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## Equilibrium Constant for the Reversible Deamination of Aspartic Acid\*

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**ABSTRACT:** The equilibrium constant for the deamination of aspartic acid to fumaric acid and ammonia has been measured between 5 and 37° with the enzyme aspartase, and between 60 and 135° by the nonenzymatic reaction.

Aspartic acid, unlike most amino acids which occur in proteins, decomposes by a reversible deamination yielding fumaric acid and ammonia (eq 1). This reaction is catalyzed by the enzyme aspartase (L-aspartate ammonia-lyase EC 4.3.1.1).

$$\text{NH}_3^+ \text{---} \text{OOCCH}_2\text{CHCOO}^- = \text{OOCCH=CHCOO}^- + \text{NH}_4^+ \quad (1)$$

tion is catalyzed by the enzyme aspartase (L-aspartate ammonia-lyase EC 4.3.1.1).

The equilibrium constant for eq 1 has been measured a number of times using aspartases from several bacteria (Quastel and Woolf, 1926; Cook and Woolf, 1928; Woolf, 1929; Jacobsohn and Tapadinhas, 1935; Williams and McIntyre, 1955; Wilkinson and Williams, 1961; Sekijo *et al.*, 1965); the values reported range from 0.008 to 0.04 at 37°. These equilibrium constants show considerable disagreement, mainly because of the difficulty in obtaining preparations of aspartase free of fumarase and because only  $\text{NH}_3$  in the equilibrium concentration mixture was measured and the other compounds were calculated by differences.

This reaction also occurs nonenzymatically at elevated

temperatures at a rate sufficient to obtain equilibrium. It has recently been proposed that this equilibrium can be used to estimate the minimum ammonium ion concentration in the oceans of the primitive earth (Bada and Miller, 1968). This estimate requires an accurate value of this equilibrium constant and its pH and temperature dependence. The equilibrium constant is also needed in an investigation of the kinetics and mechanism of the nonenzymatic reaction (J. L. Bada and S. L. Miller, unpublished results).

The equilibrium constant is given by  $\log K_{DL} = 8.188 - 2315.5/T - 0.01025T$ . The equilibrium constant for the enzyme reaction,  $K_L$ , is  $2K_{DL}$ . The pH and ionic strength dependence of this equilibrium were also investigated.

This paper reports an investigation of this equilibrium constant between 5 and 135°. The effect of pH and ionic strength was also investigated.

The reaction solutions were sealed in ampules con-

### Experimental Section

**Materials.** The L- and DL-aspartic acids and the Good buffers (Good *et al.*, 1966) were purchased from Calbiochem. The fumaric acid (Eastman) was recrystallized three times from hot water. All other chemicals were reagent grade. The aspartase enzyme from *Enterobacter aerogenes* subspecies *alvei* (formerly *Bacterium cadaveris*) was given to us by Dr. V. R. Williams. It was in the form of the frozen ammonium sulfate precipitate and had been partly purified according to the procedure of Williams and Lartigue (1967).

The reaction solutions were sealed in ampules con-

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structed from either Pyrex or alkali-resistant glass (Corning Glass 7280), the latter being used for the very basic solutions. The temperature baths at 118 and 135° were regulated to  $\pm 0.2^\circ$  by refluxing solutions of 1-butanol and water-propionic acid, respectively. The low-temperature baths were maintained by standard laboratory regulators at  $\pm 0.02^\circ$ .

**Methods.** The equilibrium concentrations of aspartic acid, ammonia, and fumaric acid were measured for each equilibrium determination. The aspartic acid concentrations were determined on a Beckman-Spinco amino acid analyzer using the method of Spackman *et al.* (1958), modified by Dus *et al.* (1967); the ammonia by Conway (1963) diffusion; and the fumaric acid by its ultraviolet absorption in 1–2 M hydrochloric acid solutions. The molar extinction coefficients determined at 250, 240, and 230 m $\mu$  were 503, 1127, and 4340 M<sup>-1</sup> cm<sup>-1</sup>, respectively. To correct for the absorbancy of the buffer, a solution with the same concentration of buffer, salts, and enzyme, was used as a blank. For the basic solutions the blank was heated for the same length of time to correct for substances dissolved from the glass walls.

A portion of the frozen enzyme-ammonium sulfate precipitate was dissolved in a minimum volume of cold 10<sup>-2</sup> M HEPES<sup>1</sup> buffer at approximately pH 7, containing 10<sup>-3</sup> M mercaptoethanol and 10<sup>-5</sup> M MgSO<sub>4</sub> and dialyzed at 4° for approximately 8 hr against the same buffer. The buffer was changed every 2 hr. After dialysis, 1.0 ml of the cold enzyme solution containing approximately 0.2 unit (6  $\mu$ g) of enzyme (Williams and Lartigue, 1967) was added to 3.0 ml of a cold solution containing a known amount of either L-aspartate or fumarate and NH<sub>4</sub><sup>+</sup>, and 2  $\times$  10<sup>-2</sup> M HEPES, 10<sup>-4</sup> M MgSO<sub>4</sub>, 10<sup>-5</sup> M EDTA, and 0.1 M NaCl; the pH of the final solution was 7.3 at 20°. At this pH, less than 0.02% of the total amount of aspartic acid is complexed by the Mg<sup>2+</sup> (Lumb and Martell, 1953). In the ionic strength investigations, NaCl was added to adjust the ionic strength to the desired values. MES buffer was used instead of the HEPES buffer for the pH 6 equilibrium determination at 36°. Immediately after addition of the enzyme solution, the reaction mixture was sealed in a glass ampule and brought to the desired temperature. The approach to equilibrium at 5 and 36° was followed by removing samples at various time intervals. The reaction was 50% completed in approximately 30 hr at 5° and in 2 hr at 36°. The 15 and 27° samples were removed from the baths after the 5° reaction had reached equilibrium. Upon removal from a bath, a sample was immediately diluted with a known volume of 1 M HCl to inactivate the enzyme. No indication of bacterial contamination was found in any of the samples.

Solutions of DL-aspartic acid and either succinate, phosphate, or borate buffers were used for the high-tem-

perature determinations. Before being placed in the temperature baths, the samples were deoxygenated and sealed under vacuum. The approach to equilibrium was observed by following the kinetics of the deamination reaction at each pH. The samples were left in the baths long enough for the reaction to have proceeded through approximately 5–6 half-lives. The high-pH samples were diluted in 1 M HCl immediately upon removal from the sealed ampules to minimize the loss of ammonia. The product of the high-temperature deamination is fumaric acid. Chromatography of several equilibrium mixtures on silica (Bulen *et al.*, 1952) showed the presence of less than 0.2% maleic acid. Amino acid analysis indicated <0.2% decarboxylation to  $\alpha$ - and  $\beta$ -alanine.

The pH values of the MES and HEPES buffers at the various temperatures were calculated from Good *et al.* (1966), while those of the phosphate and borate buffers were taken from Bates (1964). The pH values of the succinate buffers as a function of temperature were measured between 0 and 93°. These determinations were made on a Radiometer 25 SE pH meter, using Radiometer K4016 and G202CH high-temperature electrodes. The pH meter was standardized with 0.05 M potassium acid phthalate before each measurement. The pH of the standard phthalate buffer at the various temperatures was taken from Bates (1964).

The pK's of fumaric acid at an ionic strength of 0.1 were also measured by this method between 0 and 95°. These pK's were fitted by the method of least squares to give  $pK_{1\text{Fum}} = -5.697 + 1336.0/T + 0.013928T$  and  $pK_{2\text{Fum}} = -3.669 + 1114.7/T + 0.013758T$ . The aspartic acid pK's and the pK<sub>a</sub> of ammonium ion were determined at 20° and  $I = 0.1$ . The corresponding pK's at elevated temperatures and  $I = 0.1$  were calculated from these data and the temperature dependence of the pK's of aspartic acid (Smith and Smith, 1942) and the pK<sub>a</sub> of ammonium ion (Bates and Pinching, 1949). The values used are  $pK_{1\text{Asp}} = -4.056 + 1109.6/T + 0.008138T$ ,  $pK_{2\text{Asp}} = -6.864 + 1706.3/T + 0.016506T$ ,  $pK_{3\text{Asp}} = -3.011 + 2880.3/T + 0.010173T$ , and  $pK_a = -0.492 + 2835.8/T + 0.001225T$ .

## Results

**Variation of Equilibrium Constants with pH.** The equilibrium constant for eq 1 is given by

$$K_{DL}^0 = \frac{a_{\text{Fum}^{2-}} \cdot a_{\text{NH}_4^+}}{a_{\text{DL-Asp}^{--}}} = K_{DL} \frac{\gamma_{\text{Fum}^{2-}} \cdot \gamma_{\text{NH}_4^+}}{\gamma_{\text{DL-Asp}^{--}}} \quad (2)$$

$$K_{DL} = \frac{(\text{Fum}^{2-})(\text{NH}_4^+)}{(\text{DL-Asp}^{--})} \quad (3)$$

where the parentheses refer to molal concentrations, and the  $\gamma$ 's are the respective activity coefficients. The corresponding equilibrium constant in terms of the L-aspartic acid is  $K_L = (\text{Fum}^{2-})(\text{NH}_4^+)/(\text{L-Asp}^{--})$ . Since  $K_L = K_D$ , rewriting eq 3 as

$$\frac{1}{K_{DL}} = \frac{(\text{D-Asp}^{--}) + (\text{L-Asp}^{--})}{(\text{Fum}^{2-})(\text{NH}_4^+)} = \frac{1}{K_D} + \frac{1}{K_L} = \frac{2}{K_L}$$

gives  $K_L = 2K_{DL}$ . The apparent equilibrium constant,

<sup>1</sup> Abbreviations used that are not listed in *Biochemistry* 5, 1445 (1966), are: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; MES, 2-(N-morpholino)ethanesulfonic acid; Asp<sup>0+</sup>, HOOCCH<sub>2</sub>CH(NH<sub>3</sub><sup>+</sup>)COOH; Asp<sup>0+-</sup>, HOOCCH<sub>2</sub>CH(NH<sub>3</sub><sup>+</sup>)COO<sup>-</sup>; Asp<sup>+-</sup>, -OOCCH<sub>2</sub>CH(NH<sub>3</sub><sup>+</sup>)COO<sup>-</sup>; Asp<sup>-0</sup>, -OOCCH<sub>2</sub>CH(NH<sub>3</sub>)COO<sup>-</sup>; Fum<sup>00</sup>, HOOCCH=CHCOOH; Fum<sup>0-</sup>, HOOCCH=CHCOO<sup>-</sup>; Fum<sup>2-</sup>, -OOCCH=CHCOO<sup>-</sup>.

$K_{DL}^{app}$ , is defined as

$$K_{DL}^{app} = \frac{(Fum)_T(NH_4^+)_T}{(DL-Asp)_T} \quad (4)$$

where  $(Fum)_T$ ,  $(NH_4^+)_T$ , and  $(DL-Asp)_T$  are the total concentrations at equilibrium of the various ionic forms of fumarate, ammonia, and DL-aspartate, respectively.

The theoretical variation of  $K_{DL}^{app}$  with pH can be calculated by rewriting eq 4 as

$$K_{DL}^{app} = \frac{(Fum)_T(NH_4^+)_T}{(DL-Asp)_T} = \frac{(Fum^{00} + Fum^{0-} + Fum^{2-})(NH_3 + NH_4^+)}{(Asp^{0+0} + Asp^{0+-} + Asp^{1-} + Asp^{2-})} \quad (5)$$

$$K_{DL}^{app} = \frac{K_{DL} \left[ 1 + \frac{(H^+)}{K_{2Fum}} + \frac{(H^+)^2}{K_{1Fum}K_{2Fum}} \right] \left[ 1 + \frac{K_a}{(H^+)} \right]}{\left[ 1 + \frac{K_{3Asp}}{(H^+)} + \frac{(H^+)}{K_{2Asp}} + \frac{(H^+)^2}{K_{1Asp}K_{2Asp}} \right]} \quad (5)$$

A similar equation can be written for  $K_L^{app}$  for the enzyme equilibrium. For pH values greater than 10, eq 5 reduces to

$$K_{DL}^{app} = K_{DL} \frac{K_a}{K_{3Asp}} \quad (6)$$

Figure 1 shows the theoretical curves calculated from eq 5 and the experimental values determined at several pH values and  $I = 0.1$ . The uncertainty of these  $K_{DL}^{app}$  values is about  $\pm 5\%$ . The range of pH values at  $36^\circ$  was limited by the stability of the enzyme; it was found that the enzyme could only be used between pH 5 and 8. The same equilibrium constant at pH 7 and  $36^\circ$  was obtained using HEPES, MES, Tris, and phosphate buffers.

Values of  $K_{DL}^{app}$  in base can also be calculated from the ratio of the rates of the forward and reverse reactions of eq 1. The rates of deamination of aspartic acid as a function of pH have been measured by observing the rate of appearance of ammonia and fumaric acid from buffered solutions of DL-aspartic acid (Bada and Miller, 1968). The rates of addition of ammonia to fumarate were determined by measuring the rate of disappearance of fumarate in 0.01 and 0.1 M NaOH and various concentrations of ammonia (J. L. Bada and S. L. Miller, unpublished results). The rates of deamination and addition are both independent of pH for pH values greater than 10 and are given by

$$\log k_{deamination} (\text{sec}^{-1}) = 9.693 - 6248.5/T \quad (7)$$

$$\log k_{addn} (\text{sec}^{-1} M^{-1}) = 5.246 - 4247.6/T \quad (8)$$

From these expressions, values of  $K_{DL}^{app}$  in base were calculated; these are also shown in Figure 1.

The variation of  $K_{DL}^{app}$  with pH as predicted by eq 5 is within the experimental uncertainties of the observed data. The major source of error in these  $K_{DL}^{app}$  calculations is the uncertainty in the various  $pK$ 's on extrapolation from 95 to 118 and  $135^\circ$ . The close agreement between the theoretical and experimental values at these temperatures suggests that these  $pK$ 's are not substantially in error.

**Variation of  $K_{DL}$  with Temperature.** The apparent equilibrium constants between 5 and  $36^\circ$  were determined

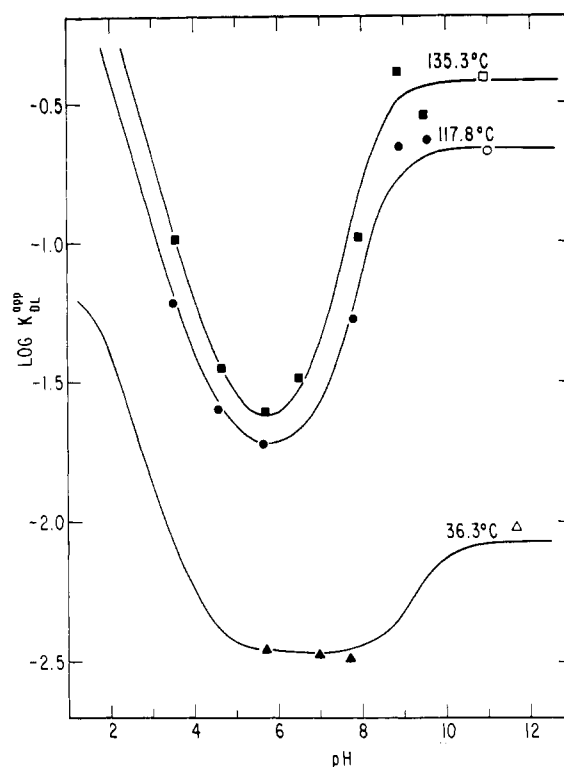


FIGURE 1: Variation of  $\log K_{DL}^{app}$  with pH. The filled symbols are equilibrium determinations and the open symbols equilibrium constants calculated from the ratio of the rates of deamination and addition. The solid curves were calculated from eq 5.

using the enzyme aspartase; the results are shown in Table I. Equilibrium was approached from both the forward and reverse directions to assure that equilibrium was attained. From eq 5 and the data in Table I, values of  $K_L$  were calculated. At the temperatures and pH of these measurements, there is very little difference between  $K_L^{app}$  and  $K_L$ . The  $K_{DL}$  values of  $1.07 \times 10^{-3}$  at  $4.9^\circ$  and  $3.36 \times 10^{-3}$  at  $36.3^\circ$  are obtained by dividing  $K_L$  by 2.

Using eq 5 and the data shown in Figure 1,  $K_{DL}$  values at  $117.8$  and  $135.3^\circ$  were also calculated. The results are  $1.78 \times 10^{-2}$  and  $2.16 \times 10^{-2}$ , respectively. The corrections of  $K_{DL}^{app}$  to  $K_{DL}$  from eq 5 are substantial at these temperatures. From the ratio of the rates of deamination and addition in base and eq 6,  $K_{DL}$  was calculated at  $65.3$ ,  $78.9$ , and  $100.5^\circ$ .

A plot of  $\log K_{DL}$  vs.  $1/T$  is shown in Figure 2. The equilibrium constants were fitted by the method of least squares to give

$$\log K_{DL} = 8.188 - 2315.5/T - 0.01025T \quad (9)$$

From eq 6 and 9, the equation for the equilibrium constant at pH values greater than 10 is given by

$$\log K_{pH>10} = 5.669 - 2271.0/T - 0.00131T \quad (10)$$

Both of these equations are for  $I = 0.1$ .

**Variation of Equilibrium Constant with Ionic Strength.** Figure 3 shows the variation of  $K_{DL}$  with ionic strength at  $36$  and  $135^\circ$ . The change in  $K_{DL}$  is small for  $I$  greater than 0.1. The variation of  $K_{DL}$  with  $I$  can be calculated

TABLE I: Enzymatically Determined Equilibrium Constants ( $K_L^{\text{app}}$ ) at pH 7.

Temp (°C)	Incubn Time (hr)	L-Asp Initial $\times 10^3$	Fumarate Initial $\times 10^3$	$\text{NH}_4^+$ Initial $\times 10^3$	L-Asp Final $\times 10^3$	Fumarate Final $\times 10^3$	$\text{NH}_4^+$ Final $\times 10^3$	$K_L^{\text{app}}$ $\times 10^3$
36.3	49	0.0	7.07	8.44	1.52	1.45	6.93	6.62
	14	8.19	0.0	0.0	1.90	2.05	6.29	6.80
	38	8.32	0.0	0.0	1.77	1.87	6.55	6.76
							Av $K_L^{\text{app}}$	6.73
27.4	135	0.0	7.40	8.74	1.69	1.16	7.05	4.83
	97	7.95	0.0	0.0	1.55	1.30	6.40	5.38
							Av $K_L^{\text{app}}$	5.11
15.3	169	0.0	7.32	8.66	1.96	0.885	6.70	3.02
	141	8.24	0.0	0.0	2.11	1.07	6.13	3.11
							Av $K_L^{\text{app}}$	3.07
4.9	118	0.0	7.25	8.59	2.39	0.752	6.20	1.95
	169	0.0	7.60	8.98	2.43	0.761	6.55	2.05
	141	8.49	0.0	0.0	2.55	1.05	5.94	2.44
							Av $K_L^{\text{app}}$	2.15

from eq 2 and the extended Debye-Hückel expression (Lewis and Randall, 1961),  $\log \gamma_i = -AZ_i^2 I^{1/2} / (1 + I^{1/2}) + CI$ , giving

$$\log K_{\text{DL}} = \log K_{\text{DL}}^0 + \frac{A(Z_{\text{Fum}^{2-}})^2 \cdot I^{1/2}}{1 + I^{1/2}} + (C_{\text{Asp}^{--}} - C_{\text{Fum}^{2-}} - C_{\text{NH}_4^+})I \quad (11)$$

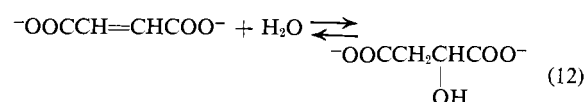
where  $A$  is the Debye-Hückel constant which varies with temperature and  $C$  is a constant which depends upon the salt.  $Z_{\text{Fum}^{2-}}$  is the effective charge on the fumarate dianion which is less than  $-1$  but greater than  $-2$  since it is not the point charge assumed in the derivation of the Debye-Hückel equation. Using the data shown in Figure 3, equations were calculated in the form of eq 11 by the method of least squares. The results are  $\log K_{\text{DL}}(36.3^\circ) = -2.835 + 1.771 I^{1/2} / (1 + I^{1/2}) - 0.41 I$  and  $\log K_{\text{DL}}(135.3^\circ) = -2.054 + 1.967 I^{1/2} / (1 + I^{1/2}) - 0.35 I$ . Using 0.522 and 0.680 for the values of  $A$  at 36 and  $135^\circ$  (Robinson and Stokes, 1959), the respective values of  $Z_{\text{Fum}^{2-}}$  calculated at these temperatures are  $-1.8$  and  $-1.7$ . If  $C_{\text{NH}_4^+}$  is assumed equal to 0.15 (Bates and Pinching, 1949), the values of  $C_{\text{Asp}^{--}} - C_{\text{Fum}^{2-}}$  at 36 and  $135^\circ$  are  $-0.26$  and  $-0.20$ , respectively.

The extrapolation to infinite dilution gives a  $K_{\text{DL}}^0$  of  $1.46 \times 10^{-3}$  at  $36^\circ$  and  $8.83 \times 10^{-3}$  at  $135^\circ$  compared with  $3.36 \times 10^{-3}$  and  $2.16 \times 10^{-2}$  at  $I = 0.1$ , a factor of about 2.4.

#### Discussion

At 5 and  $36^\circ$ , the values of  $K_L$  determined in this set of experiments using the aspartase enzyme are  $2.14 \times 10^{-3}$  and  $6.72 \times 10^{-3}$ , respectively. These are in close agreement with those determined in the experiments of Jacobsohn and Tapadinhas (1935). The higher values obtained

by other workers are not due to differences in ionic strength or pH. Difficulties were encountered in early enzymatic studies of this equilibrium because the aspartase preparations contained fumarase which catalyzes the reversible hydration of fumaric acid to L-malic acid (eq 12).



The aspartase enzyme preparation used by Jacobsohn and Tapadinhas (1935) contained fumarase, but the experiments were designed such that corrections for this activity could be made. The experiments of Williams and coworkers (Wilkinson and Williams, 1961; Williams and McIntyre, 1955) were the only ones which used an extensively purified aspartase which showed no fumarase activity.

The aspartase enzyme used in this experiment was originally thought to be free of fumarase. The mass balance on the reactants and products in Table I shows a loss of four-carbon species, suggesting the formation of malic acid. Two of the equilibrium mixtures were chromatographed on silica (Bulen *et al.*, 1952) and malic acid was found. Assuming the presence of fumarase and that equilibrium was attained between fumarate and L-malate, approximate equilibrium constants for eq 12 can be calculated from the data in Table I. These calculations yield  $K_{\text{app}} = 3.9$  at  $27.4^\circ$  and  $\Delta H = -3900$  cal mole $^{-1}$ , which are consistent with values obtained in other studies of this equilibrium using fumarase (Bock and Alberty, 1953; Krebs, 1953). These data show that either there was fumarase in the enzyme preparation or that aspartase has a small amount of fumarase activity.

At high temperatures the hydration of fumarate also takes place nonenzymatically (Rozelle and Alberty, 1957;

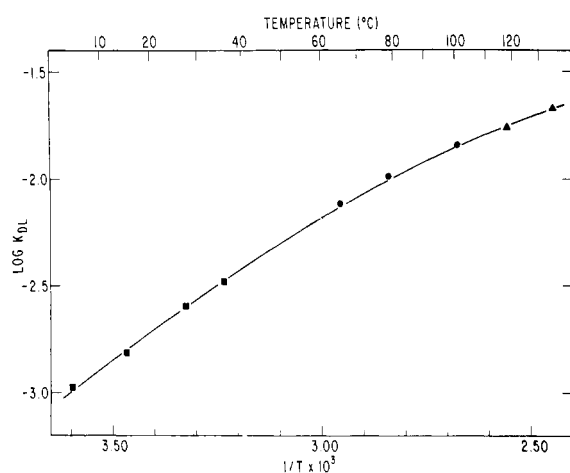
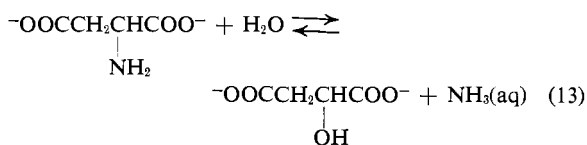


FIGURE 2: Log  $K_{DL}$  as a function of temperature. (■) Enzymatically determined equilibrium constants; (●) equilibrium constants calculated from the ratio of rates of deamination and addition; (▲) nonenzymatically determined equilibrium constants.

Erickson and Alberty, 1959; Bender and Connors, 1962). The rate of this reaction was negligible in the 118 and 135° determinations, except for the pH values less than 5. For the equilibrium determination at pH 3.5 and 4.5 the rate of this reaction was sufficiently slow that it did not interfere with the determination of the aspartic acid equilibrium constant.

The thermodynamic quantities for eq 1 can be calculated from eq 9. At 25° and  $I = 0.1$ ,  $\Delta F^\circ = 3.60$  kcal mole<sup>-1</sup>,  $\Delta H^\circ = 6.42$  kcal mole<sup>-1</sup>,  $\Delta S^\circ = 9.5$  eu, and  $\Delta C_p = -28$  cal mole<sup>-1</sup> deg<sup>-1</sup>. The large value of  $\Delta C_p$  is due to differences in the temperature variation of the various  $pK$ 's.

At 25°, the values calculated from eq 10 for the aspartate-fumarate-ammonia equilibrium for pH values greater than 10, are  $\Delta F^\circ = 3.19 \text{ kcal mole}^{-1}$ ,  $\Delta H^\circ = 9.86 \text{ kcal mole}^{-1}$ ,  $\Delta S^\circ = 22.4 \text{ eu}$ , and  $\Delta C_p = -3.6 \text{ cal mole}^{-1} \text{ deg}^{-1}$ . The values at 25° for the fumarate hydration equilibrium in base are  $\Delta F^\circ = -1.26 \text{ kcal mole}^{-1}$ ,  $\Delta H^\circ = -4.1 \text{ kcal mole}^{-1}$ , and  $\Delta S^\circ = -9.5 \text{ eu}$  (Erickson and Alberty, 1959). From these data the values for the reaction



are  $\Delta F^\circ = 1.93 \text{ kcal mole}^{-1}$ ,  $\Delta H^\circ = 5.76 \text{ kcal mole}^{-1}$ , and  $\Delta S^\circ = 12.9 \text{ eu}$ . These thermodynamic data may be compared with those for the reaction,  $\text{CH}_3\text{NH}_2(\text{aq}) + \text{H}_2\text{O}(\text{l}) = \text{CH}_3\text{OH}(\text{aq}) + \text{NH}_3(\text{aq})$ . The values calculated for this reaction are  $\Delta F^\circ = 4.75 \text{ kcal mole}^{-1}$ ,  $\Delta H^\circ = 8.09 \text{ kcal mole}^{-1}$ , and  $\Delta S = 11.5 \text{ eu}$  (Latimer, 1952; Felsing and Wohlford, 1932; Aston and Ziemer, 1946; Felsing and Phillips, 1936).

The corresponding reaction for alanine is

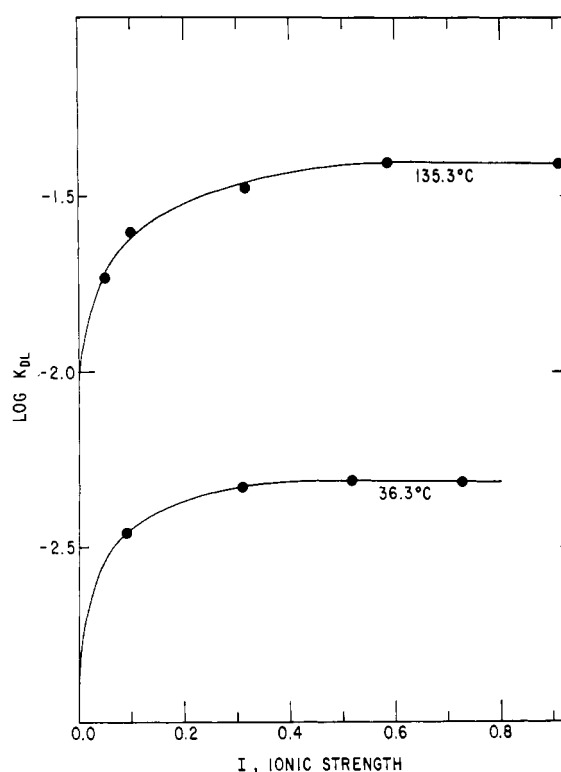
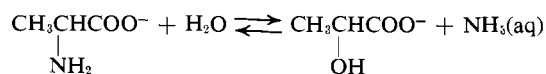


FIGURE 3: Variation of  $\log K_{DL}$  with ionic strength.

The  $\Delta F^\circ$  is 2.35 kcal mole<sup>-1</sup> calculated from the free energies of Burton and Krebs (1953) and the second ionization constant for alanine (Robinson and Stokes, 1959). This  $\Delta F^\circ$  compares favorably with the value calculated for eq 13. However, the free energies of alanine and lactate are less accurate than those for methylamine and methyl alcohol.

Aspartase is generally held to act *in vivo* as a degradative rather than as a synthetic enzyme, although the evidence for this conclusion is weak (Halpern and Umbarger, 1960). The equilibrium constants reported here are more favorable for aspartic acid formation than most of the previously determined values, making the possibility of a synthetic role for this enzyme more attractive. However, this reaction can be pushed either way by drawing off the aspartic acid or fumaric acid by a metabolic sequence, and the allosteric properties of aspartase (Williams and Lartigue, 1967) can also affect the direction of the reaction *in vivo*. It is not surprising that aspartase is absent from vertebrates since this enzyme would degrade the aspartate synthesized by various pathways, not only removing aspartate from the amino acid pool but also producing ammonia which is toxic.

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