binding site, and that protonation of the α-amino group of isoleucine-16 makes the chain terminus a less effective competitor, especially since it is tied back in the ionic bond with the side-chain carboxylate of aspartate-194. The second possibility is that protonation and formation of the ionic bond stabilize an active site conformation which is otherwise energetically unfavorable, and that deprotonation results in partial collapse of the site's critically defined structure. This latter suggestion seems more likely, based on data with nonspecific substrates of chymotrypsin. With *p*-nitrophenyl acetate or methyl hippurate[35] at high pH, catalysis rather than binding is altered;[4] that is, one observes a pH-dependent intramolecular noncompetitive inhibition phenomenon. From this one concludes that the conformational change alters the position of serine-195 relative to histidine-57. Hence, upon breaking the isoleucine-16–aspartate-194 ionic bond the polypeptide loop containing serine-195 and aspartate-194 collapses into the apolar site. Such collapse would prevent binding of molecules of a particular size, hydrophobic character, or specificity for part of the active site, but not those without such characteristics.

[35] J. Feder, unpublished results, this laboratory.

Maxwell Relations for Thermodynamic Quantities of Biochemical Reactions

Robert A. Alberty

Contribution from the Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts. *Received January 31, 1969*

Abstract: Since equilibrium calculations for biochemical reactions such as reaction 1, ATP[1] + $H_2O \rightleftharpoons$ ADP + P_i, are generally carried out with expressions of the type K_{obsd} = (ADP)(P_i)/(ATP), where the concentrations include all species, the various thermodynamic quantities are all functions of pH and concentrations of any cations present which form complexes with reactants or products as well as temperature, pressure, and electrolyte medium. In this paper the only complexing cation considered to be present is Mg^{2+}, so that the thermodynamic quantities are considered to be functions of only temperature, pH, and pMg[2] at constant ionic strength and 1 atm. The Maxwell relations give relations between rates of change of thermodynamic quantities with respect to different independent variables. For example, it is shown that the rate of change of the heat of reaction with pH is proportional to the rate of change of the production, n_H, of H^+ with temperature. The rate of change of the heat of reaction with pMg is proportional to the rate of change of the production, n_{Mg}, of Mg^{2+} with temperature. Equations are also derived for $\partial \Delta S°_{obsd}/\partial pH$, $\partial \Delta S°_{obsd}/\partial pMg$, $\partial \Delta C°_{p,obsd}/\partial pH$, and $\partial \Delta C°_{p,obsd}/\partial pMg$ in terms of n_H, n_{Mg}, $\partial n_H/\partial T$, $\partial n_{Mg}/\partial T$, $\partial^2 n_H/\partial T^2$, and $\partial^2 n_{Mg}/\partial T^2$. Contour plots are given for $\partial \Delta G°_{obsd}/\partial pH$, $\partial \Delta G°_{obsd}/pMg$, $\partial \Delta H°_{obsd}/\partial pH$, $\partial \Delta H°_{obsd}/\partial pMg$, $T(\partial \Delta S°_{obsd}/\partial pH)$, and $T(\partial \Delta S°_{obsd}/\partial pMg)$ for reaction 1 which present the dependence on pH and pMg at 25° and 0.2 ionic strength in the range pH 4–10 and pMg 1–7. The equation is derived for the change in heat capacity, $\Delta C°_{p,obsd}$, for reaction 1 and estimates of this quantity are also presented for the range pH 4–10 and pMg 1–7. In order to estimate $\Delta C°_{p,obsd}$ it has been necessary to assume values of $\Delta C°_p$ for the dissociations of ATP and ADP on the basis of analogy with structurally related compounds.

The observed equilibrium constant, K_{obsd}, for the hydrolysis of ATP written in terms of the total

$$\mathrm{ATP} + \mathrm{H_2O} \rightleftharpoons \mathrm{ADP} + \mathrm{P_i} \qquad (1)$$

$$K_{obsd} = (\mathrm{ADP})(\mathrm{P_i})/(\mathrm{ATP}) \qquad (2)$$

concentrations of reactants and products is a function of pH[3-5] and metal ion concentrations.[6-10] With data on the observed equilibrium constant at one pH and free metal ion concentration and with dissociation constants for the weak acids and complexes it is possible to calculate the standard Gibbs free energy of hydrolysis at various pH values and metal ion concentrations (eq 3). This is the change in Gibbs free energy when the

$$\Delta G°_{obsd} = -RT \ln K_{obsd} \qquad (3)$$

reactants designated in the equilibrium constant expression, each in the standard state of (hypothetical) 1 *M* solution, are converted to the products designated in the equilibrium constant expression, each in the standard state of (hypothetical) 1 *M* solution, all at the designated temperature in an electrolyte solution of the specified ionic strength having the designated pH and free metal ion concentration. With data on the heat of hydrolysis of ATP and the enthalpies of ionization and complex dissociation of the acidic forms and magnesium complexes of ATP, ADP, and P_i, the values of $\Delta H°_{obsd}$ and $\Delta S°_{obsd}$ for the over-all reaction may be obtained[11]

[1] ATP, adenosine triphosphate; ADP, adenosine diphosphate; P_i, orthophosphate.

[2] pMg is defined for Mg^{2+} in a similar way to pH for H^+ and is assumed to be obtained by use of a reversible divalent cation electrode.

[3] M. Dixon, "Multi-enzyme Systems," Cambridge University Press, Cambridge, England, 1949.

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

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

[8] P. George, R. C. Phillips, and R. C. Rutman, *Biochemistry*, **2**, 508 (1963).

[9] R. A. Alberty, *J. Biol. Chem.*, **243**, 1337 (1968).

[10] R. C. Phillips, P. George, and R. J. Rutman, *ibid.*, **244**, in press.

[11] R. A. Alberty, *ibid.*, **244**, in press.

Table I. $\Delta C°_p$ for Acid Dissociation and for Magnesium Complex Dissociation at 25°

Reaction	$\Delta C°_p$, cal deg^{-1} mol^{-1}	Temp range, °C	Ref
Acids			
$H_3PO_4 \rightleftharpoons H^+ + H_2PO_4^-$	-51	0–60	15
$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^{2-}$	-45	0–60	15
$H_2P_2O_7^{2-} \rightleftharpoons H^+ + HP_2O_7^{3-}$	-30	0–65	*b*, *c*
$HP_2O_7^{3-} \rightleftharpoons H^+ + P_2O_7^{4-}$	-30	0–65	*b*, *c*
Glycerol-1-$OPO_3H^- \rightleftharpoons H^+ +$ glycerol-1-OPO_3^{2-}	-48 ± 2[a]	5–50	*d*
Glycerol-2-$OPO_3H_2 \rightleftharpoons H^+ +$ glycerol-2-OPO_3H^-	-78 ± 7	5–50	*e*
Glycerol-2-$OPO_3H^- \rightleftharpoons H^+ +$ glycerol-2-OPO_3^{2-}	-54 ± 1	5–50	*e*
Glucose-1-$OPO_3H^- \rightleftharpoons H^+ +$ glucose-1-OPO_3^{2-}	-47 ± 1	5–50	*f*
Magnesium Complexes			
$MgHPO_4^0 \rightleftharpoons Mg^{2+} + HPO_4^{2-}$	-60 ± 7	10–50	*g*
Mg-glucose-1-$OPO_3^0 \rightleftharpoons Mg^{2+} +$ glucose-1-OPO_3^{2-}	-59 ± 16	10–50	*g*
Mg-glycerol-2-$OPO_3^0 \rightleftharpoons Mg^{2+} +$ glycerol-2-OPO_3^{2-}	-56 ± 33	10–50	*g*

[a] Standard error. [b] R. R. Irani, *J. Phys. Chem.*, **65**, 1463 (1961). [c] Calculation of ΔC_p by Alberty using method of Everett and Wynne-Jones.[14] [d] S. P. Datta and A. K. Grzybowski, *Biochem. J.*, **69**, 218 (1958). [e] J. H. Ashby, E. M. Crook, and S. P. Datta, *ibid.*, **56**, 198 (1954). [f] J. H. Ashby, H. B. Clarke, E. M. Crook, and S. P. Datta, *ibid.*, **59**, 203 (1955). [g] H. B. Clarke, D. C. Cusworth, and S. P. Datta, *ibid.*, **58**, 146 (1954).

as functions of pH and pMg so that the relative contributions of the two terms in eq 4 may be calculated.

$$\Delta G°_{\text{obsd}} = \Delta H°_{\text{obsd}} - T\Delta S°_{\text{obsd}} \tag{4}$$

The calculations reported in this paper require information on $\Delta C°_p$ for the various ionic reactions in addition to the thermodynamic quantities for these reactions given in the earlier paper.[11] We will be concerned with two types of change in heat capacity at constant pressure: the $\Delta C°_p$ for a reaction written in terms of particular ionized species (eq 5) and $\Delta C°_{p,\text{obsd}}$ for a reaction such as reaction 1 written in terms of total concentrations of reactants and products (eq 6).

$$\Delta C°_p = [\partial \Delta H°/\partial T]_p \tag{5}$$

$$\Delta C°_{p,\text{obsd}} = [\partial \Delta H°_{\text{obsd}}/\partial T]_{\text{pH,pMg}} \tag{6}$$

Harned and Embree[12] showed that for a number of weak acids p*K* passes through a minimum value in the 0–100° temperature range. Pitzer[13] showed that a value of $\Delta C°_p = -40$ cal deg^{-1} mol^{-1} gave an excellent representation of such data. Everett and Wynne-Jones[14] extended these calculations to a wide variety of weak acids and discussed the sign and magnitude of the heat capacity change in terms of the orientation of solvent molecules around the ions. For acid and complex dissociations the values of $\Delta C°_p$ seem to be quite dependent upon type of reaction and not so dependent upon the chemical natures of the acid or complex ion. For example, Harned and Owen[15] give the following summary of a good deal of data.

$$HA = H^+ + A^- \tag{7}$$

$$\Delta C°_p \cong -(36 - 41) \text{ cal deg}^{-1} \text{ mol}^{-1}$$

$$^+H_3NRCO^-_2 = H^+ + H_2NRCO^-_2 \tag{8}$$

$$\Delta C°_p \cong -16 \text{ cal deg}^{-1} \text{ mol}^{-1}$$

Existing data on orthophosphoric acid, pyrophosphoric acid, and organic phosphate compounds are summarized in Table I. All the values are for zero ionic strength except for pyrophosphoric acid where the p*K*'s were determined in 0.1 *M* $(CH_3)_4NCl$. The following generalizations are derived from this table: (a) $\Delta C°_p$ for acid dissociation is greater for the uncharged acid than for the acid with charge 1− and the $\Delta C°_p$ values for acids of charge 1− are greater than for acids with higher charges. (b) $\Delta C°_p$ for the dissociation of Mg^{2+} from an uncharged phosphate complex is about −60 cal deg^{-1} mol^{-1}.

Theory

This discussion of biochemical reactions is limited to the independent variables *T*, pH, and pMg. The pressure is held constant at 1 atm. Since the standard Gibbs free energy, $\Delta G°_{\text{obsd}}$, is a function of the state of the system, its differential may be written

$$\begin{aligned} d\Delta G°_{\text{obsd}} = {} & (\partial \Delta G°_{\text{obsd}}/\partial T)_{\text{pH,pMg}} dT + \\ & (\partial \Delta G°_{\text{obsd}}/\partial \text{pH})_{T,\text{pMg}} d\text{pH} + \\ & (\partial \Delta G°_{\text{obsd}}/\partial \text{pMg})_{T,\text{pH}} d\text{pMg} = \\ & -\Delta S°_{\text{obsd}} dT - 2.3RTn_{\text{H}} d\text{pH} - 2.3RTn_{\text{Mg}} d\text{pMg} \end{aligned} \tag{9}$$

since[11]

$$(\partial \Delta G°_{\text{obsd}}/\partial T)_{\text{pH,pMg}} = -\Delta S°_{\text{obsd}} \tag{10}$$

$$(\partial \Delta G°_{\text{obsd}}/\partial \text{pH})_{T,\text{pMg}} = -2.3RTn_{\text{H}} \tag{11}$$

$$(\partial \Delta G°_{\text{obsd}}/\partial \text{pMg})_{T,\text{pH}} = -2.3RTn_{\text{Mg}} \tag{12}$$

where $\Delta S°_{\text{obsd}}$ is the standard entropy change for the reaction written in terms of total species, n_{H} is the number of moles of H^+ produced[16] when the reactants are converted to products at constant *T*, pH, and pMg, and n_{Mg} is the number of moles of Mg^{2+} produced. Equation 9 is useful for estimating the uncertainty in $\Delta G°_{\text{obsd}}$ for small uncertainties in *T*, pH, and pMg.

Maxwell equations are obtained by setting cross derivatives equal. This yields eq 13–15. Maxwell equations

$$(\partial \Delta S°_{\text{obsd}}/\partial \text{pH})_{T,\text{pMg}} = 2.3R[n_{\text{H}} + T(\partial n_{\text{H}}/\partial T)_{\text{pH,pMg}}] \tag{13}$$

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(12) H. S. Harned and N. D. Embree, *J. Am. Chem. Soc.*, **56**, 1050 (1934).

(13) K. S. Pitzer, *ibid.*, **59**, 2365 (1937).

(14) D. H. Everett and W. F. K. Wynne-Jones, *Trans. Faraday Soc.*, **35**, 1380 (1939).

(15) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd ed, Reinhold Publishing Corp., New York, N. Y., 1958, p 644.

(16) I. Green and W. F. H. M. Mommaerts, *J. Biol. Chem.*, **202**, 541 (1953).

$$(\partial \Delta S^\circ_{\text{obsd}}/\partial \text{pMg})_{T,\text{pH}} = 2.3R[n_{\text{Mg}} + T(\partial n_{\text{Mg}}/\partial T)_{\text{pH,pMg}}] \quad (14)$$

$$(\partial n_{\text{H}}/\partial \text{pMg})_{T,\text{pH}} = (\partial n_{\text{Mg}}/\partial \text{pH})_{T,\text{pMg}} \quad (15)$$

are useful because they give relations between thermodynamic measurements that might not otherwise have been obvious. Equations of the type of (15) have been pointed out by Wyman.[17]

Since the standard enthalpy, $\Delta H^\circ_{\text{obsd}}$, of the reaction is also a function of T, pH, and pMg, its differential may be written

$$\begin{aligned} d\Delta H^\circ_{\text{obsd}} = {} & (\partial \Delta H^\circ_{\text{obsd}}/\partial T)_{\text{pH,pMg}} dT + \\ & (\partial \Delta H^\circ_{\text{obsd}}/\partial \text{pH})_{T,\text{pMg}} d\text{pH} + \\ & (\partial \Delta H^\circ_{\text{obsd}}/\partial \text{pMg})_{T,\text{pH}} d\text{pMg} = \Delta C^\circ_{\text{p,obsd}} dT + \\ & 2.3RT^2(\partial n_{\text{H}}/\partial T)_{\text{pH,pMg}} d\text{pH} + \\ & 2.3RT^2(\partial n_{\text{Mg}}/\partial T)_{\text{pH,pMg}} d\text{pMg} \quad (16) \end{aligned}$$

where the second form is obtained by inserting eq 6, 17, and 18. The derivatives of $\Delta H^\circ_{\text{obsd}}$ with respect to pH

$$(\partial \Delta H^\circ_{\text{obsd}}/\partial \text{pH})_{T,\text{pMg}} = 2.3RT^2(\partial n_{\text{H}}/\partial T)_{\text{pH,pMg}} \quad (17)$$

$$(\partial \Delta H^\circ_{\text{obsd}}/\partial \text{pMg})_{T,\text{pH}} = 2.3RT^2(\partial n_{\text{Mg}}/\partial T)_{\text{pH,pMg}} \quad (18)$$

and pMg may be obtained from the derivatives of $\Delta H^\circ_{\text{obsd}} = \Delta G^\circ_{\text{obsd}} + T\Delta S^\circ_{\text{obsd}}$ with respect to pH and pMg using eq 11–14. Equations 17 and 18 link very different types of measurements: calorimetric measurements at different pH and pMg values, on one hand, with measurements of acid and magnesium ion productions as a function of temperature, on the other. Taking the cross derivations of eq 16 yields eq 19, 20, and eq 15.

$$(\partial \Delta C^\circ_{\text{p,obsd}}/\partial \text{pH})_{T,\text{pMg}} = 2.3RT[2(\partial n_{\text{H}}/\partial T)_{\text{pH,pMg}} + T(\partial^2 n_{\text{H}}/\partial T^2)_{\text{pH,pMg}}] \quad (19)$$

$$(\partial \Delta C^\circ_{\text{p,obsd}}/\partial \text{pMg})_{T,\text{pH}} = 2.3RT[2(\partial n_{\text{Mg}}/\partial T)_{\text{pH,pMg}} + T(\partial^2 n_{\text{Mg}}/\partial T^2)_{\text{pH,pMg}}] \quad (20)$$

The differential of $\Delta S^\circ_{\text{obsd}}$ is given by eq 21, where the

$$\begin{aligned} d\Delta S^\circ_{\text{obsd}} = {} & (\partial \Delta S^\circ_{\text{obsd}}/\partial T)_{\text{pH,pMg}} dT + \\ & (\partial \Delta S^\circ_{\text{obsd}}/\partial \text{pH})_{T,\text{pMg}} d\text{pH} + \\ & (\partial \Delta S^\circ_{\text{obsd}}/\partial \text{pMg})_{T,\text{pH}} d\text{pMg} = (\Delta C^\circ_{\text{p,obsd}}/T) dT + \\ & 2.3R[n_{\text{H}} + T(\partial n_{\text{H}}/\partial T)_{\text{pH,pMg}}] d\text{pH} + \\ & 2.3R[n_{\text{Mg}} + T(\partial n_{\text{Mg}}/\partial T)_{\text{pH,pMg}}] d\text{pMg} \quad (21) \end{aligned}$$

second form is obtained by introducing eq 13, 14, and 22, which may be derived by differentiating eq 4 with

$$(\partial \Delta S^\circ_{\text{obsd}}/\partial T)_{\text{pH,pMg}} = \Delta C^\circ_{\text{p,obsd}}/T \quad (22)$$

respect to temperature and using eq 6 and 10. Taking the cross derivatives yields eq 19, 20, and 15.

The above relations are perfectly general and are quite independent of any knowledge about the underlying ionic equilibria. However, since there is presently no direct experimental data which illustrate these equations, it is of interest to estimate the magnitudes of the various derivatives from our knowledge of the ionic equilibria of reaction 1.

The Gibbs free energy of hydrolysis of ATP which corresponds with eq 1 is given by eq 23, where K is the

$$\Delta G^\circ_{\text{obsd}} = -RT \ln (Kf_{\text{ATP}}/(\text{H}^+)f_{\text{ADP}}f_{\text{P}}) \quad (23)$$

equilibrium constant for

$$\text{ATP}^{4-} + \text{H}_2\text{O} = \text{ADP}^{3-} + \text{HPO}_4{}^{2-} + \text{H}^+ \quad (24)$$

$$K = (\text{ADP}^{3-})(\text{HPO}_4{}^{2-})(\text{H}^+)/(\text{ATP}^{4-}) \quad (25)$$

The fractions f_{ATP}, f_{ADP}, and f_{P} in the forms ATP^{4-}, ADP^{3-}, and $\text{HPO}_4{}^{2-}$, respectively, are given by eq 26–28. The symbols for the dissociation constants are

$$f_{\text{ATP}} = \{1 + (\text{Mg}^{2+})/K_{\text{MgATP}} + [(\text{H}^+)/K_{1\text{ATP}}][1 + (\text{Mg}^{2+})/K_{\text{MgHATP}} + (\text{H}^+)/K_{2\text{ATP}}]\}^{-1} \quad (26)$$

$$f_{\text{ADP}} = \{1 + (\text{Mg}^{2+})/K_{\text{MgADP}} + [(\text{H}^+)/K_{1\text{ADP}}][1 + (\text{Mg}^{2+})/K_{\text{MgHADP}} + (\text{H}^+)/K_{2\text{ADP}}]\}^{-1} \quad (27)$$

$$f_{\text{P}} = \{1 + (\text{Mg}^{2+})/K_{\text{MgP}} + (\text{H}^+)/K_{2\text{P}}\}^{-1} \quad (28)$$

identified in Table II.

Table II. Estimated Values of $\Delta C^\circ_{\text{p}}$ at 25° and 0.2 Ionic Strength Tetraalkylammonium Halide

Reaction	Constant	$\Delta C^\circ_{\text{p}}$, cal $\text{deg}^{-1}\ \text{mol}^{-1}$
Acids		
$\text{H}_4\text{ATP}^0 \rightleftharpoons \text{H}^+ + \text{H}_3\text{ATP}^-$	$K_{4\text{ATP}}$	-51^a
$\text{H}_3\text{ATP}^- \rightleftharpoons \text{H}^+ + \text{H}_2\text{ATP}^{2-}$	$K_{3\text{ATP}}$	-30^a
$\text{H}_2\text{ATP}^{2-} \rightleftharpoons \text{H}^+ + \text{HATP}^{3-}$	$K_{2\text{ATP}}$	-15
$\text{HATP}^{3-} \rightleftharpoons \text{H}^+ + \text{ATP}^{4-}$	$K_{1\text{ATP}}$	-30
$\text{H}_3\text{ADP}^0 \rightleftharpoons \text{H}^+ + \text{H}_2\text{ADP}^-$	$K_{3\text{ADP}}$	-45^a
$\text{H}_2\text{ADP}^- \rightleftharpoons \text{H}^+ + \text{HADP}^{2-}$	$K_{2\text{ADP}}$	-15
$\text{HADP}^{2-} \rightleftharpoons \text{H}^+ + \text{ADP}^{3-}$	$K_{1\text{ADP}}$	-30
$\text{H}_3\text{PO}_4 \rightleftharpoons \text{H}^+ + \text{H}_2\text{PO}_4{}^-$	$K_{3\text{P}}$	$-51^{15,a}$
$\text{H}_2\text{PO}_4{}^- \rightleftharpoons \text{H}^+ + \text{H}_2\text{PO}_4{}^{2-}$	$K_{2\text{P}}$	-45^{15}
Complexes		
$\text{MgATP}^{2-} \rightleftharpoons \text{Mg}^{2+} + \text{ATP}^{4-}$	K_{MgATP}	-30
$\text{MgADP}^- \rightleftharpoons \text{Mg}^{2+} + \text{ADP}^{3-}$	K_{MgADP}	-45
$\text{MgHATP}^- \rightleftharpoons \text{Mg}^{2+} + \text{HATP}^{3-}$	K_{MgHATP}	-45
$\text{MgHPO}_4{}^0 \rightleftharpoons \text{Mg}^{2+} + \text{HPO}_4{}^{2-}$	K_{MgP}	-60^b
$\text{MgHADP}^0 \rightleftharpoons \text{Mg}^{2+} + \text{HADP}^{2-}$	K_{MgHADP}	-60

[a] These values are used only in the estimation of $\Delta C^\circ_{\text{p}}$ for reaction 24. [b] See Table I, footnote *g*.

The expression for $\Delta C^\circ_{\text{p,obsd}}$ is obtained by differentiating the expression for $\Delta H^\circ_{\text{obsd}}$ and may be written as eq 29, where $\Delta C^\circ_{\text{p,1}}$ is the change in heat capacity

$$\Delta C^\circ_{\text{p,obsd}} = \Delta C^\circ_{\text{p,1}} - C_{\text{ADP}} - C_{\text{P}} + C_{\text{ATP}} \quad (29)$$

for reaction 24 and the contributions C_{ADP}, C_{P}, and C_{ATP} to the dependence of $\Delta C^\circ_{\text{p,obsd}}$ on pH and pMg are given by terms of the type

$$\begin{aligned} C_{\text{ADP}} = {} & f_{\text{ADP}}\left[\frac{(\text{H}^+)}{K_{1\text{ADP}}} C_{1\text{ADP}} + \frac{(\text{Mg}^{2+})}{K_{\text{MgADP}}} C_{\text{MgADP}} + \frac{(\text{H}^+)(\text{Mg}^{2+})}{K_{1\text{ADP}}K_{\text{MgHADP}}} C_{\text{MgHADP}} + \frac{(\text{H}^+)^2}{K_{1\text{ADP}}K_{2\text{ADP}}} C_{2\text{ADP}}\right] + \\ & \frac{f^2_{\text{ADP}}}{RT^2}\left[\frac{(\text{H}^+)}{K_{1\text{ADP}}} \Delta H^\circ_{1\text{ADP}} + \frac{(\text{Mg}^{2+})}{K_{\text{MgADP}}} \Delta H^\circ_{\text{MgADP}} + \frac{(\text{Mg}^{2+})(\text{H}^+)}{K_{1\text{ADP}}K_{\text{MgHADP}}} (\Delta H^\circ_{1\text{ADP}} + \Delta H^\circ_{\text{MgHADP}}) + \frac{(\text{H}^+)^2}{K_{1\text{ADP}}K_{2\text{ADP}}} (\Delta H^\circ_{1\text{ADP}} + \Delta H^\circ_{2\text{ADP}})\right]^2 \quad (30) \end{aligned}$$

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(17) J. Wyman, *Advan. Protein Chem.*, **4**, 407 (1948); **19**, 223 (1964).

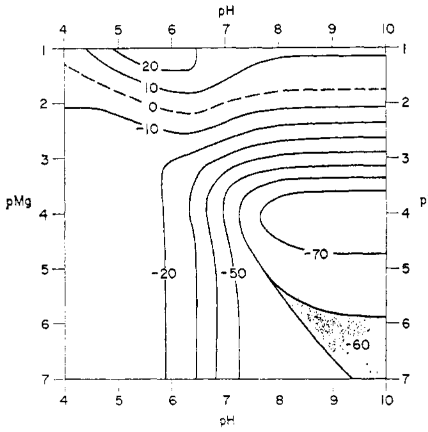

Figure 1. Contour diagram of estimates of $\Delta C^\circ_{p,\,obsd}$ for ATP + H_2O = ADP + P_i at 25° in 0.2 ionic strength tetra-*n*-propylammonium chloride. The values are given in cal deg^{-1} mol^{-1}. In the shaded region the values are within one unit of -60 cal deg^{-1} mol^{-1}.

where

$$C_{1ADP} = \Delta C^\circ_{p1ADP} - \Delta H^\circ_{1ADP}/RT^2 \quad (31)$$

$$C_{MgADP} = \Delta C^\circ_{pMgADP} - \Delta H^\circ_{MgADP}/RT^2 \quad (32)$$

$$C_{MgHADP} = \Delta C^\circ_{p1ADP} + \Delta C^\circ_{pMgHADP} - (\Delta H^\circ_{1ADP} + \Delta H^\circ_{MgHADP})^2/RT^2 \quad (33)$$

$$C_{2ADP} = \Delta C^\circ_{p1ADP} + \Delta C^\circ_{p2ADP} - (\Delta H^\circ_{1ADP} + \Delta H^\circ_{2ADP})^2/RT^2 \quad (34)$$

In order to calculate $\Delta C^\circ_{p,obsd}$ as a function of pH and pMg, it is necessary to have values of ΔC°_p for the various acid and complex ion dissociations that occur in the range of pH and pMg of interest and to have a value of ΔC°_p for one of the possible ionic reactions, or a value of $\Delta C^\circ_{p,obsd}$ at a known pH and pMg. The calculations given here are based on the values given in Table II. Experimental values are available for orthophosphate, but it has been necessary to assume values for ΔC°_p for ATP and ADP on the basis of analogy with structurally related compounds.

It would be desirable, of course, to have values of ΔC°_p for 0.2 ionic strength. Except for the two values for pyrophosphate, the values of ΔC°_p in Table I are for zero ionic strength. The value of ΔC°_p for the dissociation of H_4ATP° is assumed to be the same as for H_3PO_4. The value for the dissociation of H_3ATP^- is assumed to be the same as for $H_2P_2O_7{}^{2-}$. The values for the dissociations of H_2ATP^{2-} and H_2ADP^- are assumed to be like those for amino groups in amino acids. The values for the dissociations of $HATP^{3-}$ and $HADP^{2-}$ are assumed to be like the values for $HP_2O_7{}^{3-}$ and $H_2P_2O_7{}^{2-}$. The value for the dissociation of H_3ADP^0 is assumed to be like that for $H_2PO_4{}^-$. The value for the dissociation of $MgHADP^0$ is assumed to be like that for $MgHPO_4{}^0$, and the values for $MgHATP^-$ and $MgADP^-$ are assumed to be equal and somewhat smaller than for $MgHPO_4{}^0$ because of the trends exhibited by the acids in Table I. The value for the dissociation of $MgATP^{2-}$ is assumed to be still smaller on this basis.

The value of ΔC°_p for reaction 24 has been estimated on the basis of the assumption that $\Delta C^\circ_p = 0$ for reaction 35 of species with zero net charges. There

$$H_4ATP^0 + H_2O = H_3ADP^0 + H_3PO_4 \quad (35)$$

may be a change in heat capacity for this reaction, but it is probably small compared with that for reactions involving the creation or destruction of charges in aqueous solution. Adding and subtracting the ΔC°_p values in Table II yield $\Delta C^\circ_{p,1} = -60$ cal deg^{-1} mol^{-1} for reaction 24.

Calculations and Discussion

To illustrate the various derivatives which have been discussed above, calculations have been carried out for reaction 1. Contour diagrams of ΔG°_{obsd}, n_H, n_{Mg}, ΔH°_{obsd}, and $T\Delta S^\circ_{obsd}$ *vs.* pH and pMg have been presented earlier[11] for 25° and 0.2 ionic strength. These calculations are based on the assumption that the solutions of the various species in the electrolyte medium of tetra-*n*-propylammonium chloride are ideal. Thus the calculations apply only for dilute solutions of the reactants and products. The equations for doing these calculations and the values of the thermodynamic quantities, which have all been obtained experimentally, are given in the earlier paper.[11] The contour diagrams shown in Figures 1 and 2 have been obtained using a typewriter terminal of the M.I.T. CTSS system. Arrays of 2511 values of the desired derivatives were typed out in response to a MAD program giving the necessary equations. The desired contour diagrams were drawn in by hand.

Figure 1 gives $\Delta C^\circ_{p,obsd}$ values calculated using -60 cal deg^{-1} mol^{-1} for ΔC°_p for reaction 24. Errors in this value do not affect the shape of the surface but do affect its height. The shape of the surface is determined by the ΔG°, ΔH°, and ΔC°_p values for the various acid and magnesium complex dissociations for ATP, ADP, and P_i. These calculations indicate that ΔH°_{obsd} does not change as rapidly with temperature at low pH or low pMg as at high pH and high pMg. Because of eq 22 this same surface may be interpreted as giving $T(\partial \Delta S^\circ_{obsd}/\partial T)_{pH,pMg}$. At high pH and high pMg, ΔS°_{obsd} decreases with increasing temperature at a rate of 0.2 cal deg^{-2} mol^{-1}, but at high (Mg^{2+}) it increases at a rate as large as 0.06 cal deg^{-2} mol^{-1}. Since $\partial \Delta H^\circ_{obsd}/\partial T$ and $\partial \Delta S^\circ_{obsd}/\partial T$ are related through ΔC°_p, their values are strictly proportional to one another at a given T, pH, and pMg, and they change in the same ways with changing pH and pMg.

The diagrams in Figure 2 have been obtained by numerical differentiation. The first diagram gives $(\partial \Delta G^\circ_{obsd}/\partial pH)_{pMg}$ in kcal mol^{-1} as a function of pH and pMg. Because of eq 11 the values given here are proportional to n_H, and n_H may be obtained by dividing the values given by -1.36. A contour diagram for n_H has been given earlier,[11] but it is convenient to have this diagram to compare with the two to the right.

The second diagram gives $(\partial \Delta H^\circ_{obsd}/\partial pH)_{pMg}$ in kcal mol^{-1}. Because of eq 17 this diagram may also be interpreted as a plot of $(\partial n_H/\partial T)_{pH,pMg}$; the values given simply have to be divided by 408 to obtain $(\partial n_H/\partial T)_{pH,pMg}$ in mol deg^{-1}. This plot indicates the magnitude of the errors in ΔH°_{obsd} resulting from errors of the pH. In the neutral region the error in ΔH°_{obsd} may be as large as 0.1 kcal mol^{-1} for an error in pH of 0.1. Looked at the other way the plot gives the error in the measured value of n_H resulting from an error in tem-

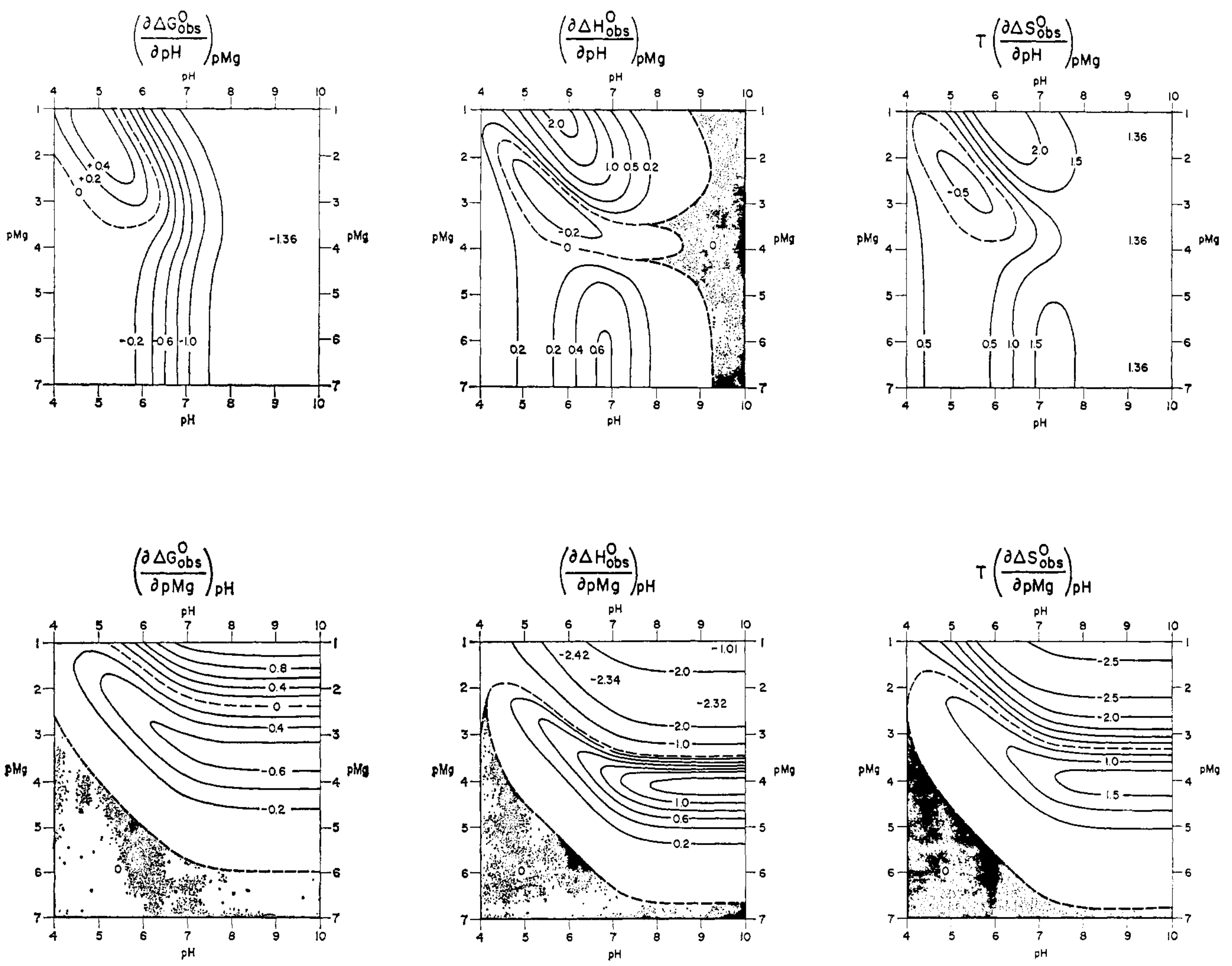

Figure 2. Contour diagrams for derivatives of standard thermodynamic functions with respect to pH and pMg for ATP + H_2O = ADP + P_i at 25° in 0.2 ionic strength tetra-*n*-propylammonium chloride. All values are in kcal mol^{-1}. In the shaded regions the values are within 0.1 kcal mol^{-1} of zero.

perature. In the neutral region the error in n_H may be as large as 0.0025 mol (0.4%) for an error of 1°.

The third diagram gives $T(\partial\Delta S^\circ_{obsd}/\partial pH)_{pMg}$ in kcal mol^{-1}. In the neutral range the error in this quantity resulting from an error of 0.1 in pH may be as large as 0.2 kcal mol^{-1}. Because of eq 13 this diagram may also be interpreted as yielding $n_H + T(\partial n_H/\partial T)_{pH,pMg}$; this quantity may be obtained by dividing the values given by 1.36.

The next row of diagrams gives the derivatives of the standard thermodynamic functions with respect to pMg. Dividing the values of $(\partial\Delta G^\circ_{obsd}/\partial pMg)_{pH}$ in kcal mol^{-1} by -1.36 gives n_{Mg}, the number of moles of magnesium ion produced per mole of ATP hydrolyzed. Dividing the values of $\partial(\Delta H^\circ_{obsd}/\partial pMg)_{pH}$ in kcal mol^{-1} by 408 gives $\partial n_{Mg}/\partial T$. It is of special interest to note that in general ΔH°_{obsd} is more seriously affected by errors in pMg than errors in pH. At pH 7 pMg 2 an error of 0.1 in pMg causes an error of 0.23 kcal mol^{-1} in ΔH°_{obsd} and an error in temperature of 1° causes an error of 0.0056 in n_{Mg}. This is an error of 3% per degree. The next figure shows that errors in pMg may produce significant errors in ΔS°_{obsd}.

The pairs of contour diagrams which are above and below each other in Figure 2 are related in an interesting way. The slope of the upper diagram in the direction of increasing pMg at a particular pH and pMg is equal to the slope of the lower diagram in the direction of increasing pH at the same pH and pMg.

These calculations illustrate the power of thermodynamics to relate different types of equilibrium measurements to each other without any information about the molecular changes involved. The relation between the experimental quantity $\partial n_H/\partial T$ and the experimental quantity $\partial\Delta H^\circ_{obsd}/\partial pH$ is not dependent upon knowledge of the underlying equilibria, such as are summarized in Table II. In the absence of experimental data on $\partial n_H/\partial T$ and $\partial\Delta H^\circ_{obsd}/\partial pH$ these quantities have been calculated using our present knowledge of the molecular changes, but it is important to understand that the relations in eq 9–22 are not dependent on the expression of ΔG°_{obsd} in terms of ionic reactions.

These calculations emphasize the need for measurements of ΔH° for ionic reactions of substances of biochemical interest and of ΔH°_{obsd} of biochemical reactions at carefully measured pH and pMg values. There is also the need for making these measurements over a range of temperature that is sufficiently large so that ΔC°_p and $\Delta C^\circ_{p,obsd}$ may be obtained.

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