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An Equilibrium and Calorimetric Investigation of the Hydrolysis of L-Tryptophan to (Indole + Pyruvate + Ammonia)[1]

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Apparent equilibrium constants and calorimetric enthalpies of reaction have been measured for the reaction L-tryptophan(aq) + $H_2O(l)$ = indole(aq) + pyruvate(aq) + ammonia(aq) which is catalyzed by L-tryptophanase. High-pressure liquid-chromatography and microcalorimetery were used to perform these measurements. The equilibrium measurements were performed as a function of pH, temperature, and ionic strength. The results have been interpreted with a chemical equilibrium model to obtain thermodynamic quantities for the reference reaction: L-tryptophan(aq) + $H_2O(l)$ = indole(aq) + pyruvate$^-$(aq) + NH_4^+(aq). At T = 25°C and I_m = 0 the results for this reaction are: $K^\circ = (1.05 \pm 0.13) \times 10^{-4}$, $\Delta_r G^\circ = (22.71 \pm 0.33)$ kJ-mol^{-1}, $\Delta_r H^\circ = (62.0 \pm 2.3)$ kJ-mol^{-1}, and $\Delta_r S^\circ = (132 \pm 8)$ J-K^{-1}-mol^{-1}. These results have been used together with thermodynamic results from the literature to calculate standard Gibbs energies of formation, standard enthalpies of formation, standard molar entropies, standard molar heat capacities, and standard transformed formation properties for the substances participating in this reaction.

KEY WORDS: Ammonia; apparent equilibrium constant; calorimetry; enthalpy; entropy; heat capacity; indole; pyruvic acid; thermodynamic properties; L-tryptophan.

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1. Introduction

The bacterial enzyme L-tryptophanase (Enzyme Commission Number[1] EC 4.1.99.1) catalyzes the reaction[4]

$$\text{L-tryptophan(aq)} + H_2O(l) = \text{indole(aq)} + \text{pyruvate(aq)} + \text{ammonia(aq)} \quad (1)$$

The terms L-tryptophan, indole, pyruvate, and ammonia are used here to represent the total amounts of the various charged and uncharged species formed from the ionization of these substances in solution. This reaction also has a catalytic requirement for pyridoxal 5-phosphate and for either K^+(aq) or NH_4^+(aq).[2] This reaction is used to manufacture L-tryptophan, which finds application as an antidepressant and is also one of the essential amino acids needed for growth. The enzyme L-tryptophanase is also used as an anti-tumor agent. Since the position of equilibrium of this reaction is a matter of general interest and since the literature does not contain any reports of apparent equilibrium constants or enthalpy changes for this reaction, we have undertaken this thermodynamic investigation. In this study, we have used high-pressure liquid-chromatography (HPLC) and microcalorimetry to determine apparent equilibrium constants and transformed enthalpy changes for this reaction. The results are interpreted in terms of a chemical equilibrium model which allows one to relate the measured quantities (apparent equilibrium constants and calorimetric enthalpies of reaction) to standard thermodynamic quantities for a reference reaction involving specific chemical species.

2. Experimental

The molar masses (g-mol^{-1}) of the substances used in this study are: L-tryptophan ($C_{11}N_2O_2H_{12}$), 204.23; indole (C_8NH_7), 117.15; sodium pyruvate ($C_3O_3H_3Na$), 110.04; pyridoxal 5-phosphate, ($C_8NO_6PH_{10}$), 247.14; water, 18.0153; K_2HPO_4, 174.18; H_3PO_4, 97.995; NaOH, 39.997; KCl, 74.551; and NH_4Cl, 53.492. The L-tryptophan, indole, potassium phosphate, sodium pyruvate, pyridoxal 5-phosphate, and L-tryptophanase from E. coli were obtained[5] from Sigma; am-

[4]The Chemical Abstract Services registry numbers of the principal substances used in this study are: ammonium chloride, 12125-02-9; L-tryptophan, 73-22-3; indole, 120-72-9; sodium pyruvate, 113-24-6; and pyridoxal 5-phosphate, 41468-25-1.

[5]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.

monium chloride was from Mallinckrodt; and phosphoric acid was from Baker. A purer and more active sample of L-tryptophanase was kindly provided by Dr. Edith Wilson Miles (National Institutes of Health). This sample, having a concentration of 11 g-dm^{-3}, was prepared from E. coli B/lt7-A[2,3] and was ≈1000 times more active than the sample of L-tryptophanase obtained from Sigma. It was stored at -80°C until ready for use. At that time it was dialyzed (molar mass cut off was 3500 g-mol^{-1} at 1°C) against the phosphate buffer used in this study. Due to the limited amount of this enzyme, it was used only for the calorimetric experiments where having a rapid reaction was more important than in the equilibrium measurements where it was possible to allow several days for the experiments. Water contents for the following substances were determined by Karl-Fischer analysis with resulting mass percents: L-tryptophan, (0.58±0.02); indole, (0.23±0.03); sodium pyruvate, (0.053±0.005); and ammonium chloride, (0.53±0.26). The L-tryptophan was reported by the vendor to have an optical rotation of −31.4° (1 g of L-tryptophan in 0.100 dm^3 of water at 25°C, wavelength of 589 nm). The purity of the L-tryptophan, indole, and sodium pyruvate were determined chromatographically (see below). Only one peak was observed for each sample.

The reaction mixtures were analyzed for L-tryptophan, indole, and pyruvate with a Hewlett-Packard model 1090 HPLC Zorbax C_{18} column thermostatted at 38°C, and an ultraviolet detector set at 220 nm. The mobile phase used for the analysis of the reaction mixtures was x volume percent (0.02 mol-dm^{-3} KH_2PO_4 at pH = 4.7) and y volume percent methanol. The following mobile-phase gradient was used: $x = 100$ and $y = 0$ at $t = 0$; $x = 60$ and $y = 40$ at $t = 15$ min; and $x = 0$ and $y = 100$ at $t = 25$ min. The flow rate was always 0.7 cm^3-min^{-1}. Typical retention times were 3.6 min for pyruvate, 12.8 min for L-tryptophan, and 26.1 min for indole. Solutions of pyruvate, L-tryptophan, and indole were prepared daily for the determination of the response factors of these substances. The amounts of total ammonia in the respective solutions were determined from the amounts of the ammonium chloride used to prepare the solutions with an adjustment for the amounts of reaction.

Equilibrium measurements were performed by approaching equilibrium from both directions of reaction. Three to five days were allowed for equilibration. The enzyme was then filtered from the solutions using Centricon concentrators (molar mass cut off = 10^4 g-mol^{-1}) in an ultracentrifuge at 30,000 r.p.m. for ≈20 min. The rotor of the ultracentrifuge was thermostatted at the temperature at which experi-

ments had been performed. This procedure served to freeze the position of equilibrium of the solutions which were being analyzed. Following filtration, the samples were diluted by a factor of 2 to 3. This was done because of the high absorbance of indole and L-tryptophan which otherwise would saturate the detector.

The calorimeters were of the heat-conduction type. The sample vessels, which were fabricated from high-density polyethylene, contained two compartments holding approximately 0.55 cm^3 and 0.45 cm^3 of solution. The vessels and their contents were allowed to equilibrate in the microcalorimeters for ≈1 h before the solutions in the vessel were mixed. Calibration of the calorimeters was done electrically with a calibrated digital voltmeter, standard resistor, and time-interval counter. Descriptions of the calorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results are given in Refs. 4 and 5. Following reaction in the calorimeter for 53 min, the calorimeter vessels were removed from the calorimeters and their contents were analyzed with the HPLC. It was found that 12.9 to 15.1 percent of the L-tryptophan had not reacted. Appropriate corrections were applied for this unreacted L-tryptophan. The average of the "blank" enthalpy changes accompanying the mixing of the substrate solution and of the enzyme solution with the buffer were $-(0.14 \pm 1.3)$ mJ. The measured enthalpies of reaction ranged from 67 to 78 mJ.

The measurement of the pH of the reaction mixtures was performed with a combination glass micro-electrode and an Orion Model 811 pH meter. All measurements were carried at the temperature at which the reactions occurred. Calibration was performed with a standard buffer prepared from potassium dihydrogen phosphate (0.009695 mol-kg^{-1}) and disodium hydrogen phosphate (0.03043 mol-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 performed with satisfactory agreement (± 0.03) in the pH of these solutions. The difference between the pH of the substrate solution in the calorimetric measurements prior to any reaction and the pH of the final reaction mixture was (0.01 ± 0.03). Thus, reaction (1) is considered to have occurred at essentially constant pH.

3. Results and Discussion

The apparent equilibrium constant for the overall biochemical reaction is

$$K' = m(\text{indole})m(\text{pyruvate})m(\text{ammonia})/m(\text{L-tryptophan}) \quad (2)$$

where m(ammonia) is the sum of the molalities of the species NH_3^0(aq) and NH_4^+(aq). Similar definitions hold for the other molalities in Eq. (2). The chemical reference reaction is selected to be

$$\text{L-tryptophan(aq)} + H_2O(l) = \text{indole(aq)} + \text{pyruvate}^-\text{(aq)} + NH_4^+\text{(aq)} \quad (3)$$

The standard equilibrium constant for this reaction is given in terms of the activities a of the species

$$K^\circ = a(\text{indole})a(\text{pyruvate}^-)a(NH_4^+)/\{a(\text{L-tryptophan})a(H_2O)\} \quad (4)$$

The thermodynamics of these reactions will be described with a model and computational procedure of the type previously used for the disproportionation reaction of adenosine 5′-diphosphate to adenosine 5′-triphosphate and adenosine 5′-monophosphate.[6] In this model, the equilibrium equations are solved to obtain the fractions of the various species in solution and the contributions of these species to the measured quantities (apparent equilibrium constants and calorimetric enthalpies of reaction) are calculated. The thermodynamic quantities which pertain to the reference reaction are calculated as parameters in the model. The calculation is made self-consistent with respect to the ionic strength and the activity of the water is calculated with a Gibbs-Duhem integration as a part of the equilibrium model.

It is necessary to know the acidity constants ($pK = -\log K$) and standard enthalpies of ionization ($\Delta_r H^\circ$) of the various substances participating in these reactions in order to calculate thermodynamic quantities for the reference reaction (3). These acidity constants, which have been obtained from the literature, are given in Table I. Where workers reported acidity constants at different temperatures, we have calculated standard enthalpies of ionization with the equation of Clarke and Glew[44] and an assumed standard heat capacity change $\Delta_r C_p^\circ = 0$. The results of the various studies shown in Table I were also adjusted to $I_m = 0$ with an extended Debye-Hückel equation[6] in which the ion-size parameter was set at 1.6 $kg^{1/2}$-$mol^{-1/2}$. Several workers failed to report the ionic strengths at which the acidity constants were determined. In these cases we assumed that the ionic strength was 0.1 mol-kg^{-1} to ob-

Table I. Acidity Constants and Associated Molar Enthalpy Changes for the Ionization of L-Tryptophan and Pyruvic Acid[a]

°C	I_m[b]	pK	pK^o	Ref.
	L-Tryptophan$^+$(aq) = L-Tryptophan(aq) + H$^+$(aq)			
25	?	2.27	≈2.3	7
26	≈0.01	2.4	2.4	8
20	≈0.01	2.20	2.19	9
20	1.0	2.39	2.38	10
25	0.10	2.46	2.46	11
25	6.0	2.20	2.71	12
25	3.0	2.75	2.75	13
37	0.15	2.46	2.48	14
20	0.37	2.46	2.45	15
25	0.50	2.38	2.38	16
25	0	2.60	2.60	17
25	?	2.38	≈2.4	18,19
23	0.1	2.32	2.32	20
°C	I_m[b]	$\Delta_r H^{oc}$	$\Delta_r H^{od}$	Ref.
25	0.10	≈0	≈0	11
25	3.0	3.3	3.3	13[e]
25	?	-1.4	≈1.4	18,19[e]
°C	I_m[b]	pK	pK^o	Ref.
	L-Tryptophan(aq) = L-Tryptophan$^-$(aq) + H$^+$(aq)			
25	?	9.37	≈9.6	7
25	≈0.02	9.39	9.51	21
26	≈0.01	9.4	9.42	8
20	≈0.005	9.55	9.58	9
20	0.01	9.57	9.62	22
20	1.00	9.43	9.69	10
25	0.10	9.41	9.62	11
25	6.0	9.55	9.71	12
25	0.16	9.28	9.53	23
25	3.0	9.92	10.4	13
37	0.15	9.09	9.62	14
20	0.37	9.63	9.82	15

Table I. Continued

°C	I_m[b]	pK	pK^o	Ref.
	L-Tryptophan(aq) = L-Tryptophan$^-$(aq) + H$^+$(aq)			
20	0.10	9.48	9.57	24
30	0.10	9.25	9.57	24
25	0.10	9.41	9.62	25
25	0.50	9.47	9.81	16
25	0.10	9.33	9.54	26
37	0.15	9.02	9.62	27
25	0.10	9.36	9.57	28
20	0.15	9.45	9.57	29
25	?	9.59	≈9.8	30
25	0	9.61	9.61	17
25	?	9.39	≈9.6	18,19
23	0.1	9.21	9.37	20
°C	I_m[b]	$\Delta_r H^{oc}$	$\Delta_r H^{od}$	Ref.
25	0.10	38	37.4	24
25	0.10	≈44	≈43	11
20	?	44.4	≈44	31[e]
25	0.16	44.6	43.9	23[e]
25	3.0	38.5	37.1	13[e]
25	?	49.9	≈50	18,19[e]
°C	I_m[b]	pK	pK^o	Ref.
	Pyruvic Acid(aq) = Pyruvate$^-$(aq) + H$^+$(aq)			
25	?	2.49	≈2.7	32
8	?	2.75	≈2.8	33
25	0	2.49	2.49	34
25	?	2.49	≈2.7	35
25	?	2.39	≈2.8	36
25	0	2.60	2.60	37
25	1.0	2.11	2.50	38
25	1.0	2.17	2.56	39
25	0.30	2.20	2.50	40
25	2.0	2.27	2.71	41

Table I. Continued

°C	I_m [b]	$\Delta_r H^{o}$ [c]	$\Delta_r H^{o}$ [d]	Ref.
25	?	8.1	≈8	42[f]
25	?	≈10	≈10	36
25	0.05	12.6	12.1	37[e]

[a] The results obtained at the indicated ionic strengths I_m were adjusted with the extended Debye-Hückel equation to $I_m = 0$ and where necessary to 25°C to obtain the results given in column 4. [b] Units: mol-kg^{-1}. [c] Units: kJ-mol^{-1}. [d] $I_m = 0$; Units: kJ-mol^{-1}. [e] Obtained from calorimetric measurements. [f] Calculated from the enthalpy of solution and the enthalpy of neutralization of pyruvic acid(l). The enthalpy of formation of H_2O(l) and OH^-(aq) were taken from Ref. 43.

Table II. Thermodynamic Quantities for the Reference Reaction for the Hydrolysis of L-Tryptophan and the Acidity Constants of These Substances[a]

K^o or pK^o	$\Delta_r H^{o}$ [b]	$\Delta_r S^{o}$ [c]	$\Delta_r C_p^{o}$ [c]
L-Tryptophan(aq) + H_2O(l) = Indole(aq) + Pyruvate$^-$(aq) + NH_4^+(aq)			
$K^o = 1.05\times10^{-4}$	62.0	132	-
L-Tryptophan$^+$(aq) = L-Tryptophan(aq) + H^+(aq)			
$pK^o = 2.40$	3	-36	-
L-Tryptophan(aq) = L-Tryptophan$^-$(aq) + H^+(aq)			
$pK^o = 9.60$	43	-40	-
Pyruvic Acid(aq) = Pyruvate$^-$(aq) + H^+(aq)			
$pK^o = 2.56$	12.1	-8	-
NH_4^+(aq) = NH_3(aq) + H^+(aq)			
$pK^o = 9.25$	52.22	-2	7
$H_2PO_4^-$(aq) = HPO_4^{2-}(aq) + H^+(aq)			
$pK^o = 7.21$	4.2	-124	-220

[a] 25°C; $I_m = 0$; and $p = 0.1$ MPa. The acidity constant and related thermodynamic quantities for the ionization of orthophosphate are also given. [b] Units: kJ-mol^{-1}. [c] Units: J-K^{-1}-mol^{-1}.

tain approximate results at $I_m = 0$. In some cases, the acidity constants had to be adjusted to 25°C. The final selected molar enthalpies of ionization (see Table II) were used for this purpose.

Table II contains a summary of the thermodynamics of ionization of L-tryptophan(aq), pyruvate(aq), NH_4^+(aq), and $H_2PO_4^-$(aq). Indole(aq) does not ionize unless taken to a high pH. The acidity constants and related quantities in this table are, for the most part, the averages of the respective quantities given in column 4 in Table I. We have dropped the acidity constant results of Nozaki and Tanford[12] and of Williams[13] which were obtained at very high ionic strengths. Also, we have not included the approximate results obtained from Böesken *et al.*,[32] Larsson,[33] Strehlow,[35] and Leussing and Shultz[36] for the pyruvate acidity constant. The result of Ojelund and Wadsö[37] for the enthalpy of ionization of pyruvic acid(aq) is judged to be the most reliable. The ionization constant and enthalpy of ionization of NH_4^+(aq) are calculated from the formation properties given in the NBS Tables;[43] the change in heat capacity is based upon the heat-capacity measurements of Allred and Woolley.[45] The standard equilibrium constant, standard enthalpy change, and standard entropy change $\Delta_r S^\circ$ for the ionization of $H_2PO_4^-$(aq) were calculated from the standard formation properties given in the NBS Tables.[43] The standard heat-capacity change for the ionization of $H_2PO_4^-$(aq) was calculated from the standard apparent molar heat capacities reported by Larson *et al.*[46] The phosphate ionizations[43] at $pK^\circ = 2.15$ and $pK^\circ = 12.34$ are neglected in the subsequent calculations as are the other ionizations that are far removed ($|pK - pH| > 2.0$) from the pH of the solutions used in this study.

Results of the equilibrium and calorimetric measurements are given in Tables III and IV. In Table IV, the calorimetrically determined molar enthalpy of reaction $\Delta_r H$(cal) is equal to the measured enthalpy of reaction divided by the amount of reaction. It has been shown[47] that

$$\Delta_r H(\text{cal}) = \Delta_r H'^\circ + \Delta_r N_H \cdot \Delta_r H^\circ(\text{Buff}) \qquad (9)$$

where $\Delta_r H'^\circ$ is the standard transformed enthalpy of reaction, $\Delta_r N_H$ is the change in binding of H^+(aq) in the reaction, and $\Delta_r H^\circ$(Buff) is the standard enthalpy of ionization of the buffer. The quantities in Eq. (9) pertain to the ionic strength at which an actual measurement is performed. The standard equilibrium constants and the standard enthalpies of reaction for the reference reaction (3), and the ionic strengths given in Tables III and IV were calculated from the experimental results with the equilibrium model and the thermodynamic quantities for the ionizations

Table III. Apparent Equilibrium Constants for the Reaction L-Tryptophan(aq) + H_2O(l) = Indole(aq) + Pyruvate(aq) + Ammonia(aq)[a]

°C	pH	m^b K_2HPO_4	m^b NaOH	m^b NH_4Cl	m^b KCl	$10^4K'$(for)	$10^4K'$(rev)	$I_m{}^b$	10^4K^o
14.3	7.98	0.1029	0.0000	0.214	0.208	0.83±0.02		0.727	0.37±0.04
14.3	7.98	0.1029	0.0000	0.214	0.208		0.89±0.01	0.726	0.39±0.04
19.0	7.90	0.1029	0.0000	0.215	0.208	1.37±0.10		0.726	0.60±0.07
19.0	7.91	0.1029	0.0000	0.214	0.208		1.54±0.06	0.725	0.68±0.07
25.0	7.76	0.1029	0.0000	0.214	0.208	2.35±0.20		0.723	1.03±0.13
25.0	7.77	0.1029	0.0000	0.214	0.208		2.28±0.20	0.722	1.00±0.13
25.0	7.84	0.1103	0.0091	0.141	0.998	2.40±0.16		1.47	0.95±0.14
25.0	7.85	0.1103	0.0091	0.141	0.998		2.77±0.30	1.47	1.10±0.18
25.0	7.89	0.1030	0.0016	0.191	0.000	2.12±0.36		0.494	0.98±0.19
25.0	7.97	0.1030	0.0016	0.190	0.000		2.22±0.97	0.494	1.03±0.46
25.0	7.93	0.1062	0.0035	0.210	0.408	2.65±0.35		0.933	1.11±0.19
25.0	7.93	0.1062	0.0035	0.210	0.409		2.61±0.18	0.934	1.10±0.14
25.0	8.85	0.1030	0.0162	0.190	0.000	2.59±0.16		0.491	1.19±0.11
25.0	8.85	0.1031	0.0162	0.189	0.000		2.23±0.12	0.491	1.03±0.09
31.1	7.71	0.1029	0.0000	0.214	0.208	4.24±0.39		0.721	1.84±0.17
31.1	7.71	0.1029	0.0000	0.214	0.208		3.59±0.46	0.721	1.55±0.20
37.0	7.81	0.1049	0.0042	0.210	0.203	5.98±0.33		0.720	2.56±0.14
37.0	7.81	0.1049	0.0043	0.210	0.203		5.71±0.36	0.720	2.44±0.16
43.0	7.94	0.1047	0.0153	0.196	0.190	9.59±0.47		0.697	4.04±0.20
43.0	7.92	0.1048	0.0121	0.200	0.194		10.1±0.27	0.703	4.26±0.12

[a] The results are paired and correspond to sets of measurements performed on the same day. Four to six measurements were performed for each apparent equilibrium constant given above. The uncertainties given in columns 7 and 8 are 95 percent confidence limits. The molalities of the K_2HPO_4, NaOH, NH_4Cl, and KCl are those at the start of the experiments. The molality of the L-tryptophan was ≈0.0026 mol-kg^{-1} and the molality of the pyruvate was ≈0.0042 mol-kg^{-1} at the initiation of those experiments started from the forward direction. The molality of the indole was ≈0.0033 mol-kg^{-1} and the molality of the pyruvate ≈0.0069 mol-kg^{-1} at the initiation of those experiments started from the reverse direction. The molality of the pyridoxal 5-phosphate was ≈0.00037 mol-kg^{-1} in all experiments. The mass fraction of the L-tryptophanase (Sigma sample) was ≈0.0086. The ionic strength I_m and the standard equilibrium constant K^o for the reference reaction (3) at the indicated temperature and at $I_m = 0$ are calculated quantities. The errors assigned to the standard equilibrium constants in column 10 include an allowance for possible systematic errors in the parameters used in the model to calculate them. [b] Units: mol-kg^{-1}.

given in Table II. The change in binding of H^+(aq) was also calculated and was used as an intermediate result in these calculations.

We judge that systematic errors in the equilibrium measurements are less than the average of the error estimates (95 percent confidence

Table IV. Results of Calorimetric Measurements for the Reaction L-Tryptophan(aq) + H_2O(l) = Indole(aq) + Pyruvate(aq) + Ammonia(aq) at 25°C[a]

m^b	m^c	m^d	m^e	I_m	$\Delta_r H$(cal)	$\Delta_r H^o$(3)
0.001518	0.1003	0.00467	0.000426	0.300	66.3	65.1
0.001559	0.1003	0.00467	0.000426	0.300	64.5	63.3
0.001630	0.1003	0.00467	0.000426	0.300	64.6	63.4
0.001498	0.1003	0.00467	0.000426	0.300	60.3	59.1
0.001533	0.1003	0.00467	0.000426	0.300	64.1	62.9
0.001434	0.1003	0.00467	0.000426	0.300	59.3	58.1

[a] Units: m and I_m, mol-kg^{-1} and $\Delta_r H$, kJ-mol^{-1}. The molality of the L-tryptophan ($C_{11}N_2O_2H_{12}$) is that prior to any reaction. The molality of the components of the phosphate buffer and of the pyridoxal 5-phosphate ($C_8NO_6PH_{10}$) are also given. The pH at which these experiments was performed was 7.82. This is the pH of the final reaction mixtures. The ionic strength I_m and the standard enthalpy of reaction $\Delta_r H^\circ$ at $I_m = 0$ for the reference reaction (3) are calculated quantities. The mass fraction of the L-Tryptophanase (National Institutes of Health sample) was ≈0.0004. [b] ($C_{11}N_2O_2H_{12}$). [c] (K_2HPO_4). [d] (H_3PO_4). [e] ($C_8NO_6PH_{10}$).

limits) assigned to the apparent equilibrium constants in columns 7 and 8 in Table III. The basis for this judgment is that the various substances have been carefully characterized both by chromatography and by Karl Fischer analysis. Also, the chromatographic response factors were determined on a daily basis. That the results obtained from both directions of reaction are generally in accord is particularly important in establishing that equilibrium was obtained and that there was no significant problem due to the presence of D-tryptophan in the sample of L-tryptophan used in this study. A simple average of the standard equilibrium constants at 25°C and $I_m = 0$ is $K^\circ = (1.05 \pm 0.05) \times 10^{-4}$. The average of the calorimetric measurements is $\Delta_r H^\circ(3) = (62.0 \pm 2.3)$ kJ-mol^{-1} at $I_m = 0$. These uncertainties are also at the 95 percent confidence limit. From the temperature dependency of K°, and with $\Delta_r C_p^\circ = 0$, we calculate $\Delta_r H^\circ = (60.8 \pm 3.3)$ kJ-mol^{-1} at 25°C and $I_m = 0$. The agreement of the standard enthalpy change for the reference reaction (3) obtained in two different ways is excellent. However, we prefer the calorimetric result and will use it in subsequent calculations.

We now consider the errors associated with the treatment of the results. To do this we assume that the pK° and enthalpy of ionization of L-tryptophan(aq) are respectively uncertain by ±0.05 and ±4 kJ-mol^{-1}, that the ion-size parameter is uncertain by ±0.3 kg$^{1/2}$-mol$^{-1/2}$, and that

the uncertainties in the thermodynamic quantities for the ionization of $NH_4^+(aq)$ and $H_2PO_4^-(aq)$ are negligible. These assumed uncertainties were then used individually to perturb the parameters in the model. The effects of these individual perturbations were then combined in quadrature and with the estimates of random error given in columns 7 and 8 in Table III to obtain the final estimated errors given in column 10 in Table III. The weighted average of the results for the standard equilibrium constants determined at 25°C is $(1.05\pm0.13)\times10^{-4}$. This is the same as that obtained above from a simple average, but the uncertainty is more than twice as large. This very substantial increase is due almost entirely to the uncertainty in the "ion-size" parameter in the extended Debye-Hückel equation, *i.e.*, in the estimated activity coefficients needed in the calculations. A similar error analysis performed on the calorimetric measurements shows that the effect of the estimated errors in the parameters in the model on $\Delta_r H^\circ$ for the reference reaction (3) is ±0.13 kJ-mol^{-1}. This is significantly less than the estimate of random error of ±2.3 kJ-mol^{-1} obtained above. Thus, the summary results for the reference reaction (3) at 25°C and $I_m = 0$ are: $K^\circ = (1.05\pm0.13)\times10^{-4}$, $\Delta_r G^\circ = (22.71\pm0.33)$ kJ-mol^{-1}, $\Delta_r H^\circ = (62.0\pm2.3)$ kJ-mol^{-1}, and $\Delta_r S^\circ = (132\pm8)$ J-K^{-1}-mol^{-1}.

The results obtained in this study can be used in conjunction with the chemical equilibrium model to calculate the apparent equilibrium constant, the standard transformed Gibbs energy change $\Delta_r G'^\circ$,[48,49] and the standard transformed enthalpy change $\Delta_r H'^\circ$ for reaction (1) under a wide variety of conditions. At 25°C, pH = 7.0, and $I_m = 0.25$ mol-kg^{-1}: $K' = 2.0\times10^{-4}$, $\Delta_r G'^\circ = 21.1$ kJ-mol^{-1}, and $\Delta_r H'^\circ = 62.9$ kJ-mol^{-1}. At 37°C, pH = 7.0, and $I_m = 0.25$ mol-kg^{-1}, $K' = 5.4\times10^{-4}$, $\Delta_r G'^\circ = 19.4$ kJ-mol^{-1}, and, assuming that $\Delta_r C'^\circ_p = 0$, $\Delta_r H'^\circ \approx 63$ kJ-mol^{-1}.

We are unaware of any results in the literature that can be used to calculate the equilibrium constant or the enthalpy change for either the overall biochemical reaction (1) or the reference reaction (3). However, the results obtained herein can be used with results from the literature to calculate the standard Gibbs energies of formation $\Delta_f G^\circ$ and standard enthalpies of formation $\Delta_f H^\circ$ of the substances in reaction (3) both in aqueous solution and in the condensed phase at 25°C. In some cases, standard molar heat capacities $\bar{C}^\circ_p$ and standard molar entropies $\bar{S}^\circ$ for the condensed phase and standard partial molar heat capacities $\bar{C}^\circ_{p,2}$ and standard partial molar entropies $\bar{S}^\circ_2$ of the solute species are also obtained. The results of these calculations are given in Table V. Details of

Table V. Standard Thermodynamic Properties of the Substances Pertinent to this Study in Aqueous Solution and in the Condensed Phase at 25°C[a]

Substance	State	Molar Mass[b]	$\Delta_f H^{\circ c}$	$\Delta_f G^{\circ c}$	$\bar{S}^{\circ d}$	$\bar{C}_p^{\circ d}$
H_2O	l	18.0153	-285.830	-237.129	69.91	75.29
NH_3	aq	17.0305	-80.29	-26.50	111.3	75
NH_4^+	aq	18.0379	-132.51	-79.31	113.4	68
$C_3O_3H_4$, Pyruvic acid	l	88.063	-587.0			
$C_3O_3H_4$, Pyruvic Acid	aq	88.063	-606.1	-488.2	191	
$C_3O_3H_3^-$, Pyruvic Ion	aq	87.056	-594.0	-473.6	183	
C_8NH_7, Indole	s	117.15	86.65	213.5	174	
C_8NH_7, Indole	aq	117.15	97.5	223.8	175	
$C_{11}N_2O_2H_{11}^-$, L-Tryptophan$^-$	aq	203.23	-362.2	-59.9	230	
$C_{11}N_2O_2H_{12}$, L-Tryptophan	s	204.23	-417.6	-121.5	251.0	238.2
$C_{11}N_2O_2H_{12}$, L-Tryptophan	aq	204.23	-405.2	-114.7	270	420
$C_{11}N_2O_2H_{13}^+$, L-Tryptophan$^+$	aq	205.23	-408.2	-128.4	306	

[a] The standard thermodynamic properties pertain to the pure substance at p = 0.1 MPa. For aqueous solutions, the standard state for the solute is the hypothetical ideal solution at m = 1 mol-kg^{-1} and the standard state for the solvent is the pure solvent. [b] Molar mass, g-mol^{-1}. [c] kJ-mol^{-1}. [d] J-K^{-1}-mol^{-1}.

these calculations now follow. In all cases the temperature is 25°C and p is 0.1 MPa.

We first consider the formation properties of the pyruvic acid species. Miller and Smith-Magowan[50] constructed a thermodynamic cycle involving reactions in the Kreb's cycle to calculate $\Delta_f G^\circ$(pyruvate$^-$, aq). We adjust their result of $\Delta_f G^\circ$(pyruvate$^-$, aq) = -474.85 kJ-mol^{-1}, which was given at I = 0.1 mol-kg^{-1}, to the standard state (the extended Debye-Hückel equation with an "ion-size" parameter equal to 1.6 kg$^{1/2}$-mol$^{-1/2}$ was used to estimate the activity coefficients at I_m = 0.1 mol-kg^{-1}) and obtain $\Delta_f G^\circ$(pyruvate$^-$, aq) = -473.63 kJ-mol^{-1}.

This is in reasonable agreement with $\Delta_f G^\circ$(pyruvate$^-$, aq) = -472.37 kJ-mol^{-1} given by Wilhoit[51] who also used a thermochemical network to calculate this property. We obtain $\Delta_f H^\circ$(pyruvic acid, l) from Domalski's[52] evaluation of the early energy of combustion study of Blaschko.[42] We use the molar enthalpy of solution of pyruvic acid(l) which was also measured by Blaschko[42] with $\Delta_f H^\circ$(pyruvic acid, l) to calculate $\Delta_f H^\circ$(pyruvic acid, aq). Since, the molar enthalpy of ionization of pyruvic acid(aq) to pyruvate$^-$(aq) is known (see Table II), $\Delta_f H^\circ$(pyruvate$^-$, aq) is obtained. Combination of $\Delta_f G^\circ$ and $\Delta_f H^\circ$ for pyruvate$^-$(aq) leads to $\Delta_f S^\circ$(pyruvate$^-$, aq) and, with the entropies of the elements,[43] to $\bar{S}^\circ_2$(pyruvate$^-$, aq) = 183 J-K^{-1}-mol^{-1}. Use of the thermodynamic quantities given in Table II yields $\Delta_f G^\circ$ and $\bar{S}^\circ_2$ for pyruvic acid(aq).

We use Domalski's evaluation of the combustion results of Tsuzuki *et al.*[53] and obtain $\Delta_f H^\circ$(L-tryptophan, s). Cole *et al.*[54] measured the heat capacity of L-tryptophan(s) as a function of temperature from which we obtain $\bar{C}^\circ_p$ (L-tryptophan, s) and $\bar{S}^\circ$(L-tryptophan, s). This standard molar entropy is used together with the standard molar entropies of the elements[43] to calculate $\Delta_f S^\circ$(L-tryptophan, s). Combination of $\Delta_f H^\circ$ and $\Delta_f S^\circ$ for L-tryptophan(s) leads to $\Delta_f G^\circ$(L-tryptophan, s). The solubility in water of L-tryptophan(s) has been measured several times[55-62] from which we obtain the molality of the saturated solution of 0.0641 mol-kg^{-1}. In the studies in which the solubility was determined[55-62] there was no mention of a hydrated form of L-tryptophan(s) and we assume that the anhydrous form of this substance is the stable form at 25°C. The solubility of 0.0641 mol-kg^{-1} is used together with an estimated activity coefficient of unity to obtain $\Delta_r G^\circ$ for the solution reaction and then $\Delta_f G^\circ$(L-tryptophan, aq). The standard enthalpy of solution of L-tryptophan(s) has been measured calorimetrically by Abu-Hamdiyyah and Shehabuddin[63] and by Rodante.[18] The results differ by 5.7 kJ-mol^{-1}. The study done by Abu-Hamdiyyah and Shehabuddin[63] appears to be the more careful of these two even though the molar enthalpy of solution of L-tryptophan(s) calculated from Dalton and Schmidt's[56] solubility measurements at several temperatures is in agreement with the calorimetric molar enthalpy of solution reported by Rodante.[18] We therefore use the result from Abu-Hamdiyyah and Shehabuddin[63] with $\Delta_f H^\circ$(L-tryptophan, s) to obtain $\Delta_f H^\circ$(L-tryptophan, aq). We then use $\Delta_f H^\circ$(L-tryptophan, aq) and $\Delta_f G^\circ$(L-tryptophan, aq) to calculate $\Delta_f S^\circ$(L-tryptophan, aq) and then

with the molar entropies of the elements[43] obtain $\bar{S}^{\circ}_2$(L-tryptophan, aq) = 270 J-K^{-1}-mol^{-1}. With the thermodynamic quantities for the ionizations of the L-tryptophan aqueous species, we calculate $\Delta_f H^{\circ}$, $\Delta_f G^{\circ}$, and for $\bar{S}^{\circ}_2$ for L-tryptophan^{+}(aq) and L-tryptophan^{-}(aq). The standard partial molar heat capacity of L-tryptophan(aq) is from Jolicoeur *et al.*[64] and $\bar{C}^{\circ}_{p,2}(NH_3, aq)$ and $\bar{C}^{\circ}_{p,2}(NH_4^+, aq)$ are from Allred and Woolley.[45]

Table VI. Standard Transformed Formation Properties at 25°C[a]

Substance	State	$\Delta_f H'^{\circ}$ [b]	$\Delta_f G'^{\circ}$ [b]
L-Tryptophan	aq	-409.9	374.5
H_2O	l	-286.65	-155.66
Indole	aq	94.6	509.2
Pyruvate	aq	-594.8	-352.1
Ammonia	aq	-133.45	82.93

[a] p = 0.1 MPa; pH = 7; and I_m = 0.25 mol-kg^{-1}. [b] Units: kJ-mol^{-1}.

We have now obtained $\Delta_f G^{\circ}$, $\Delta_f H^{\circ}$, and $\bar{S}^{\circ}_2$ for pyruvate^{-}(aq) and for L-tryptophan(aq). The standard thermodynamic properties of H_2O(l) and NH_4^+(aq) are taken from the NBS Tables[43] and used together with $\Delta_r G^{\circ}$, $\Delta_r H^{\circ}$, and $\Delta_r S^{\circ}$ for the reference reaction (3) to calculate $\Delta_f G^{\circ}$, $\Delta_f H^{\circ}$, and $\bar{S}^{\circ}_2$ for indole(aq). There is one reported solubility[65] (m = 0.0158 mol-kg^{-1}) of indole in water. In view of the hydrophobic nature of indole, we assume that the stable form of indole(s) at 25°C is the anhydrous form. This assumption and the solubility lead to $\Delta_r G^{\circ}$ for the solution reaction and then to $\Delta_f G^{\circ}$ for indole(s). There is an accurately known molar enthalpy of formation of indole(s) from Good's[66] combustion study. Combination of this $\Delta_f H^{\circ}$ with $\Delta_f G^{\circ}$ and the molar entropies of the elements[43] leads to $\bar{S}^{\circ}$ (indole, s) = 174 J-K^{-1}-mol^{-1}. While there is no direct experimental result for $\bar{S}^{\circ}$ (indole, s) with which to make a comparison, we do have an estimate from Chirico[67] of $\bar{S}^{\circ}$(indole, s) ≈190 J-K^{-1}-mol^{-1}, which differs by only 16 J-K^{-1}-mol^{-1} from the result obtained from the above thermodynamic cycle calculation. This estimate uses spectroscopic frequencies in a statistical mechanical calculation[68] to obtain $\bar{S}^{\circ}$ (indole, g). This result is then combined with the entropy of sublimation obtained from vapor pres-

sures measured as a function of temperature[69] to obtain $\bar{S}^\circ$ (indole, s). However, the molar entropy of sublimation is uncertain by ≈25 J-K^{-1}-mol^{-1} and this uncertainty is directly propagated to $\bar{S}^\circ$ (indole, s). Nevertheless, the approximate agreement of the results indicates that there are no substantive errors in the above thermodynamic cycle calculation. Additional experiments are needed to establish these property values more firmly.

The thermodynamic quantities in Table V can be used[49,70] to calculate standard transformed formation properties of the substances in reaction (1). The results of these calculations are given in Table VI. As done in earlier calculations of this type,[49,70] we have used the convention of making a transformation for all of the hydrogen atoms in these substances. However, unlike the tables produced on the adenosine 5'-triphosphate series,[70] the entries in Table VI are directly related to the elements and there is no need to assign arbitrary zeros to any of the formation properties. A check on these results is done by calculating $\Delta_r H'^\circ$ and $\Delta_r G'^\circ$ for reaction (1) at 25°C, I_m = 0.25 mol-kg^{-1}, and pH = 7 from the standard transformed formation properties in Table VI and comparing them with the values of these reaction properties obtained from the chemical equilibrium model (see above). Thus, from Table VI we have $\Delta_r H'^\circ$ = 62.9 kJ-mol^{-1} and $\Delta_r G'^\circ$ = 21.2 kJ-mol^{-1}. From the chemical equilibrium model we had obtained $\Delta_r H'^\circ$ = 62.9 kJ-mol^{-1} and $\Delta_r G'^\circ$ = 21.1 kJ-mol^{-1}. The difference of 0.1 kJ-mol^{-1} in the values of $\Delta_f G'^\circ$ obtained in two different ways is within the rounding errors inherent in these calculations. The advantage of having tables of standard transformed formation properties is that apparent equilibrium constants and transformed enthalpies of reaction can be calculated conveniently. By adopting the convention of making a transformation for all of the hydrogen atoms in these substances, these standard transformed formation properties can be used for calculations involving complex reaction sequences and metabolic pathways.

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