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THERMODYNAMICS OF THE CONVERSION OF FUMARATE TO L-(−)-MALATE

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The thermodynamics of the conversion of aqueous fumarate to L-(−)-malate has been investigated using both heat conduction microcalorimetry and a gas chromatographic method for determining equilibrium constants. The reaction was carried out in aqueous Tris-HCl buffer over the pH range 6.3–8.0, the temperature range 25–47°C, and at ionic strengths varying from 0.0005 to 0.62 mol kg^{-1}. Measured enthalpies and equilibrium ratios have been adjusted to zero ionic strength and corrected for ionization effects to obtain the following standard state values for the conversion of aqueous fumarate^{2-} to malate^{2-} at 25°C: $K = 4.20 \pm 0.05$, $\Delta G° = -3557 \pm 30$ J mol^{-1}, $\Delta H° = -15670 \pm 150$ J mol^{-1}, and $\Delta C_p^\circ = -36 \pm 10$ J mol^{-1} K^{-1}. Equations are given which allow one to calculate the combined effects of pH and temperature on equilibrium constants and enthalpies of this reaction.

1. Introduction

While equilibrium constants for the enzyme-catalyzed (fumarase or fumarate hydratase, EC 4.2.1.2) conversion of fumaric acid to L-(−)-malic acid have been reported several times [1–8], the only available calorimetric data in the literature are the approximate measurement of Ohlmeyer [9,10] and measurements by Kitzinger and Hems [11]. The latter report exists in the literature only as a personal communication and has not been documented. In view of the fundamental importance of this reaction in the Kreb's cycle and also since it is used in the food and pharmaceutical industries for the preparation of L-(−)-malic acid [12], we have undertaken an investigation of its thermodynamics.

Our investigation consisted of two parts. The first part was an investigation where equilibrium constants were determined using a gas chromatographic procedure to confirm our knowledge of the value of the equilibrium constant at 298.15 K and to demonstrate the absence of any side reactions. The second part was a series of microcalorimetric measurements of the enthalpy of reaction as a function of ionic strength at three different temperatures. These measurements were used to calculate values of the standard state enthalpy ($\Delta H°$) and heat capacity change (ΔC_p°) at 298.15 K for the process:

$$\mathrm{fum}^{2-}(\mathrm{aq}) + \mathrm{H_2O(l)} = \mathrm{mal}^{2-}(\mathrm{aq}) \qquad \text{(A)}$$

2. Experimental

Fumaric acid, disodium fumarate, L-(−)-malic acid, disodium L-(−)-malate and fumarase from

Abbreviations: fum, fumarate; mal, L-(−)-malate; BSTFA, bis(trimethylsilyl)-trifluoroacetamide.

chicken heart were purchased from Sigma *. The fumarase was supplied as a suspension in 3.2 mol l^{-1} $(NH_4)_2SO_4$ solution and was stated by the vendor to contain no measurable traces of either malic dehydrogenase or lactic dehydrogenase activity. Both fumaric and L-(−)-malic acids and L-threonine were found to be chromatographically pure using the chromatographic method described below. BSTFA and acetonitrile were purchased from Pierce, Tris was from Fisher Scientific, and L-threonine was from Vega Biochemicals. Clear Reacti-Vials with open top screw caps and Tuf-Bond Teflon-silicone disks from Pierce were used for all equilibrium constant measurements. The water contents of the fumaric acid, disodium fumarate, L-(−)-malic acid and disodium L-(−)-malate were carefully determined by Karl Fischer titration and were found to be 0.069, 0.114, 0.046, and 9.2 mass percent, respectively. Corrections for the moisture contents of the disodium fumarate and disodium L-(−)-malate were applied to the calorimetric measurements.

Equilibrium constants were measured starting with either fumaric or L-(−)-malic acid. Each acid was dissolved in Tris-HCl buffer, pH 8.00. Most experiments were carried out using 0.05 mol l^{-1} buffer, except for two sets of experiments where 0.01 and 0.005 mol l^{-1} buffer were used. Measurements of pH were made using a Markson 4503 pH meter and a Fisher Scientific combination, microelectrode which was periodically standardized using Fisher certified buffers. All starting solutions of fumaric and L-(−)-malic acids and L-threonine were prepared gravimetrically, using a balance sensitive to 1 μg.

In a typical experiment 2 μl of fumarase were added to 7 ml of solutions containing either fumaric or L-(−)-malic acid dissolved in Tris-HCl buffer. Starting concentrations of each acid were approx. 1 mmol l^{-1}. This mixture was divided between six Reacti-Vials and the mass of solution added to each vial was determined using a balance sensitive to 100 μg. These tightly closed vials were shaken for 6 h in a water bath thermostatted to ± 0.03 K. Following this equilibration period, a vial and its contents were removed from the water bath and frozen rapidly in liquid nitrogen. To each frozen sample, a weighed mass of L-threonine solution was added, and the sample was immediately frozen again. The samples were then lyophilized for periods ranging from 12 to 24 h (FTS Systems Flexi-Dry apparatus). For each series of measurements five more vials were prepared with known masses of fumaric acid, L-(−)-malic acid and L-threonine solutions. They were then frozen in liquid nitrogen and lyophilized in the same apparatus with the vials from the equilibrium experiments. These solutions were used to calculate response factors for fumaric and L-(−)-malic acid with L-threonine as the internal standard, using the gas chromatography procedure described below.

Experiments were also performed in which the equilibration times were varied from 1 to 6 h; these experiments showed no change in the measured ratio of fumaric to L-(−)-malic acid. Also, as will be seen from an examination of the results, the same ratio was measured using either pure L-(−)-malic acid or pure fumaric acid in solutions as a starting point in the equilibrium measurement. Thus, we have confirmed that the reaction under investigation is essentially at equilibrium under the experimental conditions.

Lyophilized samples were trimethylsilylated using BSTFA in acetonitrile as previously described [13]. The entire procedure for each sample from incubation to trimethylsilyl derivatization was conducted in the same vial.

A Hewlett-Packard model 5880A microprocessor-controlled gas chromatograph equipped with a flame ionization detector was used for the separation and quantitative analysis of fumaric and L-(−)-malic acids. The injection port was maintained at 250°C and the detector at 350°C. Injections were made manually. Helium was used as the carrier gas for all separations at an inlet pressure of 100 kPa. The split ratio was 1:30. Separations were performed using a Hewlett Packard cross-linked methyl silicone high performance capillary column (12 m length, 0.2 mm inner diameter). All peaks showed baseline separation and were symmetrical. A typical chromato-

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

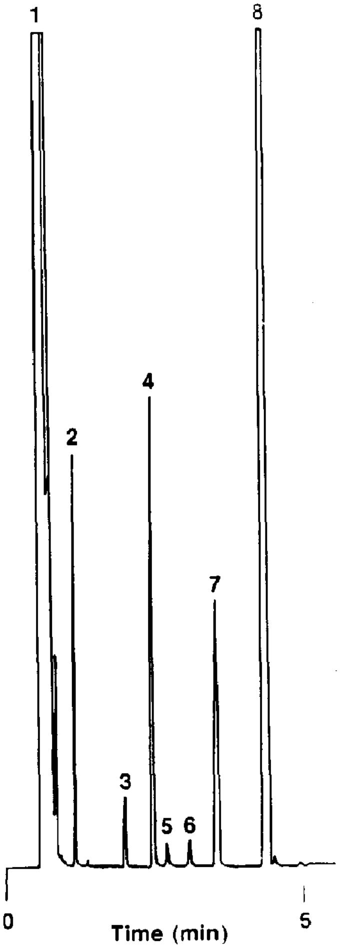

Fig. 1. Gas chromatogram obtained from the trimethylsilated derivatives of the lyophilized equilibrium mixture of L-(–)-malic and fumaric acids in 0.05 mol l^{-1} Tris-HCl buffer. The column was a Hewlett-Packard cross-linked methyl silicone high-performance capillary column, 12 m length and 0.2 mm inner diameter at a temperature of 150°C. Peaks: (1) solvent, (2) unidentified peak from enzyme preparation, (3) fumaric acid, (4) L-threonine, (5,6,8) Tris; (7) L-(–)-malic acid.

gram is shown in fig. 1. Tris shows one large peak and two smaller peaks, neither of which interfere with the peaks of the substances of interest.

The calorimetric techniques have been described [14–16]. As done previously, heat measurements were performed by mixing two separate solutions, designated as enzyme and substrate solutions, respectively, in the microcalorimeters. Heats of mixing of enzyme solutions with a (blank) 'substrate' solution containing zero substrate were also determined at each temperature at which measurements were performed with the following results: 0.0 mJ at 298.15 K, + 9.02 mJ at 313.15 K, and + 32.3 mJ at 320.15 K. These 'blank' heat effects were applied as corrections to measured heat effects. Sufficient amounts of enzyme were used in the reactions carried out in the microcalorimeters to effect essentially complete reaction within 30 min.

3. Results and discussion

3.1. Thermodynamic representation of the equilibrium

The conversion of fumarate to L-(–)-malate can be represented as

$$\Sigma\text{fum} + \text{H}_2\text{O} = \Sigma\text{mal} + n_\text{H}\text{H}^+ \qquad \text{(B)}$$

where Σ indicates that different ionic states exist (e.g., mal^{2-}, malH^-, fum^{2-}, and fumH^-) and n_H is the number of protons produced or absorbed as a part of the reaction. Application of equilibrium thermodynamics to the above reaction [17,18] * leads to the following:

$$f_\text{fum} = [\text{fum}^{2-}]/[\text{fum}]_\text{tot} = \{1 + ([\text{H}^+]/K_\text{F1}) \times (1 + [\text{H}^+]/K_\text{F2})\}^{-1} \qquad (1)$$

$$f_\text{mal} = [\text{mal}^{2-}]/[\text{mal}]_\text{tot} = \{1 + ([\text{H}^+]/K_\text{M1}) \times (1 + [\text{H}^+]/K_\text{M2})\}^{-1} \qquad (2)$$

$$n_\text{H} = \{[\text{H}^+]/K_\text{F1} + 2[\text{H}^+]^2/(K_\text{F1}K_\text{F2})\} f_\text{fum} - \{[\text{H}^+]/K_\text{M1} + 2[\text{H}^+]^2/(K_\text{M1}K_\text{M2})\} f_\text{mal} \qquad (3)$$

$$K_\text{obs} = [\text{mal}]_\text{tot}/[\text{fum}]_\text{tot} = K_\text{A} f_\text{fum}/f_\text{mal} \qquad (4)$$

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* Eqs. 31 and 32 in ref. 18 contain typographical errors; in both equations the enthalpy terms should be squared. We thank Dr. David Smith-Magowan for bringing this to our attention.

$$\begin{aligned}\Delta H^\circ_{\text{obs}} = \Delta H^\circ_{\text{A}} &+ f_{\text{fum}}\{[\text{H}^+]\Delta H^\circ_{\text{M1}}/K_{\text{M1}}\\ &+ [\text{H}^+]^2(\Delta H^\circ_{\text{M1}} + \Delta H^\circ_{\text{M2}})/(K_{\text{M1}}K_{\text{M2}})\}\\ &+ f_{\text{mal}}\{[\text{H}^+]\Delta H^\circ_{\text{F1}}/K_{\text{F1}} + [\text{H}^+]^2(\Delta H^\circ_{\text{F1}}\\ &+ \Delta H^\circ_{\text{F2}})/(K_{\text{F1}}K_{\text{F2}})\}\end{aligned} \tag{5}$$

$$\begin{aligned}\Delta C^\circ_{p,\text{obs}} = \Delta C^\circ_{p,\text{A}} &+ f_{\text{fum}}\{[\text{H}^+]C_{\text{F1}}/K_{\text{F1}}\\ &+ [\text{H}^+]^2 C_{\text{F2}}/(K_{\text{F1}}K_{\text{F2}})\}\\ &+ (f^2_{\text{fum}}/(RT^2))\{[\text{H}^+]\Delta H^\circ_{\text{F1}}/K_{\text{F1}}\\ &+ [\text{H}^+]^2(\Delta H^\circ_{\text{F1}} + \Delta H^\circ_{\text{F2}})/(K_{\text{F1}}K_{\text{F2}})]\\ &- f_{\text{mal}}\{[\text{H}^+]C_{\text{M1}}/K_{\text{M1}}\\ &+ [\text{H}^+]^2 C_{\text{M2}}/(K_{\text{M1}}K_{\text{M2}})\}\\ &- (f^2_{\text{mal}}/(RT^2))\{[\text{H}^+]\Delta H^\circ_{\text{M1}}/K_{\text{M1}}\\ &+ [\text{H}^+]^2(\Delta H^\circ_{\text{M1}} + \Delta H^\circ_{\text{M2}})/(K_{\text{M1}}K_{\text{M2}})\}\end{aligned} \tag{6}$$

where

$$C_{\text{F1}} = \Delta C^\circ_{p,\text{F1}} - (\Delta H^\circ_{\text{F1}})^2/(RT^2) \tag{7a}$$

$$C_{\text{F2}} = \Delta C^\circ_{p,\text{F1}} + \Delta C^\circ_{p,\text{F2}} - (\Delta H^\circ_{\text{F1}} + \Delta H^\circ_{\text{F2}})^2/(RT^2) \tag{7b}$$

$$C_{\text{M1}} = \Delta C^\circ_{p,\text{M1}} - (\Delta H^\circ_{\text{M1}})^2/(RT^2) \tag{7c}$$

$$C_{\text{M2}} = \Delta C^\circ_{p,\text{M1}} + \Delta C^\circ_{p,\text{M2}} - (\Delta H^\circ_{\text{M1}} + \Delta H^\circ_{\text{M2}})^2/(RT^2) \tag{7d}$$

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 quantity is the observed or experimental one; f is the fraction of a given substance existing in a specified ionic state. The equilibrium constants (K), standard state enthalpies (ΔH°), and standard state heat capacity changes (ΔC°_p) refer to process A (see Section 1) and to the following ionization equilibria:

$$\text{fumH}^-(\text{aq}) = \text{fum}^{2-}(\text{aq}) + \text{H}^+(\text{aq}),\ K_{\text{F1}} \tag{C}$$

$$\text{fumH}_2(\text{aq}) = \text{fumH}^-(\text{aq}) + \text{H}^+(\text{aq}),\ K_{\text{F2}} \tag{D}$$

$$\text{malH}^-(\text{aq}) = \text{mal}^{2-}(\text{aq}) + \text{H}^+(\text{aq}),\ K_{\text{M1}} \tag{E}$$

$$\text{malH}_2(\text{aq}) = \text{malH}^-(\text{aq}) + \text{H}^+(\text{aq}),\ K_{\text{M2}} \tag{F}$$

$$\text{Tris}(\text{aq}) + \text{H}^+(\text{aq}) = \text{Tris H}^+(\text{aq}),\ K_{\text{Tris}} \tag{G}$$

If an experiment is performed in a solution containing a buffer, the measured heat effect will also include a contribution due to the protonation of the buffer; for the experiments reported herein this contribution is equal to $n_{\text{H}}\Delta H^\circ_{\text{Tris}}$. Eqs. 1–7 summarize the standard state thermodynamic behavior of the conversion of fumarate to malate. Table 1 contains values of ΔG°, ΔH° and ΔC°_p for the relevant processes needed in these equations. Note that we have also included in table 1 values of ΔG°, ΔH° and ΔC°_p for process A which were determined as the principle objective of this investigation.

Table 1

Values of thermodynamic parameters [a] at 298.15 K relevant to the conversion of fumarate to malate and for the protonation of Tris

Process	Equilibrium constant or pK	ΔG° (J mol^{-1})	ΔH° (J mol^{-1})	ΔC°_p (J mol^{-1} K^{-1})	References
$\text{H}_2\text{O(l)} + \text{fum}^{2-}(\text{aq}) = \text{mal}^{2-}(\text{aq})$	$K_{\text{A}} = 4.20 \pm 0.05$	-3557 ± 30	-15670 ± 150	-36 ± 10	this work
$\text{fumH}^-(\text{aq}) = \text{fum}^{2-}(\text{aq}) + \text{H}^+(\text{aq})$	p$K_{\text{F1}} = 4.603$	26276	−2845	−221 [b]	[21]
$\text{fumH}^0_2(\text{aq}) = \text{fumH}^-(\text{aq}) + \text{H}^+(\text{aq})$	p$K_{\text{F2}} = 3.093$	17656	460	−155 [b]	[21]
$\text{malH}^-(\text{aq}) = \text{mal}^{2-}(\text{aq}) + \text{H}^+(\text{aq})$	p$K_{\text{M1}} = 5.096$	29090	−1181	−221	[22]
$\text{malH}^0_2(\text{aq}) = \text{malH}^-(\text{aq}) + \text{H}^+(\text{aq})$	p$K_{\text{M2}} = 3.460$	19750	2955	−155	[22]
$\text{Tris}^0(\text{aq}) + \text{H}^+(\text{aq}) = \text{Tris H}^+(\text{aq})$	p$K_{\text{Tris}} = 8.072$	−46075	−47480	−5	[23,24]

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

[b] Estimated.

Use of eqs. 1–7 with the data in table 1 allow one to predict how much measured quantities (K_{obs}, $\Delta H^\circ_{\mathrm{obs}}$ and $\Delta C^\circ_{p,\mathrm{obs}}$) will vary with pH from the desired quantities (K_{A}, $\Delta H^\circ_{\mathrm{A}}$ and $\Delta C^\circ_{p,\mathrm{A}}$) which specify the thermodynamic behavior of process A, the reference reaction for the conversion of fumarate to malate. Assuming that $(\partial \Delta C^\circ_p/\partial T)$ is negligible, ΔC°_p equals $\Delta C^\circ{}_{p,298}$ and the temperature dependency of the equilibrium constants and the enthalpies of reaction can also be calculated:

$$\Delta G^\circ_{\mathrm{T}} = \Delta H^\circ_{298} + \Delta C^\circ_p(T-298) + T(\Delta G^\circ_{298} - \Delta H^\circ_{298})/298 - T\Delta C^\circ_p \ln(T/298) \qquad (8)$$

$$K = \exp(-\Delta G^\circ_{\mathrm{T}}/RT) \qquad (9)$$

and

$$\Delta H^\circ_{\mathrm{T}} = \Delta H^\circ_{298} + \Delta C^\circ_p(T-298) \qquad (10)$$

where R is the gas constant (8.31441 J mol^{-1} K^{-1}), T the thermodynamic temperature, and 298.15 is abbreviated to 298. Eqs. 8–10 are equivalent to the equation of Clarke and Glew [19] where $\partial \Delta C^\circ_p/\partial T$ is equal to zero.

Application of the above equations (eqs. 1–5) using the data in table 1 shows that at pH 7 and 298.15 K, K_{obs} and $\Delta H^\circ_{\mathrm{obs}}$ will differ from K_{A} and $\Delta H^\circ_{\mathrm{A}}$ by 0.9 and 2.6%, respectively. At the same temperature and pH 8 these differences will be 0.1 and 0.3%, respectively. Accordingly, our experimental strategy was to keep the pH within this region and to apply these small, calculated corrections to measured quantities and also to vary the ionic strength and to extrapolate measured quantities to zero ionic strength thus essentially eliminating the effects attributable to nonideal behavior.

3.2. Equilibrium measurements

Equilibrium measurements (see table 2) were performed at 298.25 K in 0.005, 0.010 and 0.050 mol l^{-1} Tris-HCl buffer. These correspond to ionic strengths (I) of 0.48, 3.5 and 5.5 mmol kg^{-1}, respectively, and to pH values of 7.30, 7.63 and 7.99. Using eqs. 1–7 we calculate values of ($K_{\mathrm{obs}} - K_{\mathrm{A}}$) of + 0.018 at pH 7.3, + 0.009 at pH 7.63, and + 0.004 at pH 7.99. Applying those small corrections to the average of the equilibrium constants determined starting from fumaric acid and L-(−)-malic acid, the following values are obtained for K_{A}: 4.23 at $I = 0.48$ mmol kg^{-1}, 4.15 at $I = 3.5$ mmol kg^{-1} and 4.13 at $I = 5.5$ mmol kg^{-1}. Extrapolation of these measured values of K_{A} to zero ionic strength leads to a standard state value of K_{A} equal to 4.21; a simple average yields a value of 4.17. We adopt an average of these two methods of treating the data and adjust the data to 298.15 K (25°C) to obtain a value of $K_{\mathrm{A}} = 4.20 \pm 0.05$. The assigned uncertainty is large enough to encompass the average of all of the values of K_{obs}

Table 2

Equilibrium constants for the conversion of fumarate and malate to the equilibrium mixture and conditions of measurement

Uncertainties refer to 95% confidence limits. Δ is a quantity which represents the extent of material balance within a given set of measurements; it is defined as $100(n_{\mathrm{fum}} + n_{\mathrm{mal}})/n_{\mathrm{s}}$ where n_{fum} and n_{mal} are, respectively, the number of moles of fumarate and molate in the solution at equilibrium and n_{s} is the number of moles of fumarate or malate at the start of an experiment. The average value of Δ for the entire set of measurements is −0.090%.

T (K)	Tris (mol l^{-1})	pH	I (mmol kg^{-1})	No. of experiments	Starting from fumaric acid		Starting from malic acid	
					K	Δ (%)	K	Δ (%)
298.15	0.005	7.30	0.48	6	4.235 ± 0.042	−4.8	4.266 ± 0.038	−5.4
298.15	0.010	7.63	3.5	6	4.179 ± 0.013	−1.5	4.147 ± 0.035	−0.40
298.15	0.050	7.99	5.5	6	4.140 ± 0.023	4.6	4.133 ± 0.034	5.2
309.41	0.050	7.99	5.5	6	3.319 ± 0.0095	1.6	3.248 ± 0.024	3.4
320.05	0.050	7.99	5.5	6	2.670 ± 0.023	−4.2	2.650 ± 0.019	0.6

when their uncertainty intervals are also considered. The results of the equilibrium measurements, considered as a whole, demonstrate a material balance between the fumaric and malic acids. This and the absence of any unidentified peaks indicates the absence of any significant side reactions. We believe that the assigned uncertainty of ± 0.05 in K_A encompases both random and systematic errors.

Equilibrium measurements were also performed at 309.41 and 320.05 K yielding values of $K_A = 3.28$ and 2.66, respectively. If the value of $K_A = 4.20$ at 298.15 K is adjusted to these higher temperatures using the enthalpy and heat capacity data in table 1, values of 3.33 and 2.70 are calculated at these two respective temperatures. This is in agreement with the equilibrium measurements.

3.3. Enthalpy measurements

Enthalpy measurements were performed at 298.15, 313.15 and 320.15 K at ionic strengths varying from 0.10 to 0.62 mol kg^{-1}. These measured enthalpies and the conditions of measurement are given * in tables 3 and 4. Note that measurements were performed starting both from solutions containing only sodium malate and from solutions containing only sodium fumarate. The method of treatment of the data was to extrapolate (see figs. 2 and 3) measured apparent enthalpies of reaction ($\Delta H' = q_{\text{meas}}$/number of moles of substrate) to zero ionic strength to obtain apparent standard state enthalpies ($\Delta H^{\circ\prime}$). These apparent standard state enthalpies can then be combined [16,20] to yield the following values of $\Delta H^\circ_{\text{obs}}$: -15.62 kJ mol^{-1} at 298.15 K, -16.20 kJ mol^{-1} at 313.15 K, and -16.38 kJ mol^{-1} at 320.15 K. Using eqs. 1–5 with the data in table 1 the small ionization/buffer protonation corrections to $\Delta H^\circ_{\text{obs}}$ were calculated and the following values of ΔH°_A were obtained: -15.66 kJ mol^{-1} at 298.15 K, -16.24_5 kJ mol^{-1} at 313.15 K, and -16.43 kJ mol^{-1} at 320.15 K. A linear least-squares fit of these three enthalpies yields a value of $\Delta H^\circ = -15.67$ kJ mol^{-1} and $\Delta C_p^\circ = -36$ J mol^{-1} K^{-1} for process A at 298.15 K. The pooled standard deviations of the mean of the enthalpy measurements starting with only malate in solution is 28 J mol^{-1} and starting from fumarate is 32 J mol^{-1}. These two standard deviations when combined in quadrature yield a value of 43 J mol^{-1}. Twice this value would be a reasonable estimate of error if only random errors were present in our measurements. We believe however that an uncertainty

Table 3

Enthalpies of reaction of fumarate to the equilibrium mixture of fumarate and malate in Tris-HCl and conditions of measurement Uncertainties refer to 95% confidence limits.

T (K)	Enzyme solution		Substrate solution		pH	$I^{1/2}$ (mol$^{1/2}$ kg$^{-1/2}$)	No. of experiments	$\Delta H'$ (J mol^{-1})
	Tris {mmol (kg solution)$^{-1}$}	Enzyme {g (kg solution)$^{-1}$}	Tris {mmol (kg solution)$^{-1}$}	Na_2 fum {mmol (kg solution)$^{-1}$}				
298.15	46.23	75.5	49.69	38.83	7.92	0.318	5	−12873 ± 30
298.15	45.58	88.4	49.46	67.52	8.03	0.390	5	−13064 ± 7
298.15	46.85	62.9	49.36	79.73	8.23	0.411	6	−13088 ± 32
298.15	46.52	69.6	49.31	86.08	7.81	0.437	6	−13309 ± 32
298.15	45.06	98.8	48.88	139.37	8.03	0.528	6	−13435 ± 8
298.15	44.39	112.1	47.57	303.73	8.11	0.784	6	−13893 ± 35
313.15	46.86	62.9	49.72	34.57	7.97	0.318	8	−12191 ± 64
313.15	46.48	70.4	49.57	54.20	8.00	0.364	5	−12321 ± 60
313.15	44.73	105.3	49.05	118.43	8.00	0.506	9	−12649 ± 14
313.15	44.65	107.1	47.76	279.57	8.02	0.754	6	−13265 ± 59
320.15	45.29	94.3	49.44	69.78	8.06	0.408	6	−11731 ± 33
320.15	46.12	77.6	48.66	167.71	8.09	0.593	8	−12410 ± 19
320.15	44.46	110.9	47.77	279.12	8.15	0.751	6	−12762 ± 20

Table 4

Enthalpies of reaction of malate to the equilibrium mixture of fumarate and malate in Tris-HCl and conditions of measurement Uncertainties refer to 95% confidence limits.

T (K)	Enzyme solution		Substrate solution		pH	$I^{1/2}$ (mol$^{1/2}$ kg$^{-1/2}$)	No. of experi- ments	$\Delta H'$ (J mol^{-1})
	Tris {mmol (kg solution)$^{-1}$}	Enzyme {g (kg solution)$^{-1}$}	Tris {mmol (kg solution)$^{-1}$}	Na_2 fum {mmol (kg solution)$^{-1}$}				
298.15	46.23	75.5	49.57	43.93	7.92	0.331	6	3247 ± 32
298.15	46.85	62.9	49.25	76.49	8.25	0.409	6	3178 ± 10
298.15	46.52	69.6	49.23	78.73	7.79	0.425	5	2950 ± 9
298.15	45.58	88.4	49.17	84.40	8.05	0.435	6	3112 ± 7
298.15	44.15	117.0	48.44	158.73	8.05	0.585	6	2961 ± 10
298.15	45.83	83.4	46.93	313.63	8.11	0.783	5	2933 ± 5
313.15	46.26	74.8	49.62	38.30	7.99	0.324	8	4291 ± 81
313.15	47.38	52.4	48.85	117.80	7.97	0.512	9	4190 ± 36
313.15	44.65	107.1	47.07	298.87	8.03	0.773	6	3851 ± 13
320.15	46.26	74.8	49.62	38.30	7.99	0.324	8	4291 ± 36
320.15	45.29	94.3	49.25	76.91	8.05	0.427	6	5208 ± 71
320.15	44.49	110.3	48.27	176.62	8.12	0.605	5	4856 ± 17
320.15	44.46	110.9	47.18	288.12	8.13	0.767	6	4683 ± 18

of ± 150 J mol^{-1} in the enthalpy more realistically encompasses possible errors due to heat measurement, moisture determinations, and, in particular, to the extrapolation of measured enthalpies to zero ionic strength. The assigned uncertainty in the heat capacity of ± 10 J mol^{-1} K^{-1} reflects both

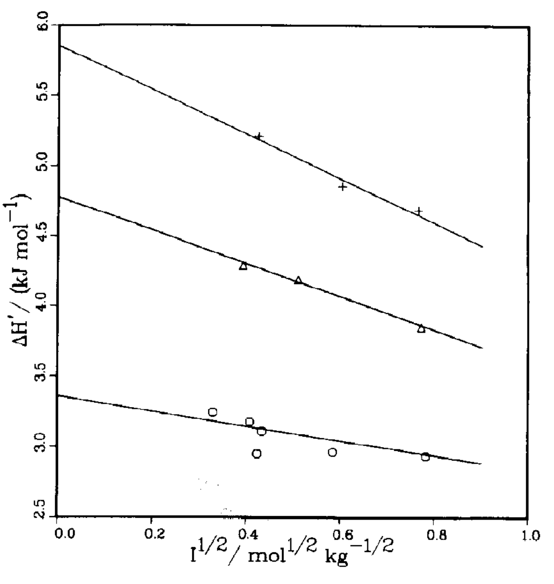

Fig. 2. Apparent enthalpies ($\Delta H'$) as a function of the square root of the ionic strength for the reaction of malate to an equilibrium mixture consisting of fumarate and malate. Temperatures of reaction: 298.15 K (○), 313.15 K (△), and 320.15 K (+), for which the extrapolated values of $\Delta H^{\circ\prime}$ are 3.37, 4.78, and 5.85 kJ mol^{-1}, respectively.

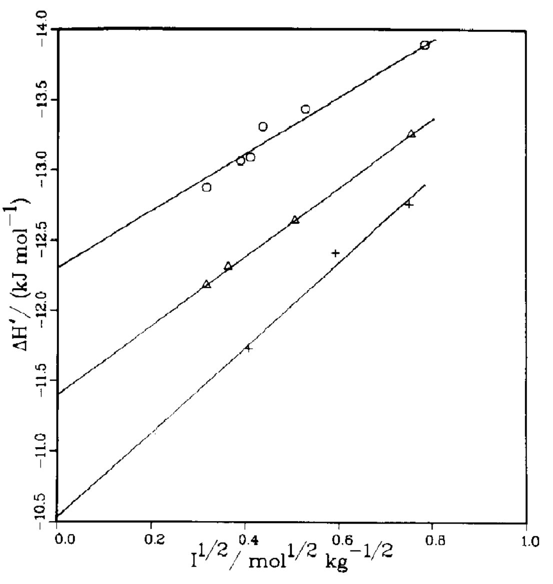

Fig. 3. Apparent enthalpies ($\Delta H'$) as a function of the square root of the ionic strength for the reaction of fumarate to an equilibrium mixture consisting of fumarate and malate. Temperatures of reaction: 298.15 K (○), 313.15 K (△), and 320.15 K (+), for which the extrapolated values of $\Delta H^{\circ\prime}$ are −12.25, −11.42, and −10.53 kJ mol^{-1}, respectively.

the allowance for random error (± 6 J mol^{-1} K^{-1} at 95% confidence limits) and an allowance of ± 4 J mol^{-1} K^{-1} for systematic errors in the enthalpies and in their temperature derivatives. In summary, we have obtained values of $K = 4.20 \pm 0.05$, $\Delta H^\circ = -15.67 \pm 0.15$ kJ mol^{-1}, and $\Delta C_p^\circ = -36 \pm 10$ J mol^{-1} K^{-1} at 298.15 K for process A.

3.4. Comparison with literature data

The available values of K and ΔH° for process A are summarized together with our results in table 5. Where possible we have adjusted the reported values to zero ionic strength and applied ionization corrections. Our experimental value of K_A is clearly in agreement with the earlier results of Bock and Alberty [1] and of Cook et al. [3]. If reasonable uncertainties are assigned to the measurements of Krebs [7], Krebs et al. [6], Kitzinger and Hems [11], and Scott and Powell [8], these measurements are also in agreement with our result. The early measurements of Borsook and Schott [2] and of Jacobsohn [5] must be considered as approximate. Our measured value of $\Delta H_\mathrm{A}^\circ$ lies between the values of ΔH° calculated from the temperature dependency of equilibrium constants obtained by Bock and Alberty [1], Krebs et al. [6], and Scott and Powell [8]. The calorimetric enthalpy reported herein is far more precise than any of the enthalpies calculated from the temperature derivative of the equilibrium constants. Kitzinger and Hems [11] measured a value of $\Delta H_\mathrm{A}^\circ$ equal to -15.48 kJ mol^{-1} in excellent agreement with our result. Unfortunately, there is little information available on their measurements. The early measurement of Ohlmeyer [9,10] is also in agreement with our calorimetrically determined enthalpy but is not as precise. Erickson and Alberty [4] studied the high temperature (90–175°C) equilibrium between fumarate and malate from which they obtained and approximate value of $\Delta C_p^\circ = -34$ J mol^{-1} K^{-1} for process A which is in excellent agreement with our result. Direct heat capacity measurements on aqueous fumarate and malate solutions would be useful in corroborating this value of ΔC_p°.

In summary, the data in table 1 in conjunction with eqs. 1–10 allow one to calculate values of K_obs as a function of pH and temperature for the conversion of fumarate to malate. The variation of K_obs with pH and temperature is not insignificant. For example, at 273 K and pH 4, K_obs is calculated to be 25.1 while at 373 K and pH 8, K_obs is calculated to be 1.07.

Table 5

Equilibrium constants and enthalpies of reaction for the conversion of fumarate to malate at 298.15 K

These values have been adjusted, where possible, to zero ionic strength and for ionization effects (see eqs. 1–5).

Worker(s)	K	ΔH° (kJ mol^{-1})	Method	Conditions of measurement
Bock and Alberty [1]	4.20	-16.57	spectrophotometry	phosphate buffer, pH 7.3
Borsook and Schott [2]	3.2	–	electrometric	pH 6.8–7.1
Cook et al. [3]	4.25	–	chemical analysis	Tris-HCl, pH 8.0
Jacobsohn [5]	5.1	–	polarimetry	barbitone buffer, pH 6.8
Krebs et al. [6]	4.05	-14.1	chemical analysis	phosphate buffer, pH 7.4 $\mathrm{NaHCO_3}$ solution
Krebs [7]	4.44	–	polarimetry and manometry	phosphate buffer, pH 7.4
Scott and Powell [8]	4.03	-14.90	chemical analysis	phosphate buffer, pH 7.29
Kitzinger and Hems [11]	4.45	-15.48	calorimetry	not known
Ohlmeyer [9,10]	–	-16.0 ± 0.7	calorimetry	veronal buffer, pH 6.8
This work	4.20 ± 0.05	-15.67 ± 0.15	gas chromatography and calorimetry	Tris-HCl buffer

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