M-2689(H)

J. Chem. Thermodynamics **1993**, 25, 73–90

Thermochemistry of the reactions between adenosine, adenosine 5′-monophosphate, inosine, and inosine 5′-monophosphate; the conversion of L-histidine to (urocanic acid + ammonia)

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(Received 26 July 1991; in final form 17 October 1991)

Results of equilibrium and calorimetric measurements are reported for a series of enzyme-catalyzed reactions involving the deamination of adenosine 5′-monophosphate (AMP), adenosine, and L-histidine, and the dephosphorylation of AMP and inosine 5′-monophosphate (IMP). These measurements have been analyzed with a model which describes the complex equilibria in solution. The results obtained are: $\Delta_r H^\circ_m = -(49.6 \pm 0.5)\,\mathrm{kJ \cdot mol^{-1}}$ for $\mathrm{AMP^{2-}(aq) + H_2O(l) + H^+(aq) = HIMP^{2-}(aq) + NH_4^+(aq)}$; $\Delta_r H^\circ_m = -(49.4 \pm 0.7)\,\mathrm{kJ \cdot mol^{-1}}$ for adenosine(aq) + $\mathrm{H_2O(l) + H^+(aq)}$ = inosine(aq) + $\mathrm{NH_4^+(aq)}$; $\Delta_r G^\circ_m = -(13.0 \pm 0.4)\,\mathrm{kJ \cdot mol^{-1}}$, $\Delta_r H^\circ_m = (0.9 \pm 0.4)\,\mathrm{kJ \cdot mol^{-1}}$, and $\Delta_r S^\circ_m = (47 \pm 2)\,\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ for $\mathrm{AMP^{2-}(aq) + H_2O(l)}$ = adenosine(aq) + $\mathrm{HPO_4^{2-}(aq)}$; $\Delta_r G^\circ_m = -(12.6 \pm 0.1)\,\mathrm{kJ \cdot mol^{-1}}$, $\Delta_r H^\circ_m = (1.2 \pm 0.2)\,\mathrm{kJ \cdot mol^{-1}}$, and $\Delta_r S^\circ_m = (48 \pm 1)\,\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ for $\mathrm{HIMP^{2-}(aq) + H_2O(l)}$ = inosine(aq) + $\mathrm{HPO_4^{2-}(aq)}$; and $\Delta_r G^\circ_m = -(2.5 \pm 0.7)\,\mathrm{kJ \cdot mol^{-1}}$, $\Delta_r H^\circ_m = (7.6 \pm 0.8)\,\mathrm{kJ \cdot mol^{-1}}$, and $\Delta_r S^\circ_m = (34 \pm 4)\,\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ for L-histidine(aq) = $\mathrm{urocanate^-(aq) + NH_4^+(aq)}$. These results are discussed in relation to both thermodynamic-cycle calculations involving these substances and other reactions of a chemically similar nature.

1. Introduction

The aim of this study is an improved knowledge of the thermodynamics of the biochemical reactions:†

$$\Sigma\mathrm{AMP(aq) + H_2O(l) = \Sigma IMP(aq) + \Sigma ammonia(aq)}, \tag{1}$$

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† Abbreviations used in this paper are: aden(aq), adenosine(aq); $\mathrm{AMP^{2-}(aq)}$, (adenosine 5′-monophosphate)$^{2-}$(aq); $\mathrm{hist^-(aq)}$, (L-histidine)$^-$(aq); $\mathrm{IMP^{3-}(aq)}$, (inosine 5′-monophosphate)$^{3-}$(aq); $\mathrm{P_i(aq)}$, (inorganic phosphate(aq); $\mathrm{inos^-(aq)}$, (inosine)$^-$(aq); Tris, tris(hydroxymethyl)aminomethane; $\mathrm{uroc^-(aq)}$, (urocanate)$^-$(aq). Protonated forms of the substrates are indicated by the addition of an appropriate number of hydrogen ions to these species. For example, the protonated forms of $\mathrm{hist^-(aq)}$ are Hhist(aq), $\mathrm{H_2hist^+(aq)}$, and $\mathrm{H_3hist^{2+}(aq)}$. The ionizations from $\mathrm{H_2AMP(aq)}$ and $\mathrm{HAMP^-(aq)}$ are from the phosphate group. The ionization from $\mathrm{Haden^+(aq)}$ is from the *N*1 position. The ionization from $\mathrm{H_2inos^+(aq)}$ and from Hinos(aq) are, respectively, from the *N*7 and *N*1 positions. The ionizations from $\mathrm{H_3hist^{2+}(aq)}$, $\mathrm{H_2hist^+(aq)}$, and from Hhist(aq) are from the carboxyl, the imidazole, and the α-amine group, respectively. The ionizations from $\mathrm{H_2uroc^+(aq)}$ and Huroc(aq) are from the carboxyl and the amine group, respectively. The ionizations from $\mathrm{H_3IMP(aq)}$ and $\mathrm{H_2IMP^-(aq)}$ are probably from the phosphate and the ionization from $\mathrm{HIMP^{2-}(aq)}$ is probably from the *N*1 position. Additional ionizations are also reported from the ribose parts of adenosine, inosine, adenosine 5′-monophosphate, and inosine 5′-monophosphate.(1)

0021-9614/93/010073+18 $08.00/0

$$\Sigma\text{adenosine(aq)} + H_2O(l) = \Sigma\text{inosine(aq)} + \Sigma\text{ammonia(aq)}, \qquad (2)$$

$$\Sigma\text{AMP(aq)} + H_2O(l) = \Sigma\text{adenosine(aq)} + \Sigma P_i\text{(aq)}, \qquad (3)$$

$$\Sigma\text{IMP(aq)} + H_2O(l) = \Sigma\text{inosine(aq)} + \Sigma P_i\text{(aq)}, \qquad (4)$$

$$\Sigma\text{L-histidine(aq)} = \Sigma\text{urocanate(aq)} + \Sigma\text{ammonia(aq)}. \qquad (5)$$

Reactions (1) and (2) are catalyzed, respectively, by AMP deaminase (EC 3.5.4.6)† and by adenosine deaminase (EC 3.5.4.4). Reactions (3) and (4) are catalyzed by alkaline phosphatase (EC 3.1.3.1) and reaction (5) is catalyzed by histidine ammonia-lyase (EC 4.3.1.3). The Σs denote the total amounts of each of these substances which can exist in a multiplicity of proton- or metal-bound forms in solution. For example, inorganic phosphate P_i exists as both HPO_4^{2-}(aq) and $H_2PO_4^-$(aq) and adenosine 5′-monophosphate exists as a mixture of the species H_2AMP(aq), $HAMP^-$(aq), AMP^{2-}(aq), and MgAMP(aq).

This multiplicity of forms of the various reactants and products is a generic problem in the treatment of complex biochemical equilibria. This matter has been discussed in detail in a recent publication(3) and we shall follow the approach and most of the terminology adopted in that paper. Thus, the treatment of the results is done with a chemical-equilibrium model which requires thermodynamic quantities for the binding of H^+ and of Mg^{2+} to the various substrates. The equilibrium model also requires the selection of a reference reaction, which involves definite ionic species, for each of the above overall reactions. The species selected for the reference reactions are those which are predominant at pH = 8.0 and at low Mg^{2+} molality. These conditions are most typical of the experimental conditions used in this study. Thus, the reference reactions for the above (overall) reactions are chosen to be

$$AMP^{2-}(aq) + H_2O(l) + H^+(aq) = HIMP^{2-}(aq) + NH_4^+(aq), \qquad (6)$$

$$\text{aden(aq)} + H_2O(l) + H^+(aq) = \text{Hinos(aq)} + NH_4^+(aq), \qquad (7)$$

$$AMP^{2-}(aq) + H_2O(l) = \text{aden(aq)} + HPO_4^{2-}(aq), \qquad (8)$$

$$HIMP^{2-}(aq) + H_2O(l) = \text{Hinos(aq)} + HPO_4^{2-}(aq), \qquad (9)$$

$$\text{Hhist(aq)} = \text{uroc}^-(aq) + NH_4^+(aq). \qquad (10)$$

The standard molality for the solute species is $1\ \text{mol} \cdot \text{kg}^{-1}$. The standard pressure is 0.1 MPa.

The above reactions are of interest for a variety of reasons which include their important role in metabolic processes(4, 5) involving purines. The thermodynamic information obtained in this study might provide understanding of the consequences that occur when the normal metabolic pathways are disrupted. Reactions (1) to (4) above form a closed thermodynamic cycle. This provides an opportunity to demonstrate both the reliability of the measurements and the methods used for data reduction to a biologically important series of reactions. Reaction (5) is currently used for the manufacture of urocanic acid which, in turn, is of importance to the pharmaceutical and cosmetic industries (*e.g.* its use in suntan lotion).(6) Finally, all of

† Enzyme commission numbers and nomenclature are from reference 2.

these processes are of interest in providing results for the correlation of the thermodynamics of these and related reactions with the common structural changes occurring in the reactions.

2. Experimental

The substances used in this study and their molar masses are: the monosodium salt of AMP, 0.36920 $\mathrm{kg \cdot mol^{-1}}$; the disodium salt of IMP, 0.39217 $\mathrm{kg \cdot mol^{-1}}$; adenosine, 0.26724 $\mathrm{kg \cdot mol^{-1}}$; inosine, 0.26823 $\mathrm{kg \cdot mol^{-1}}$; Tris, 0.12114 $\mathrm{kg \cdot mol^{-1}}$; Na_2HPO_4, 0.14196 $\mathrm{kg \cdot mol^{-1}}$; K_2HPO_4, 0.17418 $\mathrm{kg \cdot mol^{-1}}$; NaOH, 0.03997 $\mathrm{kg \cdot mol^{-1}}$; $MgCl_2$, 0.095211 $\mathrm{kg \cdot mol^{-1}}$; NH_4Cl, 0.053491 $\mathrm{kg \cdot mol^{-1}}$; $NH_4C_2H_3O_2$ (ammonium acetate), 0.077083 $\mathrm{kg \cdot mol^{-1}}$; L-histidine, 0.15516 $\mathrm{kg \cdot mol^{-1}}$; and urocanic acid, 0.13813 $\mathrm{kg \cdot mol^{-1}}$. The substances used in the equilibrium and calorimetric experiments were obtained† from Sigma Chemical Company with the following exceptions: HCl, NaOH, K_2HPO_4, Tris, and $MgCl_2$ were from Fisher Scientific; the NH_4Cl was from Mallinckrodt; and the Na_2HPO_4 was Standard Reference Material 186-IId from the National Institute of Standards and Technology. Distilled water was used without any further purification. The AMP deaminase came in a solution containing KCl (0.33 $\mathrm{mol \cdot dm^{-3}}$) and a trace amount of 2-mercaptoethanol. Prior to use, it was dialyzed for approximately 24 h at 278 K against either the (Tris + HCl) or phosphate buffers used in the equilibrium and calorimetric experiments. The adenosine deaminase, alkaline phosphatase, and histidine ammonia-lyase were received in lyophilized form. The histidine ammonia-lyase was dissolved in either the phosphate or (Tris + HCl) buffers which were used in studying reaction (5). This solution was then heated to 343 K and kept at that temperature for 30 min. This was done to destroy the activity of an enzymatic impurity present in the sample. Although this impurity was not identified, it was very likely urocanate hydratase (EC 4.2.1.49) which brings about the conversion of urocanic acid to 4,5-dihydro-4-oxo-5-imidazolepropanoate. Moisture contents were determined by Karl–Fischer analyses with resulting mass fractions: adenosine, $(0.44 \pm 0.01) \cdot 10^{-2}$; inosine, $(0.41 \pm 0.04) \cdot 10^{-2}$; NaHAMP, $(15.9 \pm 0.3) \cdot 10^{-2}$; Na_2HIMP, $(26.2 \pm 0.4) \cdot 10^{-2}$; L-histidine, $(9.55 \pm 0.48) \cdot 10^{-2}$; urocanic acid, $(0.14 \pm 0.02) \cdot 10^{-2}$; K_2HPO_4, $(0.80 \pm 0.08) \cdot 10^{-2}$; NH_4Cl, $(0.53 \pm 0.26) \cdot 10^{-2}$; $NH_4C_2H_3O_2$, $(29.5 \pm 0.4) \cdot 10^{-2}$; Tris, $(0.25 \pm 0.05) \cdot 10^{-2}$; and $MgCl_2$, $(54.2 \pm 1.5) \cdot 10^{-2}$. The moisture content of the magnesium chloride was also determined by drying it in an oven at 413 K to constant mass. The result of this analysis was a moisture mass fraction of $(53.5 \pm 0.9) \cdot 10^{-2}$, in good agreement with the result obtained by the Karl–Fischer method. However, the result obtained by drying to constant mass was judged to be more reliable than the result from the Karl–Fischer determination and was used in calculating the molalities of $MgCl_2$. Analyses of the samples of AMP, IMP, adenosine, inosine, L-histidine, and urocanic acid were done with the chromatographic procedures described below and with a refractive-index detector.

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† Certain commercial materials and products are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology.

The results of these analyses indicated that the total mole fraction of impurities in these samples was <0.001.

A Hewlett-Packard model 1090 h.p.l.c. with a Serva DEAE Si 100 anion-exchange column and an ultraviolet detector was used for all chromatographic measurements. The mobile phase used to study reactions (1), (2), and (4) was KH_2PO_4(aq, 0.01 $mol \cdot dm^{-3}$, adjusted to pH = 2.85 with concentrated phosphoric acid). The flow rate of this mobile phase was 0.027 $cm^3 \cdot s^{-1}$ when used to analyze the solutions obtained from reactions (1) and (4) and 0.008 $cm^3 \cdot s^{-1}$ when used for the solutions from reaction (2). The mobile phase used to study reaction (3) was KH_2PO_4(aq, 0.035 $mol \cdot dm^{-3}$, adjusted to pH = 2.65 with concentrated phosphoric acid). The flow rate of this mobile phase was 0.017 $cm^3 \cdot s^{-1}$. The u.v. detector was set at the wavelength 254 nm for studying the substrates in reactions (1) and (2), and the IMP in reaction (4); and at 277 nm for studying the AMP and adenosine in reaction (3) and the inosine in reaction (4). The mobile phase used to study reaction (5) consisted of 40 volume per cent acetonitrile and 60 volume per cent of a second solution which was prepared according to the following recipe. Sodium acetate trihydrate (1.36 g), triethylamine (2.0 cm^3), and acetonitrile (50 cm^3) were dissolved in water to make a total volume of 1 dm^3 of solution; this solution was then adjusted to pH = 5.9 with concentrated acetic acid. Here, the flow rate was 0.010 $cm^3 \cdot s^{-1}$ and the u.v. detector was set at 210 nm. Typical retention times for the substrates determined under the above chromatographic conditions were: 6.7 min and 16.7 min for AMP and IMP, respectively, in reaction (1); 8.0 min and 10.9 min for adenosine and inosine, respectively, in reaction (2); 3.8 min and 7.5 min for adenosine and AMP, respectively, in reaction (3); 4.3 min and 16.7 min for inosine and IMP, respectively, in reaction (4); and 5.8 min and 18.0 min for L-histidine and urocanic acid, respectively, in reaction (5). The chromatographic results were recorded and analyzed with a Dionex AI-450 computer-controlled data-acquisition system. This system converted the analog signals from the u.v. detector to digital signals which were then recorded on hard disk and accurately integrated. Examination of the chromatographic peaks showed that there were no difficulties with their base lines and shoulders. Response factors were determined on a daily basis and used to calculate the molalities of the substrates in solution.

Solutions of the substrates in a buffer containing the appropriate enzyme were allowed to equilibrate with gentle stirring in a thermostatted water bath. Equilibrium was approached from two directions: *e.g.* for reaction (3) starting with AMP (the forward direction) and starting with a mixture containing adenosine and orthophosphate (the reverse direction). Samples of the equilibrating solutions were periodically analyzed with the h.p.l.c. to monitor the changes in the concentrations of the substances in solution and to ascertain when the solutions were at equilibrium. Equilibration times were 3 d for reaction (3), 5 d for reaction (4), and 6 d for reaction (5). Following a 1 d equilibration, after which most of the reaction had occurred, the pH of the "forward direction" solution for reaction (3) was adjusted with NaOH(aq) to the same pH as the "reverse direction" solution. Additional alkaline phosphatase was then added to this solution to make certain that the reaction could continue at the pH to which the solution had been adjusted. The mass fractions of the enzymes in the equilibrium mixtures were 0.000010 for alkaline phosphatase in reactions (3) and (4) and 0.00023 for histidine ammonia-lyase in reaction (5). These solutions were analyzed by means of the chromatographic procedures described above. The chromatographic analyses showed no evidence of any side reactions accompanying the principal processes studied. The molalities of the ammonium acetate and orthophosphate were calculated from the initial molalities of these substances and the stoichiometry of the final reaction mixtures as determined chromatographically. Attempts were also made to determine apparent equilibrium constants for reactions (1) and (2). It was found, however, that the chromatographic sensitivity was inadequate to determine the amounts of AMP (reaction 1) or adenosine (reaction 2) remaining in solution from the "forward direction" solutions or which were formed from the "reverse direction" solutions. Thus, we were unable to determine apparent equilibrium constants for these two reactions.

The calorimeters were of the heat-conduction type and had calibration constants varying from 17 $W \cdot V^{-1}$ to 22 $W \cdot V^{-1}$. Each calorimeter contained two thermopiles which are solid-state (bismuth selenide + bismuth telluride + bismuth antimonide) thermoelectric modules manufactured by Cambion Corporation. The two thermopiles were connected in series and were situated in an aluminum block in an air thermostat, the temperature of which was controlled by means of a Wheatstone-bridge circuit and a controller which utilized both proportional and integral modes of action. The sample vessels, which were fabricated from high-density polyethene, contained two compartments holding approximately 0.55 cm^3 and 0.45 cm^3 of solution, respectively. The sample vessels were loaded into a copper container which was kept glued in place between the thermopile elements in a sandwich-type arrangement. The vessels and their contents were allowed to equilibrate for at least 1 h before the solutions in the vessel were mixed. The calibration of the calorimeters was done electrically with a calibrated potential-difference meter, standard resistor, and time-interval counter. The inaccuracy and imprecision of the measurements ($\approx$0.2 per cent for a total of 300 mJ) were determined with chemical reactions which produce well known enthalpy changes. Complete descriptions of the calorimeters and their performance characteristics, the data-acquisition system, and the codes used to treat the results are given in references 7 and 8.

Measurements of the enthalpy of reaction were performed by mixing a substrate solution and an enzyme solution in the calorimeter. The substrate solution was prepared by dissolving a known amount of substrate (*e.g.* AMP) in a buffer solution. The enzyme solution was prepared by adding the same buffer solution to the appropriate enzyme (*e.g.* AMP deaminase). The extent of reaction for the processes occurring in the calorimetric experiments were determined immediately following completion of the calorimetric measurements by chromatographic analyses of the solutions in the sample vessels. Appropriate corrections were applied for any unreacted substrate when calculating molar enthalpies of reaction. The calorimetric experiments typically lasted about 30 min with the exception of reaction (5) where the experiments lasted for 2 h to 3 h. The mass fractions of the enzymes in the calorimetric reaction mixtures were: 0.0006 for AMP deaminase (reaction 1); 0.0003 for adenosine deaminase (reaction 2); 0.00003 for alkaline phosphatase (reactions 3 and 4); and 0.00002 for histidine ammonia-lyase (reaction 5). The "blank" effects accompanying the mixing of the substrate solution and of the enzyme solution with the buffer were: 0.0010 J, −0.0041 J, 0.0024 J, −0.0024 J, and −0.0030 J for reactions (1) to (5), respectively. The uncertainties in these enthalpies are approximately 0.001 J. The measured enthalpies of reactions were −0.01 J to −0.39 J, −0.01 J to −0.18 J, 0.01 J to 0.03 J, −0.01 J to 0.03 J, and 0.05 J to 0.19 J for reactions (1) to (5), respectively.

The measurement of the pH of the final reaction mixtures in both the equilibrium and calorimetric experiments was done with a combination glass micro-electrode and an Orion Model 811 pH meter. All measurements were done at the temperature at which the reactions occurred, either in the microcalorimeter(s) or in the thermostats used for the equilibrium measurements. Calibration was done with a standard buffer prepared from potassium dihydrogen phosphate ($0.009695 \mathrm{~mol \cdot kg^{-1}}$) and disodium hydrogen phosphate ($0.03043 \mathrm{~mol \cdot kg^{-1}}$). These phosphates are standard reference materials 186-Id and 186-IId, respectively, from the National Institute of Standards and Technology. Intercomparisons of this "physiological" buffer against Fisher buffers certified at pH = (7.00, 8.00, and 9.00) was also done with satisfactory agreement (±0.03) in the pH of these solutions.

3. Results and discussion

The apparent equilibrium constants for reactions (1) to (5) are, respectively:

$$K'(1) = m(\Sigma\mathrm{IMP})m(\Sigma\mathrm{ammonia})/\{m(\Sigma\mathrm{AMP})m^\circ\}, \tag{11}$$

$$K'(2) = m(\Sigma\mathrm{inosine})m(\Sigma\mathrm{ammonia})/\{m(\Sigma\mathrm{adenosine})m^\circ\}, \tag{12}$$

$$K'(3) = m(\Sigma\mathrm{adenosine})m(\Sigma\mathrm{P_i})/\{m(\Sigma\mathrm{AMP})m^\circ\}, \tag{13}$$

$$K'(4) = m(\Sigma\mathrm{inosine})m(\Sigma\mathrm{P_i})/\{m(\Sigma\mathrm{IMP})m^\circ\}, \tag{14}$$

$$K'(5) = m(\Sigma\mathrm{urocanate})m(\Sigma\mathrm{ammonia})/\{m(\Sigma\textsc{l}\text{-}\mathrm{histidine})m^\circ\}, \tag{15}$$

where $m^\circ = 1 \mathrm{~mol \cdot kg^{-1}}$. Results of equilibrium measurements for reactions (3), (4), and (5) are given in table 1. The uncertainties given for the measured apparent equilibrium constants in table 1 are those calculated for the 95 per cent confidence limit based upon three to six replicate measurements. The results of the calorimetric measurements are given in tables 2 to 6. In these tables, the measured molar enthalpy changes, like the apparent equilibrium constants given above, are denoted by a prime ($'$). The uncertainties in the molar enthalpy changes given in tables 2 to 6 are also at the 95 per cent confidence limits based upon four to six replicate measurements for each set of conditions. These molar enthalpy changes also include a contribution due *to protonation of the buffer in which the reactions occurred. Appropriate corrections* for this will be discussed later. All of the molalities given in tables 1 to 6 are stoichiometric values based upon the amounts of the substances used in preparing the solutions and prior to any reaction.

TABLE 1. Results of equilibrium measurements for reactions (3) to (5). The molalities of the substrates given below are those prior to addition of the appropriate enzyme. The ionic strength I and the equilibrium constants $K°$ for the reference reactions (6) to (10) are calculated quantities. The calculated values of p(Mg) were 4.50 and 4.44 for reactions (3) and (4), respectively

Reaction (3): $\Sigma\mathrm{AMP(aq)} + \mathrm{H_2O(l)} = \Sigma\mathrm{adenosine(aq)} + \Sigma\mathrm{P_i(aq)}$

$\frac{T}{\mathrm{K}}$	pH	$\frac{m(\mathrm{NaHAMP})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{Na_2HPO_4})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{adenosine})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{MgCl_2})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{NaOH})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	K′	$K°(8)$
298.15	8.86	0.01334	0.4563	0	$2.15 \cdot 10^{-4}$	0.0107	1.40	197 ± 12	201
298.15	8.87	0	0.4680	0.01270	$2.21 \cdot 10^{-4}$	0	1.40	174 ± 8	177

Reaction (4): $\Sigma\mathrm{IMP(aq)} + \mathrm{H_2O(l)} = \Sigma\mathrm{inosine(aq)} + \Sigma\mathrm{P_i(aq)}$

$\frac{T}{\mathrm{K}}$	pH	$\frac{m(\mathrm{Na_2HIMP})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{K_2HPO_4})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{inosine})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{MgCl_2})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	K'	$K°(9)$
298.15	8.55	0.02091	0.5011	0	$2.60 \cdot 10^{-4}$	1.56	132 ± 2	163
298.15	8.56	0	0.4996	0.01988	$2.59 \cdot 10^{-4}$	1.49	125 ± 2	153

Reaction (5): ΣL-histidine(aq) = Σurocanate(aq) + Σammonia(aq)

$\frac{T}{\mathrm{K}}$	pH	$\frac{m(\text{L-histidine})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\text{urocanic acid})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{NH_4C_2H_3O_2})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{Tris})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	K'	$K°(10)$
298.25	8.70	0.02910	0	0.1185	0.101	0.145	3.41 ± 0.20	2.35
298.25	8.41	0	0.03134	0.1435	0.101	0.167	4.81 ± 0.14	3.01

TABLE 2. Results of calorimetric measurements for reaction (1): $\Sigma\mathrm{AMP(aq)} + \mathrm{H_2O(l)} = \Sigma\mathrm{IMP(aq)} + \Sigma\mathrm{ammonia(aq)}$. The ionic strength I and the molar enthalpy of reaction $\Delta_r H_m^\circ$ for the reference reaction (6) are calculated quantities

$\frac{T}{\mathrm{K}}$	pH	$\frac{m(\mathrm{NaHAMP})^{a}}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{KCl})}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{m(\mathrm{buffer})^{b}}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{-\Delta_r H'_m {}^{c}}{\mathrm{kJ \cdot mol^{-1}}}$	$\frac{-\Delta_r H_m^\circ(6)}{\mathrm{kJ \cdot mol^{-1}}}$
298.15	7.08	0.01163	0.000	0.102	0.307	44.26 ± 0.24	50.59
298.15	7.34	0.00805	0.000	0.101	0.112	7.75 ± 0.60	54.09
298.15	7.63	0.00925	0.000	0.101	0.107	4.13 ± 0.09	50.52
298.15	7.93	0.00809	0.500	0.101	0.594	4.00 ± 0.24	48.93
298.15	8.02	0.00828	0.000	0.101	0.085	3.65 ± 0.31	49.61
298.15	8.20	0.00931	0.100	0.101	0.181	4.07 ± 0.08	48.90
298.15	8.23	0.00959	0.200	0.101	0.282	4.59 ± 0.08	48.80
298.15	8.38	0.00665	0.000	0.101	0.059	4.97 ± 0.25	50.00
298.15	8.96	0.00940	0.000	0.101	0.046	7.71 ± 0.64	49.34
304.65	7.91	0.00922	0.000	0.101	0.084	5.56 ± 0.12	51.16
310.15	7.79	0.01015	0.000	0.101	0.085	5.43 ± 0.74	50.78

[a] The molality of the NaHAMP prior to any reaction.

[b] (Tris + HCl) buffer was used in all measurements with the exception of the first set done at 298.15 K and pH = 7.08, where phosphate buffer was used.

[c] The results at 298.15 K and pH = 8.96 were corrected for 5.0 moles per cent unreacted AMP. Chromatographic analyses of the reacted solutions from the other sets of measurements showed that at least 99.9 moles per cent of the AMP were converted to IMP.

TABLE 3. Results of calorimetric measurements for reaction (2): Σadenosine(aq) + H_2O(l) = Σinosine(aq) + Σammonia(aq). The ionic strength I and the molar enthalpy of reaction $\Delta_r H_m^\circ$ for the reference reaction (7) are calculated quantities

T/K	pH	m(adenosine)[a]/$\text{mol}\cdot\text{kg}^{-1}$	m(KCl)/$\text{mol}\cdot\text{kg}^{-1}$	m(buffer)[b]/$\text{mol}\cdot\text{kg}^{-1}$	I/$\text{mol}\cdot\text{kg}^{-1}$	$-\Delta_r H'_m$[c]/$\text{kJ}\cdot\text{mol}^{-1}$	$-\Delta_r H_m^\circ(7)$/$\text{kJ}\cdot\text{mol}^{-1}$
298.15	7.09	0.00439	0.000	0.102	0.277	43.72 ± 0.24	50.25
298.15	7.19	0.00745	0.000	0.101	0.099	2.07 ± 0.11	49.16
298.15	7.38	0.00832	0.000	0.101	0.095	2.16 ± 0.11	49.05
298.15	7.47	0.00718	0.206	0.101	0.299	2.06 ± 0.13	48.75
298.15	7.49	0.00801	0.109	0.101	0.202	1.73 ± 0.07	48.41
298.15	7.86	0.00728	0.000	0.101	0.075	2.88 ± 0.15	48.73
298.15	8.17	0.00747	0.000	0.101	0.057	5.50 ± 0.12	50.00
298.15	8.83	0.00731	0.000	0.101	0.023	11.71 ± 0.26	51.19
304.55	7.97	0.00850	0.000	0.101	0.060	5.20 ± 0.10	49.89
310.15	7.84	0.00933	0.000	0.101	0.060	4.77 ± 0.29	49.42

[a] The molality of the adenosine prior to any reaction.

[b] (Tris + HCl) buffer was used in all measurements with the exception of the first set done at 298.15 K and pH = 7.09, where phosphate buffer was used.

[c] These results have been corrected for unreacted adenosine (⩽3 moles per cent) as determined by chromatographic analyses of the reaction mixtures done immediately after each measurement.

TABLE 4. Results of calorimetric measurements for reaction (3): ΣAMP(aq) + H_2O(l) = Σadenosine(aq) + ΣP_i(aq). The ionic strength I and the molar enthalpy of reaction $\Delta_r H_m^\circ$ for the reference reaction (8) are calculated quantities. The calculated values of p(Mg) lie in the range 3.34 to 3.41

T/K	pH	m(NaHAMP)[a]/$\text{mol}\cdot\text{kg}^{-1}$	m(Tris)/$\text{mol}\cdot\text{kg}^{-1}$	$m(MgCl_2)$/$\text{mol}\cdot\text{kg}^{-1}$	I/$\text{mol}\cdot\text{kg}^{-1}$	$\Delta_r H'_m$[b]/$\text{kJ}\cdot\text{mol}^{-1}$	$\Delta_r H_m^\circ(8)$/$\text{kJ}\cdot\text{mol}^{-1}$
298.15	8.02	0.00856	0.101	0.0010	0.078	2.44 ± 0.24	0.96
298.15	8.42	0.00701	0.101	0.0010	0.052	1.76 ± 0.06	1.18
298.15	8.85	0.01702	0.101	0.0010	0.052	0.55 ± 0.14	0.42
304.55	7.78	0.01063	0.101	0.0010	0.086	2.53 ± 0.16	0.28
310.15	7.62	0.00921	0.101	0.0010	0.083	3.50 ± 0.29	0.60

[a] The molality of the NaHAMP prior to any reaction.

[b] Chromatographic analyses of the reaction mixtures showed that at least 99.9 moles per cent of the AMP were converted to adenosine and inorganic phosphate.

The standard equilibrium constants for the reference reactions (6) to (10) are given in terms of activities a:

$$K^\circ(6) = a(\text{HIMP}^{2-})a(\text{NH}_4^+)/\{a(\text{AMP}^{2-})a(\text{H}_2\text{O})a(\text{H}^+)\}, \tag{16}$$

$$K^\circ(7) = a(\text{Hinos})a(\text{NH}_4^+)/\{a(\text{aden})a(\text{H}_2\text{O})a(\text{H}^+)\}, \tag{17}$$

$$K^\circ(8) = a(\text{aden})a(\text{HPO}_4^{2-})/\{a(\text{AMP}^{2-})a(\text{H}_2\text{O})\}, \tag{18}$$

$$K^\circ(9) = a(\text{Hinos})a(\text{HPO}_4^{2-})/\{a(\text{HIMP}^{2-})a(\text{H}_2\text{O})\}, \tag{19}$$

$$K^\circ(10) = a(\text{H}_2\text{uroc}^+)a(\text{NH}_4^+)/\{a(\text{H}_2\text{hist}^+)a(\text{H}^+)\}. \tag{20}$$

The measured quantities are the apparent equilibrium constants (table 1) and molar enthalpies of reaction (tables 2 to 6). A chemical-equilibrium model[(3)] will be used to

TABLE 5. Results of calorimetric measurements for reaction (4): ΣIMP(aq)+H_2O(l) = Σinosine(aq)+ ΣP_i(aq). The ionic strength I and the molar enthalpy of reaction $\Delta_r H^\circ_m$ for the reference reaction (9) are calculated quantities. The calculated values of p(Mg) lie in the range 3.32 to 3.42

$\frac{T}{K}$	pH	$\frac{m(Na_2HIMP)^a}{mol \cdot kg^{-1}}$	$\frac{m(Tris)}{mol \cdot kg^{-1}}$	$\frac{m(MgCl_2)}{mol \cdot kg^{-1}}$	$\frac{I}{mol \cdot kg^{-1}}$	$\frac{\Delta_r H'_m}{kJ \cdot mol^{-1}}$ [b]	$\frac{\Delta_r H^\circ_m(9)}{kJ \cdot mol^{-1}}$
298.15	8.15	0.00961	0.101	0.0010	0.083	1.54 ± 0.10	1.65
298.15	8.55	0.01014	0.101	0.0010	0.063	-0.62 ± 0.16	1.58
298.15	9.14	0.01077	0.101	0.0010	0.045	-1.89 ± 0.98	1.91
304.55	7.92	0.00996	0.101	0.0010	0.087	1.83 ± 0.14	0.99
310.15	7.81	0.01041	0.101	0.0010	0.087	2.30 ± 0.20	1.05

[a] The molality of the Na_2HIMP prior to any reaction.

[b] Chromatographic analyses of the reaction mixtures showed that at least 99.9 moles per cent of the IMP were converted to inosine and inorganic phosphate.

TABLE 6. Results of calorimetric measurements for reaction (5): ΣL-histidine(aq) = Σurocanate(aq)+ Σammonia(aq). The ionic strength I and the molar enthalpy of reaction $\Delta_r H^\circ_m$ for the reference reaction (10) are calculated quantities

$\frac{T}{K}$	pH	$\frac{m(\text{L-histidine})^a}{mol \cdot kg^{-1}}$	$\frac{m(\text{phosphate})}{mol \cdot kg^{-1}}$	$\frac{I}{mol \cdot kg^{-1}}$	$\frac{\Delta_r H'_m}{kJ \cdot mol^{-1}}$ [b]	$\frac{\Delta_r H^\circ_m(10)}{kJ \cdot mol^{-1}}$
298.15	8.15	0.0127	0.0956	0.30	9.24 ± 0.76	7.55
310.15	8.15	0.0123	0.0956	0.29	9.44 ± 0.74	5.74

[a] The molality of the L-histidine prior to any reaction.

[b] 92 moles per cent of the L-histidine were unreacted in the first set of measurements at 298.15 K and 91 moles per cent were unreacted in the second set at 310.15 K. The apparent molar enthalpies of reaction have been corrected for the amount of unreacted L-histidine.

calculate thermodynamic equilibrium constants and standard molar Gibbs energy changes for the reference reactions from these results. The equilibrium model uses both thermodynamic values for the dissociation of H^+(aq) and Mg^{2+}(aq) from the substrates and an extended Debye–Hückel equation for the activity coefficients γ_i of the aqueous species:

$$\ln \gamma_i = -A_m z_i^2 I^{1/2}/(1 + BI^{1/2}) + 2\lambda\Sigma_{j \neq i} m_j. \qquad (21)$$

In the above equation, values of the Debye–Hückel constant A_m and its temperature derivatives have been taken from the review of Clarke and Glew.(9) The ionic strength $I = \frac{1}{2}\Sigma_i m_i z_i^2$, where m_i and z_i are, respectively, the molality and charge number of species i. In the absence of extensive equilibrium results involving variations in the ionic strength, we shall neglect the last term in equation (21) which is the product of an average pairwise interaction parameter λ and the summation over the molalities of the *other* species (designated by a subscript j). We shall also use an estimated 1.6 $kg^{1/2} \cdot mol^{1/2}$ for the "ion-size" parameter B in the above equation. A justification for the use of this value is given in our earlier paper.(3)

Thermodynamic quantities ($pK^\circ = -\lg K^\circ$, $\Delta_r H^\circ_m$, $\Delta_r S^\circ_m$, and $\Delta_r C^\circ_{p,m}$) for the hydrogens and magnesium-ion dissociation reactions relevant to the enzyme catalyzed reactions studied herein are given in table 7. The sources of these quantities will now be considered. The thermodynamic quantities for the dissociation of H^+ and Mg^{2+} from the AMP species are from our recent review(3) of these reactions. For the ionization of $HTris^+(aq)$, the acidity constant is from Bates and Hetzer(11) and the enthalpy change from Öjelund and Wadsö;(12) the heat-capacity change is calculated from the calorimetric results of Prosen and Kilday.(13) The acidity constants and enthalpies and entropies of ionization of $H_3PO_4(aq)$, $H_2PO_4^-(aq)$, and $HPO_4^{2-}(aq)$ were calculated from the standard molar Gibbs energies and enthalpies of formation and the entropies given in the "NBS Tables of Chemical Thermodynamic Properties";(14) the heat-capacity changes were calculated from the apparent molar heat capacities reported by Larson, Zeeb, and Hepler.(15) The result for the dissociation of $Mg^{2+}(aq)$ from $MgHPO_4(aq)$ are from Clarke, Cusworth, and Datta.(16,17) The acidity constant and molar enthalpy of ionization of $NH_4^+(aq)$ were calculated from the formation properties given in the NBS Tables;(14) the change in molar heat capacity is based upon the heat-capacity measurements of Allred and Woolley.(18)

The acidity constant and molar enthalpy of ionization of Haden$^+$(aq) and of Hinos(aq) are from Christensen, Rytting, and Izatt.(19) The acidity constants of the histidine species are from Leberman and Rabin(20) and the enthalpies of ionization of Hhist(aq) and H_2hist$^+$(aq) are from the calorimetric results of Christensen *et al.*(21) Jones and Williams(22) report approximate molar heat-capacity changes for the ionizations of the L-histidine species. These values pertain to a temperature of 310.15 K and an ionic strength of $3.0\ \text{mol}\cdot\text{kg}^{-1}$. We have made no adjustments of these molar heat-capacity changes to $m^\circ = 1\ \text{mol}\cdot\text{kg}^{-1}$ and $T = 298.15$ K. The molar enthalpy of ionization of H_3hist^{2+}(aq) is based on the approximate value reported by Jones and Williams.(22) This molar enthalpy change, determined at 310.15 K and an ionic strength of $3.0\ \text{mol}\cdot\text{kg}^{-1}$, has been adjusted from the reported result of $-1.1\ \text{kJ}\cdot\text{mol}^{-1}$ to the standard value of $-1.6\ \text{kJ}\cdot\text{mol}^{-1}$ at 298.15 K which is given in table 7. Here, we have used the approximate molar heat capacity of Jones and Williams(22) together with an estimated $1.6\ \text{kg}^{1/2}\cdot\text{mol}^{-1/2}$ for the B parameter in equation (21) to make this adjustment. The acidity constants for the urocanate species are based on the results of Halle, Pichon, and Terrier(23) who also cite the earlier results of Mehler and Tabor.(24) The results of Halle, Pichon, and Terrier(23) pertain to a temperature of 293.15 K and an ionic strength of $0.01\ \text{mol}\cdot\text{kg}^{-1}$. These results were adjusted to standard values at 298.15 K with the estimated enthalpies of ionization of the urocanate species (see below) and the same estimated value of the parameter B as used above for the adjustment of the molar enthalpy of ionization of H_3hist^{2+}(aq). The adjustment made to the acidity constant of H_2uroc(aq) is negligible (less than 0.05 in pK°) and the pK° of Huroc(aq) is changed by only 0.003. The values of the acidity constant and molar enthalpy of ionization of H_2IMP$^-$(aq) determined by Phillips *et al.*(25) are given in table 7. Their result(25) for the acidity constant of H_2IMP$^-$(aq) is close to the pK° result of 6.62 reported by Mäkitie and Mirttinen.(26) Clauwert and Stockx(27) report a pK° of 9.62 at 298.15 K for inosine 5′-monophosphate which we attribute to the ionization of HIMP^{2-}(aq).

Additional ionizations at extremes of pH have been reported for several of these

TABLE 7. Thermodynamic quantities at the temperature 298.15 K for the dissociation reactions to $H^+(aq)$ and $Mg^{2+}(aq)$ relevant to the enzyme-catalyzed reactions studied herein. Sources of these values are given in the text. Estimated values are given in parentheses

	$\mathrm{p}K^\circ$	$\Delta_r H^\circ_m$ / $\mathrm{kJ \cdot mol^{-1}}$	$\Delta_r S^\circ_m$ / $\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$	$\Delta_r C^\circ_{p,m}$ / $\mathrm{J \cdot K^{-1} \cdot mol^{-1}}$
$\mathrm{HAMP^-(aq) = H^+(aq) + AMP^{2-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{AMP1}} = 6.74$	-5.4	-147	(-126)
$\mathrm{H_2AMP(aq) = H^+(aq) + HAMP^-(aq)}$	$\mathrm{p}K^\circ_{\mathrm{AMP2}} = 3.99$	18.0	-16	(-63)
$\mathrm{MgAMP(aq) = Mg^{2+}(aq) + AMP^{2-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{MgAMP}} = 2.81$	-11.4	-92	(-251)
$\mathrm{HTris^+(aq) = H^+(aq) + Tris(aq)}$	$\mathrm{p}K^\circ_{\mathrm{Tris}} = 8.075$	47.48	5	-50
$\mathrm{HPO_4^{2-}(aq) = H^+(aq) + PO_4^{3-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{P1}} = 12.34$	14.7	-189	-242
$\mathrm{H_2PO_4^-(aq) = H^+(aq) + HPO_4^{2-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{P2}} = 7.21$	4.2	-124	-220
$\mathrm{H_3PO_4(aq) = H^+(aq) + H_2PO_4^-(aq)}$	$\mathrm{p}K^\circ_{\mathrm{P3}} = 2.15$	-8.0	-68	-128
$\mathrm{MgHPO_4(aq) = Mg^{2+}(aq) + HPO_4^{2-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{MgP}} = 2.70$	-12.2	-93	-251
$\mathrm{NH_4^+(aq) = H^+(aq) + NH_3(aq)}$	$\mathrm{p}K^\circ_{\mathrm{amm}} = 9.25$	52.22	-2	9
$\mathrm{Haden^+(aq) = H^+(aq) + aden(aq)}$	$\mathrm{p}K^\circ_{\mathrm{aden}} = 3.50$	16.4	-12	(0)
$\mathrm{Hinos(aq) = H^+(aq) + inos^-(aq)}$	$\mathrm{p}K^\circ_{\mathrm{inos}} = 8.96$	27.2	-80	(-160)
$\mathrm{Hhist(aq) = H^+(aq) + hist^-(aq)}$	$\mathrm{p}K^\circ_{\mathrm{hist1}} = 9.16$	43.6	-29	≈ -481
$\mathrm{H_2hist^+(aq) = H^+(aq) + Hhist(aq)}$	$\mathrm{p}K^\circ_{\mathrm{hist2}} = 6.00$	29.9	-15	≈ -628
$\mathrm{H_3hist^{2+}(aq) = H^+(aq) + H_2hist^+(aq)}$	$\mathrm{p}K^\circ_{\mathrm{hist3}} = 1.82$	≈ -1.6	-39	≈ -176
$\mathrm{Huroc(aq) = H^+(aq) + uroc^-(aq)}$	$\mathrm{p}K^\circ_{\mathrm{uroc1}} = 5.91$	(27.2)	(-22)	(-160)
$\mathrm{H_2uroc^+(aq) = H^+(aq) + Huroc(aq)}$	$\mathrm{p}K^\circ_{\mathrm{uroc2}} = 3.58$			
$\mathrm{HIMP^{2-}(aq) = H^+(aq) + IMP^{3-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{IMP1}} = 9.62$	(26.0)	(-97)	(-200)
$\mathrm{H_2IMP^-(aq) = H^+(aq) + HIMP^{2-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{IMP2}} = 6.66$	-6.0	-148	(-126)
$\mathrm{MgHIMP(aq) = Mg^{2+}(aq) + HIMP^{2-}(aq)}$	$\mathrm{p}K^\circ_{\mathrm{MgIMP}} = (2.81)$	(-11.4)	(-92)	(-251)

substances. Izatt *et al.*(10) report $\mathrm{p}K^\circ = 13.06$ for adenosine 5′-monophosphate(aq), $\mathrm{p}K^\circ = 12.35$ for adenosine(aq), and $\mathrm{p}K^\circ = 12.36$ for inosine(aq). They(10) also report molar enthalpy changes for these ionizations. Albert(28) reports an approximate p*K* of 1.5 for inosine(aq). Levene, Simms, and Bass(29) report an acidic ionization ($\mathrm{p}K \approx 1.5$) and a basic ionization ($\mathrm{p}K \approx 12.2$) for inosine 5′-monophosphate(aq). There might also be additional ionizations from several of these substances in extremely alkaline or acidic solutions. Since the p*K*s for the ionizations of these species are far removed from the pH range over which our experiments were conducted, the neglect of these ionizations has only a negligible effect (<0.1 per cent) on the subsequent calculations.

Table 7 also contains several estimates made to obtain quantities needed for the equilibrium calculations on these enzyme-catalyzed reactions. The standard molar entropy of ionization of Huroc(aq) is based upon the average of the standard molar entropies of ionization of Hhist(aq) and $\mathrm{H_2hist^+(aq)}$. The standard molar entropy of ionization of $\mathrm{HIMP^{2-}(aq)}$ is estimated from the standard molar entropy of ionization of $\mathrm{AMP^{2-}(aq)}$ reported by Izatt *et al.*(10) These estimated standard molar entropies are used with the standard molar Gibbs energy changes calculated from the respective $\mathrm{p}K^\circ$ values to obtain the estimated molar enthalpy changes for the ionization of Huroc(aq) and $\mathrm{HIMP^{2-}(aq)}$ given in table 7. There are no results in the literature for the dissociation of $\mathrm{Mg^{2+}(aq)}$ from MgHIMP(aq). The quantities for this reaction have been estimated with the quantities for the dissociation reaction of $\mathrm{Mg^{2+}(aq)}$ from MgAMP(aq), although it should be noted that Sigel(30) reports that $\mathrm{p}K = 1.7$ for the dissociation of $\mathrm{Mg^{2+}(aq)}$ from inosine-5′-monophosphate-*N*(1)-

oxide(aq) at 298.15 K and an ionic strength of $0.1\ \text{mol}\cdot\text{kg}^{-1}$. The estimated molar heat-capacity changes given in table 7 are either based upon the method used by Alberty(31) or are typical values for similar acid ionizations.(32)

Having selected a set of thermodynamic quantities for the hydrogen-ion and magnesium-ion dissociation reactions relevant to the enzyme catalyzed reactions studied herein, we are in a position to apply a chemical-equilibrium model to the experimental results in tables 1 to 6 and to calculate thermodynamic quantities for the reference reactions for each of the overall processes. The details of this calculation are given in reference 3. Note that while the equations used for equilibrium calculations on the results obtained in this study are different in detail for each system, the principles used to obtain them are the same as previously described.(3) The use of these equilibrium models with our experimental results leads to the calculated values of K° and $\Delta_r H_m^\circ$ for the reference reactions that are given in tables 1 to 6. The calculated values of the ionic strength I are also given in these tables. The quantity $\text{p(Mg)} = -\lg\{a(\text{Mg}^{2+})\}$, was also calculated for reactions (3) and (4) (see tables 1, 4, and 5).

The averages of the calculated values of K° and $\Delta_r H_m^\circ$ for the reference reactions are given in table 8. In calculating these averages we have dropped only one calorimetric result, namely the measurement at 298.15 K and $\text{pH} = 7.34$ in table 2. In this case, the adjustment from the experimental measurement to the calculated $\Delta_r H_m^\circ$ was more uncertain than for the other measurements done at higher pH values. When calculating the average values of $\Delta_r H_m^\circ$ given in table 8 we included only the calorimetric results obtained at 298.15 K. The uncertainties given in table 8 for $\Delta_r H_m^\circ$ are, in all cases, equal to two standard deviations. The uncertainties assigned to the values of K°, however, have been expanded to include the uncertainty intervals associated with the studies where equilibrium was approached from both directions. Table 8 also contains calculated values of the changes in the standard molar Gibbs energies $\Delta_r G_m^\circ$ and entropies $\Delta_r S_m^\circ$ of the reference reactions. The uncertainties in these latter quantities were calculated from the uncertainties given for the equilibrium constants and molar enthalpies of reaction. Note that the range of uncertainties in $\Delta_r G_m^\circ$ was $0.1\ \text{kJ}\cdot\text{mol}^{-1}$ to $0.7\ \text{kJ}\cdot\text{mol}^{-1}$ and in $\Delta_r H_m^\circ$ was $0.2\ \text{kJ}\cdot\text{mol}^{-1}$ to $0.8\ \text{kJ}\cdot\text{mol}^{-1}$. These uncertainties reflect the range of difficulties encountered in the experimental determinations.

In all of these calculations we have assumed $B = 1.6\ \text{kg}^{1/2}\cdot\text{mol}^{-1/2}$ in equation (21). To determine the magnitude of the uncertainties in the calculated quantities due to this assumption, we examined the effects on the calculated quantities due to changing B to $1.5\ \text{kg}^{1/2}\cdot\text{mol}^{-1/2}$. It was found that the corresponding changes in the calculated values of $\Delta_r H_m^\circ$ were negligible (less than $20\ \text{J}\cdot\text{mol}^{-1}$) in all cases. The K° values calculated for reactions (3) to (5) were changed by no more than 2 per cent due to this perturbation in the B parameter.

Reactions (6) to (10) form a thermochemical cycle which requires that the following conditions hold:

$$\Delta_r G_m^\circ(6) + \Delta_r G_m^\circ(9) - \Delta_r G_m^\circ(7) - \Delta_r G_m^\circ(8) = 0; \tag{22}$$

$$\Delta_r H_m^\circ(6) + \Delta_r H_m^\circ(9) - \Delta_r H_m^\circ(7) - \Delta_r H_m^\circ(8) = 0. \tag{23}$$

TABLE 8. Calculated values of thermodynamic quantities for the reference reactions (6) to (10) at $T = 298.15$ K

	K°	$\frac{-\Delta_r G^\circ_m}{\text{kJ} \cdot \text{mol}^{-1}}$	$\frac{\Delta_r H^\circ_m}{\text{kJ} \cdot \text{mol}^{-1}}$	$\frac{\Delta_r S^\circ_m}{\text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}}$
(6) $\text{AMP}^{2-}(\text{aq}) + \text{H}_2\text{O(l)} + \text{H}^+(\text{aq}) = \text{HIMP}^{2-}(\text{aq}) + \text{NH}_4^+(\text{aq})$			-49.6 ± 0.5	
(7) $\text{aden(aq)} + \text{H}_2\text{O(l)} + \text{H}^+(\text{aq}) = \text{Hinos(aq)} + \text{NH}_4^+(\text{aq})$			-49.4 ± 0.7	
(8) $\text{AMP}^{2-}(\text{aq}) + \text{H}_2\text{O(l)} = \text{aden(aq)} + \text{HPO}_4^{2-}(\text{aq})$	189 ± 25	13.0 ± 0.4	0.9 ± 0.4	47 ± 2
(9) $\text{HIMP}^{2-}(\text{aq}) + \text{H}_2\text{O(l)} = \text{Hinos(aq)} + \text{HPO}_4^{2-}(\text{aq})$	158 ± 7	12.6 ± 0.1	1.7 ± 0.2	48 ± 1
(10) $\text{Hhist(aq)} = \text{uroc}^-(\text{aq}) + \text{NH}_4^+(\text{aq})$	2.7 ± 0.7	2.5 ± 0.7	7.6 ± 0.8	34 ± 4

When the appropriate values of $\Delta_r H^\circ_m$ in table 8 are introduced into the left-hand side of equation (23), the calculated value is $(0.6 \pm 1.0)\ \text{kJ} \cdot \text{mol}^{-1}$. This excellent agreement lends a great deal of confidence to the overall system of calorimetric measurements and calculations.

Molar-heat capacity changes $\Delta_r C^\circ_{p,m}$ for the reference reactions (6) to (9) were calculated from the values in tables 2 to 6 to be, respectively: $(-103, -3, -22$, and $-56)\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$. The standard deviations in these quantities are $(89, 53, 42$, and $35)\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$, respectively. The molar heat-capacity change for reaction (10) is $-151\ \text{J} \cdot \text{K}^{-1} \cdot \text{mol}^{-1}$. It is based on molar enthalpies of reaction determined at only two temperatures and is even more approximate than the molar heat-capacity changes for the other reactions. Also, note that these molar heat-capacity changes are also dependent upon both the model calculation and quantities in the model from which the $\Delta_r H^\circ_m$ values were obtained. Thus, all of these heat-capacity changes have little meaning and must be considered as highly uncertain. Direct heat-capacity measurements on aqueous solutions containing these substances could yield reliable apparent molar heat capacities for the various species, which could then be used to calculate the heat-capacity changes for these reactions.

We now turn to a consideration of calorimetric and equilibrium measurements which have been previously reported for these reactions. George *et al.*(33) have reported $\Delta_r H^\circ_m = (0.42 \pm 0.42)\ \text{kJ} \cdot \text{mol}^{-1}$ for reaction (8) based upon microcalorimetric measurements performed at 298.15 K, $9 \geqslant \text{pH} \geqslant 8$, and $I \leqslant 0.01\ \text{mol} \cdot \text{kg}^{-1}$. George *et al.*(34) later give the enthalpy change for this reaction as 0 in table IV of reference 34 and state that the reaction was carried out in solutions that were not buffered. However, they(33, 34) report only a summary result and give essentially no experimental details. Nevertheless, their result of $(0.42 \pm 0.42)\ \text{kJ} \cdot \text{mol}^{-1}$ is in agreement with the result of $(0.9 \pm 0.4)\ \text{kJ} \cdot \text{mol}^{-1}$ obtained in this study. George *et al.*(34) also state that $\Delta_r G^\circ_m$ is $-9.6\ \text{kJ} \cdot \text{mol}^{-1}$ for reaction (8) at 298.15 K based upon the determination of an equilibrium constant obtained with a deaminase assay to measure the AMP concentration at equilibrium after separation of the products. This standard molar Gibbs energy change corresponds to an equilibrium constant of 48. The result of the present study is $K^\circ = (189 \pm 25)$. Since George *et al.*(34) did not specify an uncertainty in their reported result and give few experimental details, theirs must be considered an approximate value which might or might not be in agreement with the present finding.

Williams and Hiroms(35) studied the equilibrium between L-histidine and urocanic acid and ammonia (reaction 5). In this study they used spectrophotometry in two experiments to determine the concentration of urocanic acid and, in one experiment, paper chromatography to measure the concentration of L-histidine. The reaction approached equilibrium only from the urocanic acid direction in their experiments. The conditions under which these apparent equilibrium constants were determined were: $\mathrm{pH} = 8.0$; $T = 298.15$ K; $0.1\ \mathrm{mol \cdot dm^{-3}}$ (Tris + acetate) buffer; c(potassium urocanate) $= 0.2\ \mathrm{mol \cdot dm^{-3}}$; and $c(\mathrm{NH_4Cl}) = 0.5\ \mathrm{mol \cdot dm^{-3}}$. The apparent equilibrium constants they reported ranged from 2 to 5 and had a mean of 3.3. A reasonable estimate of the statistical uncertainty in this result is ± 2. We use our final result for K° for reaction (10) and the equilibrium model to calculate an apparent equilibrium constant of (5.9 ± 0.7) under the conditions used by Williams and Hiroms. Thus, our results are in agreement with the measurements of Williams and Hiroms.(35)

McClard and Kolenbrander(36) also performed equilibrium measurements on reaction (5) by spectrophotometric determination of the concentration of urocanic acid. Their results pertain to $\mathrm{pH} = 7.2$ and $0.04\ \mathrm{mol \cdot dm^{-3}}$ phosphate buffer. They(36) reported the results of their equilibrium measurements in graphical form from which we obtain the following apparent equilibrium constants for reaction (5) as a function of temperature: 0.015 at 303 K, 0.025 at 305 K, 0.027 at 308 K, 0.040 at 311 K, and 0.097 at 316 K. McClard and Kolenbrander(36) also calculate an apparent molar enthalpy change of $107.5\ \mathrm{kJ \cdot mol^{-1}}$ for this reaction from the temperature dependence of their equilibrium results. We use our final results and the equilibrium model to calculate the following apparent equilibrium constants under conditions comparable to those used by McClard and Kolenbrander:(36) 4.6 at 303 K, 4.7 at 305 K, 4.8 at 308 K, 5.0 at 311 K, and 5.3 at 316 K. Clearly, there is a serious discrepancy between their results and ours. We note that McClard and Kolenbrander determined only urocanic acid in their measurements and equilibrium was approached only from the direction of L-histidine. If they(36) had studied a reaction that was not at equilibrium, the apparent equilibrium constants which they measured would have been much lower than the true values. On this basis, and since we measured both urocanic acid and L-histidine and performed experiments in which equilibrium was approached from both directions, we believe that the results obtained by McClard and Kolenbrander are in error.

Wolfenden(37) studied the equilibrium between adenosine and inosine and ammonia (reaction 2). In his equilibrations (2 h at 298.15 K), Wolfenden started with solutions containing radioactively labeled inosine and then used paper chromatography to obtain a separation of the adenosine and inosine. He then reported the number of counts for the adenosine and inosine spots. Thus, the ratio of the radioactivity associated with the adenosine and inosine spots yielded the ratio of their concentrations. Although Wolfenden did not report the total amount of inosine used in his experiments, it was apparently much smaller than the total amount of ammonia in the solution which was known at the start of the experiments. Thus, apparent equilibrium constants K' can be calculated from the results given in the tables and figures of Wolfenden's paper. Wolfenden does not report values of apparent equilibrium constants but states that "$K_{eq} = c(\text{inosine})c(NH_3)/\{c(\text{adenosine})c(H_2O)\} = 38$ with water concentration taken as 1". He(37) did not explain how he obtained this value. In any case, we have calculated a total of 15 apparent equilibrium constants for reaction (2) from the information given in Wolfenden's table I and figures 1b and 2. These results cover the pH range 6.50 to 10.18 and were at ionic strengths of $0.5\ \text{mol} \cdot \text{kg}^{-1}$ to $2.0\ \text{mol} \cdot \text{kg}^{-1}$. We then used our equilibrium model to calculate values of K° for reaction (7) which ranged from $4.5 \cdot 10^9$ to $1.5 \cdot 10^{12}$. The average was $1.8 \cdot 10^{11}$ and the standard deviation was $0.9 \cdot 10^{11}$; although, if two of the most extreme results are discarded, the average becomes $0.89 \cdot 10^{11}$ with a standard deviation of $0.19 \cdot 10^{11}$. This yields the result $\Delta_r G_m^\circ = -64.2\ \text{kJ} \cdot \text{mol}^{-1}$ for reaction (7). This result, based upon a radioactive assay, is the only available information in the literature on the standard molar Gibbs energy change for reaction (7). However, Cohen and Wolfenden(38) have reported an apparent equilibrium constant of 78 ($T = 298.15$ K, pH = 9.2, $I \approx 2.0\ \text{mol} \cdot \text{kg}^{-1}$) for the reaction:

$$\Sigma\text{cytidine(aq)} + H_2O(l) = \Sigma\text{uridine(aq)} + \Sigma\text{ammonia(aq)}. \qquad (24)$$

We take ionization constants for uridine ($pK^\circ = 9.30$) and for cytidine ($pK^\circ = 4.08$ and 12.24) from reference 39 and use an equilibrium model for this reaction to calculate $K^\circ = 2.22 \cdot 10^{10}$ for

$$\text{cytidine(aq)} + H_2O(l) + H^+(aq) = \text{uridine(aq)} + NH_4^+(aq). \qquad (25)$$

This corresponds to $\Delta_r G_m^\circ = -59.1\ \text{kJ} \cdot \text{mol}^{-1}$ for reaction (25) which is comparable to the result of $-64.2\ \text{kJ} \cdot \text{mol}^{-1}$ found for the chemically similar reaction (7) involving the deamination of adenosine.

A possible thermodynamic pathway to these reactions involves the use of enthalpies of combustion and solution, third-law entropies, solubilities, and activity coefficients which can be combined to calculate the standard molar Gibbs energy and enthalpy of formaton and the entropy of the aqueous species. However, for the substances used in this investigation, the only existing information of this type appear to be a third-law entropy for L-histidine · HCl(cr),(40) an enthalpy of combustion for L-histidine(cr),(41) an enthalpy of solution of L-histidine(cr),(42) and solubilities of L-histidine · HCl(cr) as a function of temperature.(42) Therefore, at present, it is not possible to perform a thermodynamic-cycle calculation in which the formation properties of the substances are utilized.

We now turn to a consideration of how the thermodynamic results obtained herein relate to results obtained for other reactions that have structurally similar changes occurring in them. The $\Delta_r H_m^\circ$ obtained in this study for reaction (8), the hydrolysis of $AMP^{2-}(aq)$, was $(0.9 \pm 0.4)\ \text{kJ} \cdot \text{mol}^{-1}$. Hinz *et al.*(43) found that the molar enthalpy change for the similar reaction involving guanosine 5′-monophosphate was $2.7\ \text{kJ} \cdot \text{mol}^{-1}$. Thus, both enthalpy changes are slightly positive, in contrast to the molar enthalpies of hydrolysis of the di- and tri-phosphates which are(43,44) in the range $-20.5\ \text{kJ} \cdot \text{mol}^{-1}$ to $-23.0\ \text{kJ} \cdot \text{mol}^{-1}$. Note in table 8 that the molar entropy changes for reactions (8) and (9) are the same. Also, the molar enthalpy changes for the structurally similar reactions (6) and (7) are very nearly equal. The same situation exists for the molar enthalpy changes for reactions (8) and (9). As a result of the examination of a substantial body of thermodynamic information on the sugars and their monophosphates,[45] it was found that $\{S_2^\circ(\text{sugar, aq}) - S_2^\circ(\text{sugar phosphate}^{2-}, \text{aq})\} \approx 135\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. With the $\Delta_r S_m^\circ$ results for reactions (8) and (9), together with the standard partial molar entropy[46] of HPO_4^{2-}(aq) and the standard molar entropy[46] of H_2O(l), we find that $\{S_2^\circ(\text{adenosine, aq}) - S_2^\circ(\text{AMP}^{2-}(\text{aq})\} = \{S_2^\circ(\text{inosine, aq}) - S_2^\circ(\text{HIMP}^{2-}(\text{aq})\} = 151\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$. This result is comparable with the typical difference of $135\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ obtained from the results on the sugars and their phosphates.

The conversion of L-histidine to urocanic acid and ammonia (reaction 10), is similar to the reactions:

$$\text{phenylalanine(aq)} = \textit{trans}\text{-cinnamic acid}^-\text{(aq)} + NH_4^+\text{(aq)}, \tag{26}$$

$$\text{aspartic acid(aq)} = \text{fumarate}^-\text{(aq)} + NH_4^+\text{(aq)}. \tag{27}$$

The thermodynamic quantities for these two reactions are:[47] $\Delta_r G_m^\circ = -0.37\ \text{kJ}\cdot\text{mol}^{-1}$, $\Delta_r H_m^\circ = 24.8\ \text{kJ}\cdot\text{mol}^{-1}$, and $\Delta_r S_m^\circ = 84.4\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for reaction (26); $\Delta_r G_m^\circ = 12.2\ \text{kJ}\cdot\text{mol}^{-1}$, $\Delta_r H_m^\circ = 32.0\ \text{kJ}\cdot\text{mol}^{-1}$, and $\Delta_r S_m^\circ = 66.3\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for reaction (27). Chemical rationalization of the thermodynamics of this reaction has followed[47] the hypothesis advanced by Havir and Hanson[48] that the standard molar entropy changes for these processes were similar and that the differences in the molar enthalpies of reaction could be ordered in terms of resonance stabilization of the double bonds which are formed by the reaction. It was found in this study that $\Delta_r S_m^\circ = (34 \pm 4)\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$ for reaction (10). Thus, the first part of the above hypothesis is not valid. Clearly, additional effects underlie the thermodynamics of these reactions and, at present, we are unable to rationalize these findings.

As mentioned earlier in this paper, the conversion of L-histidine to urocanic acid (reaction 5) is used for the manufacture of urocanic acid. We have used the results obtained in this study and the equilibrium model for this reaction to calclulate the effects of changes of temperature, pH, and ionic strength on the apparent equilibrium constant K'. Thus, based on these calculations, it is found that $K' = 4.2$ at 298.15 K, pH = 7.0, and $I = 0.1\ \text{mol}\cdot\text{kg}^{-1}$. Keeping the ionic strength and pH constant, but increasing the temperature to 333.15 K, leads to $K' = 6.1$. The pH at which K' has its maximum value at 298.15 K and $I = 0.1\ \text{mol}\cdot\text{kg}^{-1}$ is 7.5. Here, $K' = 4.3$. However, if the activity-coefficient model used in this study is correct, the predicted values of K' become larger when the ionic strength is increased. Thus, at 298.15 K, pH = 7.50, and $I = 2.0\ \text{mol}\cdot\text{kg}^{-1}$, $K' = 6.7$. An additional and important consideration here is the limited solubility of urocanic acid(cr) which we have found to be approximately $0.033\ \text{mol}\cdot\text{kg}^{-1}$ in water at 298.15 K. This thermodynamic information can be used in conjunction with information on the kinetics of the reaction, the stability of the enzyme, and other practical considerations to determine an optimal set of operating conditions for the manufacture of urocanic acid from L-histidine.

It is also desirable to have values of apparent equilibrium constants K' for these reactions under approximately physiological conditions. Here, physiological conditions are taken to be:[44,49] $T = 310.15$ K, pH = 7.0, $m(Mg^{2+}) = 0.0005\ \text{mol}\cdot\text{kg}^{-1}$, and $I = 0.25\ \text{mol}\cdot\text{kg}^{-1}$. With the thermodynamic quantities obtained in this study for the reference reactions and with the equilibrium model for these complex reactions, the following values of the apparent equilibrium constant K' for reactions (3) to (5), respectively, are calculated for these conditions: 241, 210, and 5.6. The corresponding standard apparent molar Gibbs energy changes ($\Delta G_{\mathrm{m}}^{\circ\prime} = -RT \cdot \lg K'$) are: ($-14.1$, -13.8, and -4.4) $\mathrm{kJ \cdot mol^{-1}}$. Since these reactions are all unsymmetrical, the extent of reaction is related to the total molalities of these substances in solution. Under *in vivo* conditions, where the concentrations of these substances is small ($< 0.001\ \mathrm{mol \cdot dm^{-3}}$), the equilibrium position of these reactions lies very far to the right (< 0.01 mole per cent of the substances on the left-hand side of reactions (3) to (5) remaining). Thus, in much of the biological literature these reactions would be described as "irreversible".

We thank Sam Margolis of the National Institute of Standards and Technology, Robert Alberty of the Massachusetts Institute of Technology, and Todd King and Richard Veech of the National Institutes of Health for helpful discussions on the chemistry of these reactions.

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