Biophysical Chemistry 24 (1986) 13–23
Elsevier

BPC 01049

THERMODYNAMICS OF THE CONVERSION OF AQUEOUS L-ASPARTIC ACID TO FUMARIC ACID AND AMMONIA

R.N. GOLDBERG, E. GAJEWSKI, D.K. STECKLER and Y.B. TEWARI

Chemical Thermodynamics Division, Center for Chemical Physics, National Bureau of Standards, Gaithersburg, MD 20899, U.S.A.

Received 21st October 1985
Accepted 6th January 1986

Key words: *Aspartase; Aspartic acid; Calorimetry; Enthalpy; Enzyme-catalyzed reaction; Equilibrium constant; Fumaric acid; Gibbs energy; Heat capacity; HPLC; Thermodynamics*

The thermodynamics of the conversion of aqueous L-aspartic acid to fumaric acid and ammonia have been investigated using both heat conduction microcalorimetry and high-pressure liquid chromatography. The reaction was carried out in aqueous phosphate buffer over the pH range 7.25–7.43, the temperature range 13–43°C, and at ionic strengths varying from 0.066 to 0.366 mol kg^{-1}. The following values have been found for the conversion of aqueous L-aspartateH$^-$ to fumarate^{2-} and NH_4^+ at 25°C and at zero ionic strength: $K = (1.48 \pm 0.10) \times 10^{-3}$, $\Delta G^\circ = 16.15 \pm 0.16$ kJ mol^{-1}, $\Delta H^\circ = 24.5 \pm 1.0$ kJ mol^{-1}, and $\Delta C_p^\circ = -147 \pm 100$ J mol^{-1} K^{-1}. Calculations have also been performed which give values of the apparent equilibrium constant for the conversion of L-aspartic acid to fumaric acid and ammonia as a function of temperature, pH and ionic strength.

1. Introduction

The aim of this investigation is an improved understanding of the thermodynamics of the aspartase-catalyzed (fumaric aminase or L-aspartate ammonia-lyase, EC 4.3.1.1) conversion of aqueous L-aspartic acid to fumaric acid and ammonia. In addition to its inherent biochemical importance, this reaction is currently being used for the industrial production of aspartic acid [1]. Also, while equilibrium constants have been determined several times [2–9], there are no calorimetric data for this reaction in the literature.

In this investigation, we have (1) determined equilibrium constants as a function of temperature (13–43°C) using high-pressure liquid chromatography (HPLC), (2) calorimetrically measured enthalpies of reaction (25–37°C), and (3) treated the experimental data in terms of a chemical-equilibrium model which considered both the multiplicity of the species in solution and ionic strength effects. The principal objective was the determination of the Gibbs energy (ΔG°), enthalpy (ΔH°), and heat capacity (ΔC_p°) changes at 298.15 K for the reference reaction (amm, ammonia; asp, L-aspartate; fum, fumarate; mal, L-(−)-malate).

$$\text{aspH}^-(\text{aq}) = \text{fum}^{2-}(\text{aq}) + \text{NH}_4^+(\text{aq}) \qquad \text{(A)}$$

The determination of these properties was complicated by the presence of fumarase (EC 4.2.1.2) activity in the aspartase preparation used in this investigation. Thus, the results of a recent study [10] of the fumarase catalyzed reaction:

$$\text{fum}^{2-}(\text{aq}) + \text{H}_2\text{O}(\text{l}) = \text{mal}^{2-}(\text{aq}) \qquad \text{(B)}$$

were utilized in applying the needed corrections to the measured data.

0301-4622/86/$03.50

2. Experimental

2.1. Materials

Monosodium L-aspartate, disodium fumarate, disodium L-(−)-malate, L-aspartic acid, L-aspartase from *Hafnia alvei* and fumarase from chicken heart were purchased from Sigma *. The aspartase was supplied as a lyophilized powder containing 40% protein, the remainder being potassium phosphate buffer salts and stabilizer. Fumarase was supplied as a suspension in 3.2 M $(NH_4)_2SO_4$. It was dialyzed against phosphate buffer to remove NH_4^+ and lyophilized. Monosodium L-aspartate, disodium fumarate, disodium L-(−)-malate and L-aspartic acid were found to be chromatographically pure using the chromatographic method described below. KH_2PO_4 and Na_2HPO_4 were, respectively, Standard Reference Materials 186-IC and 186-IIC from NBS. $(NH_4)_2SO_4$ was from Mallinckrodt and Ca_2SO_4 from Aldrich Chemical Co. HPLC grade water was used for preparing the 0.01 M $CaSO_4$ mobile phase.

The moisture contents of the disodium fumarate, disodium L-(−)-malate and L-aspartic acid were determined by Karl Fischer titration and were found to be 0.114, 9.2, and 0.069 mass percent, respectively. The moisture content of the monosodium L-aspartate was determined using the chromatographic method described below with the L-aspartic acid having known moisture content as a reference. It was found to be 13.5 mass percent. Corrections for the moisture contents were applied in the calculations.

At the beginning of this investigation, we found that the L-aspartase preparation not only catalyzed the conversion of L-aspartate into fumarate and ammonia but also converted fumarate into L-(−)-malate. Because of that, we sought a method which would allow us to measure amounts of the three acids quantitatively. We found that L-aspartate, L-(−)-malate and fumarate were well separated using a Bio-Rad Aminex HPX-87C column with a 0.01 M $CaSO_4$ mobile phase at pH 4.7. A Hewlett-Packard HP-1090 liquid chromatograph equipped with an HP-1037A refractive index detector and an HP-3390A integrator were used. A Bio-Rad micro-guard column packed with Bio-Sil ODS-55 was placed before the analytical column which was thermostatted at 85°C.

2.2. Methods

Equilibrium constants for the conversion of L-aspartate to fumarate and ammonia were measured starting with either monosodium L-aspartate or disodium L-(−)-malate and $(NH_4)_2SO_4$. Phosphate buffer was used in all experiments. All solutions were prepared gravimetrically using a balance sensitive to 10 μg.

No other peaks were observed on the HPLC other than those attributable to L-aspartic, fumaric, L-(−)-malic acids, and the phosphate buffer. The absence of asparaginase activity was specifically confirmed in a control experiment.

Solution compositions were experimentally determined as mol substance per kg solution. This was done for both the sake of accuracy and convenience. Since equilibrium constants are more commonly expressed having molality as the measure of composition, it was necessary to perform density measurements on the solutions used in the equilibrium measurements with a pycnometer having a precision of 0.001 g ml^{-1}. Thus, equilibrium constants having as a basis either mol per kg solution (K_M), or mol per l solution (K_c), or mol per kg water (K_m) could be interrelated using the formulae: $K_M\rho_s = K_c$ and $K_m\rho_w = K_c$, where ρ_s and ρ_w are, respectively, the densities of the solution and of pure water. The maximum adjustment of a value of K_M to an equilibrium constant expressed as K_m was 0.7%. Throughout this paper, the equilibrium constants reported for the aspartase reaction are based on molalities (mol kg^{-1}).

Measurement of pH was done using an Orion Research 811 microprocessor pH/millivolt meter and a Fisher Scientific combination electrode. Recommended procedures [11] for the measurement of pH were adhered to.

In a typical experiment known amounts of L-aspartate or L-(−)-malate and ammonia were

---

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

dissolved in phosphate buffer. Then aspartase and fumarase were added, the latter being added to obtain a more rapid equilibrium. The solution was placed in a water-bath thermostatted to within ±0.05 K and was continuously stirred with a teflon-coated magnet. Equilibrium constants were measured at temperatures ranging from 286.15 to 316.25 K. Both the forward reaction (L-aspartate to fumarate, ammonia, and L-(−)-malate and the reverse reaction (L-(−)-malate plus ammonia to fumarate and L-aspartate) were carried out under the same conditions. After completion of the reaction, solutions were analyzed for fumarate, L-aspartate, and L-(−)-malate using HPLC. Response factors for these substances were determined each day in separate control experiments. At equilibrium, for the forward reaction, the total molality of ammonia was calculated as the sum of the molalities of malate and fumarate in solution; for the reverse reaction, the ammonia molality was determined as the difference between the initial molality of the $(NH_4)_2SO_4$ and the equilibrium molality of the aspartate.

The HPLC analysis required approx. 1 ml of reacted solution. The flow rate of the mobile phase was 0.7 ml min^{-1}. A typical chromatogram is shown in fig. 1. All peaks of interest showed baseline separations.

Fig. 1. HPLC chromatogram of a mixture of L-aspartic, fumaric and L-(−)-malic acids. Peaks: fumaric acid (7.8 min), L-aspartic acid (16.0 min), malic acid (21.2 min).

The calorimetric techniques have been described [12]. Heat measurements were performed by mixing two separate solutions designated as enzyme and substrate solutions, respectively, in the microcalorimeters. The enzyme solution contained both L-aspartase and fumarase. Heats of mixing of the enzyme solution with a (blank) 'substrate' solution containing zero substrate were also determined at each temperature at which heat measurements were performed. These blank heats of mixing were found to be -2.4 ± 1.2 mJ and were applied as corrections to the measured heat effects. Sufficient amounts of enzymes were used in the reactions carried out in the microcalorimeters to effect essentially complete reaction within 60 min. All measurements were carried out in phosphate buffer. The calorimetric experiments require a thermal equilibration of the reaction vessel with the calorimetric heat-sink. To minimize the thermal decomposition of the aspartase, equilibration times were held to the minimum time consistent with adequate thermal equilibration ($\approx$ 45 min). At 37°C, this technique preserved sufficient aspartase activity to carry out the desired reaction. However, at higher temperatures, adequate enzymatic activity could not be preserved.

3. Results and discussion

3.1. Thermodynamic representation of the equilibria

The overall conversion of aqueous L-aspartic acid to fumaric acid and ammonia can be represented as:

$$\sum \text{asp} = \sum \text{fum} + \sum \text{amm} + n_H H^+ \qquad \text{(C)}$$

where Σ indicates that there exist different ionic states and n_H is the number of protons produced or absorbed as a part of the reaction. A similar reaction (see process B in ref. 10) can be written for the conversion of fumarate to malate. If ideal behavior is assumed for all of the species in solution, application of equilibrium thermodynamics leads to the following:

$$f_{\text{asp}} = [\text{aspH}^-]/[\text{asp}]_{\text{tot}} = \left\{K_{A1}/[H^+] + 1 + [H^+]/K_{A2} + [H^+]^2/(K_{A2}K_{A3})\right\}^{-1} \qquad (1)$$

$$\begin{aligned} f_{\text{fum}} &= [\text{fum}^{2-}]/[\text{fum}]_{\text{tot}} \\ &= \left\{1 + [\text{H}^+]/K_{\text{F1}} + [\text{H}^+]^2/(K_{\text{F1}}K_{\text{F2}})\right\}^{-1} \end{aligned} \tag{2}$$

$$f_{\text{amm}} = [\text{NH}_4^+]/[\text{amm}]_{\text{tot}} = \{1 + K_{\text{N}}/[\text{H}^+]\}^{-1} \tag{3}$$

$$\begin{aligned} n_{\text{H}} = {} & [\text{H}^+] f_{\text{asp}}\{-K_{\text{A1}}/[\text{H}^+] + 1/K_{\text{A2}} \\ & + 2[\text{H}^+]/(K_{\text{A2}}K_{\text{A3}})\} \\ & - [\text{H}^+] f_{\text{fum}}\{1/K_{\text{F1}} + 2[\text{H}^+]/(K_{\text{F1}}K_{\text{F2}})\} \\ & + f_{\text{amm}}K_{\text{N}}/[\text{H}^+] \end{aligned} \tag{4}$$

$$\begin{aligned} K_{\text{C,obs}} &= [\text{fum}]_{\text{tot}}[\text{amm}]_{\text{tot}}/[\text{asp}]_{\text{tot}} \\ &= K_{\text{A}} f_{\text{asp}}/(f_{\text{fum}} f_{\text{amm}}) \end{aligned} \tag{5}$$

and,

$$\begin{aligned} \Delta H^{\circ}_{\text{C,obs}} = {} & \Delta H^{\circ}_{\text{A}} + f_{\text{amm}}K_{\text{N}}\Delta H^{\circ}_{\text{N}}/[\text{H}^+] \\ & - f_{\text{fum}}\{[\text{H}^+]\Delta H^{\circ}_{\text{F1}}/K_{\text{F1}} \\ & + [\text{H}^+]^2(\Delta H^{\circ}_{\text{F1}} + \Delta H^{\circ}_{\text{F2}})/(K_{\text{F1}}K_{\text{F2}})\} \\ & + f_{\text{asp}}\{-K_{\text{A1}}\Delta H^{\circ}_{\text{A1}}/[\text{H}^+] \\ & + [\text{H}^+]\Delta H^{\circ}_{\text{A2}}/K_{\text{A2}} \\ & + [\text{H}^+]^2(\Delta H^{\circ}_{\text{A2}} + \Delta H^{\circ}_{\text{A3}})/ \\ & (K_{\text{A2}}K_{\text{A3}})\} \end{aligned} \tag{6}$$

In the above equations, square brackets denote concentrations in solution which can be expressed as mol l^{-1}, mol (kg solvent)$^{-1}$, or mol (kg solution)$^{-1}$; the subscripts tot and obs denote, respectively, the total concentration of a given substance in solution and that a given parameter is an observed or bulk quantity; f is the fraction of a given substance existing in a specified ionic state. The equilibrium constants (K) and standard-state enthalpy changes which refer to the reference reaction (process A) and to the other equilibria are summarized in table 1. If an experiment is performed in a solution containing a buffer, the measured heat effect also includes a contribution due to the protonation of the buffer, namely, $n_{\text{H}}\Delta H_{\text{buff}}$, where ΔH_{buff} is the enthalpy of protonation of the buffer under the actual reaction conditions. Equations analogous to the above for the fumarase-catalyzed conversion of fumarate to malate were presented in a recent paper [10].

Assuming $(\partial \Delta C^{\circ}_p/\partial T)$ is negligible, the temperature dependency of the equilibrium constants and the enthalpies of reaction can be calculated:

$$\begin{aligned} \Delta G^{\circ}_T = {} & \Delta H^{\circ}_{298.15} + \Delta C^{\circ}_p(T - 298.15) \\ & + T(\Delta G^{\circ}_{298.15} - \Delta H^{\circ}_{298.15})/298.15 \\ & - T\Delta C^{\circ}_p \ln(T/298.15) \end{aligned} \tag{7}$$

Table 1

Values of thermodynamic parameters [a] at 298.15 K relevant to the conversion of L-aspartic acid to fumaric acid and ammonia

Thermodynamic parameters relevant to the phosphate buffer and for the conversion of fumarate to L-(−)-malate are also included.

Process	Equilibrium constant or pK	ΔG° (J mol^{-1})	ΔH° (J mol^{-1})	ΔC°_p (J mol^{-1} K^{-1})	Reference(s)
$\text{aspH}^-(\text{aq}) = \text{fum}^{2-}(\text{aq}) + \text{NH}_4^+(\text{aq})$	$K_{\text{A}} = (1.48 \pm 0.10) \times 10^{-3}$	16150 ± 160	24500 ± 1000	−147 ± 100	this paper
$\text{aspH}^-(\text{aq}) = \text{asp}^{2-}(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{A1}} = 10.006$	57112	37740	−117	[15,16]
$\text{aspH}_2^0(\text{aq}) = \text{aspH}^-(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{A2}} = 3.914$	22343	4644	−188	[15,16]
$\text{aspH}_3^+(\text{aq}) = \text{aspH}_2^0(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{A3}} = 1.994$	11380	7448	−92	[15,16]
$\text{fumH}^-(\text{aq}) = \text{fum}^{2-}(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{F1}} = 4.603$	26276	−2845	−221 [b]	[17]
$\text{fumH}_2^0(\text{aq}) = \text{fumH}^-(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{F2}} = 3.093$	17656	460	−155 [b]	[17]
$\text{NH}_4^+(\text{aq}) = \text{NH}_3^0(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{N}} = 9.243$	52810	52220	70 [b]	[18]
$\text{HPO}_4^{2-}(\text{aq}) = \text{PO}_4^{3-}(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{P1}} = 12.241$	69873	13389	−154	[16]
$\text{H}_2\text{PO}_4^-(\text{aq}) = \text{HPO}_4^{2-}(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{P2}} = 7.198$	41087	4142	−225	[16]
$\text{H}_3\text{PO}_4^0(\text{aq}) = \text{H}_2\text{PO}_4^-(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{P3}} = 2.126$	12134	−7950	−200 [b]	[16]
$\text{H}_2\text{O}(\text{l}) + \text{fum}^{2-}(\text{aq}) = \text{mal}^{2-}(\text{aq})$	$K_{\text{B}} = 4.20$	−3557	−15670	−36	[10]
$\text{malH}^-(\text{aq}) = \text{mal}^{2-}(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{M1}} = 5.096$	29090	−1181	−221	[19]
$\text{malH}_2^0(\text{aq}) = \text{malH}^-(\text{aq}) + \text{H}^+(\text{aq})$	$\text{p}K_{\text{M2}} = 3.460$	19750	2955	−155	[19]

[a] The standard state is the hypothetical ideal solution of unit molality.

[b] Estimated.

$$K = \exp(-\Delta G_T^\circ/RT) \tag{8}$$

and

$$\Delta H_T^\circ = \Delta H_{298.15}^\circ + \Delta C_p^\circ (T - 298.15) \tag{9}$$

In the above equations, R is the gas constant (8.31441 J mol^{-1} K^{-1}) and T the thermodynamic temperature.

Eqs. 1–6 are rigorously correct when all activity coefficients are equal to unity. It was found necessary, however, in the treatment of the experimental data to include activity coefficients in the calculations. In the absence of experimental information on these activity coefficients in mixtures, they have been estimated using the extended Debye-Hückel expression:

$$\ln \hat{\gamma}_i = -Az_i^2 \hat{I}^{1/2}/(1 + B\hat{I}^{1/2}) \tag{10}$$

where $\hat{\gamma}_i$ is the activity coefficient of the i-th species, A the Debye-Hückel constant [13], B an 'ion-size' parameter, z_i the charge of the i-th species, and $\hat{I}$ the ionic strength of the solution. The ^ denotes a species as distinct from a stoichiometric quantity [14]. The ionic strength ($\hat{I}$) is calculated as:

$$\hat{I} = \tfrac{1}{2}\sum_i \hat{m}_i z_i^2 \tag{11}$$

where, $\hat{m}_i$ is the molality of the i-th species and the summation extends over all of the species in solution.

3.2. Treatment of experimental data

The equilibrium and calorimetric measurements are summarized in tables 2 and 3, respectively. The aim of the data treatment was to obtain values of K_A, ΔH_A°, and $\Delta C_{p,A}^\circ$ at 298.15 K and an estimate of parameter B in eq. 10 which describes the ionic strength dependency of the equilibria in solution. The treatment of the data required an iterative, self-consistent series of calculations which at each step involved consideration of the equilibria summarized in table 1.

Table 2

Equilibrium constants for the conversion of L-aspartic acid to fumaric acid and ammonia

The forward reactions were performed using ≈ 22 (mmol Na(aspH)) kg^{-1}; the reverse reactions were performed using the same molality of Na_2mal with ≈ 16 (mmol NH_4Cl) kg^{-1} present. The quantity $K_{C,obs}$ is equal to $[\mathrm{fum}]_{tot}[\mathrm{amm}]_{tot}/[\mathrm{asp}]_{tot}$, where the subscript tot denotes total molalities of fumarate, ammonia, or L-aspartate present in the solution at equilibrium. The values of K_A given in the two right-most columns were calculated from the experimental data and refer to zero ionic strength. The values of K_A in the last column were also adjusted to 298.15 K.

Forward				Reverse			Combined,	Calculated from measurements [e]	
T (K)	$K_{C,obs}$ ($\times 10^3$)	pH	No. of measurements	$K_{C,obs}$ ($\times 10^3$)	pH	No. of measurements	$K_{C,obs}$ ($\times 10^3$)	K_A ($I = 0, T$) ($\times 10^3$)	K_A ($I = 0$, 298.15 K) ($\times 10^3$)
286.15	3.28 ± 0.13 [b]	7.43	9	3.34 ± 0.24	7.43	8	3.31 ± 0.32	1.09	1.67
292.15	3.69 ± 0.15 [b]	7.39	7	3.78 ± 0.38	7.38	6	3.74 ± 0.40	1.22	1.50
298.25	4.60 ± 0.13 [b]	7.39	8	4.81 ± 0.57	7.34	8	4.71 ± 0.72	1.52	1.52
304.25	5.22 ± 0.34 [b]	7.34	3	5.81 ± 0.25	7.32	5	5.59 ± 0.76	1.77	1.46
310.15	5.07 ± 0.28 [a]	7.30	14	[d]			5.07 ± 0.50	1.92	1.33
310.25	6.67 ± 0.22 [b]	7.25	11	6.76 ± 0.24	7.32	14	6.69 ± 0.42	2.07	1.43
310.15	8.35 ± 0.36 [c]	7.25	6	8.77 ± 0.40	7.22	5	8.54 ± 0.76	1.95	1.35
316.25	7.63 ± 0.27 [b]	7.35	13	[d]			7.63 ± 0.50	2.33	1.36

[a] KH_2PO_4, 2.9 mmol kg^{-1}; Na_2HPO_4, 10.1 mmol kg^{-1}; $I = 0.083$ mol kg^{-1}.

[b] KH_2PO_4, 8.7 mmol kg^{-1}; Na_2HPO_4, 30.3 mmol kg^{-1}; $I = 0.150$ mol kg^{-1}.

[c] KH_2PO_4, 26.1 mmol kg^{-1}; Na_2HPO_4, 90.9 mmol kg^{-1}; $I = 0.366$ mol kg^{-1}.

[d] Not performed.

[e] The average value of the equilibrium constants in the right-most column is $(1.45 \pm 0.08) \times 10^{-3}$. If the equilibrium constants in the ninth column are fitted using the Clarke and Glew model with ΔC_p° set equal to -147 J mol^{-1} K^{-1}, a value of $(1.50 \pm 0.05) \times 10^{-3}$ is obtained for K_A at 298.15 K and at zero ionic strength. We adopt a final value of $(1.48 \pm 0.10) \times 10^{-3}$.

Table 3

Enthalpies of reaction of L-aspartic acid to an equilibrium mixture of L-aspartic acid, fumaric acid, ammonia, and L-(−)-malic acid

All measurements were performed in aqueous phosphate buffer. The quantities n_{aspH} and m_{aspH} are, respectively, the number of μmol and the molality of Na(aspH) at the start of an experiment; ΔH_{meas} is $q_{\text{meas}}/n_{\text{aspH}}$; ξ_{C} and ξ_{D} are, respectively, the extent of reaction variables for processes C and D; $(n_{\text{H}}\Delta H_{\text{buff}})$ is the correction for the enthalpy of buffer protonation; $\Delta H_{\text{C,obs}}$ is calculated using eq. 12; and $\Delta H_{\text{A}}^{\circ}$ is obtained after application of ionization corrections using eq. 6.

n_{aspH} (μmol)	m_{aspH} (μmol kg^{-1})	q_{meas} (mJ)	ΔH_{meas} (J mol^{-1})	ξ_{C} (mol kg^{-1})	ξ_{D} (mol kg^{-1})	$n_{\text{H}}\Delta H_{\text{buff}}$ (kJ mol^{-1})	$\Delta H_{\text{C,obs}}$ (kJ mol^{-1})	$\Delta H_{\text{A}}^{\circ}$ (kJ mol^{-1})
T = 298.15 K, I = 0.133 mol kg^{-1}, pH = 7.27								
12.24	0.01356	−104.48	8533	0.00957	0.00773	−0.014	24.76	24.25
11.77	0.01341	−100.89	8573	0.00949	0.00767	−0.014	24.79	24.28
11.87	0.01345	−102.39	8622	0.00951	0.00768	−0.014	24.87	24.36
14.05	0.01451	−119.90	8531	0.01009	0.00815	−0.014	24.95	24.44
11.37	0.01344	−98.05	8621	0.00951	0.00768	−0.014	24.86	24.35
11.86	0.01307	−101.12	8527	0.00930	0.00751	−0.014	24.65	24.14
								average = 24.30 ± 0.09
T = 304.15 K, I = 0.133 mol kg^{-1}, pH = 7.28								
11.21	0.01292	−100.28	8948	0.00944	0.00743	−0.023	24.79	24.01
11.13	0.01282	−97.64	8772	0.00938	0.00738	−0.023	24.53	23.75
11.22	0.01261	−100.96	8999	0.00926	0.00729	−0.023	24.79	24.01
11.30	0.01279	−101.36	8971	0.00936	0.00737	−0.023	24.79	24.01
11.28	0.01301	−97.96	8685	0.00949	0.00747	−0.023	24.45	23.67
								average = 23.89 ± 0.16
T = 310.15 K, I = 0.066 mol kg^{-1}, pH = 7.30								
11.25	0.01445	−90.23	8022	0.00999	0.00766	−0.022	23.97	22.80
11.80	0.01440	−105.40	8935	0.00996	0.00763	−0.022	25.28	24.11
12.12	0.01449	−98.66	8139	0.01001	0.00767	−0.022	24.15	22.98
11.51	0.01394	−92.01	7996	0.00972	0.00745	−0.022	23.84	22.67
12.14	0.01501	−95.26	7848	0.01028	0.00788	−0.022	23.82	22.65
12.36	0.01355	−99.72	8067	0.00951	0.00729	−0.022	23.87	22.70
								average = 22.99 ± 0.46
T = 310.15 K, I = 0.134 mol kg^{-1}, pH = 7.32								
11.46	0.01523	−93.38	8150	0.01096	0.00840	−0.022	23.70	22.53
12.15	0.01547	−96.12	7914	0.01110	0.00850	−0.022	23.41	22.24
12.38	0.01542	−95.67	7727	0.01106	0.00848	−0.022	23.14	21.97
12.33	0.01566	−98.86	8020	0.01120	0.00858	−0.022	23.58	22.41
12.31	0.01580	−103.09	8376	0.01127	0.00864	−0.022	24.10	22.93
12.11	0.01496	−106.80	8821	0.01081	0.00828	−0.022	24.58	23.41
12.53	0.01456	−102.63	8187	0.01058	0.00811	−0.022	23.64	22.47
12.67	0.01393	−93.76	7400	0.01022	0.00783	−0.022	22.46	21.29
12.05	0.01311	−92.68	7689	0.00974	0.00746	−0.022	22.72	21.55
11.33	0.01474	−97.94	8643	0.01068	0.00818	−0.022	24.30	23.13
11.35	0.01433	−99.41	8760	0.01045	0.00801	−0.022	24.38	23.21
11.71	0.01473	−102.60	8762	0.01068	0.00818	−0.022	24.46	23.29
11.53	0.01440	−96.47	8367	0.01049	0.00804	−0.022	23.86	22.69
								average = 22.54 ± 0.33
T = 310.15 K, I = 0.182 mol kg^{-1}, pH = 7.28								
13.61	0.01637	−114.10	8386	0.01187	0.00910	−0.022	23.93	22.76
13.16	0.01571	−107.67	8181	0.01150	0.00881	−0.022	23.55	22.38
13.44	0.01497	−108.35	8063	0.01106	0.00848	−0.022	23.28	22.11
12.90	0.01488	−113.36	8790	0.01101	0.00844	−0.022	24.25	23.08
13.14	0.01524	−117.76	8960	0.01122	0.00860	−0.022	24.54	23.37
12.70	0.01407	−105.65	8321	0.01053	0.00807	−0.022	23.49	22.32
								average = 22.67 ± 0.40

Table 3 (continued)

n_{aspH} (μmol)	m_{aspH} (μmol kg^{-1})	q_{meas} (mJ)	ΔH_{meas} (J mol^{-1})	ξ_{C} (mol kg^{-1})	ξ_{D} (mol kg^{-1})	$n_{\mathrm{H}}\Delta H_{\mathrm{buff}}$ (kJ mol^{-1})	$\Delta H_{\mathrm{C,obs}}$ (kJ mol^{-1})	$\Delta H_{\mathrm{A}}^{\circ}$ (kJ mol^{-1})
$T = 310.15$ K, $I = 0.334$ mol kg^{-1}, pH = 7.27								
11.78	0.01410	−102.98	8738	0.01100	0.00843	−0.022	23.57	22.40
12.20	0.01407	−103.50	8485	0.01098	0.00842	−0.022	23.24	22.07
12.14	0.01430	−102.12	8410	0.01113	0.00853	−0.022	23.18	22.01
12.21	0.01413	−103.18	8453	0.01102	0.00845	−0.022	23.21	22.04
11.88	0.01397	−100.06	8420	0.01092	0.00837	−0.022	23.14	21.97
11.61	0.01425	−90.44	7788	0.01109	0.00850	−0.022	22.37	21.20
								average = 21.95 ± 0.33

Specifically, the treatment of the equilibrium data required the use of a B parameter and a value of K_{A} at a given temperature for the calculation of the ionic strength; enthalpies and heat capacities were also needed for the calculation of equilibrium constants at that temperature. The calorimetrically measured enthalpy of reaction is given by:

$$\Delta H_{\mathrm{meas}} = (\xi_{\mathrm{C}}/m_{\mathrm{asp}})\Delta H_{\mathrm{C,obs}} + (\xi_{\mathrm{D}}/m_{\mathrm{asp}})\Delta H_{\mathrm{D,obs}} + n_{\mathrm{H}}\Delta H_{\mathrm{buff}} \quad (12)$$

where m_{asp} is the total molality of aspartic acid in solution at the start of an experiment, ξ_{C} and ξ_{D} are, respectively, the extents of reaction of the conversion of L-aspartic acid to fumaric acid and ammonia and of the conversion of fumaric acid to L-(−)-malic acid. $\Delta H_{\mathrm{D,obs}}$ is the enthalpy change for the overall conversion of fumarate to L-(−)-malate:

$$\sum \mathrm{fum} + \mathrm{H_2O} = \sum \mathrm{mal} + n_{\mathrm{H}}\mathrm{H}^{+} \quad (\mathrm{D})$$

Thus, values of K_{A}, $\Delta H_{\mathrm{A}}^{\circ}$, $\Delta C_{p,\mathrm{A}}^{\circ}$ and B are needed for the calculation of ξ_{C} and ξ_{D} which in turn are needed for the calculation of $\Delta H_{\mathrm{C,obs}}$ using eq. 12. The number of protons produced (n_{H}) is calculated using eq. 4 above and eq. 3 in ref. 10. ΔH_{buff} is the enthalpy change for the protonation of phosphate buffer (see process P2 in table 1). Under the experimental conditions of reaction, the quantity $n_{\mathrm{H}}\Delta H_{\mathrm{buff}}$ makes a small contribution (less than 0.03 kJ mol^{-1}) to the measured enthalpies.

The detailed treatment of the experimental data is as follows:

(1) Initial estimates are made of K_{A}, $\Delta H_{\mathrm{A}}^{\circ}$ and $\Delta C_{p,\mathrm{A}}^{\circ}$ at 298.15 K and of parameter B in eq. 10.

(2) The ionic strength is calculated from the equilibrium data given in table 2 by solving the simultaneous non-linear equations which describe the equilibria in solution (see table 1) and by application of eq. 11. An improved value of parameter B is inferred by varying it so as to minimize the differences between the measured and calculated values of $K_{\mathrm{C,obs}}$ at 310.15 K which were determined at three different ionic strengths.

(3) Using the equilibrium data, values of K_{A} at zero ionic strength are calculated both at the temperatures at which the experiments were performed and then adjusted to 298.15 K. An average value of K_{A} at 298.15 K and at zero ionic strength is obtained. A value of $\Delta H_{\mathrm{A}}^{\circ}$ is calculated from the temperature dependency of the equilibrium constants. In that calculation, the value of $\Delta C_{p,\mathrm{A}}^{\circ}$ is constrained to be a constant over the temperature range of interest.

(4) For the conditions appropriate to the calorimetric measurements given in table 3, values of $K_{\mathrm{C,obs}}$, $K_{\mathrm{D,obs}}$, ξ_{C} and ξ_{D} are calculated. A value of n_{H} is calculated using eq. 4 above and eq. 3 in ref. 10. Values of $\Delta H_{\mathrm{C,obs}}$ are then calculated for each measurement. Examination of the ionic strength dependency of $\Delta H_{\mathrm{C,obs}}$ at 310.15 K indicated no dependency of that quantity on ionic strength. Finally, values of $\Delta H_{\mathrm{A}}^{\circ}$ are obtained after application of ionization corrections obtained using eq. 6 and the data in table 1. From the temperature dependency of $\Delta H_{\mathrm{A}}^{\circ}$ a value of $\Delta C_{p,\mathrm{A}}^{\circ}$ is calculated.

(5) Steps 1–4 above are repeated using the new

values of K_A, ΔH_A°, $\Delta C_{p,A}^\circ$ and B obtained from the previous iteration until convergence in the values of these parameters is obtained.

3.3 Results of calculations

The equilibrium and calorimetric measurements are summarized in tables 2 and 3, respectively. As a result of the calculations described above, the following values were obtained at 298.15 K and at zero ionic strength:

$$K_A = (1.48 \pm 0.10) \times 10^{-3},$$

$$\Delta G_A^\circ = 16.1_5 \pm 0.16 \text{ kJ mol}^{-1},$$

$$\Delta H_A^\circ = 24.5 \pm 1.0 \text{ kJ mol}^{-1},$$

$$\text{and } \Delta C_{p,A}^\circ = -147 \pm 100 \text{ J mol}^{-1} \text{ K}^{-1}.$$

The value of ΔH_A° is based upon all of the enthalpy data, i.e., not only that at 298.15 K. The assigned uncertainty intervals are approx. 1.5-times the 95% statistical confidence limits. This allows for additional uncertainty due to the extrapolations to zero ionic strength and due to the iterative nature of the calculation and the interdependency of the parameters. A value of B equal to 1.6 was found to describe best the ionic strength dependency of the equilibrium data at 310.15 K. This value is typical of values of this parameter which have been obtained from the treatment of activity and osmotic coefficient data of uni-univalent and uni-bivalent electrolytes [20,21]. There was no observed dependency of $\Delta H_{C,obs}$ on ionic strength.

The above value of 24.5 ± 1.0 kJ mol^{-1} for the enthalpy change for process A is based upon the calorimetric measurements. A value of 22.1 ± 1.4 kJ mol^{-1} is calculated for ΔH_A from the temperature dependency of the equilibrium constants determined at $I = 0.15$ mol kg^{-1} when $\Delta C_{p,A}^\circ$ is constrained to equal -147 J mol^{-1} K^{-1}. We believe that the direct calorimetric measurement is more reliable than the van 't Hoff enthalpy.

3.4. Comparisons with literature data

The available literature data on the conversion of aqueous L-aspartic acid to fumaric acid and ammonia are summarized in table 4. Where possible, the method and the conditions of measurement are given. Values of $K_{C,obs}$ calculated from the data in table 1 are also given for comparison with the reported literature values. Bada and Miller [9] obtained values of $\Delta H_A^\circ = 26.9$ kJ mol^{-1} and $\Delta C_{p,A}^\circ = -117$ J mol^{-1} K^{-1}. Thus, while their

Table 4

Equilibrium constants for the conversion of aqueous L-aspartic acid to fumaric acid and ammonia as reported in the literature and compared with values calculated from the data in table 1 with a B value of 1.6

Worker(s)	$K_{C,obs}$		Conditions of measurement	Method(s)
	Reported	Calculated		
Woolf [a] [4]	≈ 0.01	5.94×10^{-3}	310.15 K, pH = 7.4, $I \approx 0.1$ mol kg^{-1}, phosphate buffer	van Slyke and polarimetry
Jacobsuhn and Tapadinhas [5]	2.4×10^{-3} (278.15 K) 7.6×10^{-3} (310.15 K)	1.38×10^{-3} 4.28×10^{-3}	pH 7.0, $I \approx 0.04$, veronal buffer	van Slyke and polarimetry
Wilkinson and Williams [b] [6]	≈ 4.3×10^{-3} (302.15 K) 2.2×10^{-2} (312.15 K)	4.55×10^{-3} 6.33×10^{-3}	pH = 6.8, $I \approx 0.1$, phosphate buffer	NH_3 by Nesslerization
Sekijo et al. [8]	≈ 0.02 (310.15 K)	-	pH = ?, I = ?	?
Bada and Miller [9]	2.5×10^{-3} (278.05 K) 3.7×10^{-3} (288.45 K) 5.11×10^{-3} (300.55 K) 6.78×10^{-3} (309.45 K)	1.80×10^{-3} 2.78×10^{-3} 4.30×10^{-3} 5.85×10^{-3}	pH = 7.0, $I = 0.1$, performed in several different buffers	amino acid analysis, Conway diffusion, and ultraviolet spectroscopy

[a] Earlier papers are by Quastel and Woolf [2] and Cook and Woolf [3].

[b] Also see Williams and McIntyre [7]. The values given in the second column at 302.15 and 312.15 K are the average of the equilibrium constants determined from two different directions. The scatter in their data is large and the apparent agreement with our results is fortuitous.

equilibrium constants are systematically higher than the values obtained in this investigation, the results derived from the temperature dependency of this equilibrium data are in agreement with our values for the enthalpy and heat capacity changes. We note that Bada and Miller used Na^+ in their solutions whereas we used K^+. This may be a cause of the difference in the results.

Values of K_A and ΔH_A° at zero ionic strength and at 298.15 K were also calculated from available thermochemical data on solid-phase L-aspartic and fumaric acids [22–25], solubility and heat of solution data [26–28], and the Gibbs energy and enthalpy of formation of $NH_4^+(aq)$ [18]. The value of ΔG_A° calculated via this thermochemical cycle is 16.9 kJ mol^{-1} and has an estimated uncertainty of 12 kJ mol^{-1}. It must be considered to be in fortuitous agreement with the value of 16.15 kJ mol^{-1} obtained in this investigation. The value of ΔH_A° calculated via this thermochemical cycle is 37 ± 10 kJ mol^{-1}. It is not as reliable as our calorimetric measurement.

The aspartase reaction also enters into a thermochemical network involving other enzyme-

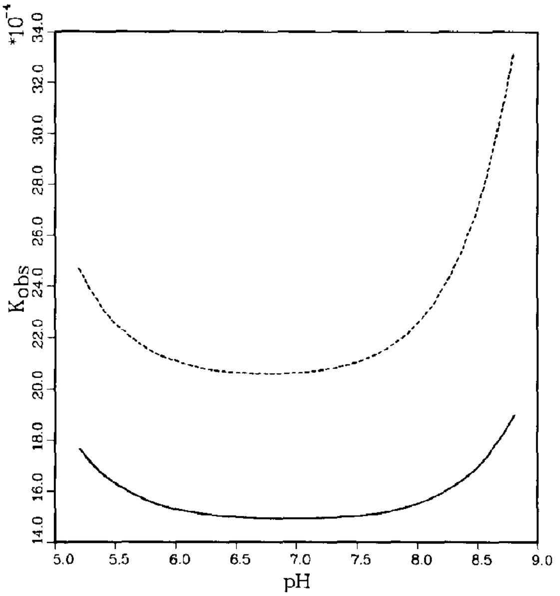

Fig. 3. The observed or bulk equilibrium constant at 298.15 K for process C, $\Sigma asp = \Sigma fum + \Sigma amm + n_H H^+$, as a function of pH at an ionic strength of zero (———) and 0.1 mol kg^{-1} (- - - - -).

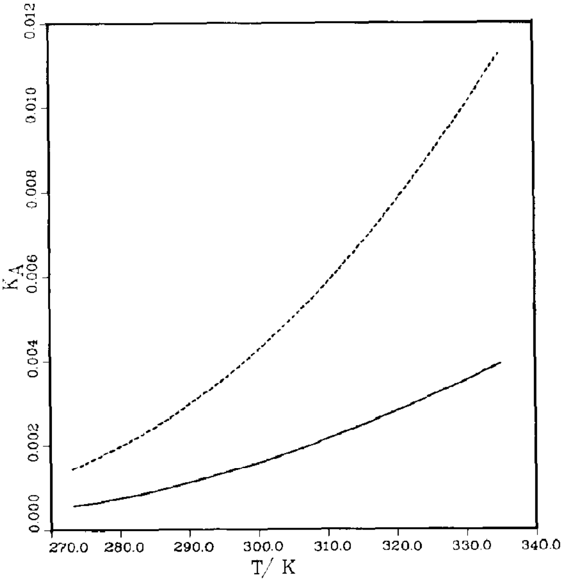

Fig. 2. The equilibrium constant for process A, $aspH^-(aq) = fum^{2-}(aq) + NH_4^+(aq)$, as a function of temperature at an ionic strength of zero (———) and 0.1 mol kg^{-1} (- - - - -).

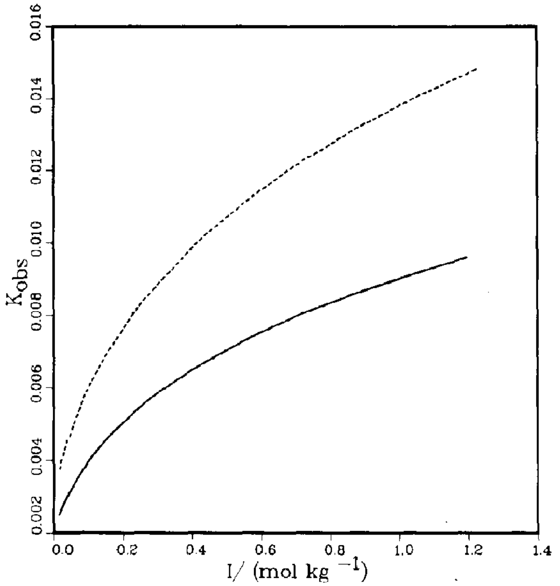

Fig. 4. The observed or bulk equilibrium constant for process C as a function of ionic strength: at pH 7.0 and 298.15 K (———); at pH 7.0 and 310.15 K (- - - - -).

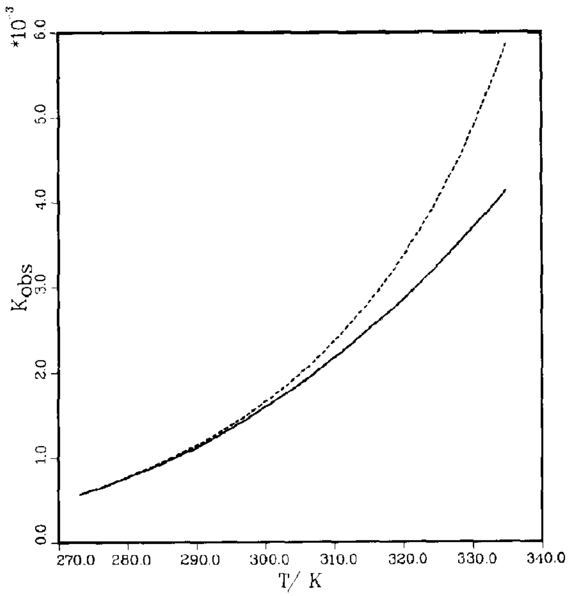

Fig. 5. The observed or bulk equilibrium constant for process C as a function of temperature at zero ionic strength at pH 7.0 (———) and pH 8.0 (- - - - - -).

catalyzed reactions related to the Krebs cycle. Examination (S.L. Miller and D. Smith-Magowan, personal communication) of this thermochemical network leads to a Gibbs energy change of 13.88 kJ mol^{-1} for process A at an ionic strength of 0.1 mol kg^{-1} and 298.15 K, in good agreement with the value of 13.70 kJ mol^{-1} obtained herein.

3.5. Calculations using summary thermochemical data

The data in table 1 summarize the thermodynamics of the aspartase reaction and can be used to calculate the variation of $K_{C,obs}$ with pH, temperature and, if values of the activity coefficients are assumed, ionic strength. These variations are not insignificant and some of them are shown in figs. 2–5 where a value of B equal to 1.6 was used to calculate the activity coefficients using eq. 10. Note that the predictions given in figs. 2–5 extend to temperatures, ionic strengths and values of the pH beyond those investigated herein. Calculated values of K_A and of $K_{C,obs}$ are also given in table 5. In general, biochemical equilibria are functions of concentrations, temperature and ionic strength and the full application of fundamental thermochemical data as given in table 1 is dependent upon being able to perform the necessary equilibrium calculations.

Acknowledgements

We thank Dr. Sam Margolis for his assistance with the Karl Fischer titrations and Drs. Stanley Miller and David Smith-Magowan for sharing the results of their calculations prior to publication.

Table 5

Values of equilibrium constants and apparent equilibrium constants for the conversion of L-aspartic acid to fumaric acid and ammonia at selected temperatures, ionic strength and pH

Process	T (K)	I (mol kg^{-1})	pH	K ($\times 10^3$)
$aspH^-(aq) = fum^{2-}(aq) + NH_4^+(aq)$ [a]	298.15	0.0	–	1.48
	298.15	0.1	–	3.98
	310.15	0.0	–	2.14
	310.15	0.1	–	5.87
$\Sigma asp = \Sigma fum + \Sigma amm + n_H H^+$ [b]	298.15	0.0	7.0	1.49
	298.15	0.1	7.0	3.98
	310.15	0.0	7.0	2.17
	310.15	0.1	7.0	5.97

[a] $K_A = a(fum^{2-})a(NH_4^+)/a(aspH^-)$, where a is the activity of the indicated species.

[b] $K_{obs} = (\Sigma fum)(\Sigma amm)/(\Sigma asp)$.

References

1 S.C. Stinson, Chem. Eng. News 63 (1985) No. 25, p. 25.
2 J.H. Quastel and B. Woolf, Biochem. J. 20 (1926) 545.
3 R.P. Cook and B. Woolf, Biochem. J. 22 (1928) 474.
4 B. Woolf, Biochem. J. 23 (1929) 472.
5 K.P. Jacobsohn and J. Tapadinhas, Biochem. Z. 282 (1935) 374.
6 J.S. Wilkinson and V.R. Williams, Arch. Biochem. Biophys. 93 (1961) 80.
7 V.R. Williams and R.T. McIntyre, J. Biol. Chem. 217 (1955) 467.
8 C. Sekijo, N. Sunahara and S. Iwakumo, Hakko To Taisha II (1965) 139.
9 J.L. Bada and S.L. Miller, Biochemistry 7 (1968) 3403.
10 E. Gajewski, R.N. Goldberg, and D.K. Steckler, Biophys. Chem. 22 (1985) 187.
11 R.A. Durst, Standardization of pH measurements, NBS special publication 260-53, (U.S. Government Printing Office, Washington, DC, 1975).
12 Y.B. Tewari and R.N. Goldberg, J. Solution Chem. 13 (1984) 523.
13 E.C.W. Clarke and D.N. Glew, J. Chem. Soc. Faraday Trans. I, 76 (1980) 1911.
14 R.N. Goldberg, J. Res. Nat. Bur. Stand. 89 (1984) 251.
15 E.R.B. Smith and P.K. Smith, J. Biol. Chem. 146 (1942) 187.
16 J.W. Larson and L.G. Hepler, in: Solute-solvent interactions, eds. J.F. Coetzee and C.D. Ritchie (Marcel-Dekker, New York, 1969) p. 1.
17 J.J. Christensen, R.M. Izatt and L.D. Hansen, J. Am. Chem. Soc. 89 (1967) 213.
18 D.D. Wagman, W.H. Evans, V.B. Parker, R.H. Schumm, I. Halow, S.M. Bailey, K.L. Churney and R.L. Nuttall, The NBS tables of chemical thermodynamic properties, J. Phys. Chem. Ref. Data 11 (1982) Suppl. 2.
19 M. Eden and R.G. Bates, J. Res. Nat Bur. Stand. 62 (1959) 161.
20 W.J. Hamer and Y.C. Wu, J. Phys. Chem. Ref. Data 1 (1972) 1047.
21 R.N. Goldberg, J. Phys. Chem. Ref. Data 10 (1981) 671.
22 G.S. Parks and H.M. Huffman, J. Am. Chem. Soc. 52 (1930) 4381.
23 G.T. Furukawa, Ninth prelimary report on a survey of thermodynamic properties of the compounds of the elements CHNOPS, NBS report 9449 (Washington, DC, 1966).
24 J.O. Hutchens, A.G. Cole, R.A. Robie and J.W. Stout, J. Biol. Chem. 238 (1963) 2407.
25 E.S. Domalski, J. Phys. Chem. Ref. Data 1 (1972) 221.
26 A. Wasserman, Z. Phys. Chem. 146A (1930) 418.
27 N.A. Lange and M.H. Sinks, J. Am. Chem. Soc. 52 (1930) 2602.
28 Hade, Thesis, University of Chicago (1962); cited by J.O. Hutchens in Handbook of Biochemistry and Molecular Biology, ed. G.D. Fasman (CRC Press, Cleveland, 1976) p. 115.