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Thermodynamics of reactions catalysed by branched-chain-amino-acid transaminase

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Apparent equilibrium constants and calorimetric enthalpies of reaction have been measured for reactions catalysed by branched-chain-amino-acid transaminase. The following biochemical reactions have been studied at the temperature 298.15 K and in the pH range (7.15 to 7.24): **l**-valine(aq) + 2-oxoglutarate(aq) = 2-oxoisovalerate(aq) + **l**-glutamate(aq); **l**-leucine(aq) + 2-oxoglutarate(aq) = 2-oxoisocaproate(aq) + **l**-glutamate(aq); and **l**-*tert*-leucine(aq) + 2-oxoglutarate(aq) = 3,3-dimethyl-2-oxobutanoate(aq) + **l**-glutamate(aq). The results have been used to calculate equilibrium constants and standard molar enthalpy $\Delta_r H^o_m$, entropy $\Delta_r S^o_m$, and Gibbs free energy $\Delta_r G^o_m$ changes for reference reactions involving specific species. Apparent equilibrium constants and standard transformed Gibbs free energy changes for these reactions under physiological conditions have also been calculated. The use of these results for optimization of product yields of the branched-chain amino acids is discussed.

KEYWORDS: apparent equilibrium constant; branched-chain-amino-acid transaminase; 3,3-dimethyl-2-oxobutanoate; enthalpy; entropy; Gibbs free energy; **l**-glutamate; **l**-leucine; 2-oxoglutarate; 2-oxoisocaproate; 2-oxoisovalerate; **l**-*tert*-leucine; **l**-valine

1. Introduction

The enzyme branched-chain-amino-acid transaminase (EC 2.6.1.42) catalyses the first step in the catabolism of branched-chain amino acids.[1] This catabolism leads to the synthesis of the important biological molecules acetylcoenzyme A and succinylcoenzyme A. Two of

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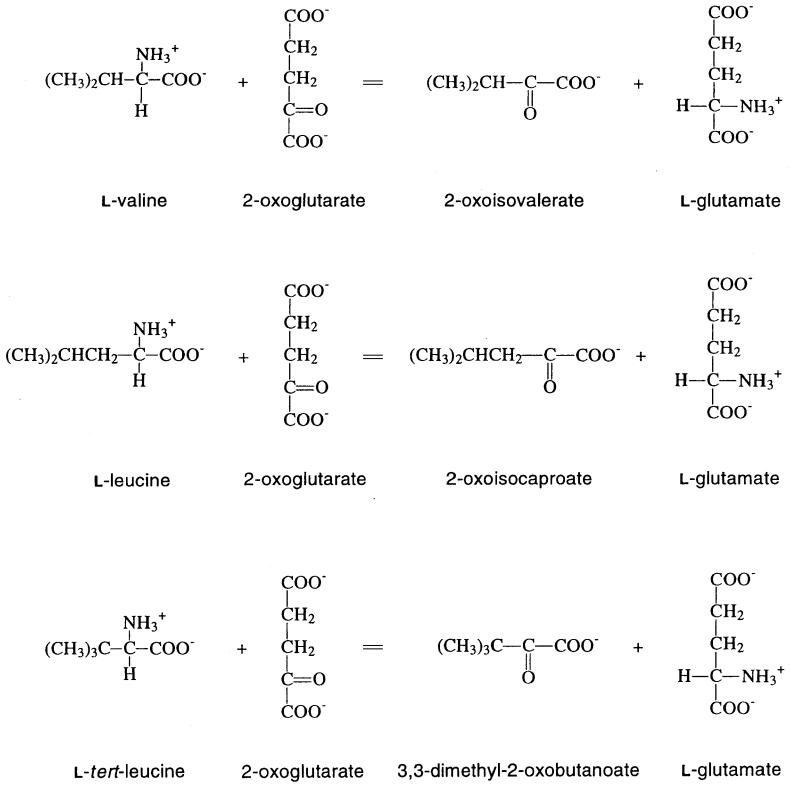

FIGURE 1. Structures of the compounds in reactions (1), (2), and (3). The predominant ionic forms at pH = 7.0 are shown.

the principal amino acids that are a substrate for this enzyme are **l**-valine and **l**-leucine; the reactions catalysed are:

$$\textbf{l}\text{-valine(aq)} + \text{2-oxoglutarate(aq)} = \text{2-oxoisovalerate(aq)} + \textbf{l}\text{-glutamate(aq)}, \quad (1)$$

$$\textbf{l}\text{-leucine(aq)} + \text{2-oxoglutarate(aq)} = \text{2-oxoisocaproate(aq)} + \textbf{l}\text{-glutamate(aq)}. \quad (2)$$

The branched-chain amino acid **l**-*tert*-leucine is also a substrate for this enzyme and undergoes the reaction

$$\textbf{l}\text{-}tert\text{-leucine(aq)} + \text{2-oxoglutarate(aq)} = \text{3, 3-dimethyl-2-oxobutanoate(aq)} + \textbf{l}\text{-glutamate(aq)}. \quad (3)$$

These three reactions involve essentially the same structural change, namely the transfer of an amino group from an amino acid to a 2-keto acid (see figure 1). Pyridoxal 5-phosphate is a required cofactor in these reactions.

These reactions are highly stereospecific and can be used to produce **l**-amino acids of high enantiomeric purity.[2, 3] This characteristic together with high catalytic rates, the

low cost of the 2-ketoacids, and lower capital costs than fermentation plants makes these reactions of considerable industrial interest.[2] Currently, **l**-valine, an intermediate that is used in drug synthesis and for the preparation of intravenous feeding solutions, is made in large quantities ($\approx 1 \cdot 10^5$ kg $\cdot$ a^{-1}) by using branched-chain-amino-acid transaminase. There is also interest in the manufacture of **l**-*tert*-leucine, a non-proteinogenic amino acid, for use as a pharmaceutical intermediate. Current production of this substance is 3000 to 5000 kg $\cdot$ a^{-1}, but demand is projected to increase by ten-fold or more within the next 3 to 5 years. However, the apparent equilibrium constants for transamination reactions have been found to fall within a very limited range: $0.016 \leqslant K' \leqslant 100$.[4,5] Thus, there is generally an incomplete conversion to the desired product, namely the branched-chain amino acid. Since knowledge of the position of equilibrium of these reactions can be extremely useful both for process optimization and for understanding the role of these reactions in metabolism,[6] we have undertaken this study that is aimed at improving knowledge of the thermodynamics of reactions (1), (2), and (3). Earlier studies[5,7] from this laboratory have dealt with transamination reactions of amino acids that were not branched, namely **l**-alanine, **l**-tyrosine, **l**-phenylalanine, and **l**-aspartate.

2. Experimental

Pertinent information on the substances used in this study is given in table 1.† The sample of 3,3-dimethyl-2-oxobutanoic acid was a gift from Dr Andreas Bommarius of Degussa-Hüls A.G. (Hannau, F.R.G.). The purities of the biochemical substances were also examined by using the chromatographic procedures described below and found to be consistent with the values given in table 1. The mass fractions of water in these substances (see table 1) were measured either by Karl-Fischer titration or by drying over phosphorous pentoxide. The Karl-Fischer titrations were done with a Metrohm Model 633 automatic titrator which was calibrated with a water-saturated octanol solution.[8] The mass fractions of water as determined by these two methods were used in all subsequent calculations that involved the amounts of these substances. The branched-chain-amino-acid transaminase was isolated and partially purified from *Escherichia coli* based on the protocol published by Monnier *et al.*[9] The enzyme used in the study of reactions (1) and (2) was in the form of lyophilized powder. However, this preparation lacked sufficient activity for the study of reaction (3). Therefore, a second preparation of this enzyme was obtained from *E. coli* in which the *ilvE* gene was cloned.[10] In this second, more active preparation, the branched-chain-amino-acid transaminase was present in the solution {K_2HPO_4 (concentration $c =$ 0.025 mol $\cdot$ dm^{-3}) + KH_2PO_4 ($c =$ 0.025 mol $\cdot$ dm^{-3}) + pyridoxal 5-phosphate ($c =$ 0.0002 mol $\cdot$ dm^{-3}), pH = 7.0} and was stored at $T \approx 278$ K until ready for use. The mass fraction of the enzyme in this solution was 0.010.

The substances pertinent to the study of reactions (1), (2), and (3) were separated by using a Hewlett-Packard 1100 h.p.l.c. equipped with a u.v. detector set at the wavelength

†Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology (NIST), nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

TABLE 1. Principal substances[a] used in this study with their Chemical Abstracts Service (CAS) registry numbers, empirical formulae, relative molecular masses M_r based on 1995 IUPAC recommendations, mass fraction water contents w, mole fraction purity x as stated by the supplier (D = Degussa-Hüls A.G., F = Fluka, M = Mallinckrodt, S = Sigma), and the method used to determine x. The mole fraction purities are exclusive of the amount of water in the samples

Substance	CAS registry number	Formula	M_r	w[b]	Supplier	x	Method(s)[c]
Branched-chain-amino-acid transaminase			[d]		[e]		
3,3-Dimethyl-2-oxobutanoic acid, sodium salt	815-17-8	$C_6H_{10}O_3$	152.13	0.0012	D	>0.99[f]	h.p.l.c.
l-Glutamic acid, sodium salt	6106-04-3	$C_5H_8NO_4Na$	169.11	0.0619	S	>0.99	t.l.c.; h.p.l.c.
l-Leucine	61-90-5	$C_6H_{13}NO_2$	131.17	0.0030	F	>0.995	non-aqueous titration; t.l.c.
l-*tert*-Leucine	20859-02-3	$C_6H_{13}NO_2$	131.17	0.0072	F	>0.99	non-aqueous titration
2-Oxoglutaric acid, sodium salt	22202-68-2	$C_5H_5O_5Na$	168.08	0.0079	S	>0.99	enzymatic assay; h.p.l.c.
2-Oxoisocaproic acid, sodium salt	4502-00-5	$C_6H_9O_3Na$	152.13	0.0073	S	≈0.98	t.l.c.; h.p.l.c.
2-Oxoisovaleric acid, sodium salt	3715-29-5	$C_5H_7O_3Na$	138.10	<0.001	F	>0.97	non-aqueous titration, h.p.l.c.
Phosphoric acid	7664-38-2	H_3PO_4	98.00		M	>0.995	(acid + base) titration
Potassium phosphate, dibasic	7758-11-4	K_2HPO_4	174.18		S	>0.995	(acid + base) titration
Pyridoxal 5-phosphate	41468-25-1	$C_8H_{10}NO_6P$	247.14	0.162	S	>0.99	t.l.c.
l-Valine	72-18-4	$C_5H_{11}NO_2$	117.15	0.0017	F	0.995	non-aqueous titration

[a] Chemical synonyms for some these substances are: 3,3-dimethyl-2-oxobutanoic acid, trimethylpyruvic acid; **l**-*tert*-leucine, **l**-2-amino-3,3-dimethylbutanoic acid; 2-oxoisocaproic acid, 4-methyl-2-oxopentanoic acid; and 2-oxoisovaleric acid, 3-methyl-2-oxobutanoic acid. [b] The mass fraction water contents of the sodium salts of 2-oxoisocaproic acid and 2-oxoisovaleric acid were determined by drying over phosphorous pentoxide. The moisture contents for the other substances were done by using Karl-Fischer titration. [c] The h.p.l.c. methods described above (see Experimental) were used as checks on the purities of the 3,3-dimethyl-2-oxobutanoic acid, **l**-glutamic acid, 2-oxoglutaric acid, 2-oxoisocaproic acid, and 2-oxoisovaleric acid. The other methods are those used by the vendor(s) to determine the purities of these substances. [d] Branched-chain-amino-acid transaminase consists of six identical subunits each of which has a relative molecular mass of $\approx 3.0 \cdot 10^4$. [e] Prepared for this study. See Experimental. [f] The mole fraction purity of 3,3-dimethyl-2-oxobutanoic acid is based upon an h.p.l.c. analysis done in our laboratory.

$\lambda = 215$ nm and a HP Hypersil BDS C-18 column (4 mm i.d., 250 mm long) thermostatted at $T = 313$ K. The mobile phase consisted of (I) [{tetrabutylammonium hydroxide ($c = 0.01$ mol $\cdot$ dm^{-3}) + ammonium phosphate ($c = 0.025$ mol $\cdot$ dm^{-3}) adjusted to pH 7.0 with NaOH} + acetonitrile (volume fraction $\phi = 0.01$)]; and (II) acetonitrile. The following gradient of these two mobile phases was formed: ϕ(I) = 0.90 and ϕ(II) = 0.10 at time $t = 0$; ϕ(I) = 0.85 and ϕ(II) = 0.15 at $t = 10$ min; ϕ(I) = 0.50 and ϕ(II) = 0.50 at $t = 20$ min. The flow rate was 0.0133 cm$^3 \cdot$ s^{-1}. Typical retention times for the substances were: **l**-glutamate, 3.7 min; **l**-valine, 3.9 min; **l**-leucine, 3.9 min; **l**-*tert*-leucine, 4.3 min; 2-oxoglutarate, 8.7 min; 2-oxoisovalerate, 11.3 min; pyridoxal-5-phosphate, 12.8 min; 3,3-dimethyl-2-oxobutanoate, 17.6 min; and 2-oxoisocaproate, 18.6 min.

The absorbances of 3,3-dimethyl-2-oxobutanoate, 2-oxoglutarate, 2-oxoisocaproate, and 2-oxoisovalerate (the compounds containing keto groups) are much larger than those of **l**-glutamate and of the amino acids, *i.e.* **l**-valine, **l**-leucine, and **l**-*tert*-leucine. Therefore, in performing the h.p.l.c. analyses, it was most advantageous to rely upon the areas of the chromatographic peaks of the compounds containing keto groups. Accordingly, the chromatographic response factors of 3,3-dimethyl-2-oxobutanoate, 2-oxoglutarate, 2-oxoisocaproate, and 2-oxoisovalerate were carefully determined by using solutions having known molalities of these substances. The molalities of **l**-glutamate and of the amino acids were then determined by using a mass balance calculation which was based upon their known initial molalities and the chromatographically determined molalities of the compounds containing keto groups.

The procedure used for the determination of the apparent equilibrium constants K' of reactions (1), (2), and (3) is now described. For each reaction, the position of equilibrium was approached from opposite directions. Thus, the solution used for the forward direction of reaction (1) contained (**l**-valine + 2-oxoglutarate) in a phosphate buffer containing pyridoxal 5-phosphate. The solution used for the reverse direction of reaction contained (2-oxoisovalerate + **l**-glutamate) in the same phosphate buffer with pyridoxal 5-phosphate. Branched-chain-amino-acid transaminase was then added to these two solutions which were contained in 4 cm^3 Teflon-capped glass bottles. These solutions were shaken gently at $\approx$50 r.p.m. in a water bath thermostatted at $T = 298.15$ K and allowed to equilibrate for $\approx$4 h. For reaction (2), the respective reaction mixtures were (**l**-leucine + 2-oxoglutarate) and (2-oxoisocaproate + **l**-glutamate); and for reaction (3) the respective reaction mixtures were (**l**-*tert*-leucine + 2-oxoglutarate) and (3,3-dimethyl-2-oxobutanoate + **l**-glutamate). The protocol used for reactions (2) and (3) was essentially the same as used for reaction (1). The times allowed for the equilibration of reaction (2) and (3) were $\approx$24 h and $\approx$4 h, respectively.

Three heat-conduction microcalorimeters were used for the enthalpy of reaction measurements. They were calibrated electrically with a high stability d.c. power supply, calibrated digital voltmeter, standard resistor, and time-interval counter. Descriptions of the microcalorimeters and their performance characteristics, the data-acquisition system, and the computer programs used to treat the results have been given by Steckler *et al.*[11, 12] At present, the voltages of the thermopiles of the microcalorimeters are measured with Hewlett-Packard 34420A Nanovolt Meters. These voltages are then recorded on a micro-

computer and the areas of the thermograms are calculated by means of a numerical integration.

The calorimetric sample vessels are fabricated from high-density polyethene. Each vessel has two compartments that hold, respectively, ≈0.55 cm^3 and ≈0.40 cm^3 of solution. In the experiments, the substrate solutions were placed in the 0.55 cm^3 compartment and the enzyme solutions were placed in the 0.40 cm^3 compartment. The substrate solutions for reactions (1), (2), and (3) initially contained, respectively, (**l**-valine + 2-oxoglutarate), (**l**-leucine + 2-oxoglutarate), and (**l**-*tert*-leucine + 2-oxoglutarate) dissolved in phosphate buffer containing pyridoxal 5-triphosphate. The 2-oxoglutarate was the limiting reactant for all three reactions. The enzyme solutions were prepared by the addition of branched-chain-amino-acid transaminase to the respective stock buffer solutions that were used for the preparation of the substrate solutions. The purpose of this procedure was to minimize the "blank" enthalpies (see below).

The vessels and their contents were allowed to equilibrate in the microcalorimeters for ≈60 min before the enzyme and substrate solutions were mixed. After this mixing, approximately 67 min, 60 min, and 38 min were allowed for reactions (1), (2), and (3), respectively. Following reaction, the vessels were removed from the microcalorimeters and the h.p.l.c. was promptly used to determine the amounts of 2-oxoglutarate remaining in solution. Thus, the mole fractions of unreacted 2-oxoglutarate were in the ranges 0.421 to 0.427, 0.401 to 0.410, and 0.230 to 0.253 for reactions (1), (2), and (3), respectively. The "blank" enthalpy changes for mixing of the substrate solutions with the buffer were in statistical agreement with each other and were pooled to obtain the value $\Delta_{mix}H = 0.29$ mJ. The "blank" enthalpy change for the mixing of the branched-chain-transaminase solution with the buffer was −0.24 mJ. These "blank" enthalpies of mixing were applied as corrections to the measured calorimetric enthalpies which were ≈ −8 mJ for reactions (1), (2), and (3).

Measurement of pH was done with an Orion Model 811 pH meter and a Radiometer combination glass micro-electrode. The pH meter was calibrated with Radiometer standard buffers that bracketed the pHs of the reaction mixtures.

3. Results and discussion

The apparent equilibrium constant[13] for reactions (1), (2), and (3), respectively, are

$$K' = m(\text{2-oxoisovalerate}) \cdot m(\textbf{l}\text{-glutamate})/\{m(\textbf{l}\text{-valine}) \cdot m(\text{2-oxoglutarate})\}, \quad (4)$$

$$K' = m(\text{2-oxoisocaproate}) \cdot m(\textbf{l}\text{-glutamate})/\{m(\textbf{l}\text{-leucine}) \cdot m(\text{2-oxoglutarate})\}, \quad (5)$$

$$K' = m(\text{3, 3-dimethyl-2-oxobutanoate}) \cdot m(\textbf{l}\text{-glutamate})/ \{m(\textbf{l}\text{-}\textit{tert}\text{-leucine}) \cdot m(\text{2-oxoglutarate})\}. \quad (6)$$

The molalities m in the above equation are the total molalities of the various charged and uncharged species that are formed from the dissociation of the various substances in solution. In discussing the thermodynamics of overall biochemical reactions, it is useful to introduce a reference reaction that pertains to specific ionic forms. Here we shall use:

$$\textbf{l}\text{-valine}^{\pm}(\text{aq}) + \text{2-oxoglutarate}^{2-}(\text{aq}) = \text{2-oxoisovalerate}^{-}(\text{aq}) + \textbf{l}\text{-glutamate}^{-}(\text{aq}), \quad (7)$$

$$\textbf{l}\text{-leucine}^{\pm}(\text{aq}) + \text{2-oxoglutarate}^{2-}(\text{aq}) = \text{2-oxoisocaproate}^{-}(\text{aq}) + \textbf{l}\text{-glutamate}^{-}(\text{aq}), \tag{8}$$

$$\textbf{l}\text{-}\textit{tert}\text{-leucine}^{\pm}(\text{aq}) + \text{2-oxoglutarate}^{2-}(\text{aq}) = \text{3, 3-dimethyl-2-oxobutanoate}^{-}(\text{aq}) + \textbf{l}\text{-glutamate}^{-}(\text{aq}). \tag{9}$$

The species selected are the predominant ionic forms at pH = 7.0. Since the choice of the reference reaction is arbitrary, other ionic forms could also have been chosen. The equilibrium constants for reactions (7), (8), and (9), respectively, are

$$K = m(\text{2-oxoisovalerate}^{-}) \cdot m(\textbf{l}\text{-glutamate}^{-})/\{m(\textbf{l}\text{-valine}^{\pm}) \cdot m(\text{2-oxoglutarate}^{2-})\}, \tag{10}$$

$$K = m(\text{2-oxoisocaproate}^{-}) \cdot m(\textbf{l}\text{-glutamate}^{-})/\{m(\textbf{l}\text{-leucine}^{\pm}) \cdot m(\text{2-oxoglutarate}^{2-})\}, \tag{11}$$

$$K = m(\text{3, 3-dimethyl-2-oxobutanoate}^{-}) \cdot m(\textbf{l}\text{-glutamate}^{-})/\{m(\textbf{l}\text{-}\textit{tert}\text{-leucine}^{\pm}) \cdot m(\text{2-oxoglutarate}^{2-})\}. \tag{12}$$

The standard state used in this study is the hypothetical ideal solution of unit molality ($m^{\circ} = 1\ \text{mol} \cdot \text{kg}^{-1}$). Since all of the above reactions are symmetrical, the values of K' and K are independent of the choice of the scale (*i.e.* molality, concentration, and mole fraction) used to express the composition of the solution.

The results of the equilibrium measurements are given in table 2. Equilibrium was approached from opposite directions for all three reactions leading to the values of the apparent reaction quotients Q' given in column 11 in this table. The uncertainties immediately following the values of Q' represent only the random errors inherent in the measurements and do not reflect possible systematic errors which are now considered. First, we judge that a reasonable estimate of the standard uncertainty[14] due to possible systematic errors in the chromatographic analyses is $0.02 \cdot Q'$ for all three reactions. Estimates of the standard uncertainties attributable to possible sample impurity are obtained by assuming that all of the samples had mole fraction impurities of 0.01 with the exception of the sample of 2-oxoisovaleric acid which was assumed to have a mole fraction impurity of 0.03. Propagation of error then leads to the following estimates of the standard uncertainties attributable to possible sample impurities: $0.094 \cdot Q'$ (forward) and $0.046 \cdot Q'$ (reverse) for reaction (1); $0.093 \cdot Q'$ (forward) and $0.043 \cdot Q'$ (reverse) for reaction (2); and $0.053 \cdot Q'$ (forward) and $0.030 \cdot Q'$ (reverse) for reaction (3). The standard uncertainties attributable to possible errors in the chromatography and to sample impurities are combined in quadrature together with the statistical uncertainty in the measured values of Q', expressed as one estimated standard deviation of the mean, to obtain combined standard uncertainties.[14] These combined standard uncertainties are then multiplied by two to arrive at the uncertainties given in parentheses given below the values of Q' in table 2. It is seen that these final error estimates are two to three times larger than the estimates of random error (two estimated standard deviations of the mean). Also, after consideration of possible systematic errors in the measurements, the value of Q' obtained from both directions of reaction are seen to be in agreement. On this basis, these quantities

TABLE 2. Results of equilibrium measurements at $T = 298.15$ K for biochemical reactions (1), (2), and (3). The molalities m given in columns 3 to 9 are those of the substances in solution at equilibrium and are equal to the sums of the molalities of the indicated substances in their various ionic forms. Abbreviations used in this table are: Glut, **l**-glutamate; DMOB, 3,3-dimethyl-2-oxobutanoate; Isocap, 2-oxoisocaproate; Isoval, 2-oxoisovalerate; Leu, **l**-leucine; t-Leu, **l**-*tert*-leucine; Oxogl, 2-oxoglutarate; PLP, pyridoxal 5-phosphate; and Val, **l**-valine. The measured reaction quotients Q' are given in column 11. The value of the ionic strength I_m is calculated. The calculated values of the equilibrium constants K for the reference reactions (7), (8), and (9) are given in the right-most column. The mass fraction w of branched-chain-amino-acid transaminase in solution was 0.0002, 0.0006, and 0.0003 for reactions (1), (2), and (3), respectively. The uncertainties immediately following the values of Q' are based on two estimated standard deviations of the mean and include the random errors in the determination of both the response factors and the reaction quotients. Estimates of total error (see Results and discussion) in the values of Q' are given in parentheses below the values of Q'. The values of Q' from both directions of reaction are combined as a weighted average to obtain the values of the apparent equilibrium constant K' pertinent to the average pH at which the measurements were performed

Reaction (1): **l**-valine(aq) + 2-oxoglutarate(aq) = 2-oxoisovalerate(aq) + **l**-glutamate(aq)

Direction of reaction	pH	$m(K_2HPO_4)$ / mol · kg^{-1}	$m(H_3PO_4)$ / mol · kg^{-1}	$10^3 \cdot m$(PLP) / mol · kg^{-1}	$10^3 \cdot m$(Val) / mol · kg^{-1}	$10^3 \cdot m$(Oxogl) / mol · kg^{-1}	$10^3 \cdot m$(Isoval) / mol · kg^{-1}	$10^3 \cdot m$(Glut) / mol · kg^{-1}	I_m / mol · kg^{-1}	Q'	K(7)
Forward	7.18	0.1006	0.0199	0.230	3.757	1.636	2.912	2.912	0.31	1.38 ± 0.08 (± 0.28)	2.76 ± 0.59
Reverse	7.24	0.1006	0.0199	0.230	2.061	2.061	2.584	3.023	0.31	1.84 ± 0.10 (± 0.21)	3.69 ± 0.45

$K' = \langle Q' \rangle = 1.67 \pm 0.17$ at $\langle$pH$\rangle = 7.21$ and $I_m = 0.31$ mol · kg^{-1}

Reaction (2): **l**-leucine(aq) + 2-oxoglutarate(aq) = 2-oxoisocaproate(aq) + **l**-glutamate(aq)

Direction of reaction	pH	$m(K_2HPO_4)$ / mol · kg^{-1}	$m(H_3PO_4)$ / mol · kg^{-1}	$10^3 \cdot m$(PLP) / mol · kg^{-1}	$10^3 \cdot m$(Leu) / mol · kg^{-1}	$10^3 \cdot m$(Oxogl) / mol · kg^{-1}	$10^3 \cdot m$(Isocap) / mol · kg^{-1}	$10^3 \cdot m$(Glut) / mol · kg^{-1}	I_m / mol · kg^{-1}	Q'	K(8)
Forward	7.15	0.1006	0.0199	0.232	0.931	2.118	2.063	2.063	0.30	2.16 ± 0.13 (± 0.43)	4.31 ± 0.91
Reverse	7.23	0.1006	0.0199	0.232	1.753	1.753	3.505	2.238	0.31	2.56 ± 0.18 (± 0.30)	5.13 ± 0.65

$K' = \langle Q' \rangle = 2.42 \pm 0.25$ at $\langle$pH$\rangle = 7.19$ and $I_m = 0.31$ mol · kg^{-1}

Reaction (3): **l**-*tert*-leucine(aq) + 2-oxoglutarate(aq) = 3,3-dimethyl-2-oxobutanoate(aq) + **l**-glutamate(aq)

Direction of reaction	pH	$m(K_2HPO_4)$ / mol · kg^{-1}	$m(H_3PO_4)$ / mol · kg^{-1}	$10^3 \cdot m$(PLP) / mol · kg^{-1}	$10^3 \cdot m$(t − Leu) / mol · kg^{-1}	$10^3 \cdot m$(Oxogl) / mol · kg^{-1}	$10^3 \cdot m$(DMOB) / mol · kg^{-1}	$10^3 \cdot m$(Glut) / mol · kg^{-1}	I_m / mol · kg^{-1}	Q'	K(9)
Forward	7.15	0.1012	0.0218	0.348	1.857	1.326	3.756	3.756	0.31	6.23 ± 0.32 (± 0.77)	12.5 ± 1.8
Reverse	7.23	0.1012	0.0218	0.348	1.341	1.341	2.770	3.846	0.31	5.93 ± 0.18 (± 0.47)	11.9 ± 1.2

$K' = \langle Q' \rangle = 6.01 \pm 0.40$ at $\langle$pH$\rangle = 7.19$ and $I_m = 0.31$ mol · kg^{-1}

are now considered to be apparent equilibrium constants. For each reaction, the values of the apparent equilibrium constants from both directions of reaction are combined as a weighted average to obtain the values of K' for reactions (1), (2), and (3) given in table 2.

The results of the calorimetric experiments are given in table 3. We judge that reasonable estimates of error in the values of $\Delta_r H_m$(cal) for reactions (1), (2), and (3) are: $0.01 \cdot \Delta_r H_m$(cal) due to impurities in the 2-oxoglutarate (the limiting reactant); $0.01 \cdot \Delta_r H_m$(cal) due to a possible error in the chromatographic determination of the extent of reaction; $10^{-3} \cdot \Delta_r H_m$(cal) due to possible errors in the calorimetric calibrations; and $0.04 \cdot \Delta_r H_m$(cal) due to uncertainties in the "blank" enthalpies. Combining these standard uncertainties with the statistical uncertainties in the measured values of $\Delta_r H_m$(cal) as described above, leads to final estimates of possible total error in $\Delta_r H_m$(cal) of (± 0.63, ± 0.53, and ± 0.47) $kJ \cdot mol^{-1}$ for reactions (1), (2), and (3), respectively.

The pKs and standard molar enthalpies $\Delta_r H_m^o$ for the proton dissociation reactions of the reactants and of the other solutes are needed to relate the experimental results for reactions (1), (2), and (3) to thermodynamic quantities for the respective references reactions (7), (8), and (9). These pKs and standard molar enthalpies $\Delta_r H_m^o$ are given in table 4. The values of the pKs and $\Delta_r H_m^o$s for the ionization of **l**-glutamic acid, **l**-leucine, 2-oxoglutaric acid, pyridoxal 5-phosphate, and **l**-valine are from Martell and Smith.[15] In two cases (2-oxoglutaric acid and pyridoxal 5-phosphate), the values of the pKs and $\Delta_r H_m^o$s were adjusted to ionic strength $I_m = 0$ by using an "ion-size" parameter of 1.6 $kg^{1/2} \cdot mol^{-1/2}$ in the extended Debye–Hückel equation used to estimate the activity coefficients of the aqueous species in solution. The thermodynamic quantities for the ionization of $H_2PO_4^-$ are calculated from the CODATA tables.[16] However, there are no experimentally determined values of the pKs and $\Delta_r H_m^o$s for the ionizations of **l**-*tert*-leucine, 3,3-dimethyl-2-oxobutanoate, 2-oxoisocaproic acid, and 2-oxoisovaleric acid. In the absence of direct measurements, we have estimated values for these quantities by using pKs and $\Delta_r H_m^o$s for structurally similar compounds. Accordingly, the value of the pKs and $\Delta_r H_m^o$s for **l**-*tert*-leucine are based on the values of the corresponding quantities for **l**-leucine and for **l**-valine. The pKs of 2-oxopropanoic acid and of 2-oxobutanoic acid are 2.48 and 2.50, respectively, at $T = 298.15$ K and $I_m = 0$.[15] On the basis we estimate the pKs of 2-oxoisocaproic acid, 3,3-dimethyl-2-oxobutanoic acid, and 2-oxoisovaleric acid to be ≈ 2.5. The values of the pKs of all of the reactants are all well removed (*i.e.* $|pK - pH| \geqslant 2.0$) from the pHs at which the measurements were performed. Thus, any errors in the values of the pKs (measured or estimated) will have only a small effect on the calculated values of K and $\Delta_r H_m^o$ for the reference reactions (7), (8), and (9).

We are now able to calculate equilibrium constants K and standard molar enthalpies of reaction $\Delta_r H_m^o$ for the reference reactions (7), (8), and (9). This is done by using our previously described equilibrium model[17] in its present implementation[18] together with the experimental results for K' and $\Delta_r H_m$(cal) and the thermodynamic quantities for the proton dissociation reactions given in table 4. An "ion-size" parameter of 1.6 $kg^{1/2} \cdot mol^{-1/2}$ in the extended Debye–Hückel equation was used to estimate the activity coefficients of the aqueous species in solution. Accordingly, we have obtained the values of the equilibrium constants for the reference reactions given in the right-

TABLE 3. Results of the calorimetric measurements at $T = 298.15$ K for biochemical reactions (1), (2), and (3). The molalities m are those obtained after mixing of the enzyme and substrate solutions and prior to any reaction. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms. Abbreviations used in this table are: Leu, **l**-leucine; t-Leu, **l**-*tert*-leucine; Oxogl, 2-oxoglutarate; PLP, pyridoxal 5-phosphate; and Val, **l**-valine. $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction. The values of the ionic strength I_m are calculated. The mass fraction w of branched-chain-amino-acid transaminase in solution was 0.0007, 0.0014, and 0.0003 for reactions (1), (2), and (3), respectively. The uncertainties given in this table are equal to two estimated standard deviations of the mean. Estimates of total error of (± 0.63, ± 0.53, and ± 0.47) kJ · mol^{-1} are assigned to the values of $\Delta_r H_m$(cal) for reactions (1), (2), and (3), respectively (see Results and discussion)

Reaction (1): **l**-valine(aq) + 2-oxoglutarate(aq) = 2-oxoisovalerate(aq) + **l**-glutamate(aq)

Experiment	pH	$m(K_2HPO_4)$ / mol · kg^{-1}	$m(H_3PO_4)$ / mol · kg^{-1}	$10^3 \cdot m$(PLP) / mol · kg^{-1}	$10^3 \cdot m$(Val) / mol · kg^{-1}	$10^3 \cdot m$(Oxogl) / mol · kg^{-1}	I_m / mol · kg^{-1}	$\Delta_r H_m$(cal) / kJ · mol^{-1}
1	7.21	0.0909	0.0180	0.254	3.111	2.707	0.31	4.94
2	7.21	0.0907	0.0179	0.253	3.042	2.647	0.31	3.92
3	7.21	0.0906	0.0179	0.253	3.005	2.615	0.31	3.54
4	7.21	0.0909	0.0180	0.254	3.109	2.705	0.31	5.09
5	7.21	0.0905	0.0179	0.252	2.994	2.605	0.31	4.82
6	7.21	0.0907	0.0179	0.253	3.029	2.636	0.31	5.60
7	7.21	0.0908	0.0180	0.253	3.063	2.665	0.31	5.52
8	7.21	0.0903	0.0179	0.252	2.934	2.553	0.31	5.32
9	7.21	0.0907	0.0179	0.253	3.031	2.638	0.31	4.79

$\langle \Delta_r H_m(\text{cal}) \rangle = (4.84 \pm 0.47)$ kJ · mol^{-1}

Reaction (2): **l**-leucine(aq) + 2-oxoglutarate(aq) = 2-oxoisocaproate(aq) + **l**-glutamate(aq)

Experiment	pH	$m(K_2HPO_4)$ / mol · kg^{-1}	$m(H_3PO_4)$ / mol · kg^{-1}	$10^3 \cdot m$(PLP) / mol · kg^{-1}	$10^3 \cdot m$(Leu) / mol · kg^{-1}	$10^3 \cdot m$(Oxogl) / mol · kg^{-1}	I_m / mol · kg^{-1}	$\Delta_r H_m$(cal) / kJ · mol^{-1}
1	7.20	0.1005	0.0199	0.309	2.911	2.595	0.31	4.87
2	7.20	0.1005	0.0199	0.309	2.883	2.569	0.31	5.31
3	7.20	0.1005	0.0199	0.309	2.923	2.605	0.31	5.10
4	7.20	0.1005	0.0199	0.309	2.911	2.595	0.31	5.71
5	7.20	0.1005	0.0199	0.309	2.883	2.569	0.31	5.09
6	7.20	0.1005	0.0199	0.309	2.923	2.605	0.31	5.42
7	7.20	0.1005	0.0199	0.309	3.010	2.682	0.31	5.53
8	7.20	0.1004	0.0199	0.309	2.826	2.518	0.31	4.85
9	7.20	0.1005	0.0199	0.309	2.923	2.606	0.31	4.19

$\langle \Delta_r H_m(\text{cal}) \rangle = (5.12 \pm 0.30)$ kJ · mol^{-1}

Reaction (3): **l**-*tert*-leucine(aq) + 2-oxoglutarate(aq) = 3,3-dimethyl-2-oxobutanoate(aq) + **l**-glutamate(aq)

Experiment	pH	$m(K_2HPO_4)$ / mol · kg^{-1}	$m(H_3PO_4)$ / mol · kg^{-1}	$10^3 \cdot m$(PLP) / mol · kg^{-1}	$10^3 \cdot m$(t-Leu) / mol · kg^{-1}	$10^3 \cdot m$(Oxogl) / mol · kg^{-1}	I_m / mol · kg^{-1}	$\Delta_r H_m$(cal) / kJ · mol^{-1}
1	7.20	0.0981	0.0211	0.296	3.897	2.996	0.31	4.05
2	7.20	0.0980	0.0211	0.296	3.730	2.868	0.31	3.72
3	7.20	0.0980	0.0211	0.296	3.742	2.877	0.31	4.46
4	7.20	0.0980	0.0211	0.296	3.753	2.886	0.31	4.12
5	7.20	0.0979	0.0211	0.295	3.700	2.842	0.31	3.39
6	7.20	0.0981	0.0211	0.296	3.825	2.941	0.31	3.62
7	7.20	0.0981	0.0211	0.296	3.901	3.000	0.31	4.94
8	7.20	0.0980	0.0211	0.296	3.803	2.924	0.31	3.87
9	7.20	0.0981	0.0211	0.296	3.895	2.995	0.31	3.80

$\langle \Delta_r H_m(\text{cal}) \rangle = (4.00 \pm 0.32)$ kJ · mol^{-1}

TABLE 4. The pKs and standard molar enthalpy changes $\Delta_r H_m^o$ for the aqueous proton dissociation reactions of substances pertinent to this study at $T = 298.15$ K and $I_m = 0$. See Results and discussion for the basis of these values

Reaction	pK	$\frac{\Delta_r H_m^o}{kJ \cdot mol^{-1}}$
l-glutamic acid$^+$ = **l**-glutamic acid + H^+	2.16	3.0
l-glutamic acid = **l**-glutamate$^-$ + H^+	4.30	3.0
l-glutamate$^-$ = **l**-glutamate^{2-} + H^+	9.96	40.0
l-leucine$^+$ = **l**-leucine$^\pm$ + H^+	2.33	1.6
l-leucine$^\pm$ = **l**-leucine$^-$ + H^+	9.74	45.4
l-*tert*-leucine$^+$ = **l**-*tert*-leucine$^\pm$ + H^+	≈2.3[a]	≈1.0[a]
l-*tert*-leucine$^\pm$ = **l**-*tert*-leucine$^-$ + H^+	≈9.7[a]	≈4.5[a]
3,3-dimethyl-2-oxobutanoic acid = 3,3-dimethyl-2-oxobutanoate$^-$ + H^+	≈2.5[a]	
2-oxoglutaric acid = 2-oxoglutarate$^-$ + H^+	2.24	
2-oxoglutarate$^-$ = 2-oxoglutarate^{2-} + H^+	5.12	
2-oxoisocaproic acid = 2-oxoisocaproate$^-$ + H^+	≈2.5[a]	
2-oxoisovaleric acid = 2-oxoisovalerate$^-$ + H^+	≈2.5[a]	
pyridoxal 5-phosphate = pyridoxal 5-phosphate$^-$ + H^+	3.72	17.0
pyridoxal 5-phosphate$^-$ = pyridoxal 5-phosphate^{2-} + H^+	6.46	−5.2
pyridoxal 5-phosphate^{2-} = pyridoxal 5-phosphate^{3-} + H^+	8.89	23.8
l-valine$^+$ = **l**-valine$^\pm$ + H^+	2.29	0.7
l-valine$^\pm$ = **l**-valine$^-$ + H^+	9.72	44.7
$H_2PO_4^-$ = HPO_4^{2-} + H^+	7.212	3.6

[a] Estimated value.

most column of table 2. The uncertainties in these calculated equilibrium constants have two components: errors in the measured values of the apparent equilibrium constants and possible errors in the quantities used in the equilibrium model. Since the difference $|pK - pH|$ was $\geqslant 2.0$ for all reactions studied herein, perturbation of the pK values had only a small effect ($< \pm 0.01 \cdot K$) on the calculated values of the equilibrium constants. However, it was found that the uncertainty in the activity coefficient was the major factor in the component of uncertainty attributable to the equilibrium model. Since reactions (7), (8), and (9) are charge asymmetric, this is not unexpected. The effects of these possible errors on the calculated thermodynamic quantities were determined by perturbing first the measured apparent equilibrium constants by the final experimental uncertainties (see above discussion) and then the "ion-size" parameter used in the activity coefficient model by ± 0.3 $kg^{1/2} \cdot mol^{-1/2}$. The effects due to these perturbations were then combined in quadrature to obtain the final estimated uncertainties assigned to the equilibrium constants for the reference reactions (7), (8), and (9) in table 2. For each reaction, the values of the equilibrium constant obtained from both directions of reaction are combined as a weighted average to obtain the following values for the reference reactions at $T = 298.15$ K and $I_m = 0$: $K = (3.33 \pm 0.35)$ for reaction (7), $K = (4.85 \pm 0.53)$ for reaction (8), and $K = (12.1 \pm 0.98)$ for reaction (9).

TABLE 5. Equilibrium constants K, standard molar Gibbs free energy changes $\Delta_r G^o_m$, standard molar enthalpy changes $\Delta_r H^o_m$, and standard molar entropy changes $\Delta_r S^o_m$ at $T = 298.15$ K and $I_m = 0$ for the reference reactions (7), (8), and (9) in aqueous solution. The standard state for the solutes is the hypothetical ideal solution of unit molality; the activity of water $a_w \to 0$ as the sum of the molalities of the solutes $\to 0$. The basis of the uncertainties is given in the text (see Results and discussion)

Reaction	K	$\frac{\Delta_r G^o_m}{kJ \cdot mol^{-1}}$	$\frac{\Delta_r H^o_m}{kJ \cdot mol^{-1}}$	$\frac{\Delta_r S^o_m}{kJ \cdot mol^{-1}}$
(7) **l**-valine$^{\pm}$ + 2-oxoglutarate^{2-} = 2-oxoisovalerate$^-$ + **l**-glutamate$^-$	3.33 ± 0.4	-2.98 ± 0.3	5.7 ± 0.7	29.1 ± 2.6
(8) **l**-leucine$^{\pm}$ + 2-oxoglutarate^{2-} = 2-oxoisocaproate$^-$ + **l**-glutamate$^-$	4.85 ± 0.6	-3.91 ± 0.3	6.0 ± 0.6	33.2 ± 2.2
(9) **l**-*tert*-leucine$^{\pm}$ + 2-oxoglutarate^{2-} = 3,3-dimethyl-2-oxobutanoate$^-$ + **l**-glutamate$^-$	12.1 ± 1.0	-6.18 ± 0.2	4.9 ± 0.5	37.2 ± 1.8

Similarly we use the equilibrium model[17,18] to calculate $\Delta_r H^o_m = (5.71 \pm 0.64)$ kJ · mol^{-1} for reaction (7), $\Delta_r H^o_m = (5.99 \pm 0.54)$ kJ · mol^{-1} for reaction (8), and $\Delta_r H^o_m = (4.87 \pm 0.48)$ kJ · mol^{-1} for reaction (9). The uncertainties in these calculated enthalpies were obtained in the same way as was done for the equilibrium constants. Thus, they reflect the uncertainties both in the experimentally determined values of $\Delta_r H_m$(cal) and in the equilibrium model. In this case, it was found that perturbation of the value of the "ion-size" parameter by ± 0.3 kg · mol^{-1} resulted in changes in the calculated values of $\Delta_r H^o_m$ of only ± 0.08 kJ · mol^{-1}. Thus, the final uncertainties in the values of the $\Delta_r H^o_m$s are primarily due to the experimental factors outlined above. The equilibrium model also yielded the following values for the change in binding of the hydrogen ion: $\Delta_r N(H^+) = (-0.00266, -0.00288,$ and $-0.00288)$ for reactions (1), (2), and (3), respectively. Thus, the buffer protonation corrections were small ($\leqslant 0.01$ kJ · mol^{-1}) for all three reactions. The final values of the equilibrium constants, standard molar enthalpy, standard molar Gibbs free energy, and standard molar entropy changes for the reference reactions (7), (8), and (9) are given in table 5.

We now compare the results of this study with those from the literature. Jenkins and Taylor[19] reported $K' = 1.75$ for reaction (2) at $T = 310.15$ K and pH = 8.3 (Tris buffer). From the information given by Jenkins and Taylor,[19] we calculate the ionic strength of the solution they used to be ≈ 0.056 mol · kg^{-1}. Use of the equilibrium model (see above) leads to $K = 2.4$ for reaction (8) at $T = 298.15$ K and $I_m = 0$. In performing this calculation, we used the value of $\Delta_r H^o_m$ obtained in this study for reaction (8) as well as the values p$K = 8.075$ and $\Delta_r H^o_m = 47.48$ kJ · mol^{-1} for the ionization of Tris · H$^+$(aq). These latter two values were taken, respectively, from the studies of Bates and Hetzer[20] and of Öjelund and Wadsö.[21] While Jenkins and Taylor[19] did not assign an uncertainty to their result, the value $K = 2.4$ for reaction (8) that was obtained from their study is proximate to the value $K = (4.85 \pm 0.6)$ obtained in this study.

There are no other direct measurements of thermodynamic quantities in the literature for biochemical reactions (1), (2), and (3). However, it is possible to calculate a value

of K for reaction (8), the reference reaction corresponding to reaction (2), by using a thermochemical cycle which is now described. The first step in this calculation uses the result of Sanwal and Zink[22] who reported $K' = 0.011$ at $T = 303.15$ K and pH = 11.0 (glycine buffer) for the overall biochemical reaction

$$\textbf{l}\text{-leucine(aq)} + \text{NAD}_{\text{ox}}\text{(aq)} + \text{H}_2\text{O(l)} = \text{2-oxoisocaproate(aq)} + \text{NAD}_{\text{red}}\text{(aq)} + \text{ammonia(aq)}. \quad (13)$$

NAD_{ox} and NAD_{red} are, respectively, the oxidized and reduced forms of β-nicotinamide-adenine dinucleotide. From the information given by Sanwal and Zink,[22] we calculate the ionic strength of the solution they used to be ≈ 0.11 mol · kg^{-1}. Use of our equilibrium model leads to $K = 1.6 \cdot 10^{-14}$ and $\Delta_r G^o_m = 78.75$ kJ · mol^{-1} for the chemical reference reaction

$$\textbf{l}\text{-leucine}^{\pm}\text{(aq)} + \text{NAD}^-_{\text{ox}}\text{(aq)} + \text{H}_2\text{O(l)} = \text{2-oxoisocaproate}^-\text{(aq)} + \text{NAD}^{2-}_{\text{red}}\text{(aq)} + \text{NH}_4^+\text{(aq)} + \text{H}^+\text{(aq)}. \quad (14)$$

In performing this calculation, we used the values $pK = 9.778$ and $\Delta_r H^o_m = 44.35$ kJ · mol^{-1} for the ionization of glycine$^{\pm}$(aq) to {glycine$^-$(aq) + H$^+$(aq)}. The values $pK = 9.25$ and $\Delta_r H^o_m = 52.2$ kJ · mol^{-1} for the ionization of NH_4^+(aq) were calculated from the CODATA tables.[16] The second step in this thermochemical cycle calculation uses $\Delta_r G^o_m = 22.65$ kJ · mol^{-1} at $T = 298.15$ K for the reaction.

$$\text{NAD}^-_{\text{ox}}\text{(aq)} + \text{H}_2\text{(g)} = \text{NAD}^{2-}_{\text{red}}\text{(aq)} + \text{H}^+\text{(aq)}. \quad (15)$$

This value was obtained by adjustment of the value $\Delta_r G^o_m = 20.2$ kJ · mol^{-1} given by Miller and Smith-Magowan[23] from $I_c = 0.1$ mol · dm^{-3} to $I_m = 0$ as described above. Subtraction of reaction (15) from reaction (14) gives the reaction

$$\textbf{l}\text{-leucine}^{\pm}\text{(aq)} + \text{H}_2\text{O(l)} = \text{2-oxoisocaproate}^-\text{(aq)} + \text{NH}_4^+\text{(aq)} + \text{H}_2\text{(g)}. \quad (16)$$

Thus, by using the values of $\Delta_r G^o_m$ for reactions (14) and (15), we obtain $\Delta_r G^o_m = 56.1$ kJ · mol^{-1} for reaction (16) at $T = 298.15$ K and $I_m = 0$. Since the values of $\Delta_f G^o_m$ of NH_4^+(aq) and of $\text{H}_2\text{O(l)}$ are well known,[16] the difference in the standard molar Gibbs free energies of formation $\{\Delta_f G^o_m(\text{2-oxoisocaproate}^-) - \Delta_f G^o_m(\text{leucine}^{\pm})\} = -101.64$ kJ · mol^{-1} is obtained. The third and final step in the thermochemical cycle calculation uses tabulated values from the literature for $\Delta_f G^o_m$ for **l**-glutamate$^-$(aq) and 2-oxoglutarate^{2-}(aq). Thus, using Wilhoit's[24] values of $\Delta_f G^o_m$ for these two species, we obtain $\Delta_r G^o_m = -5.7$ kJ · mol^{-1} and $K = 10.0$ for reaction (8) at $T = 298.15$ K and $I_m = 0$. Alternatively, use of the values of $\Delta_f G^o_m$ for **l**-glutamate$^-$(aq) and 2-oxoglutarate^{2-}(aq) tabulated by Miller and Smith-Magowan[23] leads to $\Delta_r G^o_m = 0.44$ kJ · mol^{-1} and $K = 0.83$ for reaction (8). We note that the values of $\Delta_f G^o_m$ tabulated by Miller and Smith-Magowan pertain to $I_c = 0.1$ mol · dm^{-3} and were therefore adjusted to $I_m = 0$ in performing this calculation. In any case, the mean result of this thermochemical cycle calculation is $\langle K \rangle = 5.4$ which is close to the experimental result $K = (4.85 \pm 0.6)$ from our present study.

It is useful to have values of the apparent equilibrium constant K' for biochemical

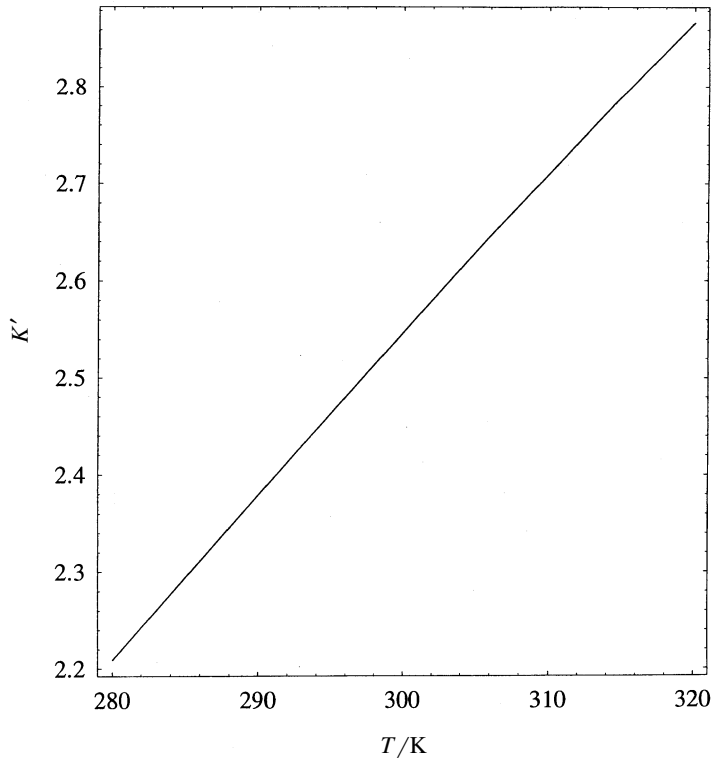

FIGURE 2. Plot of the apparent equilibrium constant K' for reaction (2) as a function of temperature at pH = 7.0 and $I_m = 0.25\ \text{mol} \cdot \text{kg}^{-1}$.

reactions under approximately physiological conditions. Here, physiological conditions are taken to be:[25] $T = 311.15$ K, pH = 7.0, pMg = 3.0, and $I_m = 0.25\ \text{mol} \cdot \text{kg}^{-1}$. In performing these calculations, it was assumed that the neutral species and those having charge numbers of -1 do not bind to Mg^{2+}(aq) and that the binding constant for Mg^{2+}(aq) with 2-oxoglutarate^{2-}(aq) is equal to the known[15] binding constant for Ca^{2+}(aq) with 2-oxoglutarate^{2-}(aq), namely $K(T = 298.15,\ \text{and}\ I_c = 0.1\ \text{mol} \cdot \text{dm}^{-3}) = 19.5$. Since only a tiny fraction of the total amount of 2-oxoglutarate^{2-} will be complexed with Mg^{2+}, even the complete neglect of this binding constant causes a negligible error ($< 0.01 \cdot K'$) in the calculated value of K'. Thus, by using the equilibrium model with the thermodynamic quantities obtained in this study for the reference reactions (see table 5), we calculate the values of K' and the standard molar transformed Gibbs free energy change $\Delta_r G'^{o}_{m}$ for reactions (1), (2), and (3) given in table 6. Brosnan *et al.*[26] reported that the transamination reaction {**l**-alanine(aq) + 2-oxoglutarate(aq) = pyruvate(aq) + **l**-glutamate(aq)} functions *in vivo*, either at, or in the vicinity of, equilibrium. It would be interesting to find out how far from equilibrium reactions (1), (2), and (3) operate *in vivo*.

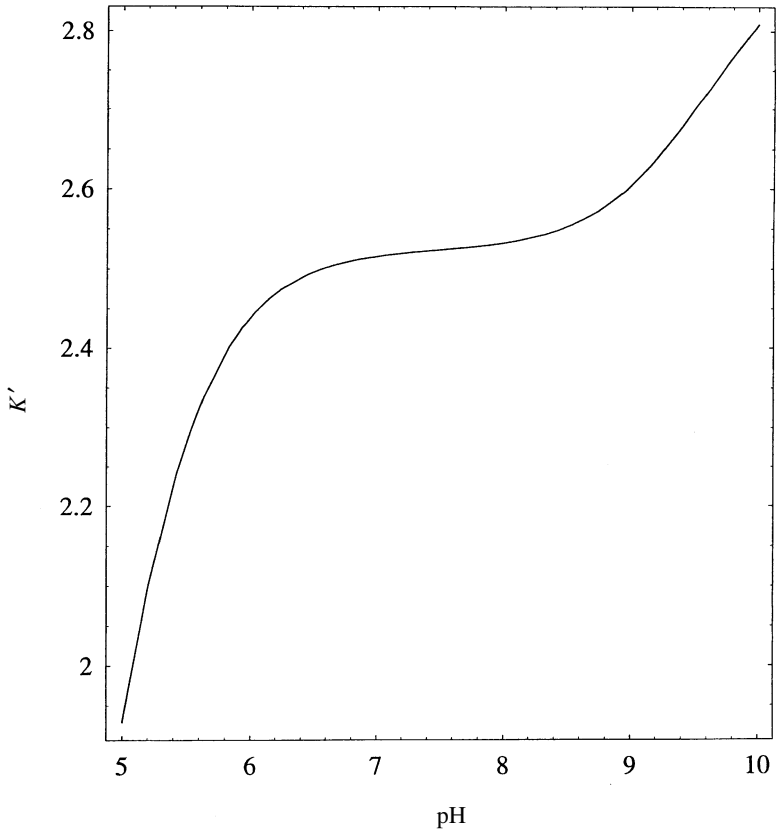

FIGURE 3. Plot of the apparent equilibrium constant K' for reaction (2) as a function of pH at $T = 298.15$ K and $I_m = 0.25$ mol · kg^{-1}.

TABLE 6. Apparent equilibrium constants K' and standard transformed molar Gibbs free energy changes $\Delta_r G'^{o}_m$ at approximately physiological conditions ($T = 311.15$ K, pH = 7.0, pMg = 3.0, and $I_m = 0.25$ mol · kg^{-1}) for the biochemical reactions studied herein

Reaction	K'	$\frac{\Delta_r G'^{o}_m}{\text{kJ} \cdot \text{mol}^{-1}}$
(1) **l**-valine + 2-oxoglutarate = 2-oxoisovalerate + **l**-glutamate	1.9	−1.6
(2) **l**-leucine + 2-oxoglutarate = 2-oxoisocaproate + **l**-glutamate	2.7	−2.6
(3) **l**-*tert*-leucine + 2-oxoglutarate = 3,3-dimethyl-2-oxobutanoate + **l**-glutamate	6.2	−4.7

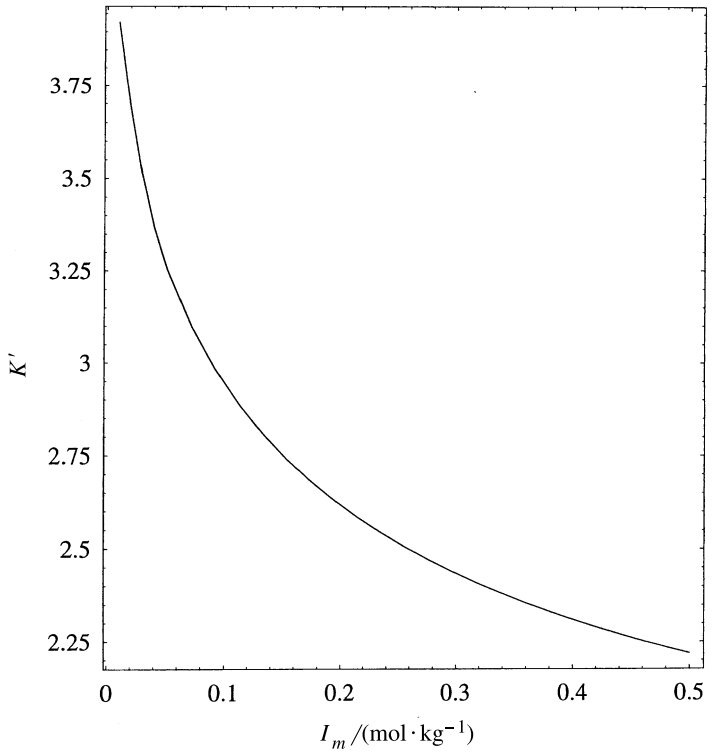

FIGURE 4. Plot of the apparent equilibrium constant K' for reaction (2) as a function of ionic strength I_m at $T = 298.15$ K and pH = 7.0.

Tewari *et al.*[5] have pointed out that nearest neighbor interactions are unchanged in transamination reactions. Therefore, any estimation method that considers only nearest neighbor interactions must necessarily lead to the prediction that the thermodynamic reaction quantities $\Delta_r G^o_m$, $\Delta_r H^o_m$, $\Delta_r S^o_m$, and $\Delta_r C^o_{p,m}$ are zero for these reactions. This prediction is consistent with the results obtained in the present study, *i.e.* the thermodynamic reaction quantities are all small. The common structural changes in these reactions is also reflected in the narrow range of values of $\Delta_r S^o_m$ for these reactions. Specifically, the values of $\Delta_r S^o_m$ of the transamination reactions involving **l**-alanine, **l**-tyrosine, **l**-phenylalanine (see reference 5) and the three reactions studied herein are in the range 22 J · K^{-1} · mol^{-1} to 39 J · K^{-1} · mol^{-1} ($\langle \Delta_r S^o_m \rangle = 33$ J · K^{-1} · mol^{-1}). This characteristic value of $\Delta_r S^o_m$ can be useful in estimating thermodynamic quantities for transamination reactions where either $\Delta_r G^o_m$ or $\Delta_r H^o_m$ have been determined.

As mentioned in the Introduction, there is significant interest in the use of transamination reactions for the manufacture of amino acids. The thermodynamic results obtained in this study can be used for process optimization. This is illustrated in figures 2, 3, and 4 where

the dependency of the apparent equilibrium constant K' of reaction (2) on temperature, pH, and ionic strength, respectively, are shown. Essentially identical trends of K', on T, pH, and I_m as seen for reaction (2) in these figures are also exhibited by reactions (1) and (3). If one desires to manufacture **l**-leucine, a lower value of K' translates to an increase in the product yield. Interestingly, there is a very significant decrease in the value of K' if this reaction is carried out at high ionic strengths. This could be accomplished by addition of a salt to the reaction mixture; this assumes that this does not interfere adversely with the kinetics or the chemistry of the reaction. There is also a decrease in the value of K' as the pH is lowered—particularly below ≈6.0. However, the activity of the enzyme may not be optimal at such pHs. Additionally, if the enzyme can still maintain sufficient activity, there is a clear indication that operating at lower temperatures will lead to higher product yields. Thus, an appropriate combination of shifts in the temperature, pH, and ionic strength could lead to improved product yields for these branched-chain amino acids. While the relative trends with pH and ionic strength (see figures 3 and 4) can be predicted without a knowledge of the equilibrium constants for the chemical references reactions (7), (8), and (9), one does need the values of $\Delta_r H^o_m$ for these reference reactions in order to predict the effect of temperature on K'. The equilibrium constants for the three reference reactions are needed to calculate the optimal product yields.

Also, the possibility of using Le Chatelier's principle to derive these reactions to completion should not be overlooked. In fact, this principle has been used[10, 27–29] in transamination reactions catalysed by aspartate aminotransferase. In this case, the oxaloacetate that is produced is converted to (pyruvate + carbon dioxide) thus increasing the product yield of the desired **l**-amino acid. The predicted shifts in the position of equilibrium (see figure 3) with pH can also be viewed in terms of Le Chatelier's principle.

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REFERENCES

1. Voet, D.; Voet, J. G. *Biochemistry*. John Wiley: New York. **1990**.
2. Rozzell, J. D. *Methods Enzymol.* **1987**, 136, 479–497.
3. Stirling, D. I. *Chirality in Industry*. Collins, A. N.; Sheldrake, G. N.; Crosby, J.: editors. John Wiley: Chichester. **1992**, 209–222.
4. Goldberg, R. N.; Tewari, Y. B. *J. Phys. Chem. Ref. Data* **1994**, 23, 547–617.
5. Tewari, Y. B.; Kishore, N.; Goldberg, R. N.; Luong, T. N. *J. Chem. Thermodynamics* **1998**, 30, 777–793.
6. Krebs, H. A.; Kornberg, H. L.; Burton, K. *A Survey of the Energy Transformations in Living Matter*. Springer-Verlag: Berlin. **1957**.
7. Kishore, N.; Tewari, Y. B.; Goldberg, R. N. *J. Chem. Thermodynamics* **1998**, 30, 1373–1384.
8. Margolis, S. *Certificate of Analysis: Standard Reference Material 2890*. National Institute of Standards and Technology: Gaithersburg, MD. **1998**.
9. Monnier, N.; Montmitonnet, A.; Chesne, S.; Pelmont, J. *Biochimie* **1976**, 58, 663–675.
10. Crump, S. P.; Rozzell, J. D. *Biocatalytic Production of Amino Acids and Derivatives: New Developments and Process Considerations*. Rozzell, J. D.; Wagner, F.: editors. Hanser: Munich. **1992**, 43–58.
11. Steckler, D. K.; Goldberg, R. N.; Tewari, Y. B.; Buckley, T. J. *J. Res. Natl. Bur. Stand (U.S.)* **1986**, 91, 113–121.

12. Steckler, D. K.; Goldberg, R. N.; Tewari, Y. B.; Buckley, T. J. Computer Software for the Acquisition and Treatment of Calorimetric Data. *Natl. Bur. Stand. (U.S.) Tech. Note 1224*. U.S. Government Printing Office: Washington, DC. **1986**.
13. Alberty, R. A.; Cornish-Bowden, A.; Gibson, Q. H.; Goldberg, R. N.; Hammes, G. G.; Jencks, W.; Tipton, K. F.; Veech, R.; Westerhoff, H. V.; Webb, E. C. *Pure Appl. Chem.* **1994**, 66, 1641–1666.
14. Taylor, B. N.; Kuyatt, C. E. Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results. *NIST Technical Note 1297*. U.S. Government Printing Office: Washington, DC. **1994**.
15. Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critical Stability Constants of Metal Complexes Database: NIST Standard Reference Database 46*. National Institute of Standards and Technology: Gaithersburg, MD. **1998**.
16. Cox, J. D.; Wagman, D. D.; Medvedev, V. A. *CODATA Key Values for Thermodynamics*. Hemisphere: New York. **1989**.
17. Goldberg, R. N.; Tewari, Y. B. *Biophys. Chem.* **1991**, 40, 241–261.
18. Akers, D. L.; Goldberg, R. N. "BioEqCalc: A package for performing equilibrium calculations on biochemical reactions". *Mathematica J.* (in press).
19. Jenkins, W. T.; Taylor, R. T. *Methods Enzymol.* **1970**, 17A, 802–807.
20. Bates, R. G.; Hetzer, H. B. *J. Phys. Chem.* **1961**, 65, 667–671.
21. Öjelund, G.; Wadsö, I. *Acta Chem. Scand.* **1968**, 22, 2691–2699.
22. Sanwal, B. D.; Zink, M. W. *Archiv. Biochem. Biophys.* **1961**, 94, 430–435.
23. Miller, S. L.; Smith-Magowan, D. *J. Phys. Chem. Ref. Data* **1990**, 19, 1049–1073.
24. Wilhoit, R. *Biochemical Microcalorimetry*. Brown, H. D.: editor. Academic Press: New York. **1969**, 33–81, 305–317.
25. Veloso, D.; Guynn, R. W.; Oskarsson, M.; Veech, R. L. *J. Biol. Chem.* **1973**, 248, 4811–4819.
26. Brosnan, J. T.; Krebs, H. A.; Williamson, D. H. *Biochem. J.* **1970**, 117, 91–96.
27. Rozzell, J. D. *US Patent*, 4,518,692, **1985**.
28. Rozzell, J. D. *US Patent*, 4,826,766, **1989**.
29. Rozzell, J. D. *US Patent*, 4,880,738, **1989**.

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