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Thermochemistry of the hydrolysis of L-arginine to (L-citrulline + ammonia) and of the hydrolysis of L-arginine to (L-ornithine + urea)

Y. B. Tewari, N. Kishore, S. A. Margolis, R. N. Goldberg,

Biotechnology Division, National Institute of Standards and Technology,
Gaithersburg, MD 20899, U.S.A.

and T. Shibatani

Tanabe Seiyaku Company, Ltd., 16–89 Kashima-3-Chome,
Yadogwa-Ku, Osaka 532, Japan

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Molar enthalpies of reaction for the hydrolysis of L-arginine(aq) to {L-ornithine(aq) + urea(aq)} and for the hydrolysis of L-arginine(aq) to {L-citrulline(aq) + ammonia(aq)} have been measured by microcalorimetry. These reactions are catalyzed, respectively, by arginase and by arginine deiminase. The effects of variations in pH, temperature, and ionic strength on the molar enthalpies of reaction were studied. The results have been analyzed with a model which accounts for the complex equilibria in solution. The results obtained at $T = 298.15$ K for the reference reactions are: $\Delta_r H_m^\circ = -(21.4 \pm 0.5)\ \mathrm{kJ \cdot mol^{-1}}$ for L-arginine$^+$(aq) + H_2O(l) = L-ornithine$^+$(aq) + urea(aq) and $\Delta_r H_m^\circ = -(31.9 \pm 0.8)\ \mathrm{kJ \cdot mol^{-1}}$ for L-arginine$^+$(aq) + H_2O(l) = L-citrulline(aq) + NH_4^+(aq). These results are discussed in terms of thermodynamic-cycle calculations and in terms of the metabolic urea cycle.

1. Introduction

The reactions studied herein are:[†]

$$\Sigma\mathrm{arg(aq)} + \mathrm{H_2O(l)} = \Sigma\mathrm{orn(aq)} + \Sigma\mathrm{urea(aq)}, \tag{1}$$

$$\Sigma\mathrm{arg(aq)} + \mathrm{H_2O(l)} = \Sigma\mathrm{cit(aq)} + \Sigma\mathrm{ammonia(aq)}. \tag{2}$$

Reactions (1) and (2) are catalyzed, respectively, by arginase (EC 3.5.3.1) and by arginine deiminase (EC 3.5.3.6).[‡] Here, the Σs denote the total amounts of each of

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[†] Abbreviations used in this paper are: arg, L-arginine; cit$^-$, L-citrulline$^-$; orn, L-ornithine; ATP, adenosine 5′-triphosphate; AMP, adenosine 5′-monophosphate; FMOC, 9-fluoroenylmethylchloroformate. The protonated forms of the amino acids are indicated by the addition of a hydrogen-ion to these species. Chemical Abstract Service registry numbers for the principal compounds used in this study are: L-arginine monohydrochloride, 119-34-2; L-ornithine monohydrochloride, 3184-13-2; L-citrulline, 627-77-0; and urea, 57-13-6.

[‡] Enzyme commission numbers and nomenclature are from Webb, E. C. *Enzyme Nomenclature*. Academic Press: Orlando. **1984**.

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the substances which may exist in a variety of ionized forms in solution. Reaction (1) is the final step in the metabolic urea cycle. Reaction (2) is employed for the industrial manufacture of L-citrulline which, in turn, is used medicinally for the treatment of asthenia. A thermodynamic study of these reactions is of value in understanding the direction of these reactions under a variety of possible conditions. As will be seen below, it will also serve to clarify the role of reaction (1) in the metabolic urea cycle.

2. Experimental

The substances used in this study and their molar masses were: L-citrulline, $0.17519\ \mathrm{kg \cdot mol^{-1}}$; L-arginine monohydrochloride, $0.21066\ \mathrm{kg \cdot mol^{-1}}$; L-ornithine monohydrochloride, $0.16862\ \mathrm{kg \cdot mol^{-1}}$; urea, $0.060056\ \mathrm{kg \cdot mol^{-1}}$; K_2HPO_4, $0.17418\ \mathrm{kg \cdot mol^{-1}}$; $MnCl_2$, $0.12584\ \mathrm{kg \cdot mol^{-1}}$; KCl, $0.074551\ \mathrm{kg \cdot mol^{-1}}$; H_2O, $0.0180153\ \mathrm{kg \cdot mol^{-1}}$; and H_3PO_4, $0.097995\ \mathrm{kg \cdot mol^{-1}}$. Samples of L-citrulline, L-arginine monohydrochloride, L-ornithine monohydrochloride, $MnCl_2$, and urea were obtained from Fluka.† The K_2HPO_4, the concentrated phosphoric acid, and the arginase were, respectively, from Fisher Scientific, Baker, and Sigma. The arginine deiminase was prepared from *Pseudomonas putida* according to a procedure previously described.⁽¹⁾ Both enzymes were in the form of lyophilized powders. Moisture contents for the following substances were determined by Karl-Fischer analyses with resulting mass fractions: L-citrulline, 0.01; L-arginine monohydrochloride, 0.011; L-ornithine monohydrochloride, 0.011; and urea, 0.0057. Moisture contents for the following salts were determined by drying in an oven at the temperature $T = 413$ K with resulting mass fractions: $MnCl_2$, 0.029; KCl, 0.00036; and K_2HPO_4, 0.0080. Analyses of the L-citrulline, L-arginine monohydrochloride, L-ornithine monohydrochloride, and urea were done chromatographically (Hewlett-Packard model 1090 h.p.l.c., Bio-Rad Aminex HPX-72S column thermostatted at $T = 328$ K, mobile phase $0.2\ \mathrm{mol \cdot dm^{-3}}$ $(NH_4)_2SO_4(aq)$ at pH = 8.5, flow rate of $0.013\ \mathrm{cm^3 \cdot s^{-1}}$, and a refractive-index detector). The results of these analyses indicated that the total mole fraction of impurities in these samples was <0.001.

The primary method used for the analysis of the reaction mixtures is based upon a method developed by Cunico *et al.*⁽²⁾ A Hewlett-Packard model 1090 h.p.l.c. with a Varian C_{18} Aminotag column thermostatted at $T = 308$ K and an ultraviolet detector set at the wavelength 264 nm was used for most of the chromatographic measurements. A fluorescence detector (wavelength of 264 nm for excitation and 313 nm for emission) was used instead of the u.v. detector when additional sensitivity was needed in the attempts to determine the apparent equilibrium constants for the two reactions under study. The mobile phase (flow rate of $0.022\ \mathrm{cm^3 \cdot s^{-1}}$) was produced as a gradient mixture of two solutions designated as solutions I and II, respectively. The gradient was set at 100 volume per cent of solution I at the start; 66

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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.

volume per cent of solution I and 34 volume per cent of solution II at 14 min; and 100 volume per cent of solution II at 29 min. Solution I was 25 volume per cent of acetonitrile and 75 volume per cent of a second solution {(0.01 $\mathrm{mol \cdot dm^{-3}}$ tetramethylammonium chloride + 0.02 $\mathrm{mol \cdot dm^{-3}}$ sodium citrate) adjusted to $\mathrm{pH} = 3.75$ with concentrated phosphoric acid}. Solution II was 72 volume per cent of acetonitrile and 28 volume per cent of a second solution. In this case, the second solution was 90 volume per cent of an aqueous solution (0.01 $\mathrm{mol \cdot dm^{-3}}$ tetramethylammonium chloride + 0.02 $\mathrm{mol \cdot dm^{-3}}$ sodium citrate adjusted to $\mathrm{pH} = 4.25$ with concentrated phosphoric acid) and 10 volume per cent of methanol.

The analytical procedure required the addition of 2.0 g of an aqueous solution (0.2 $\mathrm{mol \cdot dm^{-3}}$ boric acid + 0.2 $\mathrm{mol \cdot dm^{-3}}$ sodium bicarbonate adjusted to $\mathrm{pH} = 8.5$ with concentrated NaOH) to 0.1 g of the solution being analyzed. A second solution (3 mg of 9-fluoroenylmethylchloroformate (FMOC) in 1 $\mathrm{cm^3}$ of dry acetone) was immediately added to this solution in the volume ratio 1. The resulting mixture was shaken briefly and allowed to stand for 10 min at room temperature (295 K). Samples of this final solution were then injected into the chromatograph. With this derivatization procedure and the chromatographic conditions described above, typical retention times were 11.4 min for L-arginine, 13.9 min for L-citrulline, and 29.6 min for L-ornithine. All of the dilutions of the samples being analyzed were done gravimetrically so that the dilution factors would be well known.

For the analysis of some of the reaction mixtures obtained from the hydrolysis of L-arginine(aq) to {L-ornithine(aq) + urea(aq)}, a Hewlett-Packard model 1090 h.p.l.c. with a Bio-Rad Aminex HPX-72S column thermostatted at $T = 328$ K was used. The mobile phase was ammonium sulfate (0.2 $\mathrm{mol \cdot dm^{-3}}$ adjusted to $\mathrm{pH} = 8.5$ with aqueous ammonia), the flow rate was 0.013 $\mathrm{cm^3 \cdot s^{-1}}$, and the u.v. detector was set at a wavelength of 210 nm. Typical retention times were 7.4 min for L-ornithine, 8.2 min for L-arginine, 9.6 min for L-citrulline, and 13.2 min for urea. The earlier-mentioned chromatographic procedure with the derivatization procedure had the advantage of an enhanced sensitivity over this second chromatographic method.

The calorimeters were of the heat-conduction type. The sample vessels, which were fabricated from high-density polyethene, contained two compartments holding approximately 0.55 $\mathrm{cm^3}$ and 0.45 $\mathrm{cm^3}$ of solution, respectively. The vessels and their contents were allowed to equilibrate for at least 1 h before the solutions in the vessel were mixed. Calibration of the calorimeters was done electrically with a calibrated potential-difference meter, standard resistor, and time-interval counter. In recent years we have used the reaction of aqueous Tris with aqueous HCl (which is kept at a molality greater than that of the Tris) as a test reaction. The result of 31 measurements of this molar enthalpy of neutralization at $T = 298.15$ K is $\Delta_r H_m^\circ = -(47.475 \pm 0.058)\ \mathrm{kJ \cdot mol^{-1}}$. The uncertainty is equal to two standard deviations of the mean. This result is in excellent agreement with the result of $\Delta_r H_m^\circ = -(47.48 \pm 0.03)\ \mathrm{kJ \cdot mol^{-1}}$ reported by Öjelund and Wadsö.(3) Complete descriptions of the calorimeters and their performance characteristics, the acquisition system, and the computer programs used to treat the results are given in references 4 and 5.

Measurements of enthalpies 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 L-arginine monohydrochloride in a buffer solution. Reaction (1) required the addition of $MnCl_2$ as a cofactor. The enzyme solution was prepared by adding the same buffer solution to the appropriate enzyme, *i.e.* either arginase or arginine deiminase. The mass fractions of the enzymes in the calorimetric reaction mixtures were 0.0025 to 0.0040 for arginase (reaction 1) and 0.013 to 0.016 for arginine deiminase (reaction 2). The extents 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. Note that the activity of the enzyme is destroyed by the addition of the (FMOC + acetone) to the solutions removed from the calorimeters. Since this addition was done within 60 s of the end of a calorimetric experiment, the position of the reaction was frozen and the amount of unreacted substrate(s) determined by this method of analysis should have accurately reflected the amounts of these substances remaining in the calorimeter at the end of a measurement. Thus, only a small amount of unreacted L-arginine {(0.16 to 0.41) mole per cent} was found for reaction (1) and less (0.01 mole per cent to 0.07 mole per cent) for reaction (2). Appropriate corrections were applied for any unreacted substrate when calculating the molar enthalpies of reaction. The chromatography indicated that no side reactions occurred during the hydrolysis of L-arginine · HCl(aq) to {L-ornithine(aq) + urea(aq)}. However, it was found that some L-ornithine(aq) was also formed when L-arginine · HCl(aq) was hydrolyzed to {L-citrulline(aq) + ammonia(aq)}, *i.e.* reaction (2). The mole fraction formed depended upon the conditions of reaction and varied from 0.006 to 0.140. Corrections for this side reaction were made (see below) to determine the molar enthalpy of reaction (2). The two chromatographic methods described above indicated the absence of additional side reactions and showed that there was a mass balance between the amount of L-arginine(aq) reacted and the amounts of the products formed.

The calorimetric experiments typically lasted about 30 min. The "blank" enthalpy changes accompanying the mixing of the substrate solution and of the enzyme solution with the buffer were determined for each set of calorimetric conditions. These blank values varied with the conditions of measurement and were -0.00033 J to 0.0119 J for reaction (1) and -0.0024 J to 0.0222 J for reaction (2). The reproducibility of the blank values for a particular set of conditions was in all cases within ± 0.002 J. The measured enthalpies of reactions were -0.203 J to -0.290 J for reaction (1) and -1.90 J to -2.10 J for reaction (2).

Attempts were also made to determine the apparent equilibrium constants for reactions (1) and (2) by approaching the position of equilibrium from both directions for each reaction. In these attempts, a fluorescence detector was used to obtain a substantially increased sensitivity over the u.v. detector which was used to monitor the extent of reaction in the calorimetric experiments. In spite of this substantially increased sensitivity, we are presently unable to state that the signal attributable to the remaining L-arginine in solution is above the chromatographic background. Therefore, it was not possible to determine apparent equilibrium constants for reactions (1) and (2).

The measurement of the pH of the final reaction mixtures in the 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. Calibration was performed 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 pHs of these solutions.

3. Results and discussion

The analysis of the experimental results was done in terms of an equilibrium model(6) which accounts for the contributions of the various ionizations of the reacting substances to the measured quantities, *i.e.* the molar enthalpies in the case of this study. Thus, from measured quantities which pertain to an overall reaction, it is possible to calculate quantities which pertain to reference reactions involving specific species. The reference reactions for reactions (1) and (2) are, respectively, chosen to be:

$$\mathrm{Harg^{+}(arg) + H_2O(l) = Horn^{+}(aq) + urea(aq)}, \tag{3}$$

$$\mathrm{Harg^{+}(aq) + H_2O(l) = Hcit(aq) + NH_4^{+}(aq)}. \tag{4}$$

The analysis of the results of this investigation requires a knowledge of the acidity constants and molar enthalpies of ionization of the various substances. Table 1 contains the acidity constants and associated molar enthalpy and molar heat-capacity changes from the literature for L-arginine(aq), L-citrulline(aq), and L-ornithine(aq). Included in table 1 are results for the ionization reactions of these substances which have pKs that lie within 3 of the pH range in which our measurements were performed. The results of the various studies shown in table 1 have also been adjusted to the standard state with an extended Debye–Hückel expression in which the "ion-size" parameter was set(6) at $1.6\,\mathrm{kg^{1/2}\cdot mol^{-1/2}}$. Here, and throughout this paper, the standard molality for a solute species is $1\,\mathrm{mol\cdot kg^{-1}}$. The standard pressure is 0.1 MPa. We now turn to a discussion of the selection of the results which will be used in subsequent calculations.

Datta and Grzybowski(11) used electrochemical cells without liquid junctions to determine acidity constants for L-arginine(aq). This method is generally more accurate than the methods used in the other studies and we have therefore adopted their results for use in our calculations. Datta and Grzybowski(11) also report acidity constants for L-arginine(aq) at several temperatures. We have used their results to calculate the standard molar enthalpy and standard molar heat-capacity changes for the ionization of L-arginine(aq). For the standard acidity constants of L-ornithine(aq) and L-citrulline(aq), we have adopted an average of the results shown in table 1 and have discarded only the result of Trikha *et al.*(18) for L-citrulline(aq). The standard

TABLE 1. Acidity constants and associated molar enthalpy and molar heat-capacity changes for the ionization of L-arginine(aq), L-citrulline(aq), and L-ornithine(aq) from the literature. The results have been adjusted to $m^\circ = 1\ \mathrm{mol \cdot kg^{-1}}$ and $T = 298.15$ K

$\mathrm{Harg^+(aq) = H^+(aq) + arg(aq)}$

$\frac{T}{\mathrm{K}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	pK	pK°	Reference
293.15	≈0.01	9.08	9.03	Albert(7)
298.15	0.0	9.01	9.01	Batchelder and Schmidt(8)
296.15	≈0.10	9.09	9.04	Birch and Harris(9)
298.15	0.10	9.36	9.57	Clarke and Martell(10)
298.15	0.0	8.991	8.99	Datta and Grzybowski(11)
298.15	0.10	9.07	9.28	Ellenbogen(12)
310.15	0.15	8.82	9.37	Hallman *et al.*(13)
292.15	≈0.01	9.11	9.04	Perkins(14)
293.15	1.0	9.21	9.47	Perrin(15)
298.15	0.1	9.07	9.28	Sakurai *et al.*(16)
298.30	0.15	9.11	9.36	Tanford and Shore(17)

$\frac{T}{\mathrm{K}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{\Delta_r H_m}{\mathrm{kJ \cdot mol^{-1}}}$	$\frac{\Delta_r H_m^\circ}{\mathrm{kJ \cdot mol^{-1}}}$	Reference
298.15	0.0	44.89	44.89	Datta and Grzybowski(11)

$\frac{T}{\mathrm{K}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{\Delta_r C_{p,m}}{\mathrm{kJ \cdot mol^{-1}}}$	$\frac{\Delta_r C_{p,m}^\circ}{\mathrm{kJ \cdot mol^{-1}}}$	Reference
298.15	0.0	−38	−38	Datta and Grzybowski(11)

$\mathrm{Hcit(aq) = H^+(aq) + cit^-(aq)}$

$\frac{T}{\mathrm{K}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	pK	pK°	Reference
298.15	0.10	9.71	9.92	Clarke and Martell(10)
298.15	0.10	9.41	9.62	Ellenbogen(12)
293.15	≈0.01	9.69	9.63	Perkins(14)
298.15	0.10	8.64	8.85	Trikha *et al.*(18)

$\mathrm{Horn^+(aq) = H^+(aq) + orn(aq)}$

$\frac{T}{\mathrm{K}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	pK	pK°	Reference
293.15	≈0.01	8.62	8.57	Albert(7)
298.15	0.0	8.69	8.69	Batchelder and Schmidt(8)
298.15	0.10	8.98	9.19	Clarke and Martell(10)
298.15	0.15	8.80	9.04	Griffith *et al.*(19)
310.15	0.15	8.55	9.11	Hallman *et al.*(13)
298.15	0.10	8.74	8.95	Hay and Morris(20)
293.15	1.0	8.93	9.18	Perrin(15)
298.15	0.3	8.86	9.16	Sayer and Rabenstein(21)
≈298.15	0.2	8.83	9.10	Gergely *et al.*(22)

$\frac{T}{\mathrm{K}}$	$\frac{I}{\mathrm{mol \cdot kg^{-1}}}$	$\frac{\Delta_r H_m}{\mathrm{kJ \cdot mol^{-1}}}$	$\frac{\Delta_r H_m^\circ}{\mathrm{kJ \cdot mol^{-1}}}$	Reference
≈298.15	0.2	47.1	46.3	Gergely *et al.*(22)

TABLE 2. Thermodynamic quantities at $T = 298.15$ K and $p = 0.1$ MPa for the hydrolysis of L-arginine(aq) to {L-ornithine(aq) + urea(aq)} and for the hydrolysis of L-arginine(aq) to {L-citrulline(aq) + ammonia(aq)}. The acidity constants and related thermodynamic quantities for L-arginine(aq), L-citrulline(aq), L-ornithine(aq), ammonia(aq), and orthophosphate(aq) are also given. The results for the hydrolysis reactions of L-arginine(aq) are from this study

Reaction	$K°$ or p$K°$	$\Delta_r H_m^\circ$ / $\text{kJ}\cdot\text{mol}^{-1}$	$\Delta_r S_m^\circ$ / $\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$	$\Delta_r C_{p,m}^\circ$ / $\text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$
$\text{Harg}^+(\text{aq}) + \text{H}_2\text{O(l)} = \text{Horn}^+(\text{aq}) + \text{urea(aq)}$	$K° > 2 \cdot 10^4$	(-21.4 ± 0.5)		
$\text{Harg}^+(\text{aq}) + \text{H}_2\text{O(l)} = \text{Hcit(aq)} + \text{NH}_4^+(\text{aq})$	$K° > 2.6 \cdot 10^4$	$-(31.9 \pm 0.8)$		
$\text{Harg}^+(\text{aq}) = \text{H}^+(\text{aq} + \text{arg(aq)}$	p$K° = 8.99$	44.89	−21.5	−38
$\text{Hcit(aq)} = \text{H}^+(\text{aq}) + \text{cit}^-(\text{aq})$	p$K° = 9.72$	49.8[a]	−19[a]	
$\text{Horn}^+(\text{aq}) = \text{H}^+(\text{aq}) + \text{orn(aq)}$	p$K° = 9.00$	46.3	−17.0	
$\text{NH}_4^+(\text{aq}) = \text{H}^+(\text{aq}) + \text{NH}_3(\text{aq})$	p$K° = 9.25$	52.22	−2	9
$\text{H}_2\text{PO}_4^-(\text{aq}) = \text{H}^+(\text{aq}) + \text{HPO}_4^{2-}(\text{aq})$	p$K° = 7.21$	4.2	−124	−220

[a] These quantities were estimated.

molar enthalpy of ionization of L-ornithine(aq) is based upon the acidity constants determined as a function of temperature by Gergely *et al.*(22) The standard molar entropy change for the ionization of L-citrulline(aq) has been estimated as $-19\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$, based upon the similar results for L-arginine(aq) and L-citrulline(aq). These selected values are given in table 2.

Ionizations of L-arginine(aq), L-citrulline(aq), L-ornithine(aq), and urea(aq) under highly acidic and basic conditions have also been reported. Thus, L-arginine(aq) also has a p$K° \approx 1.8$,(7-9,11 13,15) and ≈ 12.6;(7,9,16,23,24) L-citrulline(aq) also has a p$K° \approx 2.3$;(12,18) L-ornithine(aq) also has a p$K° \approx 2.0$,(7,15,22) and ≈ 11.1;(7,13,15,19-23,25) and for urea(aq) p$K° \approx 0.15$.(26-30) As mentioned above, these ionizations are far removed from the conditions under which the experiments were performed and their neglect has only a negligible (<0.1 per cent) effect in the subsequent calculations.

The standard equilibrium constant, standard molar enthalpy, and standard molar entropy change for the ionization of $\text{H}_2\text{PO}_4^-(\text{aq})$ and of $\text{NH}_4^+(\text{aq})$ were calculated from the formation properties given in the NBS Tables.(31) The standard molar heat-capacity change for the ionization of $\text{H}_2\text{PO}_4^-(\text{aq})$ is calculated from the standard apparent molar heat capacities reported by Larson, Zeeb, and Hepler.(32) The standard molar heat-capacity change for the ionization of $\text{NH}_4^+(\text{aq})$ is based upon the heat-capacity measurements of Allred and Woolley.(33) The phosphate ionizations at p$K° = 2.15$ and 12.34,(31) are neglected in the subsequent calculations.

The positions of equilibrium for reactions (1) and (2) are too far to the right to allow us to determine apparent equilibrium constants for these reactions. Based upon our attempts to perform these equilibrium measurements, we found that $K'(1)$ is greater than $2 \cdot 10^4$ at $T = 298.15$ K, $I \approx 0.1\ \text{mol}\cdot\text{kg}^{-1}$, and pH = 7.75. This corresponds to $K°(3)$ being greater than $2 \cdot 10^4$. Similarly, $K'(2)$ was found to be greater than $2 \cdot 10^4$ at $T = 298.15$, $I \approx 0.1\ \text{mol}\cdot\text{kg}^{-1}$, and pH = 6.0. This corresponds to $K°(4)$ being greater than $2.6 \cdot 10^4$.

TABLE 3. Results of calorimetric measurements for reaction (1): Σarg(aq)+H_2O(l) = Σorn(aq)+urea(aq). The molality of the $C_6H_{15}N_4O_2Cl$ (L-arginine · HCl) is that prior to any reaction. The ionic strength I and the standard molar enthalpy of reaction $\Delta_r H^\circ_m(3)$ for the reference reaction are calculated quantities. The uncertainties given for $\Delta_r H_m(1)$ are 95 per cent confidence limits

$\frac{T}{K}$	pH	$\frac{m(C_6H_{15}N_4O_2Cl)}{mol \cdot kg^{-1}}$	$\frac{m(K_2HPO_4)}{mol \cdot kg^{-1}}$	$\frac{m(H_3PO_4)}{mol \cdot kg^{-1}}$	$\frac{m(MnCl_2)}{mol \cdot kg^{-1}}$	$\frac{m(KCl)}{mol \cdot kg^{-1}}$	$\frac{I}{mol \cdot kg^{-1}}$	$\frac{\Delta_r H_m(1)}{kJ \cdot mol^{-1}}$	$\frac{\Delta_r H^\circ_m(3)}{kJ \cdot mol^{-1}}$
298.15	6.47	0.01299	0.09451	0.05265	0.0002695	0	0.254	-21.83 ± 0.20	−21.83
298.15	6.94	0.01377	0.09286	0.02471	0.0001824	0	0.271	-21.51 ± 0.07	−21.51
298.15	7.46	0.01293	0.09602	0.009598	0.0002887	0	0.294	-21.22 ± 0.05	−21.23
298.15	7.67	0.01328	0.09780	0.006363	0.0001855	0	0.303	-21.15 ± 0.05	−21.17
298.15	7.79	0.01357	0.09609	0.01926	0.0002811	0.4884	0.818	-21.53 ± 0.08	−21.56
298.15	7.83	0.01227	0.09704	0.002547	0.0002772	0.1994	0.493	-21.31 ± 0.04	−21.34
298.15	7.94	0.01320	0.09821	0.002543	0.0004160	0	0.304	-20.94 ± 0.05	−20.97
298.15	8.27	0.01478	0.09829	0	0.0004644	0	0.306	-21.01 ± 0.07	−21.08
304.65	7.87	0.01366	0.09628	0.0002942	0.0002989	0	0.298	-21.47 ± 0.04	−21.57
310.25	7.86	0.01271	0.09628	0.0002942	0.0002989	0	0.297	-22.03 ± 0.21	−22.22

TABLE 4. Results of calorimetric measurements for reaction (2): Σarg(aq)+H_2O(l) = Σcit(aq)+Σammonia(aq). The molality of the $C_6H_{15}N_4O_2Cl$ (L-arginine · HCl) is that prior to any reaction. Corrections to the measured molar enthalpy $\Delta_r H_m$(meas.) have been applied for the side reaction of L-arginine(aq) to {L-ornithine(aq) + urea(aq)} to obtain the (corrected) molar enthalpy $\Delta_r H_m(2)$ for reaction (2) given in column 10. The mole fraction of L-ornithine(aq) formed x_1 is given in column 8. The ionic strength I and the standard molar enthalpy of reaction for the reference reaction $\Delta_r H^\circ_m(4)$ are calculated quantities. The uncertainties given for $\Delta_r H_m$(meas.) are 95 per cent confidence limits

$\frac{T}{K}$	pH	$\frac{m(C_6H_{15}N_4O_2Cl)}{mol \cdot kg^{-1}}$	$\frac{m(K_2HPO_4)}{mol \cdot kg^{-1}}$	$\frac{m(H_3PO_4)}{mol \cdot kg^{-1}}$	$\frac{m(KCl)}{mol \cdot kg^{-1}}$	$\frac{I}{mol \cdot kg^{-1}}$	x_1	$\frac{\Delta_r H_m(meas.)}{kJ \cdot mol^{-1}}$	$\frac{\Delta_r H_m(2)}{kJ \cdot mol^{-1}}$	$\frac{\Delta_r H^\circ_m(4)}{kJ \cdot mol^{-1}}$
298.15	5.70	0.09902	0.09421	0.08650	0	0.305	0.0197	-31.82 ± 0.03	−32.03	−31.60
298.15	5.87	0.09977	0.09502	0.07296	0.5806	0.896	0.0146	-31.75 ± 0.05	−31.90	−31.35
298.15	5.92	0.09939	0.09490	0.07497	0.2034	0.519	0.0251	-31.70 ± 0.07	−31.96	−31.47
298.15	6.15	0.09833	0.09494	0.07433	0	0.329	0.0260	$-31.95 + 0.06$	−32.23	−31.79
298.15	6.55	0.09774	0.09450	0.05265	0	0.352	0.0467	-32.00 ± 0.12	−32.52	−32.08
298.15	7.21	0.09785	0.09795	0.02418	0	0.394	0.1398	-31.86 ± 0.07	−33.56	−33.15
304.65	6.10	0.09986	0.09410	0.07220	0	0.324	0.0062	-32.35 ± 0.06	−32.42	−31.95
310.15	6.13	0.10099	0.09410	0.07212	0	0.329	0.0441	-32.45 ± 0.15	−32.92	−32.42

The results of the calorimetric measurements for the hydrolysis of L-arginine(aq) to {L-ornithine(aq) + urea(aq)} are given in table 3. Note that in table 3, the calculated results for $\Delta_r H_m^\circ(3)$ at $T = 298.15$ K vary from $-21.83\ \mathrm{kJ \cdot mol^{-1}}$ at pH = 6.47 to $-21.08\ \mathrm{kJ \cdot mol^{-1}}$ at pH = 8.27. This essentially monotonic trend is explicable in terms of the uncertainties in the $\mathrm{p}K^\circ$ and $\Delta_r H_m^\circ$ for the ionizations of L-arginine(aq) and L-ornithine(aq). For example, a change in the $\mathrm{p}K^\circ$ of L-arginine(aq) from 8.99 to 8.94 leads to a change of $0.49\ \mathrm{kJ \cdot mol^{-1}}$ in the calculated $\Delta_r H_m^\circ(3)$ at pH = 8.27. A similar change of 0.05 in the $\mathrm{p}K^\circ$ of L-ornithine(aq) leads to a change of $0.50\ \mathrm{kJ \cdot mol^{-1}}$ in the calculated $\Delta_r H_m^\circ(3)$ at pH = 8.27. An increase of $3\ \mathrm{kJ \cdot mol^{-1}}$ in the standard molar enthalpies of ionization of L-arginine(aq) and of L-ornithine(aq) each lead to changes of $0.35\ \mathrm{kJ \cdot mol^{-1}}$ in the calculated $\Delta_r H_m^\circ(3)$ at pH = 8.27. Since it is furthest removed from the ionizations of L-arginine(aq) and L-ornithine(aq), the result obtained at pH = 6.47 is changed by no more than $0.01\ \mathrm{kJ \cdot mol^{-1}}$ by the aforementioned perturbations. The uncertainties due to these perturbations are combined in quadrature and lead to approximate uncertainties of $0.02\ \mathrm{kJ \cdot mol^{-1}}$ and $0.86\ \mathrm{kJ \cdot mol^{-1}}$ for the results at pH = 6.47 and at pH = 8.27, respectively. We assume that this uncertainty varies linearly from pH = 6.47 to pH = 8.27. The uncertainty obtained for each result obtained on this basis is then added in quadrature to the statistical uncertainty given for each $\Delta_r H_m(1)$ at $T = 298.15$ K in table 3 to obtain a final estimated uncertainty for each $\Delta_r H_m^\circ(3)$ in that table. These results are then used to obtain a weighted average of $-(21.45 \pm 0.23)\ \mathrm{kJ \cdot mol^{-1}}$ for $\Delta_r H_m^\circ(3)$. A simple average of all of the calculated results for $\Delta_r H_m^\circ(3)$ at $T = 298.15$ K is $-(21.34 \pm 0.20)$ $\mathrm{kJ \cdot mol^{-1}}$. This latter uncertainty is equal to two standard deviations of the mean. We adopt a final result of $\Delta_r H_m^\circ(3) = -(21.4 \pm 0.5)\ \mathrm{kJ \cdot mol^{-1}}$. The assigned uncertainty includes an allowance for possible errors due to sample impurity, calorimetry, and chromatography.

As mentioned above, the arginine deiminase used in this study had some arginase activity present in it. Because of this, some of the L-arginine(aq) was converted to {L-ornithine(aq) + urea(aq)}. Thus, the measured molar enthalpy of reaction is:

$$\Delta_r H_m(\text{meas.}) = \Delta H / n(\text{arg}) = x_1 \Delta_r H_m(1) + x_2 \Delta_r H_m(2). \qquad (5)$$

Here, ΔH is the enthalpy of reaction, n(arg) is the amount of L-arginine(aq) which has reacted, and x_1 and x_2 are the mole fractions of L-ornithine(aq) and L-citrulline(aq) formed in the reaction; $(x_1 + x_2) = 1$. Since x_1 and x_2 were determined chromatographically and since $\Delta_r H_m(1)$ can be calculated with the equilibrium model from the above result for $\Delta_r H_m^\circ(3)$ and the thermodynamic quantities given in table 2, the molar enthalpy of reaction (2), $\Delta_r H_m(2)$, can be obtained with equation (5). The measured molar enthalpy of reaction, the mole fraction of L-ornithine(aq) formed, and the (corrected) molar enthalpy for reaction (2) are given in table 3. There is also an approximately monotonic trend in the results for $\Delta_r H_m^\circ(4)$ with increasing pH. However, this trend does not appear to be explicable in terms of the uncertainties in the $\mathrm{p}K^\circ$ and $\Delta_r H_m^\circ$ for the ionizations of L-arginine(aq) and L-citrulline(aq). However, the trend is not as well defined as in the previous set of results and we take a simple average of the results at $T = 298.15$ K and obtain $\Delta_r H_m^\circ(4) = -(31.91 \pm 0.54)$ $\mathrm{kJ \cdot mol^{-1}}$. The uncertainty here is two standard deviations of the mean. Because of uncertainties of the type discussed above, the error estimate is increased slightly and we adopt a final result of $-(31.9 \pm 0.8)\ \mathrm{kJ \cdot mol^{-1}}$ for $\Delta_r H_m^\circ(4)$ at $T = 298.15$ K.

From the calorimetric measurements performed at three different temperatures, we calculate $\Delta_r C_{p,m}^\circ(3) = -(72 \pm 46)\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ and $\Delta_r C_{p,m}^\circ(4) = -(41 \pm 45)\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$. These results pertain to the temperature interval 298.15 K to 310.15 K. Since the uncertainties given here are only the statistical uncertainties, these results for the standard molar heat-capacity changes for reactions (3) and (4) must be considered as approximate. The apparent molar heat capacities of L-arginine(aq) and urea(aq) have been measured, respectively, by Jolicoeur *et al.*(34) and by Phillip, Perron, and Desnoyers.(35) However, the apparent molar heat capacities of L-ornithine(aq) and L-citrulline(aq) have not been measured. We therefore cannot calculate $\Delta_r C_{p,m}^\circ$ for reactions (3) and (4) from the apparent molar heat capacities C_ϕ° of the reactants and products. If one makes the simple estimates: $\{C_\phi^\circ(\mathrm{Horn^+}) + C_\phi^\circ(\mathrm{urea}) \approx C_\phi^\circ(\mathrm{Harg^+})\}$ and $\{C_\phi^\circ(\mathrm{Hcit}) + C_\phi^\circ(\mathrm{NH_4^+}) \approx C_\phi^\circ(\mathrm{Harg^+})\}$, one obtains $\Delta_r C_{p,m}^\circ = -C_\phi^\circ(\mathrm{H_2O}) = -75\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ for reactions (3) and (4). This simple estimate is in agreement with the approximate $\Delta_r C_{p,m}^\circ$ results for both reactions (3) and (4).

There are no thermochemical or equilibrium results in the literature for the reactions studied here. We have explored the possibility of performing thermodynamic-cycle calculations to obtain standard molar Gibbs free energy and molar enthalpy changes for these reactions. Examination of the literature showed that molar enthalpies of combustion have been measured for L-arginine(s), DL-ornithine(s), and urea(s).(35,37) Third-law molar entropies have been determined for L-arginine(s), DL-citrulline(s), DL-ornithine(s), and ornithine dihydrochloride(s).(38) The solubility and molar enthalpy of solution of L-arginine monohydrochloride(s) and the molar enthalpy of solution of L-arginine(s) have been measured.(39) Also, the solubility of urea(s) and its molar enthalpy of solution have been measured.(40,41) Nevertheless, several key pieces of thermodynamic information are needed before thermodynamic-cycle calculations can be performed to obtain the desired standard molar enthalpy and standard molar Gibbs free energy changes for these reactions. We note that thermodynamic-cycle calculations which are based upon molar enthalpies of combustion and third-law standard molar entropies generally yield molar enthalpies of reaction which are less accurate than direct calorimetric measurements on the reaction(s) of interest. This is also generally the situation in regards to equilibrium measurements. However, when the equilibrium constants are very large, as for the reactions studied herein, this general rule may not hold.

The standard equilibrium constant for reaction (4) can be estimated with the result for $\Delta_r H_m^\circ(4)$ determined in this study and with an approximate value for $\Delta_r S_m^\circ(4)$. Larson *et al.*(42) have examined the standard molar entropy changes for several ammonia-elimination reactions of the generic type:

$$\mathrm{X(aq) = Y^-(aq) + NH_4^+(aq)}. \qquad (6)$$

The observation was that $\Delta_r S_m^\circ(6)$ should be in the range $34\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ to $84\ \mathrm{J \cdot K^{-1} \cdot mol^{-1}}$ at $T = 298.15$ K. With the standard molar entropy changes for the ionizations of Hcit(aq) and of $\mathrm{Harg^+}$ (see table 2) and with the standard molar entropy of $H_2O(l)$, $\Delta_r S_m^\circ(4)$ is estimated to be in the range $-39\ J \cdot K^{-1} \cdot mol^{-1}$ to $12\ J \cdot K^{-1} \cdot mol^{-1}$ at $T = 298.15\ K$. With this and with the experimental result of $-31.9\ kJ \cdot mol^{-1}$ for $\Delta_r H_m^\circ(4)$, we estimate that $\Delta_r G_m^\circ(4)$ should be in the range $-35.5\ kJ \cdot mol^{-1}$ to $-20.3\ kJ \cdot mol^{-1}$ at $T = 298.15\ K$. This corresponds to an estimate for $K^\circ(4)$ in the range $3.6 \cdot 10^3$ to $1.7 \cdot 10^6$. With these estimated values for $K^\circ(4)$ and the equilibrium model for reaction (2), the apparent equilibrium constant $K'(2)$ should be in the range $1.5 \cdot 10^3$ to $7.3 \cdot 10^5$ at $T = 310.15\ K$, $pH = 7$, and $I = 0.25\ mol \cdot kg^{-1}$. We have found that $K'(2)$ was greater than $2 \cdot 10^4$.

An alternative way of obtaining this information uses the thermodynamic cycle:

$$\Sigma\text{L-citrulline(aq)} + \Sigma\text{aspartate(aq)} + \Sigma\text{ATP(aq)} = \Sigma\text{L-arginosuccinate(aq)} + \Sigma\text{AMP(aq)} + \Sigma\text{pyrophosphate(aq)}, \quad (7)$$

$$\Sigma\text{ATP(aq)} + H_2O(l) = \Sigma\text{AMP(aq)} + \Sigma\text{pyrophosphate(aq)}, \quad (8)$$

$$\Sigma\text{L-arginosuccinate(aq)} = \Sigma\text{L-arginine(aq)} + \Sigma\text{fumarate(aq)}, \quad (9)$$

$$\Sigma\text{aspartate(aq)} = \Sigma\text{fumarate(aq)} + \Sigma\text{ammonia(aq)}. \quad (10)$$

Thus:

$$\Delta_r G_m'^\circ(2) = -\Delta_r G_m'^\circ(7) + \Delta_r G_m'^\circ(8) - \Delta_r G_m'^\circ(9) + \Delta_r G_m'^\circ(10). \quad (11)$$

This pathway has been used by Schuegraf *et al.*(43) to obtain $\Delta_r G_m'^\circ(2) = -36.0\ kJ \cdot mol^{-1}$ at $T = 311.15\ K$, $pH = 7.5$, and $I = 0.2\ mol \cdot kg^{-1}$. This corresponds to $K'(2) = 1.1 \cdot 10^6$ under these conditions. With this result and $\Delta_r H_m^\circ(4) = -31.9\ kJ \cdot mol^{-1}$ and with the equilibrium model, we calculate $K^\circ(4) = 1.1 \cdot 10^6$ and $\Delta_r S_m^\circ(4) = 9\ J \cdot K^{-1} \cdot mol^{-1}$ at $T = 298.15\ K$. The results for reactions (7), (9), and (10) are, respectively, based upon the equilibrium measurements of Schuegraf *et al.*,(43) Ratner and Anslow,(44) and Williams and McIntyre.(45) By far the most serious problem in this calculation is the value for $\Delta_r G_m'^\circ(8)$ which, unfortunately, also rests upon an estimate which is uncertain by at least several $kJ \cdot mol^{-1}$. In any case, our finding that $K^\circ(4)$ was greater than $2.6 \cdot 10^4$ at $T = 298.15\ K$ is consistent with the result obtained from this thermodynamic pathway.

Reaction (2) is used for the manufacture of L-citrulline. The results of this study show that, for practical purposes, this reaction can be said to proceed to completion.

The apparent equilibrium constants for the reactions in the metabolic urea cycle(46,47) are summarized in table 5. The carbamoyl phosphate(aq) is formed as a product of the reaction of ATP(aq) and carbon dioxide(aq) with either ammonia(aq) or glutamine(aq). Examination of these results show that the position of equilibrium lies very far to the right for all but the third reaction in this cycle. The apparent equilibrium constant for the first reaction is very large and helps to commit the {L-ornithine(aq) + carbamoyl phosphate(aq)} to this metabolic pathway. Also, since the apparent equilibrium constant for the final reaction is very large, it serves to remove the products of the third reaction in the cycle and to make the overall urea cycle move in the direction of the synthesis of urea(aq). The L-ornithine(aq) which is produced in the final reaction re-enters the cycle at the first reaction shown in table 5. Not shown in table 5 is the fact that the urea cycle involves the transport of L-ornithine(aq) and L-citrulline(aq) across the mitochondrial membrane. Thus, a

TABLE 5. *Apparent equilibrium constants for the reactions in the metabolic urea cycle*

K'	$\frac{\Delta_r G_m'^{\circ}}{\text{kJ}\cdot\text{mol}^{-1}}$	$\frac{T}{\text{K}}$	pH	$\frac{I}{\text{mol}\cdot\text{kg}^{-1}}$	Reference
carbamoyl phosphate(aq) + L-ornithine(aq) = L-citrulline(aq) + orthophosphate(aq)					
$\approx 1\cdot 10^5$	-29.8	311.15	6.7 to 7.4	≈ 0.1	Reichard(48)
citrulline(aq) + aspartate(aq) + ATP(aq) = arginosuccinate(aq) + pyrophosphate(aq) + AMP(aq)					
2.14[a]	-2.0	311.15	6.91	≈ 0.01	Schuegraf *et al.*(43)
arginosuccinate(aq) = L-arginine(aq) + fumarate(aq)					
0.0114	11.6	311.15	7.5	≈ 0.01	Ratner *et al.*(44)
L-arginine(aq) + H_2O(l) = ornithine(aq) + urea(aq)					
$>2\cdot 10^4$	<-23.8	298.15	7.75	≈ 0.1	This study

[a] Schuegraf *et al.*(43) also give apparent equilibrium constants at pH = 5.90, pH = 7.70, and pH = 7.79.

complete picture of the energetics of the urea cycle must involve a consideration of this transport as well as an understanding of the kinetics of these reactions.

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