Equilibrium Constants under Physiological Conditions for the Reactions of Polynucleotide Phosphorylase and RNA Polymerase*

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The observed equilibrium constants ($K_{obs}$) for the polynucleotide phosphorylase reaction (EC 2.7.7.8) have been determined under physiological conditions of temperature (38°C) and ionic strength (0.25 M) and physiological ranges of pH and free [Mg$^{2+}$]. Using Σ and square brackets to indicate total concentration:

$$K_{obs} = \frac{[\Sigma poly(A)_{n+1}][\Sigma P]}{[\Sigma poly(A)_{n}][\Sigma ADP]}$$

The value of $K_{obs}$ has been found to be sensitive to variations in free [Mg$^{2+}$] but relatively insensitive to pH. At pH 7.0 and 38°C, $K_{obs}$ = 4.08 at free [Mg$^{2+}$] = 0 (Δ$G_{obs}^{o}$ = -3.64 kJ/mol (-0.87 kcal/mol)), 2.99 at free [Mg$^{2+}$] = 10$^{-3}$ M, and 1.04 at free [Mg$^{2+}$] = 10$^{-2}$ M. At pH 8.0, $K_{obs}$ = 3.69 at free [Mg$^{2+}$] = 0, 2.54 at free [Mg$^{2+}$] = 10$^{-3}$ M, and 0.83 at free [Mg$^{2+}$] = 10$^{-2}$ M. The value of $K$ at 38°C and $I$ = 0.25 M is calculated to be 6.10 ± 0.21 (Δ$G^{o}$ = -1.67 kJ/mol (-0.12 kcal/mol)).

The K for the polynucleotide phosphorylase reaction has been combined with the K for the reactions of adenylate kinase, the hydrolysis of ATP to ADP and P, and the hydrolysis of ATP to AMP and PP that under the same physiological conditions to calculate a value of 2.89 × 10$^{-7}$ M (Δ$G^{o}$ = 38.9 kJ/mol (9.30 kcal/mol)) for the K of the RNA polymerase reaction using formation of pA as the model reaction.

$$K_{obs} = \frac{[\Sigma poly(A)_{n+1}][\Sigma PP]}{[\Sigma poly(A)_{n}][\Sigma ATP]}$$

Values of $K_{obs}$ for the RNA polymerase reaction at 38°C, pH 7.0, and $I$ = 0.25 M have been calculated to be 1.49 × 10$^{2}$ (Δ$G_{obs}^{o}$ = -12.9 kJ/mol (-3.09 kcal/mol)) at free [Mg$^{2+}$] = 0; 1.17 × 10$^{2}$ (Δ$G_{obs}^{o}$ = -12.3 kJ/mol (-2.94 kcal/mol)) at free [Mg$^{2+}$] = 10$^{-3}$ M and 2.68 × 10$^{2}$ (Δ$G_{obs}^{o}$ = -14.4 kJ/mol (-3.45 kcal/mol)) at free [Mg$^{2+}$] = 10$^{-2}$ M. Unlike the polynucleotide phosphorylase reaction, the RNA polymerase reaction is sensitive to pH. At pH 8.0 the corresponding values of $K_{obs}$ are 2.59 × 10$^{2}$ (Δ$G_{obs}^{o}$ = -14.4 kJ/mol (-3.43 kcal/mol)) at free [Mg$^{2+}$] = 0; 84.0 × 10$^{2}$ (Δ$G_{obs}^{o}$ = -17.4 kJ/mol (-4.16 kcal/mol)) at free [Mg$^{2+}$] = 10$^{-3}$ M.

The equilibrium constant for the hydrolysis of polynucleotide phosphorylase can be determined by combining the value of K for the RNA polymerase reaction with the value of K for the hydrolysis of ATP to AMP and PP.$^{4}$ The value of $K_{obs}$ for free [Mg$^{2+}$] = 0 is 7.30 × 10$^{4}$ (Δ$G_{obs}^{o}$ = -28.9 kJ/mol (-6.92 kcal/mol)) and at free [Mg$^{2+}$] = 10$^{-3}$ M is 7.63 × 10$^{4}$ (Δ$G_{obs}^{o}$ = -29.1 kJ/mol (-6.95 kcal/mol)).

Polynucleotide phosphorylase (EC 2.7.7.8), present in both bacteria (1,2) and eukaryotic cells (3), catalyzes the reversible polymerization of 5'-nucleoside diphosphates with the release of P. (4,5). The enzyme can catalyze the polymerization of either a mixture of nucleoside diphosphates or of a single species such as the polymerization of ADP (Equation 1) (1):

$$\text{poly(A)}_{n} + \text{ADP} \rightleftharpoons \text{poly(A)}_{n+1} + \text{P}, \quad (1)$$

The polymer formed from mixtures of nucleoside diphosphates cannot be distinguished chemically from natural RNA (6).

The equilibrium constant of this reaction has been suggested to be approximately 1.5 to 2 (30°C, pH 8.1, MgCl$_2$ = 10$^{-2}$ M) (1) and sensitive to variations in magnesium concentration (2). Unfortunately, however, no actual data on the equilibrium constant of this reaction seem to be available and there seem to be no estimates of the constant under physiological conditions. Therefore, in the current study using the synthesis and degradation of poly(A) as a model, we have examined in detail the observed equilibrium constant ($K_{obs}$)$^{1}$ of the polynucleotide phosphorylase reaction under physiological ranges of pH and free [Mg$^{2+}$]. Combining these results with the equilibrium constant determined previously under the same conditions for the reactions of adenylate kinase (7), the hydrolysis of ATP to ADP and P (8), and the hydrolysis of ATP to AMP and PP (9) has allowed the calculation of the $K_{obs}$ for the RNA polymerase reaction (EC 2.7.7.6) (Equation 2):

$$\text{poly(A)}_{n} + \text{ATP} \rightleftharpoons \text{poly(A)}_{n+1} + \text{PP}, \quad (2)$$

Not only is the $K_{obs}$ of Reactions 1 and 2 different under physiological conditions, but there is significant difference in response of the equilibrium constant to variation in pH and free [Mg$^{2+}$]. Therefore, under physiological conditions, the synthesis and degradation of polynucleotides can be achieved by two pathways of different equilibrium characteristics.

$^{*}$ This work was supported by Grant AU-631 from the Robert A. Welch Foundation, Bank of the Southwest Building, Houston, TX 77001. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
EXPERIMENTAL PROCEDURES

Enzymes—A unit of activity is the amount of enzyme which will convert 1 umol of a substrate per min under standard assay conditions. Polynucleotide phosphorylase from *Micrococcus lysodeikticus* (0.54 unit/mg) was obtained from Sigma Chemical Co., St. Louis, MO. Other commercially prepared enzymes for assays were obtained from Boehringer Mannheim, New York.

Substrates and Reagents—Na<sub>2</sub>ADP, 1.5H<sub>2</sub>O, AMP, H<sub>2</sub>O free acid, type I, 5'-polyadenylic acid (molecular weight approximately 10<sup>5</sup>), and the substrates for the assays were obtained from Sigma. Analytical grade MgCl<sub>2</sub>, KCl, potassium phosphate, and other reagents used in the metabolite assays were obtained from Mallinckrodt, St. Louis, MO.

Assays—ADP and AMP were assayed by the method of Adam (10); P<sub>i</sub> was measured by the method of Gynnn et al. (11); ATP, by the methods of Lambrecht (12) and Lowry and Passonneau (13).

Instruments—Metabolite assays were performed on a Zeiss PMQ III spectrophotometer; pH measurements were made at 25°C or 38°C with a Radiometer Copenhaggen microelectrode G2971G2 and Radiometer PFM 71MK2 pH meter with Radiometer precision buffers as standards.

Methods—The details of the concentrations of the reaction mixtures used for the determination of the equilibrium constant are given in Tables I, II, and III. Since adenylate kinase contaminates the polynucleotide phosphorylase, AMP was added to the reaction mixtures to maintain the adenylate kinase reaction close to equilibrium (7). Since AMP is a product of the reaction, contaminating adenylate kinase would cause a loss of ADP to the side reaction. Methods-The details of the concentrations of the reaction mixtures used for the determination of the equilibrium constant are given in Tables I, II, and III. Since adenylate kinase contaminates the polynucleotide phosphorylase, AMP was added to the reaction mixtures to maintain the adenylate kinase reaction close to equilibrium (7). Near complete loss of ADP to the side reaction.

Time Course—Aliquots of 0.25 ml of the forward or reverse reaction mixtures were pipetted into each of eight tubes (13 x 100 mm). The tubes and the reaction mixture remaining were sealed and placed in a 38°C water bath. At time intervals ranging from 20 min to 6 h, 50 µl of ice cold 7.2 M HClO<sub>4</sub> was added to one of the tubes which was then chilled rapidly to 0°C. After standing on ice for 20 min, the tubes were centrifuged at 0°C and 900 x g for 15 min. A 0.2-ml aliquot of the clear supernatant was transferred to a fresh tube and 20 µl of 6 M K<sub>2</sub>CO<sub>3</sub> were added to bring the pH to 5 to 6. After 20 min at 0°C, the precipitate was removed by centrifugation. The clear supernatant was assayed for ATP, ADP, AMP, and P<sub>i</sub>. Recovery of substrates and products was complete. After all the samples had been taken, the pH was measured in the reaction mixture remaining in the original tube. Equilibrium was reached at 5 h in the forward direction and 6 h in the reverse direction. Based upon these results, all remaining experiments were handled similarly except that single points were taken for convenience at 6 h for both the forward and reverse reactions. The enzyme obtained the ability to maintain equilibrium of the reaction for at least 12 h.

Determination of Apparent Binding Constant of Pol(A)—The spectrophotometric method of Burton (14) was used to determine the apparent association constants between magnesium and the polymer at 38°C pH 7.0, and I = 0.25 M (K<sub>s</sub> as the monovalent cation). Aliquots (20 µl) of MgCl<sub>2</sub> (0.02 to 0.2 M) were added to 3 ml temperature-controlled cuvettes with or without 2 mM polyadenylic acid. The total number of binding sites per molecule containing 1.055 x 10<sup>5</sup> x 10<sup>-10</sup> M<sup>-1</sup> was 140 ± 20. The total number of binding sites per molecule containing 1.055 x 10<sup>5</sup> x 10<sup>-10</sup> M<sup>-1</sup> was 140 ± 20. The total number of binding sites per molecule containing 1.055 x 10<sup>5</sup> x 10<sup>-10</sup> M<sup>-1</sup> was 140 ± 20.

Calculations — The values of the appropriate constants for ATP, ADP, AMP, and P<sub>i</sub> have been determined or cited previously (8, 9, 17). Only acid groups with a pK<sub>a</sub> between 5 and 9 have been considered. For calculations at 25°C, the ΔH<sup>o</sup> values cited by Alberty (18) were used to adjust the binding and acid dissociation constants.

Since the polymer is both the product and the substrate for the polynucleotide phosphorylase reaction, the ratio of [poly(A)<sub>-</sub>] to [poly(A)<sub>+</sub>] is equal to 1. K<sub>pol</sub> is therefore numerically equal to [poly(A)<sub>-</sub>]/[poly(A)<sub>+</sub>] and K is equal to [HPO<sub>4</sub>]<sup>-2</sup>/[ADP]<sup>-</sup> in analogy to the glycogen phosphorylase reaction (19).

For all calculations, unit activity has been taken to be the standard state of liquid water.

RESULTS AND DISCUSSION

Apparent Binding Constants of Magnesium to Poly(A)—The Scatchard plot of the interaction of magnesium with the poly(A) polymer is shown in Fig. 1. A curve rather than a straight line results probably reflecting cooperativity of the multiple binding sites on the molecule, although exact interpretation of such plots seems to be complicated (16). As an approximation, however, the graph can be interpreted as demonstrating roughly two types of binding sites. From the initial slope, it can be estimated that there are 40 binding sites/molecule each with an intrinsic binding constant of 2.3 x 10<sup>10</sup> M<sup>-1</sup>. Extrapolation of the terminal portion of the curve also predicts 100 sites/molecule of lower affinity (K<sub>s</sub> = 2.2 x 10<sup>10</sup> M<sup>-1</sup>). The total number of binding sites per molecule (estimated at 140) is in reasonable agreement with the 150 sites expected assuming 2 AMP residues per site and assuming a molecular weight of 10<sup>5</sup> for the polymer. The binding of magnesium to the polymer under the current conditions is slightly lower than that found by Sanders and Ts'o in low

\[ [\Sigma ADP] - [ADP<sup>-2</sup>] + [HIADP<sup>-2</sup>] + [MgADP<sup>-</sup>] \]
\[ + [MgHADP<sup>-</sup>] + [KADP<sup>-</sup>] \]  (4)
\[ [\Sigma AMP] = [AMP<sup>-2</sup>] + [HAMP<sup>-</sup>] + [MgAMP<sup>-</sup>] + [KAMP<sup>-</sup>] \]  (5)
\[ [\Sigma ATP] = [ATP<sup>-4</sup>] + [HATP<sup>-4</sup>] \]
\[ + [MgATP<sup>-4</sup>] + [MgHATP<sup>-4</sup>] \]
\[ [\Sigma Mg] = [Mg<sup>-2</sup>] + [MgHPO<sub>4</sub><sup>-2</sup>] + [MgADP<sup>-2</sup>] + [MgAMP<sup>-2</sup>] \]
\[ + [MgP]<sub>A</sub> + [MgATP<sup>-2</sup>] + [MgHATP<sup>-2</sup>] \]
\[ [K<sup>-</sup>] = [K<sup>-</sup>] + [KHPO<sub>4</sub><sup>-2</sup>] + [KHPO<sub>4</sub><sup>-2</sup>] + [KHPO<sub>4</sub><sup>-2</sup>] \]  (10)

Fig. 1. Determination of the apparent binding constant of poly(A). For methodology and results, see text. The dashed line extrapolation to the x axis yields n, the number of sites. The y intercept is nK<sub>s</sub>.

H. Thames, and R. W. Guynn, unpublished work.
ionic strength (5 mM) sodium phosphate buffer (20). The high K concentration under the physiological conditions used in this study may account for the decreased affinity between magnesium and the polymer. The number or concentration of polymer-associated magnesium binding sites at equilibrium has been taken to be equal to the initial number of sites corrected for any change in total adenine nucleotides recovered. The proportion of high affinity sites ($\sum \text{poly(A) sites}$) to low affinity sites ($\sum \text{poly(A)}$) has been assumed to be constant (40:100) under the current conditions for purposes of calculating the free [Mg$^{2+}$] in the equilibrium mixtures.

**Equilibrium Constant of the Polynucleotide Phosphorylase Reaction**—The time course for the forward and reverse reactions is presented in Table I; and the experimental data for the other runs, in Tables II and III. In each case, Equations 3 to 10 and the definitions and values of the acid dissociation and cation binding constants have been used to calculate the concentrations of the ionic species HPO$_4^{2-}$ and ADP$^3-$ from the total equilibrium concentrations of phosphate and ADP. In turn, the value of the ionic constant K for Equation (14) has been calculated. There is good agreement between the average value of K determined in the forward direction (5.78 ± 0.31) and in the reverse direction (6.36 ± 0.27) (Table II). Since there is no significant difference between the results from the two directions, an average of 6.10 ± 0.21 has been taken corresponding to a $\Delta G^0$ of -4.67 kJ/mol (1.12 kcal/mol). At 25°C the corresponding value of $K$ is 9.56 ± 0.63 (5.84 kJ/mol (1.39 kcal/mol)) (Table III).

### TABLE I

| Time (Minutes) | Trial | P | ADP | P | ADP | AMP | ATP | Mg$^{2+}$ | ZMg$^{2+}$ |
|---------------|-------|----------|----------|----------|----------|----------|----------|-------------|-------------|
| 20            | forward | 4.27 | 6.99 | 1.36 | 2.99 | 2.70 |
| 50            | forward | 6.71 | 4.11 | 1.63 | 7.73 | 8.65 |
| 95            | reverse | 3.68 | 1.72 | 0.53 | 0.76 | 0.78 |
| 120           | reverse | 8.19 | 2.60 | 3.12 | 2.60 | 3.06 |
| 180           | reverse | 8.26 | 2.65 | 3.12 | 2.60 | 3.06 |
| 240           | reverse | 8.01 | 2.60 | 3.12 | 2.60 | 3.06 |
| 300           | reverse | 7.06 | 2.37 | 2.98 | 2.60 | 3.06 |
| 360           | reverse | 8.30 | 2.38 | 2.98 | 2.60 | 3.06 |

### TABLE II

**Experimental Determination of $K_{eq}$ for the Polynucleotide Phosphorylase Reaction at 38°C**

Details of the procedure are as described in Table I and under "Experimental Procedures." All forward reactions consisted of 0.25 mM MgCl$$_2$$ and 0.5 mM MnCl$$_2$$ in varying proportions. Ionic strength was brought to 0.25 mM with K$^{+}$ as the monovalent cation.

#### Table II

| Initial Concentrations (mM) | Equilibrium Concentrations (mM) | Mg$^{2+}$ in | P | ADP | ATP | Mg$^{2+}$ | ZMg$^{2+}$ |
|-----------------------------|---------------------------------|-------------|----------|----------|----------|-------------|-------------|
| 2.50 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.750 | 1.94 | 15.0 | 6.89 | 221 | 7.2 | 1.06 | 5.37 |
| 5.00 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 1.36 | 3.33 | 15.0 | 6.89 | 210 | 7.2 | 1.25 | 6.68 |
| 9.5 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.206 | 0.512 | 15.0 | 6.89 | 210 | 7.2 | 0.78 | 4.45 |
| 12.5 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.801 | 0.190 | 15.0 | 6.89 | 210 | 7.2 | 1.18 | 6.33 |
| 16.25 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.512 | 2.19 | 11.3 | 6.58 | 210 | 7.2 | 1.16 | 5.52 |
| 20.0 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.723 | 1.80 | 10.5 | 3.72 | 210 | 7.2 | 1.15 | 5.45 |
| 25.0 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.987 | 2.45 | 10.5 | 4.21 | 210 | 7.2 | 1.14 | 5.38 |
| 30.0 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.497 | 1.23 | 7.77 | 4.25 | 240 | 1.10 | 6.65 |
| 35.0 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.811 | 2.01 | 7.77 | 4.25 | 240 | 1.10 | 6.65 |
| 40.0 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.592 | 1.24 | 7.77 | 4.25 | 240 | 1.10 | 6.65 |
| 45.0 | 9.30 | 4.26 | 4.04 | 2.91 | 1.83 | 0.592 | 1.24 | 7.77 | 4.25 | 240 | 1.10 | 6.65 |

### Table III

| Time coarse direction | Mg$^{2+}$ in | P | ADP | ATP | Mg$^{2+}$ | ZMg$^{2+}$ |
|-----------------------|-------------|----------|----------|----------|-------------|-------------|
| 120 reverse | 8.75 | 2.09 | 4.19 |
| 180 reverse | 7.57 | 2.04 | 3.71 |
| 240 reverse | 7.97 | 2.27 | 3.96 |
| 300 reverse | 7.30 | 2.15 | 3.39 |
| 360 reverse | 7.17 | 2.15 | 3.33 |
The value of \( K \) can be used to back-calculate the value of \( K_{\text{obs}} \) for the reaction at a specified pH, \([K^+]\), and free \([Mg^{2+}]\) concentration (Fig. 2). Equation 3 can be rewritten as Equation 11.

\[
[S] = [\text{Pi}]^2/\bar{f}_r
\]

where

\[
\bar{f}_r = 1 + \frac{[H^+]}{K_{\text{aPiHPO}_4^{-}}} + \frac{[Mg^{2+}]K_{\text{KMPHPO}_4^{-}}}{[K^+]K_{\text{HMPHPO}_4^{-}}}
\]

Likewise, Equation 4 can be rewritten as Equation 12.

\[
[SADP] = [\text{ADP}]^2/\bar{f}_{ADP}
\]

\[
\bar{f}_{ADP} = 1 + \frac{[H^+]}{K_{\text{aADP}}} + \frac{[Mg^{2+}]K_{\text{KADP}}}{[K^+]K_{\text{HADP}}} + \frac{[Mg^{2+}][H^+]K_{\text{KADP}}}{K_{\text{aKADP}}} + (K^+)K_{\text{KADP}}
\]

Dividing Equation 11 by Equation 12 yields Equation 13 which in turn yields Equation 14.

\[
\frac{[S]}{[SADP]} = \frac{[\text{Pi}]^2}{[\text{ADP}]^2} \bar{f}_r/ \bar{f}_{ADP}
\]

\[
K_{\text{obs}} = K \bar{f}_r/ \bar{f}_{ADP}
\]

Using Equation 14, \( K_{\text{obs}} \) for the polynucleotide phosphorylase reaction has been calculated at 25°C and 38°C, pH 7.0, \( I = 0.25 \text{ M} \) and \([K^+] = 0.2 \text{ M}\). At 38°C (Fig. 2), the value of \( K_{\text{obs}} \) is 4.08 at free \([Mg^{2+}] = 0\), 2.99 at free \([Mg^{2+}] = 10^{-2} \text{ M} \), and 1.04 at free \([Mg^{2+}] = 10^{-3} \text{ M} \). At 25°C, the corresponding values of \( K_{\text{obs}} \) are 4.40 at free \([Mg^{2+}] = 0\), 3.40 at free \([Mg^{2+}] = 10^{-2} \text{ M} \), and 1.36 at free \([Mg^{2+}] = 10^{-3} \text{ M} \). The calculated values of \( K_{\text{obs}} \) at 25°C are higher at all free \([Mg^{2+}]\) than the values at 38°C and \( \Delta H_{\text{obs}} \) for the reaction depends upon the free \([Mg^{2+}]\). \( \Delta H_{\text{obs}} \) can be calculated to be -5.23 kJ/mol (I = 0.25 M) and -1.25 kcal/mol (I = 0.25 M). At free \([Mg^{2+}] = 0\), 8.12 kcal/mol (I = 0.25 M) at free \([Mg^{2+}] = 10^{-2} \text{ M} \), and -16.0 kcal/mol (I = 0.25 M) at free \([Mg^{2+}] = 10^{-3} \text{ M} \). The dependence of \( \Delta H_{\text{obs}} \) upon the free \([Mg^{2+}]\) reflects the differences of the \( \Delta H_{\text{obs}} \) for the binding constants of the substrates (19).

The relative insensitivity of \( K_{\text{obs}} \) of the polynucleotide phosphorylase reaction to pH (Fig. 3) is predicted from the very similar wild dissociation constants for ADP and P (Equation 13). It can be calculated at pH 8.0 (38°C, I = 0.25 M, \([K^+] = 0.2 \text{ M}\)); the value of \( K_{\text{obs}} \) is 2.54 at free \([Mg^{2+}] = 10^{-3} \text{ M} \). The value of \( K_{\text{obs}} \) is also relatively insensitive to the \([K^+]\). For example at pH 7.0, 38°C, \([Mg^{2+}] = 0\), lowering the \([K^+]\) from 0.2 to 0.1 M changes the value of \( K_{\text{obs}} \) from 4.08 to 4.68. Likewise \( K_{\text{obs}} \) shows relative insensitivity to ionic strength. At 38°C, pH 7.0, and \([Mg^{2+}] = 10^{-2} \text{ M} \); \( K_{\text{obs}} = 2.64 \pm 0.21 \) (four trials) at \( I = 0.04 \text{ M} \) and 3.64 \pm 0.17 (four trials) at \( I = 1.0 \text{ M} \).

**Equilibrium Constant of the RNA Polymerase Reaction under Physiological Conditions**—The synthesis and degradation of a polynucleotide chain by the RNA polymerase reaction (EC 2.7.7.6) differs from that of polynucleotide phosphorylase only in the use of ATP and PPi (Equation 2) rather than ADP and Pi (Equation 1). It is possible to calculate the value of the equilibrium constant (Equation 19) for the synthesis of p(A) by the RNA polymerase reaction from the \( K \) for the polynucleotide phosphorylase reaction (Equation 15) and the values of \( K \) previously determined under the same physiological conditions for the hydrolysis of ATP to ADP and Pi (EC 3.6.1.4) (Equation 16) (\( K = 1.23 \times 10^{12} \text{ M}^{-2} \text{ (as written)} \) (8), the hydrolysis of ATP to AMP and PPi (EC 3.6.1.8) (Equation 17) (\( K = 1.51 \times 10^{12} \text{ M}^{-2} \) (9), and the adenylyl kinase reaction (EC 2.7.4.3) (Equation 18) (\( K = 2.39 \text{ (as written)} \) (7)).
The value of $K$ for the RNA polymerase reaction, therefore, can be calculated to be $2.89 \times 10^{-7}$ M ($\Delta G^o = -38.91$ kJ/mol (9.30 kcal/mol)). The value at pH 7.0 of $K/[H^+]$ (2.89) is not very different from that of polynucleotide phosphorylase reaction ($K = 6.10$). The values for $K_{obs}$ under physiological conditions, and the sensitivity to the free $[Mg^{2+}]$ (Fig. 4) and the pH (Fig. 3) are significantly different, however. $K_{obs}$ for the RNA polymerase reaction is calculated from Equation 20. The definition of $f_{ATP}$ (Equation 22), is derived from Equation 21; the definition of $f_{PP_i}$ from Equations 6 and 23. The values for the constants used with Equations 22 and 23 have been cited and discussed previously (8, 9).

Values of $K_{obs}$ for the RNA polymerase reaction at 38°C and pH 7.0 are shown in Fig. 2. Values of $K_{obs}$ are calculated to be 149 at free $[Mg^{2+}] = 0$ ($\Delta G_{obs}^o = -12.9$ kJ/mol (3.09 kcal/mol)), 117 at free $[Mg^{2+}] = 10^{-3}$ M, and 268 at free $[Mg^{2+}] = 10^{-2}$ M. At pH 8.0, the corresponding values are 259 at free $[Mg^{2+}] = 0$, 840 at free $[Mg^{2+}] = 10^{-3}$ M, and 2423 at free $[Mg^{2+}] = 10^{-2}$ M.

Although the synthesis of poly(A) has been used in this case as a model for the RNA polymerase reaction, the value of $K$ would not be expected to be very different when the base composition of the polymer varies since there is no evidence for any but minimal difference among the bond energies of the various nucleoside triphosphates. There are, however, significant differences among the stacking energies of RNA and DNA of differing base pair compositions (21, 22) which would have to be taken into account in describing the overall thermodynamics of RNA or DNA synthesis.

The value found in the current work for the $K_{obs}$ of the RNA polymerase reaction is very close to that found by Kato et al. (23) under similar conditions (99 at pH 7.0, 30°C, and probably 8 mM total MgCl$_2$) for the DNA polymerase reaction as might be expected since the phosphate bonds are identical.

Equilibrium Constant of the Hydrolysis of Polynucleotide Polymer under Physiological Conditions—The equilibrium constant for the hydrolysis of ribonucleotide can be calculated from the reciprocal of the value of $K$ for the RNA polymerase reaction ($K = 3.46 \times 10^{-4}$) and the value of $K$ for Equation 17. The sum of these reactions is given by Equation 24.

$$\text{poly}(A)_{n+m} + H_2O \rightleftharpoons K_{obs} \text{poly}(A)_{n+m} + AMP^2- + H^+$$

Values of $K_{obs}$ are calculated using Equation 27 which is derived from Equations 25 and 26.

$$f_{AMP} = 1 + \frac{[H^+]}{K_{obs}[AMP]} + [Mg^{2+}]K_{obs}[AMP]$$

$$f_{ATP} = 1 + \frac{[H^+]}{K_{obs}[ATP]} + [Mg^{2+}]K_{obs}[ATP]$$

where

$$f_{ATP} = \frac{1 + [H^+]}{K_{obs}[ATP]} + [Mg^{2+}]K_{obs}[ATP]$$

$K_{obs}$ for hydrolysis of the polymer at free $[Mg^{2+}] = 0$ is $7.30 \times 10^4$ ($\Delta G_{obs}^o = -29.1$ kJ/mol (-6.93 kcal/mol)) and at free $[Mg^{2+}] = 10^{-2}$ M is $7.63 \times 10^5$ ($\Delta G_{obs}^o = -29.1$ kJ/mol (-6.94 kcal/mol)). $K_{obs}$ and $\Delta G_{obs}^o$ do not vary widely

For these calculations, potassium binding to AMP has been ignored in order to be internally consistent with previous data (9).
Equilibrium Constant of Polynucleotide Phosphorylase

FIG. 5. Effect of $pMg$ on the $K_{obs}$ for the hydrolysis of polynucleotide polymer. $K_{obs}$ was calculated using Equation 27. At 38°C, pH 7.0, $I = 0.25$ M, $[K^+] = 0$, $pMg = -\log [Mg^{2+}]$.

Fig. 6. Effect of pH on the $K_{obs}$ for the hydrolysis of polynucleotide polymer. Values were calculated for $K_{obs}$ at a fixed free $[Mg^{2+}]$ using Equation 27. At 38°C, $I = 0.25$ M, free $[Mg^{2+}] = 10^{-7}$ M.

with either Mg$^{2+}$ or pH in physiological ranges (Figs. 5 and 6). At free $[Mg^{2+}] = 10^{-7}$ M, $\Delta G_{obs}^0 = -29.9$ kJ/mol ($-7.14$ kcal/mol); at pH 6.5, $\Delta G_{obs}^0 = -27.1$ kJ/mol ($-6.48$ kcal/mol), and at pH 8.0, $\Delta G_{obs}^0 = -34.4$ kJ/mol ($-8.23$ kcal/mol).

Poly(A) chains are present in mammalian liver and the presence of polynucleotide phosphorylase in the mitochondria of mammals is well established although its significance is not entirely clear (3). It may be that polynucleotide phosphorylase serves entirely a degradative function since the $[2P:][2nu-

Acknowledgments—Appreciation is expressed to Dr. Howard Thanes (Department of Biomathematics, M.D. Anderson Hospital, Houston, TX 77030) for devising the computer program to analyze the data and to Ellen Eschenbrenner for assistance with some of the assays.

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