Potentiometric Studies of the Secondary Phosphate Ionizations of AMP, ADP, and ATP, and Calculations of Thermodynamic Data for the Hydrolysis Reactions*

ROGER C. PHILLIPS, S.J., PHILIP GEORGE, AND ROBERT J. RUTMAN

From the John Harrison Laboratory of Chemistry, University of Pennsylvania, Philadelphia

Received December 4, 1962

Values of pK' for the secondary phosphate ionization of AMP, ADP, and ATP have been measured over a range of ionic strength and temperature by a simple potentiometric procedure. To minimize complex formation, alkali metal ions in the phosphates were first replaced by the tetra-*n*-propyl ammonium ion by an exchange reaction on a resin column, and the corresponding bromide salt was used to vary the ionic strength. Extrapolation of pK' to zero ionic strength gave pK^0 values from which the standard free energies, enthalpies, and entropies of ionization were obtained. These data have been used to calculate better values for the various fundamental thermodynamic quantities that appear in the "chemical potential" and "isotherm" equations for the observed standard free energies of hydrolysis of AMP, ADP, and ATP.

Accurate pK values and thermodynamic data for the ionization of groups in biologically important molecules are essential for a complete understanding of the nature of the chemical driving force in their reactions. The role that ionization plays and its thermodynamic significance are particularly well exemplified by the reactions of the high-energy phosphate compounds. The salient points may be summarized as follows.

The different numbers of ionizing groups in reactants and products and their different pK values lead to a concomitant participation of H^+ in the reactions at most hydrogen ion concentrations, including the biological pH range about neutrality. It has become customary to use a standard free energy change evaluated without regard to the participation of H^+: this is the quantity which is of direct practical importance in biosynthesis since the reactions occur naturally in a buffered environment and are usually studied *in vitro* under similar conditions. This "observed" standard free energy change may be conveniently referred to as ΔF_{obs}.

Simple and useful as this practice may be, ΔF_{obs} is an unusual standard free energy change from the point of view of chemical thermodynamic data in general. The very omission of $[H^+]$ in its evaluation carries with it the implicit adoption of the hydrogen ion concentration of the experimental observations as the standard state for H^+ instead of the conventional 1 molal standard state. Over a range of pH the values of ΔF_{obs} thus involve a changing standard state for H^+, and, at a particular pH, the entropy change calculated from the general expression, $\Delta F = \Delta H - T\Delta S$, is not directly comparable with those for other ionic reactions since the partial molal entropies of ionic species are evaluated on the basis of the 1 molal standard state.

We have shown (George and Rutman, 1960) that ΔF_{obs} is related to the more familiar standard free energy change based upon the 1 molal standard state for H^+, denoted by $\Delta F'$, by a "chemical potential" equation (equation 1), where ϕ is the number of

$$\Delta F_{obs} = \Delta F' + \phi RT ln[H^+] \qquad (1)$$

moles of H^+ required as product to complete the stoichiometry of the reaction. ϕ is a complicated function of $[H^+]$ and the ionization constants for the reactants and products. This equation expresses quantitatively the chemical driving force in terms of the contribution from the prevailing $[H^+]$ and from the partial molal free energies of all the other species of reactants and products actually present at the pH concerned, *i.e.*,

$$\Delta F' = \Sigma \bar{F}^0 \text{ (products)} - \Sigma \bar{F}^0 \text{ (reactants)}.$$

Another equation can be derived which relates ΔF_{obs} to the standard free energy change for some reference reaction, for example the reaction at an experimental $[H^+]$ of 1 molal at which the majority of reactants and products would be un-ionized. In this "isotherm" equation (equation 2), f(H) is also

$$\Delta F_{obs} = \Delta F^0 + RT ln f(\text{H}) \qquad (2)$$

a complicated function of $[H^+]$ and the ionization constants for the reactants and the products, namely, the product of the fractions of the products present in the forms appropriate to 1 molal H^+, divided by the product of the fractions of the reactants present in the forms appropriate to 1 molal H^+. This equation is more useful in calculating the pH dependence of ΔF_{obs}: when its value at one pH is known, the value at another pH can readily be obtained.

Ionization constants for reactants and products are thus an integral part of both fundamental thermodynamic equations, and, for this reason, we have undertaken a potentiometric study of the secondary ionizations of the terminal phosphate groups of ATP, ADP, and AMP so that more reliable values for the various thermodynamic terms can be calculated for hydrolysis and group transfer reactions in the biological pH range.

For the determination of the pK values we were faced with a choice between a very accurate and rigorous method using a cell without liquid junction, and a less accurate and less rigorous method using glass electrode, calomel electrode, and pH meter. It was decided to test the accuracy of a very simple and rapid procedure based on the second method. A solution of the substance is titrated precisely to the half-way point of the secondary ionization, and, at a carefully controlled temperature, the pH is measured and the measurement repeated after each of a series of additions of neutral salt to increase the ionic strength over a suitable range. Both the base and neutral salt are chosen so that complex formation between their cation component and the anionic phosphate species is minimized. Similar measurements are then made at other temperatures.

The data obtained in this way for the second ionization of orthophosphoric acid are reported in detail below. It will be seen that the extrapolated pK value at zero ionic strength at 25° is within 0.02 units of the value obtained by Bates and Acree (1943)

---

* This work was undertaken as part of a research program on the thermodynamics of phosphate reactions, supported by USPHS grant AM-04764.

using a more accurate and rigorous method, and the limits of uncertainty in the enthalpy and entropy of ionization are 0.3 kcal/mole and 1 e.u. respectively. Thus, although in principle this simple procedure does not furnish true thermodynamic data because of the lack of strict correspondence between *p*H reading and −log (hydrogen ion activity), it would appear that the values obtained are sufficiently close to the true values for most calculations, and so we adopted the procedure for the other phosphate compounds. For very few biochemical reactions have thermodynamic data been established with this degree of accuracy, and, with the exception of a small number of structurally unrelated compounds, more accurate ionization studies have been carried out systematically only on a few types of substituted carboxylic acids and aliphatic amines.

Abbreviations and Symbols.—The usual abbreviations AMP, ADP, and ATP for adenosine mono-, di-, and triphosphate, respectively, and "ortho" for orthophosphate, are used when there is no need to specify the ionic species precisely. When this is essential amp^{2-}, adp^{3-}, and atp^{4-} are used to designate the nucleotide species in which the phosphate groups are fully ionized: likewise the completely un-ionized forms are represented by ampH$_2$, adpH$_3$, and atpH$_4$, and the partially ionized forms by ampH^{1-}, adpH$_2{}^{1-}$, atpH$_3{}^{1-}$, *etc.*

The abbreviations tma, tea, and tpa stand for the tetramethyl, tetraethyl, and tetra-*n*-propyl ammonium cations respectively.

The symbols pK' and pK^0 refer, as usual, to the negative logarithm of the ionization constant at finite ionic strengths and at zero ionic strength: the standard free energy, enthalpy, and entropy of ionization are denoted by $\Delta F_i{}^0$, $\Delta H_i{}^0$, and $\Delta S_i{}^0$ respectively.

EXPERIMENTAL

The compounds were obtained from Sigma Chemical Company in the following forms: monosodium salt of orthophosphoric acid, free acid of AMP extracted from yeast, monosodium salt of ADP extracted from horse muscle, and disodium salt of ATP extracted from rabbit muscle. The Sigma Company assay for inorganic phosphate indicated that the ATP and ADP were 99% and 98–100% pure, respectively. The crystalline AMP melted in the melting point range of the pure compound (196–200°). Titration curves also indicated high purity. The AMP and ATP were used within a month of arrival; ADP was used immediately because it decomposes to the extent of 1–3% per month below 0°.

Since these phosphate compounds form complexes with all cations of significant charge density, *e.g.*, metal ions, it was necessary to replace such ions in the system with an electrostatically weak cation before carrying out the measurements. The tetra-*n*-propyl ammonium ion was chosen for this purpose, and replacement was achieved by ion exchange in the following way.

A column of 50W-X8 Dowex resin 8 mm in diameter and 50 cm long was converted to the acidic form with 3 M HCl. An excess of 10% aqueous tetra-*n*-propyl ammonium hydroxide was then allowed to run through the column several times to give the tpa form of the resin, *i.e.*, $RSO_3N(n\text{-}C_3H_7)_4$. This indirect method for making the tpa resin was adopted because the tpa ion will not readily exchange with other cations bound to the resin on account of its very weak electrostatic properties: hence it has to be introduced by way of an exchange reaction in which the other product has a very favorable free energy of formation—in this case water, by neutralization. A liter of 0.5 mM solution of each of the sodium salts of the phosphates was then allowed to run through a column of the tpa resin 8 mm in diameter and 7.5 cm in length. Flame tests showed that no sodium was present in the eluent. The solutions were stored at 5–10°.

Fifty-ml samples of these 0.5 mM salt solutions were titrated with 4.00 mM tpaOH with use of an E336 Metrohm Potentiograph. The inflection points in the titration curves corresponding to the neutralization of the secondary phosphate hydrogen were clear and reproducible to within 0.5% and were taken to be the equivalence points (Mattock, 1961). The titration curves also indicated that partial neutralization of the remaining primary phosphate group of ADP and ATP had occurred in the exchange column, making a comparison with the titration of a sample from the original solution necessary to determine the amount of tpaOH necessary to bring the tpa phosphate solution to the half-neutralization point of the secondary phosphate ionization. It was assumed that the concentration of phosphate did not change in the column.

Fifty-ml samples of these stock 0.5 mM tpa phosphate solutions were then placed in a temperature-controlled vessel of a Beckman Model GS *p*H meter, which had been calibrated with three standard buffers, boric acid–potassium chloride–sodium hydroxide buffer (*p*H 9.00 ± 0.02), monopotassium phosphate–sodium hydroxide buffer (*p*H 7.00 ± 0.02), and potassium biphthalate buffer (*p*H 4.00 ± 0.02). The glass and saturated calomel electrodes of the *p*H meter had previously been allowed to come to thermal equilibrium for a day in a water bath at the temperature to be used (Wingfield and Acree, 1937). The solutions were flushed out with nitrogen and held under a nitrogen atmosphere to eliminate carbon dioxide absorption. The exact quantity of base was added to bring the secondary phosphate ionization to the half-neutralization point, and the *p*H was recorded. Twelve to fifteen increments of tpaBr were then added, and the *p*H was again recorded after each addition. This procedure was carried out in triplicate at 10°, 25°, and 38° for each solution.

RESULTS

The *p*H of the half-neutralized solution was taken to be equal to pK' at each ionic strength. In some cases this is a very good assumption while in others it is a poor assumption. The exact equation for K' must be consulted in each case. This equation is given by Kumler (1938) (equation 3), where M is

$$K' = \frac{[H^+]\left[m + [H^+] - \dfrac{kw}{[H^+]}\right]}{M - \left[m + [H^+] - \dfrac{kw}{[H^+]}\right]} \qquad (3)$$

the concentration of acid before the beginning of the titration, and m the concentration of anion released by added base. The second term in the expression for the anion concentration in square brackets takes account of the ionization of unneutralized acid and is significant only when $pK' < 4$. The third term in the same expression takes account of the hydrolysis of the anion produced in the titration and becomes significant only when $pK' > 10$. The assumption that *p*H (half titration) = pK' is thus fully justified for the present titrations since in all cases $pK' \approx 7$.

The values of pK' for each compound at the three temperatures were plotted against $\sqrt{\mu}$ and extrapolated to zero ionic strength to get thermodynamic pK values, as illustrated in Figure 1 for the data at 25°. (The μ term is, of course, accurately known over the entire range of measurement, since the concentrations of ions produced in the titration are accurately known.) When these pK^0 values are plotted against $1/T$, as shown in Figure 2, it appears that a linear relationship holds within experimental error over the temperature range concerned. Values for the enthalpy of ionization, ΔH_i^0, can thus be calculated from the slopes according to the Van't Hoff Isochore. These values are listed in Table I together with the standard free energies and entropies of ionization calculated from the equations $\Delta F_i^0 = -RTlnK^0$, and $\Delta F_i^0 = \Delta H_i^0 - T\Delta S_i^0$.

TABLE I

THERMODYNAMIC DATA FOR THE SECONDARY PHOSPHATE IONIZATION OF ORTHOPHOSPHATE, AMP, ADP, AND ATP AT 25°

Compound	$pK^0 \pm$ 0.01	$\Delta F_i^0 \pm$ 0.02 kcal/mole	$\Delta H_i^0 \pm$ 0.30 kcal/mole	$\Delta S_i^0 \pm$ 1.0 e.u.
H_3PO_4	7.18	9.80	+0.76	−30.4
AMP	6.67	9.10	−0.85	−33.4
ADP	7.20	9.80	−1.37	−37.4
ATP	7.68	10.48	−1.68	−40.7

It can be seen from Figure 1 that, as in the case of orthophosphate, pK' for AMP, ADP, and ATP becomes a linear function of $\sqrt{\mu}$ only at ionic strengths less than about 0.01. However the values fit the simple empirical equation, (4), quite closely over the entire

$$pK' = pK^0 - a\sqrt{\mu} + b\mu \qquad (4)$$

range of ionic strength employed, $0 < \mu < 0.2$, and individual equations for all four compounds at 25° and 37° are given in Table II, where the constants a and b have been determined graphically. The present values of pK' and pK^0 agree quite closely in most instances with values reported in the literature as shown in Table III; but in some cases a strict comparison cannot be made because the tetramethyl and tetraethyl ammonium salts were used.

The good agreement with Bates and Acree's (1943) value for pK^0 of orthophosphoric acid, obtained with a cell without liquid junction, was noted in the introduction, and suggests that the pK^0 values for the other compounds are equally reliable to within the experimental uncertainty of ± 0.01 units. For this same ionization, Elliot *et al.* (1958) give equation (5) at

$$pK_2' = 7.198 - 1.566\sqrt{\mu} + 0.937\mu \qquad (5)$$

38°, based on potentiometric measurements in the rather high ionic strength range 0.1 to 0.5. The coefficient "b," 0.937, is appreciably different from the value of 1.22 obtained in the present work at 37°, but this can be attributed to their use of sodium salts.

The values for the activity coefficients of ATP determined by Snell (1955) cannot readily be compared with the present data because they relate to mixtures of $atpH^{-3}$ and atp^{-4} at different concentrations in the presence of Na^+ and K^+ ions rather than the electrostatically weak cation, tpa.

DISCUSSION

Thermodynamic Data for the Secondary Phosphate Ionizations.—For the experimental procedure employed, the relationship between pK' at finite ionic strength and its extrapolated value at zero ionic strength, pK^0, is given by equation (6), where γ_A and γ_{HA} are the

$$pK' = pK^0 + \log(\gamma_A/\gamma_{HA}) \qquad (6)$$

activity coefficients of conjugate base and acid species respectively. The term γ_{H^+} does not appear because, as indicated by the close agreement with the pK^0 value of orthophosphate obtained by Bates and Acree (1943) using a cell without liquid junction, the approximation that pH reading is equal to −log hydrogen ion activity is acceptable. This approximation would,

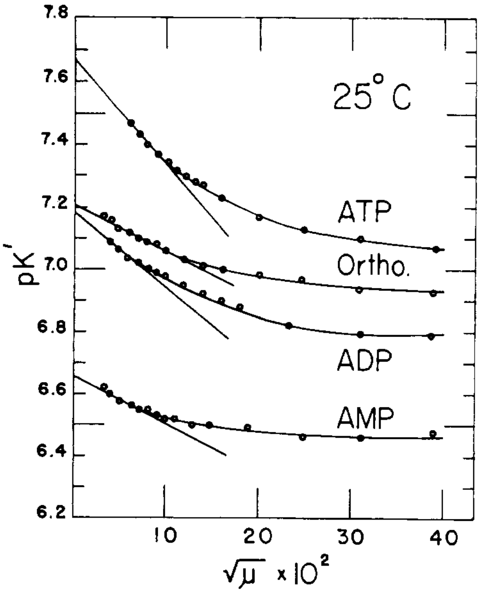

FIG. 1.—pK' for the secondary phosphate ionizations of AMP, ADP, ATP, and orthophosphoric acid plotted against $\sqrt{\mu}$: temperature 25°, titration with tetra-n-propyl ammonium hydroxide, and ionic strength adjusted with the corresponding bromide. The limiting slopes drawn in have the following values: AMP 1.5, ADP 2.5, ATP 3.5, and orthophosphate 1.5.

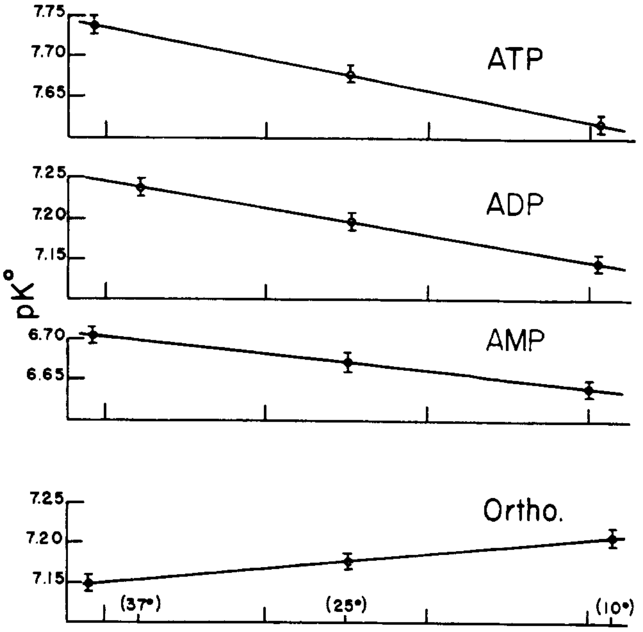

FIG. 2.—pK^0 for the secondary phosphate ionization of ATP, ADP, AMP, and orthophosphoric acid plotted against $1/T$.

TABLE II

pK' FUNCTIONS FOR THE SECONDARY PHOSPHATE IONIZATION OF ORTHOPHOSPHATE, AMP, ADP, AND ATP, WITH TETRA-*n*-PROPYL AMMONIUM ION AS THE CATION

	25°	37°
pK' H_3PO_4 ± 0.03	$7.18 - 1.52\sqrt{\mu} + 1.96\mu$	$7.15 - 1.56\sqrt{\mu} + 1.22\mu$
pK' AMP ± 0.03	$6.67 - 1.52\sqrt{\mu} + 2.52\mu$	$6.70 - 1.56\sqrt{\mu} + 2.28\mu$
pK' ADP ± 0.04	$7.20 - 2.54\sqrt{\mu} + 3.84\mu$	$7.24 - 2.60\sqrt{\mu} + 3.34\mu$
pK' ATP ± 0.04	$7.68 - 3.56\sqrt{\mu} + 4.90\mu$	$7.73 - 3.64\sqrt{\mu} + 4.25\mu$

TABLE III

COMPARISON OF pK DATA FOR SECONDARY PHOSPHATE IONIZATIONS

	Literature	This Paper
pK^0 H_3PO_4 (extrapolated)	7.20[a]	7.18
pK' H_3PO_4 in 0.2 molar tpaBr	6.92[b]	6.93
pK' AMP in 0.2 molar tpaBr	6.45[b]	6.47
pK' ADP in 0.2 molar tpaBr	6.68[b]	6.78
pK' ADP in 0.22 molar teaBr	6.65[c]	6.78
pK^0 ADP (calculated from data with tma_2 ADP and tma_3 ADP salts)	7.00[d]	7.20
pK' ATP in 0.2 molar tpaBr	6.95[b]	7.05
pK' ATP in 0.15 molar teaBr	6.98[c]	7.07

[a] Bates and Acree (1943). [b] Smith and Alberty (1956) [c] Melchior (1954). [d] Izatt and Christensen (1962).

of course, then depend also on the assumptions made by Bates in defining the National Bureau of Standards pH scale (Mattock, 1961).

According to the simple Debye-Hückel theory the relationship between pK' and pK^0 takes the form of equation (7), the slope of the pK' *vs.* $\sqrt{\mu}$ plot in

$$pK' = pK^0 - 0.509(Z_A^2 - Z_{HA}^2)\sqrt{\mu} \qquad (7)$$

the limit as $\mu \rightarrow 0$ being determined by the change in charge that accompanies the ionization.

For the secondary phosphate ionization of AMP, ADP, and ATP the change in total charge is respectively from −1 to −2, −2 to −3, and −3 to −4: the limiting slopes according to equation (7) are thus 1.5, 2.5, and 3.5, respectively, at 25°. As can be seen in Figure 1, the slope for AMP is very close to 1.5, like that for the orthophosphate ionization, which has the same charge change. Moreover the limiting slopes for ADP and ATP are very close to the theoretical values of 2.5 and 3.5 respectively. This has been taken to be indicative of Debye-Hückel behavior in the very dilute region. Accordingly, the extrapolations and the "*a*" constants in the pK' expressions of Table II have been refined to the extent of ±0.02 units with Debye-Hückel theory. This assumption should be kept in mind in evaluating the ±0.01 uncertainties given in Table I.

This close agreement is unexpected because the complicated chemical structures of AMP, ADP, and ATP are in sharp contrast to the simple electrostatic model upon which the Debye-Hückel theory is based. In particular, in the case of ADP and ATP, the charge is distributed over two and three phosphate groups, respectively, and for the terminal group, where the ionization occurs, the change in charge is from −1 to −2, like that for AMP and orthophosphate. A factor which might contribute to this simulated centralization of charge on these bulky anionic species is the large size of the cation present, namely, the tetra-*n*-propyl ammonium ion. Nevertheless, although the charges on the phosphate chain are not concentrated at a single point in space, neither are they completely isolated from each other, as indicated by the fact that the charges on adjacent phosphate groups influence the entropy change for the secondary ionization of the terminal group. Reference to Table I shows that, while the charge change on the terminal group in ADP and ATP is from −1 to −2, the entropies of ionization, −37 and −41 e.u., are appreciably more negative than the values for the corresponding ionization in AMP and orthophosphate, *i.e.*, −34 and −30 e.u. However, further comparisons show that the values for ADP and ATP by no means reflect the change in total ionic charge in its entirety. The value of −37 e.u. for ADP, total charge change from −2 to −3, is to be compared with that for the final ionization of orthophosphoric acid, $HPO_4^{2-} \rightarrow PO_4^{3-} + H^+$, *i.e.*, −43 e.u. Furthermore, the value of −41 e.u. for ATP, total charge change from −3 to −4, is even less negative than the value for the −2 to −3 charge change for orthophosphate. If there were an oxyanion ionization, $HXO_4^{3-} \rightarrow XO_4^{4-} + H^+$, then, judging from the trend in ΔS_i^0 for the successive ionizations of orthophosphoric acid, $H_3PO_4 \xrightarrow{-20.7 \text{ e.u.}} H_2PO_4^{1-} \xrightarrow{-29.9 \text{ e.u.}} HPO_4^{2-} \xrightarrow{-43.4 \text{ e.u.}} PO_4^{3-}$, ΔS_i^0 for this ionization would be much more negative, probably about −60 e.u.

These new data call for a reappraisal of the views based on pK' values which were expressed in a previous publication (George and Rutman, 1960), namely that "... in the phosphate anhydrides a single negative charge on one phosphate group scarcely affects the (first or) second ionization of the adjacent phosphate group," and "the approximate constancy of the pK for the second ionization does not result from gross compensation between the ΔH and ΔS terms." For the series AMP, ADP, and ATP the pK^0 values for the secondary ionization span a slightly greater range than the pK' values at $\mu = 0.2$, namely, about 1 pH unit compared to 0.4 units: moreover there is a perceptible compensation between ΔH_i^0 and ΔS_i^0. This effect was masked in the pK' data because of the manner in which the enthalpy of ionization varies with ionic strength. For the successive addition of the phosphate residues to AMP the increments in ΔH_i^0 are −0.5 and −0.3 kcal/mole, and the increments in ΔS_i^0 are −3 and −4 e.u. Were it not for the experimental uncertainty attached to the values, these increments in themselves would suggest roughly linear relationships. The trend in the entropy values has already been discussed, and the trend in the enthalpies of ionization is no less striking. The secondary phosphate ionization becomes progressively weaker from AMP to ATP because of the preponderance of the increasingly unfavorable (negative) entropy term: the enthalpy term, in its compensatory role, becomes more favorable (negative).

This behavior is contrary to the simple generalization that acid strength parallels the heat of neutralization, which is true for certain more common acid species such as the aliphatic carboxylic acids as compared to the aliphatic amines, although it is to be noted that these classes of acids differ markedly in structure and charge type. Somewhat analogous behavior is shown by the successive ionizations of the —COOH groups in citric and *iso*-citric acids, in which the decrease in acid strength is due to the more adverse entropies of ionization, the enthalpies of ionization becoming more favorable (Bates and Pinching, 1949; Datta and Grzybowski, 1961). A consideration of the Born-Haber cycle for the ionization process indicates that neither ΔH_i^0 nor ΔS_i^0 should necessarily be expected to govern acid strength. In fact, from this point of view, it is rather surprising that structural factors such as the addition of phosphate groups in going from AMP to ATP and the accumulation of negative charge on citrate influence the ionization characteristics to such a small extent. It would seem that adjacent parts of these molecules are pretty much insulated from each other as far as the properties that determine the magnitude of the relevant thermodynamic terms are concerned.

In this context it is interesting to compare the thermodynamic data for the secondary phosphate ionization of orthophosphoric acid with the values obtained for AMP and those for two other biochemically important monoesters, glucose-1-phosphate and glycerol-2-phosphate (Ashby *et al.*, 1954, 1955). As can be seen in Table IV the values for all three monoesters are very similar. The ionization is a little stronger in the monoesters, $\delta pK^0 \approx 0.6$, an effect that is due to the more favorable values of ΔH_i^0, which are small and negative in contrast to the small positive value for the parent acid. This effect, however, is partially offset by the more favorable value of ΔS_i^0. Hence esterification has only a slight influence on the secondary phosphate ionization, and the variation in the organic structure of these monoesters has even less influence, which again suggests that there can be little interaction between the phosphate group and the remainder of the molecules in compounds of this nature.

TABLE IV

COMPARISON OF THERMODYNAMIC DATA AT 25° FOR THE SECONDARY PHOSPHATE IONIZATIONS OF ORTHOPHOSPHORIC ACID AND SOME MONOESTERS

Compound	pK^0	ΔF_i^0 kcal/mole	ΔH_i^0 kcal/mole	ΔS_i^0 e.u.
H_3PO_4	7.18	9.80	+0.76	−30.4
AMP	6.67	9.10	−0.85	−33.4
Glycerol-2-phosphate	6.65[a]	9.07	−0.41	−31.8
Glucose-1-phosphate	6.50[b]	8.87	−0.43	−31.2

[a] Ashby *et al.* (1954). [b] Ashby *et al.* (1955).

Calculation of ϕ and f(H) for Hydrolysis and Transfer Reactions of AMP, ADP, and ATP.—As explained in the introduction, ϕ and f(H) are complicated functions of H$^+$ and the ionization constants of the reactants and products that enter into the two thermodynamic equations which express the "observed" standard free energy change in terms of more fundamental thermodynamic quantities.

Taking the hydrolysis of ATP yielding ADP and orthophosphate to illustrate the various relationships, the reaction at any pH is represented by equation (8).

$$\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{Ortho} + \phi\text{H}^+ \qquad (8)$$

The "observed" standard free energy change is given by equation (9), where $[\text{ADP}]_e$, $[\text{Ortho}]_e$, and $[\text{ATP}]_e$

$$\Delta F_{\text{obs}} = -RTln\left\{\frac{[\text{ADP}]_e \times [\text{Ortho}]_e}{[\text{ATP}]_e}\right\} \qquad (9)$$

denote the total concentrations of the ADP, orthophosphate, and ATP species, respectively, at equilibrium. The more fundamental standard free energy change, $\Delta F'$, with 1 molal as the standard state for H$^+$, is given by equation (10).

$$\Delta F' = -RTln\left\{\frac{[\text{ADP}]_e \times [\text{Ortho}]_e \times [\text{H}^+]^\phi}{[\text{ATP}]_e}\right\} \qquad (10)$$

ϕ, which is given by equation (11),

$$\phi = \frac{[\text{adpH}_2{}^{1-}] + 2[\text{adpH}^{2-}] + 3[\text{adp}^{3-}]}{[\text{total ADP}]} + \frac{[\text{H}_2\text{PO}_4{}^{1-}] + 2[\text{HPO}_4{}^{2-}] + 3[\text{PO}_3{}^{3-}]}{[\text{total orthophosphate}]} - \frac{[\text{atpH}_3{}^{1-}] + 2[\text{atpH}_2{}^{2-}] + 3[\text{atpH}^{3-}] + 4[\text{atp}^{4-}]}{[\text{total ATP}]} \qquad (11)$$

is calculated from equation (12), where K_1, K_2, *etc.*

$$\phi = \left\{\frac{K_1\text{H}^2 + 2K_1K_2\text{H} + 3K_1K_2K_3}{\text{H}^3 + K_1\text{H}^2 + K_1K_2\text{H} + K_1K_2K_3}\right\}_{\text{ADP}} + \left\{\frac{K_1\text{H}^2 + 2K_1K_2\text{H} + 3K_1K_2K_3}{\text{H}^3 + K_1\text{H}^2 + K_1K_2\text{H} + K_1K_2K_3}\right\}_{\text{orthophosphate}} - \left\{\frac{K_1\text{H}^3 + 2K_1K_2\text{H}^2 + 3K_1K_2K_3\text{H} + 4K_1K_2K_3K_4}{\text{H}^4 + K_1\text{H}^3 + K_1K_2\text{H}^2 + K_1K_2K_3\text{H} + K_1K_2K_3K_4}\right\}_{\text{ATP}} \qquad (12)$$

are the successive ionization constants of ADP, orthophosphate, and ATP specified by the respective subscripts outside the large brackets.

Taking the reaction between the species present at an experimental [H$^+$] of 1 molal as the reference reaction, *i.e.*, involving predominantly un-ionized species (equation 13), the corresponding standard

$$\text{atpH}_4 + \text{H}_2\text{O} \rightleftharpoons \text{adpH}_3 + \text{H}_3\text{PO}_4 \qquad (13)$$

free energy change, ΔF^0, is given by equation (14).

$$\Delta F^0 = -RTln\left\{\frac{[\text{adpH}_3]_e \times [\text{H}_3\text{PO}_4]_e}{[\text{atpH}_4]_e}\right\} \qquad (14)$$

The function f(H) relating ΔF_{obs} and ΔF^0 through the isotherm equation (2) is thus given by equation (15)

$$f(\text{H}) = \frac{[\text{adpH}_3]_e}{[\text{ADP}]_e} \times \frac{[\text{H}_3\text{PO}_4]_e}{[\text{Ortho}]_e} \div \frac{[\text{atpH}_4]_e}{[\text{ATP}]_e} \qquad (15)$$

and is calculated from equation (16).

$$f(\text{H}) = \left\{\frac{\text{H}^3}{\text{H}^3 + K_1\text{H}^2 + K_1K_2\text{H} + K_1K_2K_3}\right\}_{\text{ADP}} \times \left\{\frac{\text{H}^3}{\text{H}^3 + K_1\text{H}^2 + K_1K_2\text{H} + K_1K_2K_3}\right\}_{\text{orthophosphate}} \div \left\{\frac{\text{H}^4}{\text{H}^4 + K_1\text{H}^3 + K_1K_2\text{H}^2 + K_1K_2K_3\text{H} + K_1K_2K_3K_4}\right\}_{\text{ATP}} \qquad (16)$$

In order to establish the general trends in $\phi RTln$H, $\Delta F'$, $RTln$(H), and ΔF^0, approximate calculations were reported in a previous paper (George and Rutman, 1960) which were based on an assortment of ionization constants, namely, thermodynamic values for the pK's of orthophosphoric acid, finite ionic strength values for pK_2, pK_3, and pK_4 of AMP, ADP, and ATP, respectively, and assumed thermodynamic values for the primary phosphate ionizations of 1 for AMP, 1 and 2 for ADP, and 1, 1, and 2 for ATP. Experimental values for the primary ionizations are still

TABLE V

THERMODYNAMIC DATA IN KCAL/MOLE FOR ATP, ADP, AND AMP REACTIONS AT pH 7.5 AND 25° BASED ON pK^0 VALUES FOR THE SECONDARY PHOSPHATE IONIZATIONS OF ATP, ADP, AND AMP, COMPARED WITH SIMILAR DATA (IN BRACKETS) BASED ON pK' VALUES AT $\mu = 0.2$

Reaction	ΔF_{obs}	ϕ	$\phi RTln$H	$\Delta F'$	$RTlnf$(H)	ΔF^0
ATP + $H_2O \rightleftharpoons$ ARP + Ortho	−8.3	+0.92 (+0.68)	−9.4 (−7.0)	+1.1 (−1.3)	+0.6 (+0.6)	−8.9 (−8.9)
ADP + $H_2O \rightleftharpoons$ AMP + Ortho	−7.8	+0.87 (+0.67)	−8.9 (−6.9)	+1.1 (−0.9)	−1.0 (−0.8)	−6.8 (−7.0)
AMP + $H_2O \rightleftharpoons$ Adenosine + Ortho	∼−3.0	−0.21 (−0.31)	+2.2 (+3.2)	∼−5.2 (∼−6.2)	+2.1 (+2.9)	∼−5.1 (∼−5.9)
ATP + AMP $\rightleftharpoons$ 2 ADP	−0.5	+0.05 (+0.01)[a]	−0.5 (−0.1)	0 (−0.4)	+1.6 (+1.4)	−2.1 (−1.9)

[a] A misprint in the previous paper (George and Rutman, 1960) gave +0.07.

lacking, but, with the experimental values established above for the secondary ionizations, more reliable calculations can now be presented. In this respect it is important to realize that exact values for the primary ionizations are not essential for accurate estimations of ϕ throughout the biological pH range because all the primary ionizations are complete at a much lower pH. Likewise reliable estimates of ΔF_{obs} at neighboring pH values can be made from that at a given pH value within the biological pH range by use of equation (2). It is only in quite acidic solutions of $p\text{H} < 3$ that the exact values of the primary pK's become critical; even so it is unlikely, in view of the compensation between the primary ionizations on reactants and products, that the values calculated for ΔF^0 are in error by much more than 1 kcal/mole. As in the previous calculations the effect due to the ionization of the adenine $NH_3{}^+$ group in ATP, ADP, and AMP has been neglected because the variation in pK is quite small, the values being 4.0, 3.94, and 3.74, respectively, at 25° and $\mu = 0.2$ (Alberty *et al.*, 1951).

The values of ϕ, $\phi RTln$H, and $RTlnf$(H) incorporating the new thermodymamic values for the secondary phosphate ionization of AMP, ADP, and ATP, together with the previous values for their primary phosphate ionizations and for orthophosphate, are listed in Table V; the values of $\Delta F'$ and ΔF^0 have been calculated with use of the previous values adopted for ΔF_{obs}, so that the effect on $\Delta F'$ and ΔF^0 of changing the secondary phosphate pK's to the thermodynamic values can be assessed. It can be seen that although the effect is quite perceptible—up to 2.4 kcal/mole in $\phi RTln$H, and up to 0.8 kcal/mole in ΔF^0—the general trends in the values for the "high-energy" compounds ATP and ADP, compared to those for the "low-energy" compound AMP, are quite unaltered. In particular, the hydrogen ion driving force $\phi RTln$H plays a slightly less unfavorable role in the hydrolysis of AMP, while on the other hand it plays a rather more favorable role in the hydrolysis of ADP and ATP. For these two latter reactions, $\Delta F'$ at pH 7.5 actually reaches positive, *i.e.*, unfavorable, values.

Estimation of the Standard Free Energy Change for the Hydrolysis of ATP Yielding ADP and Orthophosphate at Zero Ionic Strength.—An examination of the expressions for the equilibrium constants shows that the standard free energy change at $\mu = 0$ can be calculated from the value at finite ionic strength from equation (17). Except in very acidic solution ATP always carries a higher negative charge than either ADP or orthophosphate, and so, for the fairly dilute solutions usually employed with an ionic strength of about 0.2, γ_{ADP}, γ_{ortho}, and γ_{ATP} are fractions, but γ_{ATP} is the smallest. Some compensation within the activity coefficient term is thus to be expected.

$$(\Delta F_{obs})_{\mu=0} = (\Delta F_{obs})_{\text{finite } \mu} - RTln\left[\frac{\gamma_{ADP}\cdot\gamma_{ortho}}{\gamma_{ATP}}\right] \qquad (17)$$

To estimate $(\Delta F_{obs})_{\mu=0}$ for the ATP hydrolysis the newer value of −9.1 kcal/mole at pH 7.5, 25°, and $\mu = 0.2$ (Benzinger *et al.*, 1959) will be adopted instead of the previous value of −8.3 kcal/mole for the same conditions calculated from Benzinger and Hem's (1956) data. However the calculations cannot be made using the data directly because at pH 7.5 both reactant and products are present as mixtures of ionic species—ATP about 60% atpH^{3-} and 40% atp^{4-}, ADP and orthophosphate about 34% adpH^{2-} and $H_2PO_4{}^{1-}$, and 66% adp^{3-} and $HPO_4{}^{2-}$—with the consequence that it is difficult to assign activity coefficients. But, using the isotherm equation, it is an easy matter to determine the value of ΔF_{obs} appropriate to pH 5, at which pH the hydrolysis involves predominantly single ionic species (equation 18),

$$\text{atpH}^{3-} + H_2O \rightleftharpoons \text{adpH}^{2-} + H_2PO_4{}^{1-} \qquad (18)$$

and the activity coefficient term takes on the simple form $(\gamma_{\text{adpH}^{2-}} \times \gamma_{H_2PO_4{}^{1-}})/\gamma_{\text{atpH}^{3-}}$. Now as a first approximation it can be assumed that $\gamma_{\text{adp}^{3-}} = \gamma_{\text{atpH}^{3-}}$. The total charges on the phosphate moieties are identical, and if one bears in mind the similar over-all sizes of the molecules and the finding that the Debye-Hückel limiting law is obeyed in dilute solution, this would appear to be a reasonable assumption. It is thus possible to write the activity coefficient term as $(\gamma_{\text{adpH}^{2-}} \times \gamma_{H_2PO_4{}^{1-}})/\gamma_{\text{adp}^{3-}}$, where $\gamma_{\text{adpH}^{2-}}/\gamma_{\text{adp}^{3-}}$ can be obtained from the appropriate plot of pK' against $\sqrt{\mu}$. Not only is temperature an important parameter but also the nature of the salt used to establish the ionic strength. In additional experiments, not reported above, it was found that pK' decreases more rapidly as μ is increased when Na^+ salts are used, owing to specific complex formation (see also Melchior, 1954; Smith and Alberty, 1956). However, the way in which the glutamine synthetase and glutamine hydrolysis data used to calculate the value for the observed free energy change were obtained justifies using the plot of pK' against $\sqrt{\mu}$ in Figure 1 to estimate the required activity coefficient ratio.

Very briefly, four sets of data are involved. First, in the determination of the equilibrium constant for the glutamine synthetase reaction (Levintow and Meister, 1954) (equation 19) alkali metal ions made a

$$\text{ATP + ammonia + glutamate} \rightleftharpoons \text{ADP + orthophosphate + glutamine} \qquad (19)$$

significant contribution to the over-all ionic strength of about 0.2; but Mg^{2+} ions were also present in such a concentration as to complex the ATP and ADP completely, so the concentrations of free ATP and ADP do not appear in the expression for the relevant equilibrium constant. Secondly, ATP and ADP do not participate in the hydrolysis of glutamine, so the nature of the cations present in this system is not at issue (Benzinger *et al.*, 1959). Thirdly and fourthly, in the determination of the affinity constants for the formation of the Mg^{2+} complexes and ATP and ADP, the concentration of Na^+ and K^+ ions was kept low and the ionic strength was established with tributylethylammonium bromide (Burton, 1959). Thus the crucial equilibrium constants that contain the concentration of free ATP and ADP, respectively, were measured in an ionic medium in which an electrostatically weak cation very similar to tpa predominanted, hence the value of $\gamma_{adpH^{2-}}/\gamma_{adp^{3-}}$ at $\mu = 0.2$ can appropriately be obtained from the plot in Figure 1 as 2.63 (*i.e.*, antilog 0.42).

With regard to the activity coefficient for $H_2PO_4^{1-}$, only the mean values for Na^+ and K^+ salts are available. Specific complex formation with $H_2PO_4^{1-}$ is far weaker than with ATP and ADP, and, since the mean activity coefficients for the Cl^-, Br^-, and SO_4^{2-} salts of Na^+, K^+, and the electrostatically weaker cation NH_4^+ are very similar in magnitude, a reasonable estimate for $\gamma_{H_2PO_4^{1-}}$ can be obtained by averaging the mean values for NaH_2PO_4 and KH_2PO_4. The value 0.66 at $\mu = 0.2$ and 25° is obtained from the data reported by Stokes (1945) and Scatchard and Breckenridge (1954).

As anticipated, there is quite an appreciable compensation within the activity coefficient term: $(\gamma_{adpH^{2-}} \times \gamma_{H_2PO_4^{1-}})/\gamma_{adp^{3-}} = 2.63 \times 0.66 = 1.74$; and the free energy increment, $RTln\ [\gamma_{ADP} \times \gamma_{ortho}]/\gamma_{ATP}$, in equation (17) thus makes a relatively small contribution of 0.33 kcal/mole at *p*H 5, $\mu = 0.2$ and 25°.

The remaining terms required for the calculation of $(\Delta F_{obs})_{\mu=0}$ for the ATP hydrolysis are the values of $RTln(H)$ at *p*H 5 and 7.5 for $\mu = 0$ and $\mu = 0.2$ at 25°. These have been evaluated as +1.53 and +0.55 at $\mu = 0$, and +1.52 and +0.31 at $\mu = 0.2$, respectively, with use of the pK' values interpolated from Figure 1, ATP 7.06, ADP 6.78, and orthophosphate 6.92. For the primary phosphate ionizations the values previously adopted for pK^0 were also used for pK' at $\mu = 0.2$ because the corrections in the case of ATP and ADP cannot be made with any certainty, and in any event, as noted above, extensive compensation occurs due to the form of equation (16) for $f(H)$.

The data having been assembled, the steps in the estimation of $(\Delta F_{obs})_{\mu=0}$ at *p*H 7.5 can be summarized as follows:

(i) With equation (2), $(\Delta F^0)_{\mu=0.2}$ is calculated from $(\Delta F_{obs})_{\mu=0.2}$ at *p*H 7.5, $-9.1 = (\Delta F^0)_{\mu=0.2} + 0.31$, *i.e.*, $(\Delta F^0)_{\mu=0.2} = -9.41$.

(ii) With equation (2), $(\Delta F_{obs})_{\mu=0.2}$ at *p*H 5 is calculated from $(\Delta F^0)_{\mu=0.2}$ at *p*H 7.5, $(\Delta F_{obs})_{\mu=0.2} = -9.41 + 1.52 = -7.89$.

(iii) With equation (17), $(\Delta F_{obs})_{\mu=0}$ at *p*H 5 is calculated from $(\Delta F_{obs})_{\mu=0.2}$ at *p*H 5, $(\Delta F_{obs})_{\mu=0} = -7.89 - 0.33 = -8.22$.

(iv) With equation (2), $(\Delta F^0)_{\mu=0}$ is calculated from $(\Delta F_{obs})_{\mu=0}$ at *p*H 5, $-8.22 = (\Delta F^0)_{\mu=0} + 1.53$, *i. e*, $(\Delta F^0)_{\mu=0} = -9.75$.

(v) With equation (2), $(\Delta F_{obs})_{\mu=0}$ at *p*H 7.5 is calculated from $(\Delta F^0)_{\mu=0}$, $(\Delta F_{obs})_{\mu=0} = -9.75 + 0.55 = -9.20$

The second decimal place has been retained for purpose of calculation only, and in Table VI the zero ionic strength values for ΔF^0 and ΔF_{obs} at *p*H 7.5 have been rounded off to −9.8 and −9.2 kcal/mole respectively. It is interesting to note what a small difference, 0.1 kcal/mole, the extrapolation to zero ionic strength has made in the value of ΔF_{obs} at *p*H 7.5.

TABLE VI

THERMODYNAMIC DATA FOR THE HYDROLYSIS OF ATP YIELDING ADP AND ORTHOPHOSPHATE AT ZERO IONIC STRENGTH AND 25°

All values are expressed in kcal/mole, except $\Delta S'$, which is given in e.u.

	ΔF_{obs}	$\phi RTln$H	$\Delta F'$	$\Delta H'$	$T\Delta S'$	$\Delta S'$
*p*H 5	− 8.2	0	−8.2	−5.4	+2.8	+ 9
*p*H 7.5	− 9.2	− 9.4	+0.2	−4.8	−5.0	−17
*p*H 10.0	−12.7	−13.7	+1.0	−4.4	−5.4	−18

$H^+ = 1$ molal, $\Delta F^0 = -9.8$, $\Delta H^0 \approx -5.2$, $T\Delta S^0 \approx +4.6$, $\Delta S^0 \approx +15$

Similar calculations can be carried out based on the reaction at *p*H 10, where single ionic species again predominate (equation 20). The values of ΔF_{obs}

$$atp^{4-} + H_2O \text{ —— } adp^{3-} + HPO_4^{2-} + H^+ \quad (20)$$

at $\mu = 0.2$ and $\mu = 0$ are −12.5 and −12.7 kcal/mole respectively. But these calculations do not provide an independent estimate for the standard free energy changes at $\mu = 0$ because the values of ΔF_{obs} at *p*H 5 and 10 are interrelated through the difference between the standard free energies for the secondary phosphate ionization in reactant and products.

Thermodynamic Data for the Hydrolysis of ATP Yielding ADP and Orthophosphate.—The values estimated above for ΔF_{obs} at *p*H 5, 7.5, and 10 at zero ionic strength are listed in Table VI together with the corresponding values for $\phi RTln$H and $\Delta F'$. Values of $\Delta H'$ for *p*H 5 and *p*H 10 have been calculated with the use of Benzinger and Hems' (1956) value of −4.75 kcal/mole, determined at *p*H 7.5, and the enthalpy changes for the secondary phosphate ionizations given in Table I. The entropy contribution to the driving force, $T\Delta S'$, has been evaluated from the equation $\Delta F' = \Delta H' - T\Delta S'$, and from this the entropy of hydrolysis. Values for the thermodynamic quantities at $H^+ = 1$ molal have also been included in the table, incorporating the new value for ΔF^0, −9.8 kcal/mole, and the value of −5.2 kcal/mole previously adopted for ΔH^0 (Rutman and George, 1961).

We have already emphasized that the favorable value of ΔF_{obs} for the hydrolysis of ATP originates in a complicated manner through the interplay of the more fundamental thermodynamic quantities $\phi RTln$H and $\Delta F'$, and we have shown how $\phi RTln$H and $\Delta F'$ vary a great deal as the *p*H is changed, even though ΔF_{obs} is maintained between fairly close limits (Rutman and George, 1961). The new and more reliable data in Table VI fully confirm these conclusions, although further numerical revisions will be needed, especially when values for the primary ionizations of the phosphate groups become available.

These conclusions, however, also have an important theoretical bearing. It would appear that there can be no unique explanation for the favorable free energy of hydrolysis. A theory might be set up to account for the reaction at *p*H 5 where H^+ does not participate, *i.e.*, equation (18), thus avoiding the issue of the standard

$$atpH^{3-} + H_2O \longleftrightarrow adpH^{2-} + H_2PO_4^{1-} \quad (18)$$

state for H^+. But, even if in addition this proved to be the kinetic pathway, such a theory would still be inadequate, because it would not explain the features of the hydrolysis at biological *p*H where other ionic species are present, and H^+ unavoidably participates as a product.

Furthermore, a theory that specifically accounted for any one of the reactions involving ionic species would leave unexplained the very similar standard free energy change for the reaction between un-ionized species, *i.e.*, equation (13). Likewise no theory would be complete

$$\text{atpH}_4 + \text{H}_2\text{O} \longleftrightarrow \text{adpH}_3 + \text{H}_3\text{PO}_4 \qquad (13)$$

that explained specifically this particular reaction. The missing element in all instances is a consideration of the ionization processes.

The standpoint thus reached is that no single reaction such as (18) or (13) is sufficient for an adequate theoretical treatment of the reactivity of ATP: the reactions involving both ionized and un-ionized species have to be accounted for, and hence the associated ionization equilibria assume a theoretical significance equal to that of the hydrolysis reactions themselves.

REFERENCES

Alberty, R. A., Smith, R. M., and Bock, R. M. (1951), *J. Biol. Chem. 193*, 425.

Ashby, J. H., Clarke, H. B., Crook, E. M., and Datta, S. P. (1955), *Biochem. J. 59*, 203.

Ashby, J. H., Crook, E. M., and Datta, S. P. (1954), *Biochem. J. 56*, 198.

Bates, R. G., and Acree, S. F. (1943), *J. Res. Nat. Bur. Std. 30*, 129.

Bates, R. G., and Pinching, G. (1949), *J. Am. Chem. Soc. 71*, 1274.

Benzinger, T., Hems, R., Burton, K., and Kitzinger, C. (1959), *Biochem. J. 71*, 400.

Benzinger, T., and Hems, R. (1956), *Proc. Nat. Acad. Sci. U. S. 42*, 896.

Burton, K. (1959), *Biochem. J. 71*, 388.

Datta, S. P., and Grzybowski, A. K. (1961), Biochemist's Handbook, Long, C., editor, Princeton, N. J., Van Nostrand, Table 8, p. 45.

Elliot, J. S., Sharp, R. F. and Lewis, L. (1958) *J. Phys. Chem. 62*, 686.

George, P., and Rutman, R. J. (1960), *Progr. Biophys. Biophys. Chem. 10*, 1.

Izatt, R. M., and Christensen, J. J. (1962), *J. Phys. Chem. 66*, 359.

Kumler, W. D. (1938), *J. Am. Chem. Soc. 60*, 859.

Levintow, L., and Meister, A. (1954), *J. Biol. Chem. 209*, 265.

Mattock, G. (1961), pH Measurement and Titration, New York, Macmillan Co., Chap. 13.

Melchior, N. C. (1954), *J. Biol. Chem. 208*, 615.

Rutman, R. J., and George, P. (1961), *Proc. Nat. Acad. Sci. U. S. 47*, 1094.

Scatchard, G., and Breckenridge, P. C. (1954), *J. Phys. Chem. 58*, 596.

Smith, R. M., and Alberty, R. A. (1956), *J. Am. Chem. Soc. 78*, 2376.

Snell, F. M. (1955) Electrochemistry in Biology and Medicine, Shedlorsky, T., editor, New York, John Wiley, Chap. 15, p. 284.

Stokes, J. M. (1945), *Trans. Faraday Soc. 41*, 685.

Wingfield, B., and Acree, S. F. (1937), *J. Res. Nat. Bur. Std. 19*, 163.

Estimates of Thermodynamic Data for the Formation of the Mg^{2+} Complexes of ATP and ADP at Zero Ionic Strength*

PHILIP GEORGE, ROGER C. PHILLIPS, S.J., AND ROBERT J. RUTMAN

From the John Harrison Laboratory of Chemistry, University of Pennsylvania, Philadelphia

Received December 4, 1962

In order to calculate thermodynamic data for the formation of the Mg^{2+} complexes of ATP and ADP at zero ionic strength from stability constant measurements at finite ionic strengths, an empirical extrapolation procedure is proposed, based on the assumption that the activity coefficient ratios for nucleotide species of the same charge type are identical at the same ionic strength. Calculations of the standard free energy, enthalpy, and entropy changes have been carried out with data in the literature, and quantitative aspects of the influence of complex formation on the hydrolysis of ATP yielding ADP and orthophosphate are discussed.

The determination of stability constants for the formation of the Mg^{2+} complexes of ATP and ADP[1] presents considerable experimental problems, and precise values are particularly difficult to obtain in the low ionic strength region which would permit extrapolation to zero ionic strength and hence the evaluation of the true thermodynamic constants. Considering the change in charge in the two reactions shown in equations (1) and (2), a very pronounced ionic strength effect is to be

$$\text{atp}^{4-} + \text{Mg}^{2+} \longrightarrow \text{atpMg}^{2-} \qquad (1)$$

$$\text{adp}^{3-} + \text{Mg}^{2+} \longrightarrow \text{adpMg}^{1-} \qquad (2)$$

anticipated, with the stability constant increasing as the ionic strength is decreased throughout the Debye-Hückel range.

In the absence of the desired experimental data at low ionic strengths, we propose an empirical extrapolation procedure which appears to give reasonable estimates for the thermodynamic values. It is based upon the assumption that the activity coefficient ratios for nucleotide species of the same charge type are identical at the same ionic strength. In the calculations which are presented below, we have obtained the required activity coefficient ratios from our previous studies of the secondary ionization of the terminal phosphate groups in AMP, ADP, and ATP (Phillips *et al.*, 1963).

EQUILIBRIUM CONSTANTS UPON WHICH THE CALCULATIONS ARE BASED

A survey of the literature shows that a variety of values have been obtained for the apparent stability constants of atpMg^{2-} and adpMg^{1-}. These have been collected in Table I. Although the empirical extrapo-

---

* This work was undertaken as part of a research program on the thermodynamics of phosphate reactions supported by USPHS grant AM-04764.

[1] The usual abbreviations AMP, ADP, and ATP for adenosine mono-, di-, and triphosphate, respectively, are used when there is no need to specify the ionic species precisely. When this is essential amp^{2-}, adp^{3-}, and atp^{4-} are used to designate the nucleotide species in which the phosphate groups are fully ionized; the corresponding conjugate acids are thus ampH^{1-}, adpH^{2-}, atpH^{3-}, *etc.*