AMP found was no detectable activity of acetate kinase or phosphate acetyltransferase. The samples were taken, the pH was measured in the reaction mixture remaining in the original tube. After standing in ice for 20 min the tubes were centrifuged at 0°C at 700 $\times$ g for 15 min. A 1.3-ml portion of the clear supernatant was transferred to fresh tubes and 150 $\mu$l of 6 M K$_2$CO$_3$ was added slowly to the cold tube. The concentration of the K$_2$CO$_3$ was previously adjusted so that 150 $\mu$l would neutralize the extract to pH 5.5 to 6.0. After standing for 20 min in ice, the tubes were centrifuged at 700 $\times$ g at 0°C for 20 min and the clear supernatant was assayed for ATP, ADP, acetyl-CoA, and CoA. This extract contained no detectable activity of acetate kinase or phosphate acetyltransferase. AMP was also assayed in some tubes. The AMP found was no detectable activity of acetate kinase or phosphate acetyltransferase. The samples were taken, the pH was measured in the reaction mixture remaining in the original tube. After standing in ice for 20 min the tubes were centrifuged at 0°C at 700 $\times$ g for 15 min. A 1.3-ml portion of the clear supernatant was transferred to fresh tubes and 150 $\mu$l of 6 M K$_2$CO$_3$ was added slowly to the cold tube.
for each of the assays. This eliminated potential errors in the ratios of the metabolites due to multiple pipettings of the reaction mixture and extract.

The final concentrations of phosphate and acetate were taken to be equal to the initial concentrations corrected on the assumption that the change in acetate and phosphate was stoichiometric with the change in ATP and acetyl-CoA. A potential error in a reaction mixture containing acetyl phosphate is the spontaneous hydrolysis of this compound to acetate and phosphate. However, since large and nearly equal concentrations of acetate and phosphate were initially added, it can be estimated from Table II that complete hydrolysis of the acetyl phosphate not converted to ATP and acetyl-CoA would result in a maximum error of 0.2% in the [phosphate]/[acetate] ratio.

Recovery Experiments—Reaction mixtures without enzymes have been carried through the procedure to determine the stability of ATP, ADP, acetyl-CoA, and CoA to incubation at 38° and perchloric acid extraction. ATP and acetyl-CoA were recovered from the procedure in 103% yield, ADP in 99% yield, and CoA in 98% yield.

Calculation of Free [Mg2+]—If the magnesium binding constants of the substrates at equilibrium are known, the free [Mg2+] can be calculated. Table I lists the constants from the literature which best approximate the conditions under which the equilibrium constant has been determined. In some cases the constants have been adjusted to 38° using enthalpy data of Alberty (16). The calculation of the free [Mg2+] has been performed via a computer program based upon solving simultaneous equations (21). Calculations have also been carried out with constants used by Rosing and Slater (5) including corrections for potassium binding. The calculations based on these constants were not very different from the calculations based on the constants of Table I but were less compatible with the experimentally determined points.

Calculations—The ΔG°" for the hydrolysis of ATP was calculated from the relationship ΔG°" = -RT ln Kobs, in which the gas constant R is 1.987 Cal per Kg mole (8.314 kJ per Kg mole). For all calculations, unit activity has been taken to be the standard state of liquid water.

The equilibrium constant at [Mg2+] = 0 has been calculated using equations similar to the equations derived elsewhere (6) for the citrate synthase and citrate lyase constants.

\[ \frac{K_{obs}([Mg^{2+}])}{K_{obs}([Mg^{2+}])} = \frac{([MgATP^{2+}]([MgADP^{2+}] + [MgHATP^{2+}])}{([MgATP^{2+}] + [MgADP^{2+}] + [MgHATP^{2+}])} \]

where the Kobs constants are the observed magnesium binding constants at a given pH and potassium ion concentration.

\[ \frac{K_{obs}([Mg^{2+}])}{K_{obs}([Mg^{2+}])} = \frac{([MgATP^{2+}]([MgADP^{2+}] + [MgHATP^{2+}])}{([MgATP^{2+}] + [MgADP^{2+}] + [MgHATP^{2+}])} \]

which becomes, by substitution of the constants of Table I

\[ \frac{K_{obs}([Mg^{2+}])}{K_{obs}([Mg^{2+}])} = \frac{([MgATP^{2+}]([MgADP^{2+}] + [MgHATP^{2+}])}{([MgATP^{2+}] + [MgADP^{2+}] + [MgHATP^{2+}])} \]

Kobs([Mg2+]) and Kobs([Mg2+]) are analogous to Equations 7 and 8.

The observed equilibrium constant at [Mg2+] = 0 can also be represented as

\[ \frac{K_{obs}([Mg^{2+}])}{K_{obs}([Mg^{2+}])} = \frac{([MgATP^{2+}]([MgADP^{2+}] + [MgHATP^{2+}])}{([MgATP^{2+}] + [MgADP^{2+}] + [MgHATP^{2+}])} \]

which becomes by substitution of the constants of Table I

\[ \frac{K_{obs}([Mg^{2+}])}{K_{obs}([Mg^{2+}])} = \frac{([MgATP^{2+}]([MgADP^{2+}] + [MgHATP^{2+}])}{([MgATP^{2+}] + [MgADP^{2+}] + [MgHATP^{2+}])} \]

Rearranging and multiplying through by [H+], Equation 10 becomes Equation 11. Equation 11 allows the calculation of the equilibrium constant for the ionic reaction.

**Table I**

**Acid dissociation constants and magnesium-binding constants**

The magnesium-binding constants have been adjusted to 38° using the van't Hoff equation and ΔH° values supplied by Alberty (16).

| Acid dissociation constants | Value of Constant | Temp | I | Monovalent Cation | Reference |
|-----------------------------|-------------------|------|---|------------------|-----------|
| K_a(ADP) = [H+] [ADP^-] / [ADP^-] | 1.08 x 10^-7 M | 38° | 0.25 | (nC_5H_9)_4N+ | (17) |
| K_a(AMP) = [H+] [AMP^-] / [AMP^-] | 1.20 x 10^-7 M | 38° | 0.2 | (nC_5H_9)_4N+ | (18, 19) |
| K_a(H_2PO_4^-) = [H+] [HPO_4^-] / [H_2PO_4^-] | 2.4 x 10^-7 M | 38° | 0.25 | (nC_5H_9)_4N+ | (17) |

**Magnesium binding constants**

| K_b(ADP^3-) = [MgADP^-] / [Mg^2+] [ADP^-] | 1.32 x 10^-3 M | 38° | 0.2 | (nC_5H_9)_4N+ | (18, 19) |
| K_b(HATP^-) = [MgHATP^-] / [Mg^2+] [HATP^-] | 3.55 x 10^-3 M | 38° | 0.2 | (nC_5H_9)_4N+ | (18, 19) |
| K_b(HPO_4^-) = [MgHPO_4^-] / [Mg^2+] [HPO_4^-] | 3.24 x 10^-3 M | 38° | 0.2 | (nC_5H_9)_4N+ | (18, 19) |

| K_b(AMP^2-) = [MgAMP^-] / [Mg^2+] [AMP^-] | 2.61 x 10^-3 M | 38° | 0.2 | (nC_5H_9)_4N+ | (18, 19) |
Using a similar derivation it can be shown that

\[
\frac{[\text{HADP}^2-][\text{H}_2\text{P}O_4^-]}{[\text{HATP}^3-][\text{H}_2\text{O}]} = K_{\text{obs}}(\text{Mg}^{2+}) = \frac{(K_{\text{ATP}}[\text{H}^+]) (K_{\text{ADP}}) (K_{\text{P}i})}{(K_{\text{ATP}}[\text{H}^+]) (K_{\text{ADP}}) (K_{\text{P}i})}
\]

Equation 12 allows the calculation of the equilibrium constant of the ionic reaction

\[
\text{HATP}^3- + \text{H}_2\text{O} \rightleftharpoons \text{HADP}^2^- + \text{H}_2\text{P}O_4^-
\]

**RESULTS**

**Equilibrium Constant of Combined Reactions of Acetate Kinase and Phosphate Acetyltransferase**

- Table II shows the details of the determination of the constant at one magnesium concentration. The reaction has been studied starting with either CoA, ADP, and acetyl phosphate (the kinetically more favored or "forward" reaction), or with ATP and acetyl-CoA (the "reverse" reaction). Equilibrium was reached by 15 min at 38°. It should be noted that once equilibrium was reached in the forward direction, the ATP and acetyl-CoA concentrations (the products) tended to slowly decrease, reflecting the spontaneous hydrolysis of the acetyl phosphate with reversal of the reactions to maintain equilibrium.

- The variation of the \( K_{\text{obs}} \) of the combined reaction of acetate kinase and phosphate acetyltransferase with magnesium is summarized in Table III. Attempts to achieve equilibrium with the reverse reaction at the lowest magnesium concentrations (0.32 and 0.02 mm total) were unsuccessful even with prolonged incubations. Failure to achieve equilibrium resulted from the fact that the rate of the acetate kinase reaction in the direction of acetyl phosphate production is slower at very low magnesium concentrations than the spontaneous hydrolysis of acetyl phosphate. In the other trials of the reverse reaction equilibrium was achieved rapidly, being complete within 15 min.

\[ K_{\text{obs}} \text{ varies from } 0.164 \pm 0.003 \text{ at } 10 \text{ mm total magnesium (2.7 mm free) to } 0.998 \pm 0.019 \text{ at } 0.02 \text{ mm total magnesium (0.002 mm free). The values for } K_{\text{obs}} \text{ calculated for } [\text{Mg}^{2+}] = 0 \text{ are very consistent. The average in the forward direction is...} \]

**Table II**

**Combined equilibrium constant of acetate kinase and phosphate acetyltransferase**

- In addition to the initial concentrations listed below, the reaction mixtures contained 1 mM dithiothreitol, sufficient KCl to adjust the ionic strength to 0.25, 1.5 units per ml of acetate kinase, and 8 units per ml of phosphate acetyltransferase. A unit of enzyme is that amount of enzyme which will catalyze the conversion of 1 pmole of substrate per min at 25°. Temperature = 38°, pH = 7.0 ± 0.01; values are given as means ± standard error.

\[
K_{\text{obs}} = \frac{[\text{Acetyl-CoA}][\text{CoA}][\text{Acetate}]}{[\text{ATP}][\text{ADP}][\text{P}i]}
\]

**Concentrations (mM)**

| Trial | Time (min) | Acetyl Phosphate | ATP | ADP | Acetyl-CoA | CoA | Acetate | Phosphate | Total | Free | \( K_{\text{obs}} \) Calculated for \([	ext{Mg}^{2+}] = 0\) |
|-------|------------|------------------|-----|-----|-----------|-----|---------|-----------|-------|------|----------------------------------|
| 1     | 0          | 1.0              | 0.01| 1.0 | -         | 0.30| 44.91   | 47.86     | 9.99  |      | 0.164 ± 0.003                    |
|       | 15         |                  | 0.717| 0.216| 0.103    | 0.203| 45.63   | 47.96     | 9.99  | 2.71 | 0.172                             |
|       | 30         |                  | 0.697| 0.239| 0.100    | 0.210| 45.51   | 47.95     | 9.99  | 2.72 | 0.172                             |
|       | 45         |                  | 0.707| 0.252| 0.093    | 0.216| 45.51   | 47.95     | 9.99  | 2.71 | 0.161                             |
| 2     | 0          | 1.0              | 0.01| 1.0 | -         | 0.38| 63.95   | 47.96     | 10.01 |      |                                   |
|       | 15         |                  | 0.616| 0.314| 0.110    | 0.268| 65.57   | 47.97     | 10.01 | 2.72 | 0.170                             |
|       | 30         |                  | 0.607| 0.362| 0.110    | 0.269| 65.56   | 47.97     | 10.01 | 2.72 | 0.179                             |
|       | 45         |                  | 0.588| 0.371| 0.103    | 0.271| 65.54   | 47.96     | 10.01 | 2.72 | 0.175                             |
| 3     | 0          | 1.0              | 0.01| 1.0 | -         | 0.42| 65.57   | 47.86     | 10.01 |      |                                   |
|       | 30         |                  | 0.658| 0.332| 0.119    | 0.303| 66.23   | 47.98     | 10.01 | 2.64 | 0.144                             |
|       | 45         |                  | 0.692| 0.336| 0.123    | 0.310| 66.22   | 47.98     | 10.01 | 2.64 | 0.118                             |
| 4     | 0          | -                | 1.0 | 0.02| 0.62     | 0.01| 65.65   | 47.86     | 10.01 |      |                                   |
|       | 15         |                  | 0.797| 0.258| 0.171    | 0.258| 65.38   | 47.60     | 10.01 | 2.68 | 0.156                             |
|       | 30         |                  | 0.791| 0.260| 0.173    | 0.262| 65.37   | 47.61     | 10.01 | 2.68 | 0.168                             |
|       | 45         |                  | 0.768| 0.268| 0.168    | 0.265| 65.37   | 47.60     | 10.01 | 2.69 | 0.161                             |

Mean ± S.E. 0.164 ± 0.003
The procedure is the same as that described in Table II. Varying amounts of magnesium as the chloride or acetate salt were added and the ionic strength was adjusted to 0.25 with KCl. The constant is defined by:

$$K_{obs} = \frac{[Acetyl-CoA][GTP]}{[Acetyl-CoA][ATP]}$$

Temperature was 38°, pH was 7.01 ± 0.01; values are given as means ± standard error.

| Condition | pH | Mg | ΔG° | Time (s) |
|-----------|----|----|-----|---------|
| Zero Mg   | 7.0| 0  | -8.53| 0.067 ± 0.007 |
| 10^{-3} M | 7.0| 0  | -8.53| 0.067 ± 0.007 |
| 10^{-2} M | 7.0| 0  | -8.53| 0.067 ± 0.007 |

The value of ΔG° was calculated from the combined equilibrium constant of the acetyl kinase and phosphate acetyltransferase reactions (Table III) and the ΔG° for the hydrolysis of acetyl-CoA under the same conditions. The value of ΔG° was calculated from the combined equilibrium constant of the acetate kinase and phosphate acetyltransferase reactions and the values of Benzinger et al. (1) using the glutaminase and glutamine synthetase reactions.

Calculation of Equilibrium Constant of ATP-Citrate Lyase Reaction under Physiological Conditions—Fig. 2 shows the $K_{obs}$ of the ATP hydrolysis reaction plotted with the $K_{obs}$ of the citrate synthase reaction determined elsewhere under the same conditions (6). Both are graphed as a function of free [Mg**]. The $K_{obs}$ of the citrate synthase reaction is defined as:

$$K_{obs} = \frac{[ICoA][ICoA]}{[ICoA][ICoA][ICoA]}$$

The $K_{obs}$ of the ATP-citrate lyase reaction will be:

$$K_{obs} = \frac{[ICoA][ICoA][ICoA]}{[ICoA][ICoA][ICoA]}$$

Fig. 3 shows the calculated variation of the constant of ATP-
The values from this paper obtained from the combined constant of acetate kinase and phosphate acetyltransferase (at 38°) are compared with values from the literature all of which were obtained from the glutaminase and glutamine synthetase reactions (at 37°). Values for this paper have been calculated from the data of Table II and Equations 11 and 12. All values are for pH 7.0 and unit activity has been taken to be the standard state of liquid water. In some cases the values have been calculated from data given in the original paper.

| Reaction | $\Delta G^\circ$ -Cal/mole(-kJ/mole) |
|----------|-----------------------------------|
| this paper | Benzingher et al. (1) | Rosing and Slater (5) | Phillips et al. (4) |
| $\text{ATP}^4^- + \text{H}_2\text{O} = \text{ADP}^3^- + \text{HPO}_4^{2-} + \text{H}^+$ | 8.41(35.19) | 8.6(36.0) | 7.90(33.0)* | 9.87(41.31)* |
| $\text{MgATP}^4^- + \text{H}_2\text{O} = \text{MgADP}^3^- + \text{HPO}_4^{2-} + \text{H}^+$ | 6.95(29.08) | 7.0(29.3) | 6.3(26.4)+ | 6.3(26.4)+ |
| $\text{HATP}^3^- + \text{H}_2\text{O} = \text{HADP}^2^- + \text{HPO}_4^{2-}$ | 7.67(32.10) | 7.0(30.84)§ | 9.34(39.00) | 9.34(39.00) |
| $\text{EATP} + \text{H}_2\text{O} = \text{EADP} + \text{EP}_1$ (free $[\text{Mg}^{2+}]=0$) | 8.53(35.69) | 7.93 33. 8 | 10.06(42.09) | 10.06(42.09) |
| $\text{EATP} + \text{H}_2\text{O} = \text{EADP} + \text{EP}_1$ (free $[\text{Mg}^{2+}]=10^{-3} \text{ M}$) | 7.60(31.80) | 6.79(28.8) | 8.74(36.57) | 8.74(36.57) |

* Calculated from the value of $\Delta G^\circ_{\text{b,c}}$ at $[\text{Mg}^{2+}] = 0$ and pH 9.0.
† Calculated from the data and the difference between $K_{k_{\text{ATP}}}$ and $K_{k_{\text{ADP}}}$.
§ Average of values given.

**FIG. 2.** Comparison of the equilibrium constants of the citrate synthase and ATP hydrolysis reactions as a function of the free $[\text{Mg}^{2+}]$. The curve for the hydrolysis of ATP is calculated from the data of Tables II and III and Fig. 1. The curve for the $K_{\text{obs}}$ of the citrate synthase reaction is calculated from previous data (6). Both constants are at 38°, pH 7.0, and $I = 0.25$.

**FIG. 3.** Equilibrium constant of the ATP-citrate lyase reaction as a function of the free $[\text{Mg}^{2+}]$. The curve (---) is calculated from the data of Fig. 2 and Equation 14 and represents the values of $K_{\text{obs}}$ for the ATP-citrate lyase reaction at 38°, pH 7.0, and $I = 0.25$.

**DISCUSSION**

Combined Constant of Acetate Kinase and Phosphate Acetyltransferase—The fact that the combined equilibrium constant of the acetate kinase and phosphate acetyltransferase reactions (Equation 5) is approximately 1 at $[\text{Mg}^{2+}] = 0$ (Table III) dem-
onstrates that the free energy of hydrolysis of the terminal pyrophosphate bond of ATP is nearly identical with the free energy of hydrolysis of the thioester bond of acetyl-CoA under these conditions. However, while the \( \Delta G^\circ_{o, \text{obs}} \) for the hydrolysis of the two types of bonds is virtually the same at [Mg\(^{2+}\)]= 0, the \( \Delta G^\circ_{o, \text{obs}} \) for the hydrolysis of the thioester bond of acetyl-CoA can be up to 1.1 Cal per mol (4.57 kJ per mol) more negative than that of the terminal pyrophosphate bond of ATP depending upon the free [Mg\(^{2+}\)]. The difference in the free energy of hydrolysis in the presence of Mg\(^{2+}\) is due entirely to the sensitivity of the hydrolysis of the pyrophosphate bond of ATP to the free [Mg\(^{2+}\)] (Fig. 1), the free energy of hydrolysis of acetyl-CoA being insensitive to the free [Mg\(^{2+}\)] under these conditions (6).

**Free Energy of Hydrolysis of ATP**—The experimental value for the \( \Delta G^\circ_{o, \text{obs}} \) for the ATP hydrolysis reaction has been found to vary with the free [Mg\(^{2+}\)] in a manner predictable from the acid dissociation constants and magnesium binding constants of the components of the reaction (Equation 6). The experimental values obtained for physiological conditions show good agreement with the values reported by Benzinger et al. (1) (Table IV) but are considerably lower than those of Phillips et al. (4) and higher than those of Rosing and Slater (5).

The calculation of the free energy of hydrolysis of ATP using the glutaminate and glutamine synthetase reactions is complicated by corrections which must be made in the experimental data including considerations of the activity coefficient of ammonium glutamate and the magnesium binding constant of glutamate. The high values for \( \Delta G^\circ_{o, \text{obs}} \) calculated by Phillips et al. (4) result in large part from the correction for magnesium binding by glutamate. However, there is recent evidence that the magnesium-binding constant of glutamate is, in fact, low (5), as Benzinger originally suggested (1). An overestimation of the binding by glutamate would yield values which are too high. On the other hand, the low values for the \( \Delta G^\circ_{o, \text{obs}} \) for ATP hydrolysis of Rosing and Slater (5) depend in large part upon their determination of the equilibrium constant of the glutamine synthetase reaction. The value they obtained is a factor of 5 lower than the value obtained by either Levintow and Meister (9) or Varner and Webster (23). The validity of the lower value obtained by Rosing and Slater (5) cannot be adequately judged since no analytical details were given.

It is concluded that the use of the combined equilibrium constant of the acetate kinase and phosphate acetyltransferase reactions and the equilibrium constant of acetyl-CoA hydrolysis is a very satisfactory system for the study of the ATP hydrolysis reaction, preventing fewer difficulties than the glutaminase and glutamine synthetase reactions.

Another system for studying the ATP hydrolysis reaction has been proposed using the combined equilibria of phosphoglucomutase (EC 2.7.5.1), galactokinase (EC 2.7.1.6) and glucose-6-phosphatase (EC 3.1.3.9) (24-26). Unfortunately, the constants the authors report were arrived at under significantly different free [Mg\(^{2+}\)] and therefore the value for \( \Delta G^\circ_{o, \text{obs}} \) which they obtained cannot be directly compared with the results in this paper.

**Equilibrium Constant of the ATP-Citrate Lyase Reaction**—The equilibrium constant of the ATP-citrate lyase reaction has been estimated experimentally from kinetic data to be 1 to 1.5 x 10\(^{-4}\) at 25°C, pH 5.1 in Tris buffer and approximatively 0.4 in free [Mg\(^{2+}\)] (27). The effect of varying the magnesium concentration was not studied. For our purposes, the system of Plowman and Cleland (27) was unsatisfactory for studying the equilibrium constant of the ATP-citrate lyase reaction since Tris buffer binds oxaloacetate (28). We have attempted to determine the equilibrium constant of this reaction under more physiological conditions both directly by analysis of the components of the reaction mixture and indirectly by coupling the enzyme with malate dehydrogenase (EC 1.1.1.37). Neither approach was successful, even in the presence of large amounts of enzyme, probably, in part, because one of the products ADP is an inhibitor of the reaction in vitro (29).

The \( K_{\text{obs}} \) of this reaction has been calculated, however, from the citrate synthase and ATP hydrolysis reactions (Equation 14). The calculated constant (Fig. 3) illustrates the pronounced sensitivity of the constant to the free [Mg\(^{2+}\)] in the physiological range. This sensitivity of the constant to the free [Mg\(^{2+}\)] has not been realized previously but can be understood since the two powerful magnesium binding agents, ATP and citrate, lie on the same side of the reaction.

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