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A thermodynamic investigation of reactions catalyzed by tryptophan synthase

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Abstract

Microcalorimetry and high-performance liquid chromatography have been used to conduct a thermodynamic investigation of the following reactions catalyzed by the tryptophan synthase $\alpha_2\beta_2$ complex (EC 4.2.1.20) and its subunits: indole(aq) + L-serine(aq) = L-tryptophan(aq) + H_2O(l); L-serine(aq) = pyruvate(aq) + ammonia(aq); indole(aq) + D-glyceraldehyde 3-phosphate(aq) = 1-(indol-3-yl)glycerol 3-phosphate(aq); L-serine(aq) + 1-(indol-3-yl)glycerol 3-phosphate(aq) = L-tryptophan(aq) + D-glyceraldehyde 3-phosphate(aq) + H_2O(l). The calorimetric measurements led to standard molar enthalpy changes for all four of these reactions. Direct measurements yielded an apparent equilibrium constant for the third reaction; equilibrium constants for the remaining three reactions were obtained by using thermochemical cycle calculations. The results of the calorimetric and equilibrium measurements were analyzed in terms of a chemical equilibrium model that accounted for the multiplicity of the ionic states of the reactants and products. Thermodynamic quantities for chemical reference reactions involving specific ionic forms have been obtained. These quantities permit the calculation of the position of equilibrium of the above four reactions as a function of temperature, pH, and ionic strength. Values of the apparent equilibrium constants and standard transformed Gibbs free energy changes $\Delta_r G'^{\circ}_m$ under approximately physiological conditions are given. Le Châtelier's principle provides an explanation as to why, in the metabolic pathway leading to the synthesis of L-tryptophan, the third reaction proceeds in the direction of formation of indole and D-glyceraldehyde 3-phosphate even though the apparent equilibrium constant greatly favors the formation of 1-(indol-3-yl)glycerol 3-phosphate.
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1. Introduction

The reactions catalyzed by the tryptophan synthase $\alpha_2\beta_2$ complex (EC 4.2.1.20) and its subunits have been the subject of extensive investigations [1–5]. A plausible basis as to how the enzyme operates has emerged from a knowledge of the crystal structure [6] of the tryptophan synthase $\alpha_2\beta_2$ complex found in *Salmonella typhimurium*. Specifically, this complex contains a 25 Å long tunnel that connects the active sites of the α and β subunits. This hydrophobic tunnel has a diameter of ≈ 9 Å matching that of indole. Indole is first formed from 1-(indol-3-yl)glycerol 3-phosphate at the active site of the α subunit. The indole is then transferred via the hydrophobic tunnel to the active site of the β subunit where it reacts with L-serine to form L-tryptophan (see Fig. 1). Interestingly, although the mechanisms of these reactions have been much studied, thermodynamic information on the overall biochemical reactions is relatively limited [7,8]. This is unfortunate since this data is required both for a quantitative discussion of the position of equilibrium of these reactions and for a full understanding of the energetics of both the individual reactions and the metabolic pathways in which these reactions occur [9,10].

In carrying out this investigation it was found useful to write the biochemical reactions in the way that they were actually performed under in vitro conditions:

$$\text{indole(aq)} + \text{L-serine(aq)} = \text{L-tryptophan(aq)} + \text{H}_2\text{O(l)} \quad (1)$$

$$\text{L-serine(aq)} = \text{pyruvate(aq)} + \text{ammonia(aq)} \quad (2)$$

$$\text{indole(aq)} + \text{D-glyceraldehyde 3-phosphate(aq)} = \text{1-(indol-3-yl)glycerol 3-phosphate(aq)} \quad (3)$$

$$\text{L-serine(aq)} + \text{1-(indol-3-yl)glycerol 3-phosphate(aq)} = \text{L-tryptophan(aq)} + \text{D-glyceraldehyde3-phosphate(aq)} + \text{H}_2\text{O(l)}. \quad (4)$$

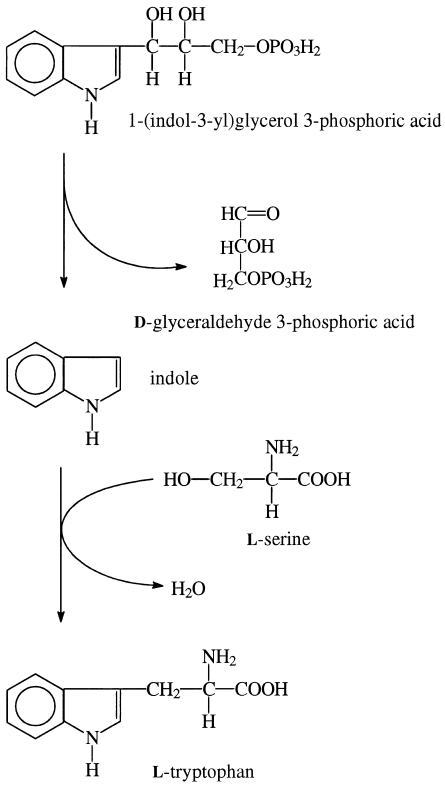

Fig. 1. The conversion of 1-(indol-3-yl)glycerol 3-phosphate to L-tryptophan under in vivo conditions.

In vivo (see Fig. 1), reaction (3) proceeds in the opposite direction to that shown above. This behavior is explained later. The $\alpha_2\beta_2$ complex was used for carrying out reactions (1), (3), and (4). The β_2 subunit was used for carrying out reaction (2) because it has a much higher activity in this reaction than the $\alpha_2\beta_2$ complex. CsCl was also used for reaction (2) since it significantly increases the activity of the β_2 subunit [11].

The literature contains values of calorimetrically determined enthalpies for the above four reactions [8] and a value for the apparent equilibrium constant for reaction (3) [7]. Apparent equilibrium constants and calorimetric enthalpies have also been reported [12] for the tryptophanase (EC 4.1.99.1) catalyzed reaction

$$\text{L-tryptophan(aq)} + \text{H}_2\text{O(l)} = \text{indole(aq)} + \text{pyruvate(aq)} + \text{ammonia(aq)} \quad (5)$$

Since these biochemical reactions can be formally added and subtracted just like algebraic equations, we have the following relationship(s): reaction (1) = {reaction (3) + reaction (4)} = {reaction (2) − reaction (5)}. Thus, one has two thermochemical cycles that can be used to check the consistency of results. These checks serve as important tests of the accuracy of the measurements. However, as will be seen below, when these checks are done in terms of the chemical reference reactions, these thermochemical cycles do not close when the previously reported results [8,12] are used. Additionally, there do not appear to be any equilibrium data in the literature for reactions (1), (2), and (4). Accordingly, it was decided to perform a series of measurements aimed both at gaining additional information on the thermodynamics of these reactions and in resolving the aforementioned problem in the failure of the thermochemical cycle to close. Reactions (1) and the reverse of reaction (3) are, respectively, the sixth (final) and fifth steps in the metabolic pathway that leads from chorismate to L-tryptophan [13]. This pathway involves the synthesis of aromatic substances from aliphatic compounds and its chemistry has evoked substantial interest. This pathway is also of industrial interest because of its potential for the large scale production of aromatic amino acids and other useful products [14,15].

2. Materials and methods

2.1. Materials

Relevant information on the substances used in this study is given in Table 1.[2] The methods used for the preparation of the $\alpha_2\,\beta_2$ complex and the β_2 subunit of tryptophan synthase from *Salmonella typhimurium* have been previously described [16,17]. The mass fractions of the enzymes (mass of an enzyme divided by the total mass of the solution) were determined from the absorbances determined at the wavelength $\lambda = 278$ nm [18]. The specific activities of the $\alpha_2\,\beta_2$ complex and the β_2 subunit in reaction (1) were, respectively, 5.2 and 0.1 μmol min^{-1} mg^{-1} under standard assay conditions [11]. In the presence of CsCl (concentration $c = 0.2$ mol dm^{-3}), the activity of the β_2 subunit in this reaction was 2.4 μmol min^{-1} mg^{-1}.

The DL-glyceraldehyde 3-phosphate was received from the vendor as the free acid dissolved in water. The stock solution was divided into 10 small vials and stored at the temperature $T \approx 253$ K until ready for use. The concentration of D-glyceraldehyde 3-phosphate in the stock solution was determined by using the enzymatic assay (glyceraldehyde 3-phosphate dehydrogenase) described by Veech et al. [19]. In this spectrophotometric assay, the molar absorption coefficient ϵ of NADH was taken to be 6292 dm^3 mol^{-1} cm^{-1} [20] at $T = 298.15$ K and $\lambda = 340$ nm. The density of the aqueous solution of the DL-glyceraldehyde 3-phosphate was 1.026 kg dm^{-3}. This density was used to convert the measured concentration ($c = 0.1133$ mol dm^{-3}) of the D-glyceraldehyde 3-phosphate to a gravimetric basis which was used for the preparation of all solutions pertinent to the calorimetric and equilibrium experiments. Unlike the other substances used in this study, the D-glyceraldehyde 3-phosphate was found to be relatively labile in solution.

Specifically, by using the aforementioned enzymatic assay [19], it was found that the fraction of the D-glyceraldehyde 3-phosphate that decomposed per hour in Na_2HPO_4 buffer ($c = 0.1$ mol dm^{-3}, pH = 7.3) was ≈ 0.054.

The 1-(indol-3-yl)glycerol 3-phosphate was prepared enzymatically by using the method of Kawasaki et al. [21], which is an improvement of the method of Hardman and Yanofsky [22]. The molality of this substance was determined by using the following procedure. First, reaction (3) was carried out with a solution having a known molality of indole and an excess amount of D-glyceraldehyde 3-phosphate. Since the response factor (molality divided by chromatographic peak

[2] 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, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

Table 1
Principal substances used in this study with their Chemical Abstracts Service (CAS) registry numbers, empirical formulae, relative molecular masses M_r, mass fraction moisture contents w determined by Karl Fischer analysis, mole fraction purity x as stated by supplier, and method used to determine x

Substance	CAS registry number	Formula	M_r	w	Supplier	x	Method[a]
Ammonium chloride	12125-02-9	NH_4Cl	53.492		S	> 0.99	$AgNO_3$ titration
Cesium chloride	76417-17-8	CsCl	168.36		BRL	> 0.999	Trace metal analysis
DL-glyceraldehyde 3-phosphate	591-59-3	$C_3H_7O_6P$	170.06		S	[b]	HPLC and enzymatic assay
Indole	120-72-9	C_8H_7N	117.15	0.00261	S	0.999	HPLC; GC
1-(indol-3-yl)glycerol 3-phosphate	4220-97-7	$C_{11}H_{14}O_6P$	189.13		[c]	[b]	HPLC and enzymatic assay
Phosphoric acid	7664-38-2	H_3PO_4	97.995		M	> 0.995	(acid + base) titration
Pyridoxal 5-phosphate	41468-25-1	$C_8H_{10}NO_6P$	247.14	0.1624	S	> 0.99	TLC
Pyruvic acid, sodium salt	113-24-6	$C_3H_3O_3Na$	110.04	0.0073	S	0.994	$HClO_4$ titration
L-serine	56-45-1	$C_3H_7NO_3$	105.09	0.00178	S	> 0.99	HPLC; TLC
Sodium phosphate, dibasic	7558-79-4	Na_2HPO_4	141.96		S	> 0.997	(acid + base) titration
L-tryptophan	73-22-3	$C_{11}H_{12}N_2O_2$	204.23	0.0058	S	> 0.99	HPLC; TLC
Tryptophan synthase, $\alpha_2\,\beta_2$ complex	9014-52-2		1.43×10^5		[c]		
Tryptophan synthase, β_2 subunit	35402-93-8		8.60×10^4		[c]		

BRL, Bethesda Research Laboratories; M, Mallinckrodt; S, Sigma.
[a] The HPLC methods described below (see Section 2.2) were used as checks on the purities of the DL-glyceraldehyde 3-phosphate, indole, 1-(indol-3-yl)glycerol 3-phosphate, L-serine, and L-tryptophan. The other methods are those used by the vendor(s) to determine the purities of these substances.
[b] The molalities of the DL-glyceraldehyde 3-phosphate and 1-(indol-3-yl)glycerol 3-phosphate were determined by enzymatic assays (see Section 2.1).
[c] Prepared for this study (see Section 2.1).

area) of indole was already known, the final molality of indole could be calculated from the area of its chromatographic peak (see below). Additionally, there was no evidence of any side reactions or chromatographic interference from the enzyme. Thus, the difference between the initial and final molalities of the indole was taken to be equal to the molality of the 1-(indol-3-yl)glycerol 3-phosphate that was formed in the reaction. This molality was then used with the chromatographic area corresponding to 1-(indol-3-yl)glycerol 3-phosphate to calculate its response factor. A knowledge of this response factor made it possible to determine chromatographically the molality of the stock solution(s) of 1-(indol-3-yl)glycerol 3-phosphate.

2.2. HPLC

A Hewlett-Packard 1050 HPLC equipped with a UV detector set at the wavelength $\lambda = 215$ nm and a Hewlett-Packard Hypersil C-18 column (2.1 mm i.d. $\times$ 100 mm long) thermostatted at $T = 318$ K were used for the analysis of L-tryptophan and indole in reaction (1). The mobile phase consisted of (I) KH_2PO_4 ($c = 0.01$ mol dm^{-3}) adjusted to pH = 3.3 with H_3PO_4 and (II) acetonitrile. The following gradient of these two mobile phases was formed: volume fraction $\phi(\mathrm{I}) = 1.00$ and $\phi(\mathrm{II}) = 0$ at time $t = 0$; $\phi(\mathrm{I}) = 1.00$ and $\phi(\mathrm{II}) = 0$ at $t = 4$ min; and $\phi(\mathrm{I}) = 0.20$ and $\phi(\mathrm{II}) = 0.80$ at $t = 20$ min. The flow rate was 0.8 cm^3 min^{-1}. Typical retention times were: L-serine, 0.8 min; L-tryptophan, 9.4 min; and indole, 15.8 min. The procedure used for the analysis of 1-(indol-3-yl)glycerol 3-phosphate and indole used for reactions (3) and (4) was similar to the above except that a Hewlett-Packard 1100 HPLC was used, the pH of the mobile phase (I) was 3.4, and the gradient was only slightly different. Namely, $\phi(\mathrm{I}) = 0.4$ and $\phi(\mathrm{II}) = 0.6$ at $t = 20$ min. Typical retention times

for 1-(indol-3-yl)glycerol 3-phosphate and indole were 3.3 min and 14.9 min, respectively.

Since the L-serine has a low molar absorption coefficient at $\lambda = 215$ nm and since the phosphate buffer used in this study interfered with the determination of L-serine, an alternative method of analysis was used for this substance. In this method, 2.0 g of a (H_3BO_3 ($c = 0.2$ mol dm^{-3}) + $NaHCO_3$ ($c = 0.2$ mol dm^{-3})) solution adjusted to pH = 8.5 with concentrated NaOH was added to 0.1 g of the solution that was being analyzed. A second solution (3.0 mg of 9-fluoroenylmethylchloroformate (FMOC) in 1.0 cm^3 of dry acetone) was immediately added to this solution in the volume ratio 1:1. The resulting mixture was shaken briefly and allowed to stand for 10 min at room temperature. Samples of this final solution were then injected into a Varian Vista 5500 HPLC (fluorescence detector set at $\lambda = 260$ nm for excitation and $\lambda = 313$ nm for emission; Varian Amino Tag C-18 column (4.6 mm i.d. × 150 mm long)). The mobile phase consisted of (I) ($NaC_2H_3O_2$ ($c = 0.015$ mol dm^{-3}) + tetramethylammonium chloride ($c = 0.01$ mol dm^{-3}) + NaN_3 ($c = 3.85 \times 10^{-4}$ mol dm^{-3})) solution adjusted to pH = 2.85 with concentrated H_3PO_4 and (II) acetonitrile. The following gradient of these two mobile phases was formed: ϕ(I) = 0.73 and ϕ(II) = 0.27 at $t = 0$; ϕ(I) = 0.58 and ϕ(II) = 0.42 at $t = 11.5$ min; and ϕ(I) = 0.58 and ϕ(II) = 0.42 at $t = 13$ min. The flow rate was 1.4 cm^3 min^{-1}. A typical retention time for the FMOC derivative of L-serine was 11.5 min.

2.3. Determination of equilibrium constants

Attempts were made to measure apparent equilibrium constants for the reactions pertinent to this study. For reactions (1) and (2), equilibrium was approached from both directions of reaction. The solution used for the forward direction of reaction (1) contained indole (molality $m = 0.0049$ mol kg^{-1}) and L-serine ($m = 0.0056$ mol kg^{-1}) in Na_2HPO_4 buffer ($m = 0.10$ mol kg^{-1}, pH = 7.01). The solution used for the reverse direction of reaction contained L-tryptophan ($m = 0.0048$ mol kg^{-1}) in the same phosphate buffer. The $\alpha_2\beta_2$ complex of tryptophan synthase was added to these two solutions so that the mass fraction of the enzyme in these solutions was $\approx 3 \times 10^{-4}$. These solutions were placed in 20 cm^3 Teflon capped glass bottles and gently shaken at ≈ 50 rpm in a water bath thermostatted at $T = 298.15$ K. Following equilibration for 25 h, the molalities of indole and L-tryptophan were measured by using the chromatographic procedure described above. A similar procedure was used for reaction (2). In this case, the solution used for the forward direction of reaction contained L-serine ($m =$ 0.020 mol kg^{-1}) and ammonium chloride ($m =$ 0.094 mol kg^{-1}) in Na_2HPO_4 buffer ($m = 0.10$ mol kg^{-1}, pH = 6.90). The solution used for the reverse direction of reaction contained pyruvic acid ($m = 0.019$ mol kg^{-1}) and ammonium chloride ($m = 0.094$ mol kg^{-1}) in the same phosphate buffer. The mass fraction of the enzyme (β_2 subunit of tryptophan synthase) in these solutions was $\approx 6 \times 10^{-4}$. These solutions were allowed to equilibrate for 8 days at $T = 308.15$ K, after which time the molality of L-serine was determined chromatographically. For reaction (4), the solution used for the reverse direction of reaction contained L-tryptophan ($m = 0.0016$ mol kg^{-1}) and D-glyceraldehyde 3-phosphate ($m = 0.0023$ mol kg^{-1}) in *N*,*N*-bis(2-hydroxyethyl)glycine (Bicine) buffer ($m = 0.10$ mol kg^{-1}, pH = 7.35). The $\alpha_2\beta_2$ complex of tryptophan synthase was added to this solution so that the mass fraction of the enzyme was $\approx 6 \times 10^{-4}$. Following equilibration for 1 day at $T = 298.15$ K, the solution was analyzed for 1-(indol-3-yl)glycerol 3-phosphate.

The approach used for the measurement of the apparent equilibrium constant for reaction (3) is complicated by the instability of the D-glyceraldehyde 3-phosphate. Therefore, we followed a method similar to that used by Weischet and Kirschner [7]. A series of synthetic solutions having known molalities of the reactants indole, 1-(indol-3-yl)glycerol 3-phosphate, and D-glyceraldehyde 3-phosphate were prepared. The molalities of the indole and 1-(indol-3-yl)glycerol 3-phosphate were kept very nearly constant (0.00095 mol kg^{-1} and 0.00098 mol kg^{-1}, respectively) and a variation in the apparent reaction quotient was obtained by varying the molality of

the D-glyceraldehyde 3-phosphate. Each of the synthetic solutions containing the three reactants was prepared immediately prior to use and then injected promptly into the HPLC. The ratio $R =$ {A(1-(indol-3-yl)glycerol 3-phosphate)/A(indole)} was then calculated from the areas A of the chromatographic peaks corresponding to 1-(indol-3-yl)glycerol 3-phosphate and indole. The known molalities of the indole and the 1-(indol-3-yl)glycerol 3-phosphate together with the areas corresponding to their peaks also gave values of the response factors and served as a check on the accuracy of the measurements. Each synthetic solution was kept in an ice bath until completion of the aforementioned chromatographic experiment. The $\alpha_2\,\beta_2$ complex of tryptophan synthase was then added to the remaining synthetic mixture. This reaction mixture was then placed in a thermostat set at $T = 298.15$ K and gently shaken for 10 min. This solution was then injected into the HPLC and the ratio R determined for this solution. With this procedure, one would expect R to remain unchanged for a solution that is already at equilibrium prior to addition of the enzyme. By working rapidly, the fraction of the D-glyceraldehyde 3-phosphate that has decomposed is expected to be < 0.01 and the problem of the instability of the D-glyceraldehyde 3-phosphate is minimized.

2.4. Microcalorimetry

Three heat-conduction microcalorimeters were used for the enthalpy of reaction measurements. They were calibrated electrically with a high stability DC 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. [23,24]. The data acquisition system has recently undergone significant modernization. The voltages of the thermopiles of the microcalorimeters are now measured with Hewlett-Packard model 34420A Nanovolt Meters. These voltages are then recorded on a microcomputer with a data acquisition program written in Hewlett-Packard HP-VEE. The integration of the areas of the thermograms is done by using code written in C++.

The sample vessels, fabricated from high-density polyethylene, contained two compartments that held ≈ 0.55 cm^3 and ≈ 0.40 cm^3 of solution. The substrate solutions were placed in the 0.55 cm^3 compartment of the microcalorimeter vessel and the enzyme solutions were placed in the 0.40 cm^3 compartment. The substrate solutions contained: (indole + L-serine) for reaction (1); L-serine for reaction (2); (indole + D-glyceraldehyde 3-phosphate) for reaction (3); and (1-(indol-3-yl)glycerol 3-phosphate + L-serine) for reaction (4). The limiting reactants were indole in reactions (1) and (3) and L-serine in reactions (2) and (4). Pyridoxal 5-phosphate was added to the stock phosphate buffer solutions used for reactions (1) and (2); CsCl was also added to the stock buffer solution used for reaction (2). The $\alpha_2\,\beta_2$ complex of tryptophan synthase was used to catalyze reactions (1), (3), and (4); the β_2 subunit was used for reaction (2). These enzymes were dissolved in the same respective stock phosphate buffer solutions that were used for the preparation of the substrate solutions. The aim of this procedure was to minimize the 'blank' enthalpy changes (see below). Reactions (1), (3), and (4) were carried out at $T = 298.15$ K. However, since the rate of reaction (2) was too slow at this temperature, it was necessary to carry out this reaction at a higher temperature ($T = 308.15$ K).

The vessels and their contents were allowed to equilibrate in the microcalorimeters for ≈ 60 min before the enzyme and substrate solutions were mixed. Reaction times of ≈ 32 min, ≈ 66 min, ≈ 36 min, and ≈ 22 min were allowed for reactions (1), (2), (3), and (4), respectively. Following reaction, the vessels were removed from the microcalorimeters and their contents were promptly analyzed to determine the amount of indole (reactions (1) and (3)), L-serine (reaction (2)), or L-tryptophan (reaction (4)) in solution. In this way the amount of substance that had undergone reaction was obtained. The fractions of the limiting reactants that had undergone reaction were 0.999, 0.940, 0.955, and 1.000, for reactions (1), (2), (3), and (4), respectively. The 'blank' enthalpy

changes for the mixing of the substrate solutions with the buffer ranged from −0.612 mJ to 0.30 mJ while for the mixing of the enzyme solutions with the buffer these enthalpies ranged from −1.4 mJ to 0.50 mJ. The pooled uncertainty (two estimated standard deviations of the mean) in these 'blank' enthalpies was 0.62 mJ. These 'blank' enthalpies of mixing were applied as corrections to the measured calorimetric enthalpies $\Delta_r H$(cal) which were ≈ −188 mJ, ≈ −55 mJ, ≈ −42 mJ, and ≈ −32 mJ for reactions (1), (2), (3), and (4), respectively.

2.5. pH measurements

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 and with a standard phosphate buffer (pH = 7.42 at $T = 298.15$ K). This latter buffer was prepared from KH_2PO_4 and Na_2HPO_4, standard reference materials 186-Id and 186-IId, respectively, from the National Institute of Standards and Technology.

3. Results and discussion

3.1. Thermodynamic formalism

The apparent equilibrium constants corresponding to reactions (1), (2), (3), (4) and (5) are, respectively,

$$K' = m(\text{L-tryptophan}) \cdot m^\circ / \{m(\text{indole}) \cdot m(\text{L-serine})\} \quad (6)$$

$$K' = m(\text{pyruvate}) \cdot m(\text{ammonia}) / \{m(\text{L-serine}) \cdot m^\circ\} \quad (7)$$

$$K' = m(\text{1-(indol-3-yl)glycerol 3-phosphate}) \cdot m^\circ / \{m(\text{indole}) \cdot m(\text{D-glyceraldehyde 3-phosphate})\} \quad (8)$$

$$K' = m(\text{L-tryptophan}) \cdot m(\text{D-glyceraldehyde 3-phosphate}) / \{m(\text{L-serine}) \cdot m(\text{1-(indol-3-yl)glycerol 3-phosphate})\} \quad (9)$$

$$K' = m(\text{indole}) \cdot m(\text{pyruvate}) \cdot m(\text{ammonia}) / \left\{m(\text{L-tryptophan}) \cdot (m^\circ)^2\right\} \quad (10)$$

The molalities m in the above equations are the total molalities of the various charged and uncharged species that are formed from the dissociation of the various substances in solution. The standard molality ($m^\circ = 1$ mol kg^{-1}) has been used in the above equations to keep the apparent equilibrium constants dimensionless.

With D-glyceraldehyde 3-phosphate, one is also dealing with a mixture of the aldehyde and diol forms [25,26], with the diol being the predominant form under the experimental conditions used in this study. Since, the interconversion of these two forms is relatively rapid [25,26], it seems safe to assume that these two forms are at equilibrium in our experiments. Thus, it will be understood that when we refer to D-glyceraldehyde 3-phosphate in this study, we are considering the equilibrium mixture of the aldehyde and the diol.

It will also be seen to be useful to introduce the following chemical reference reactions that correspond to the above overall biochemical reactions:

$$\text{indole}^0(\text{aq}) + \text{L-serine}^{\pm}(\text{aq}) = \text{L-tryptophan}^{\pm}(\text{aq}) + \text{H}_2\text{O(l)} \quad (11)$$

$$\text{L-serine}^{\pm}(\text{aq}) = \text{pyruvate}^-(\text{aq}) + \text{NH}_4^+(\text{aq}) \quad (12)$$

$$\text{indole}^0(\text{aq}) + \text{D-glyceraldehyde 3-phosphate}^{2-}(\text{aq}) = \text{1-(indol-3-yl)glycerol 3-phosphate}^{2-}(\text{aq}) \quad (13)$$

$$\text{L-serine}^{\pm}(\text{aq}) + \text{1-(indol-3-yl)glycerol 3-phosphate}^{2-}(\text{aq}) = \text{L-tryptophan}^{\pm}(\text{aq}) + \text{D-glyceraldehyde 3-phosphate}^{2-}(\text{aq}) + \text{H}_2\text{O(l)} \quad (14)$$

$$\text{L-tryptophan}^{\pm}(\text{aq}) + \text{H}_2\text{O(l)} = \text{indole}^0(\text{aq}) + \text{pyruvate}^-(\text{aq}) + \text{NH}_4^+(\text{aq}) \quad (15)$$

The equilibrium constants corresponding to reactions (11), (12), (13), (14), and (15), respectively, are:

$$K = m(\text{L-tryptophan}^{\pm}) \cdot m^{\circ} / \{m(\text{indole}^{0}) \cdot m(\text{L-serine}^{\pm})\} \quad (16)$$

$$K = m(\text{pyruvate}^{-}) \cdot m(NH_4^{+}) / \{m(\text{L-serine}^{\pm}) \cdot m^{\circ}\} \quad (17)$$

$$K = m(\text{1-(indol-3-yl)glycerol 3-phosphate}^{2-}) \cdot m^{\circ} / \{m(\text{indole}^{0}) \cdot m(\text{D-glyceraldehyde 3-phosphate}^{2-})\} \quad (18)$$

$$K = m(\text{L-tryptophan}^{\pm}) \cdot m(\text{D-glyceraldehyde 3-phosphate}^{2-}) / \{m(\text{L-serine}^{\pm}) \cdot m(\text{1-(indol-3-yl)glycerol 3-phosphate}^{2-})\} \quad (19)$$

$$K = m(\text{indole}^{0}) \cdot m(\text{pyruvate}^{-}) \cdot m(NH_4^{+}) / \left\{m(\text{L-tryptophan}^{\pm}) \cdot (m^{\circ})^{2}\right\} \quad (20)$$

The standard state used in this study is the hypothetical ideal solution of unit molality (m° = 1 mol kg^{-1}).

3.2. Equilibrium constants

The attempts to directly measure apparent equilibrium constants for reactions (1), (2), and (4) were unsuccessful. This was due to a lack of sufficient instrumental sensitivity to measure the very small amounts of the pertinent substrates. However, by using the limits of detectability for these substrates, it was possible to set the following lower limits on the values of the apparent equilibrium constants: K' (T = 298.15 K, pH = 7.01) $> 9 \times 10^7$ for reaction (1); K' (T = 308.15 K, pH = 6.9) > 330 for reaction (2); and K' (T = 298.15 K, pH = 7.01) $> 4 \times 10^6$ for reaction (4). Nevertheless, we shall later obtain values of equilibrium constants for these reactions by use of thermochemical cycle calculations.

It was possible, however, to measure the apparent equilibrium constant for reaction (3). As discussed in Section 2.3, these measurements required particular care because of the instability of the D-glyceraldehyde 3-phosphate. The results of these measurements are given in Table 2. The apparent reaction quotient Q' for reaction (3) is equal to

$$Q' = m(\text{1-(indol-3-yl)glycerol 3-phosphate}) \cdot m^{\circ} / \{m(\text{indole}) \cdot m(\text{D-glyceraldehyde 3-phosphate})\} \quad (21)$$

A linear fit of Q' as a function of ΔR was found to represent the results adequately; the

Table 2
Results of equilibrium measurements for biochemical reaction (3): indole(aq) + D-glyceraldehyde 3-phosphate(aq) = 1-(indol-3-yl)glycerol 3-phosphate(aq) at T = 298.15 K, pH = 7.54, and ionic strength I_m = 0.37 mol kg^{-1}

$10^3 \times m$(indole) (mol kg^{-1})	$10^3 \times m$(D-G3P)[a] (mol kg^{-1})	$10^3 \times m$(IGP) (mol kg^{-1})	Q'	ΔR
0.9370	0.2084	0.9914	5077	0.1698
0.9438	0.1263	0.9798	8220	0.1188
0.9747	0.1021	0.9745	9792	0.0078
0.9554	0.1017	0.9796	10 082	0.0488
0.9565	0.0826	0.9775	12 372	−0.0064
0.9595	0.0728	0.9731	13 931	−0.0033

The molalities m of the Na_2HPO_4 and H_3PO_4 were 0.0953 mol kg^{-1} and 0.00872 mol kg^{-1}, respectively. All molalities are equal to the sums of the molalities of the indicated substances in their various ionic forms and are those after mixing with the enzyme and prior to any reaction. The substances 1-(indol-3-yl)glycerol 3-phosphate and D-glyceraldehyde 3-phosphate are designated as IGP and D-G3P, respectively. Q' is the apparent reaction quotient (see Eq. (21)). The quantity ΔR is the change in the ratio {A(1-(indol-3-yl)glycerol 3-phosphate)/A(indole)}, where A(1-(indol-3-yl)glycerol 3-phosphate) and A(indole) are the areas of the chromatographic peaks corresponding to 1-(indol-3-yl)glycerol 3-phosphate and indole, respectively. The mass fraction of the enzyme ($\alpha_2\beta_2$ complex of tryptophan synthase) was 0.00013. The apparent equilibrium constant for reaction (3) is derived from these results (see Section 3.2).

[a] m(D-glyceraldehyde 3-phosphate) = 0.5m(DL-glyceraldehyde 3-phosphate).

intercept was $(1.21 \pm 0.15) \times 10^4$ and the slope was $-(3.9 \pm 1.7) \times 10^4$. Since the value of Q' which corresponds to $\Delta R = 0$ is equal to the apparent equilibrium constant, $K' = (1.21 \pm 0.15) \times 10^4$ for reaction (3) at $T = 298.15$ K, pH = 7.54, and $I_m = 0.37$ mol kg^{-1}. Here the uncertainties are equal to two estimated standard deviations. We judge that reasonable estimates of possible systematic errors in the measurements of K' are: decomposition of the D-glyceraldehyde 3-phosphate, $0.01 \times K'$; uncertainties in the molalities of the 1-(indol-3-yl)glycerol 3-phosphate and D-glyceraldehyde 3-phosphate stock solutions, $0.04 \times K'$; chromatography, $0.02 \times K'$; and weighing errors, $0.01 \times K'$. These estimates of possible systematic error are combined in quadrature together with the statistical uncertainty in the measured value of K', expressed as one estimated standard deviation of the mean, to obtain a combined standard uncertainty [27] of $0.08 \times K'$. This combined standard uncertainty is then multiplied by 2 to arrive at the final result $K' = (1.2 \pm 0.2) \times 10^4$ for reaction (3) at $T = 298.15$ K, pH = 7.54, and $I_m = 0.37$ mol kg^{-1}.

3.3. Calorimetric results

The results of the calorimetric measurements are given in Table 3. In these tables, the uncertainties in the values of the calorimetrically determined molar enthalpies of reaction $\Delta_r H_m$(cal) indicate only the random errors inherent in the measurements and do not reflect the possible systematic errors which we now consider. We judge that reasonable estimates of possible systematic errors in the measurements of $\Delta_r H_m$(cal) for reactions (1), (2), (3), and (4), respectively, are: heat measurements and corrections for 'blank' enthalpies, $0.0034 \times \Delta_r H_m$(cal), $0.0064 \times \Delta_r H_m$(cal), $0.0080 \times \Delta_r H_m$(cal), and $0.010 \times \Delta_r H_m$(cal); corrections for incomplete reaction, $0.002 \times \Delta_r H_m$(cal), $0.01 \times \Delta_r H_m$(cal), $0.01 \times \Delta_r H_m$(cal), and $0.01 \times \Delta_r H_m$(cal); moisture content and impurities in the limiting reactants $0.002 \times \Delta_r H_m$(cal), $0.01 \times \Delta_r H_m$(cal), $0.002 \times \Delta_r H_m$(cal), and $0.01 \times \Delta_r H_m$(cal); and correction for the amount of the side reaction (3) that accompanied reaction (4), $0.02 \times \Delta_r H_m$(cal). These estimates of possible systematic error are combined as was done previously (see above) in order to arrive at the final results: $\Delta_r H_m$(cal) $= -(74.5 \pm 0.7)$ kJ mol^{-1} for reaction (1); $\Delta_r H_m$(cal) $= -(12.1 \pm 0.6)$ kJ mol^{-1} for reaction (2); $\Delta_r H_m$(cal) $= -(46.9 \pm 1.2)$ kJ mol^{-1} for reaction (3); and $\Delta_r H_m$(cal) $= -(27.8 \pm 2.0)$ kJ mol^{-1} for reaction (4).

3.4. Ionization constants

The pKs and standard molar enthalpies for the proton dissociation reactions of the reactants and of the other solutes in solution are needed to relate the experimental results for reactions (1), (2), (3), and (4) to thermodynamic quantities for the respective reference reactions (11), (12), (13), and (14). These pKs and standard molar enthalpies $\Delta_r H_m^\circ$ are given in Table 4. Where necessary, the values of the pKs and $\Delta_r H_m^\circ$s were adjusted to $I = 0$ by using an 'ion-size' parameter of 1.6 kg$^{1/2}$ mol$^{-1/2}$ in the extended Debye-Hückel equation [28] to estimate the activity coefficients of the aqueous species in solution. The sources of the values for the various ionizations are: the evaluation of Tewari and Goldberg [12] for pyruvic acid^0, L-tryptophan$^+$, and L-tryptophan$^\pm$; Wagman et al. [29] for $H_2PO_4^-$ and NH_4^+; and Martell et al. [30] for the L-serine and the pyridoxal 5-phosphate species. In the absence of a value of the pK or $\Delta_r H_m^\circ$ for either 1-(indol-3-yl)glycerol 3-phosphate$^-$ or D-glyceraldehyde 3-phosphate$^-$, we have estimated values for these quantities by using the existing tabulated results [30] for glycerol 1-phosphate and glycerol 2-phosphate. It is important to note that the structures of the regions of the D-glyceraldehyde 3-phosphate and 1-(indol-3-yl)glycerol 3-phosphate from which the ionization(s) occur are very similar. Specifically, these structures differ only in that different groups (aldehyde and hydroxyl) are attached to the third carbon atoms removed from the respective phosphate groups where the relevant ionizations occur. Thus, one would expect the values of the pKs and standard molar enthalpies for the ionizations of these two phosphates to be very nearly equal. This expectation is supported by the closeness of the values of the

pKs of two structurally related pairs of substances: (4-oxopentanoic acid (pK = 4.50) and 4-hydroxybutanoic acid (pK = 4.57)) and (3-aminopropylphosphonic acid (pK = 6.89) and DL-2-amino-4-phosphobutanoic acid (pK = 6.90)). These pKs, which are from Martell et al. [30], pertain to $T = 298.15$ K and $I_c = 0.1$ mol dm^{-3}.

3.5. Heat capacities

Table 4 also contains standard molar heat-capacity changes $\Delta_r C^\circ_{p,m}$ for three ionizations. The values of $\Delta_r C^\circ_{p,m}$ for the ionization of $H_2PO_4^-$ and NH_4^+ are based, respectively, on the heat-capacity measurements of Allred and Woolley [31] and of Larson et al. [32]. The value of $\Delta_r C^\circ_{p,m}$ for the ionization of L-serine$^+$ is an estimate based on the result of Allred and Woolley [31] for the ionization of acetic acid. Since reaction (2) was carried out at $T = 308.15$ K, a value of $\Delta_r C^\circ_{p,m}$ for reaction (12) is also needed for the adjustment of the results to $T = 298.15$ K. For L-serine$^\pm$, $C^\circ_{p,2,m} = 114$ J K^{-1} mol^{-1} from Hakin et al. [33] and for NH_4^+, $C^\circ_{p,2,m} = 68$ J K^{-1} mol^{-1} from Allred

Table 3
Results of calorimetric measurements for biochemical reactions (1), (2), (3), and (4) in phosphate buffer

T (K)	pH	$m(Na_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	$10^3 \times m$(PLP) (mol kg^{-1})	$10^3 \times m$(L-serine) (mol kg^{-1})	$10^3 \times m$(indole) (mol kg^{-1})	I_m (mol kg^{-1})	$\Delta_r H_m$(cal) (kJ mol^{-1})
Reaction (1): indole(aq) + L-serine(aq) = L-tryptophan(aq) + H_2O(l)								
298.15	7.01	0.09913	0.02275	0.0984	3.127	2.951	0.32	−74.15
298.15	7.01	0.09915	0.02275	0.0984	3.168	2.989	0.32	−74.53
298.15	7.01	0.09913	0.02275	0.0984	3.126	2.949	0.32	−74.28
298.15	7.01	0.09913	0.02275	0.0984	3.124	2.948	0.32	−74.84
298.15	7.01	0.09915	0.02275	0.0984	3.172	2.993	0.32	−74.75
				$\langle \Delta_r H_m(\text{cal}) \rangle = -(74.5 \pm 0.3)$ kJ mol^{-1}				

Reaction (2): L-serine(aq) = pyruvate(aq) + ammonia(aq)								
T (K)	pH	$m(Na_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	$10^3 \times m$(PLP) (mol kg^{-1})	m(CsCl) (mol kg^{-1})	$10^3 \times m$(L-serine) (mol kg^{-1})	I_m (mol kg^{-1})	$\Delta_r H_m$(cal) (kJ mol^{-1})
308.15	6.90	0.09679	0.02530	0.0980	0.0400	5.736	0.39	−10.97
308.15	6.90	0.09677	0.02530	0.0980	0.0407	5.661	0.39	−11.89
308.15	6.90	0.09667	0.02527	0.0979	0.0435	5.380	0.39	−12.32
308.15	6.90	0.09668	0.02527	0.0979	0.0431	5.419	0.39	−12.89
308.15	6.90	0.09674	0.02529	0.0979	0.0415	5.582	0.39	−12.76
308.15	6.90	0.09669	0.02528	0.0979	0.0428	5.448	0.39	−11.16
308.15	6.90	0.09673	0.02529	0.0979	0.0418	5.554	0.39	−12.35
308.15	6.90	0.09669	0.02528	0.0979	0.0427	5.457	0.39	−12.31
				$\langle \Delta_r H_m(\text{cal}) \rangle = -(12.1 \pm 0.5)$ kJ mol^{-1}				

Reaction (3): indole(aq) + L-glyceraldehyde 3-phosphate(aq) = 1-(indol-3-yl)glycerol 3-phosphate(aq)							
T (K)	pH	$m(Na_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	$10^3 \times m$(indole) (mol kg^{-1})	$10^3 \times m$(DL-G3P) (mol kg^{-1})	I_m (mol kg^{-1})	$\Delta_r H_m$(cal) (kJ mol^{-1})
298.15	7.26	0.09770	0.01692	1.127	4.344	0.37	−46.85
298.15	7.26	0.09769	0.01692	1.147	4.421	0.37	−46.90
298.15	7.26	0.09769	0.01692	1.155	4.451	0.37	−47.08
298.15	7.26	0.09769	0.01692	1.147	4.421	0.37	−47.03
298.15	7.26	0.09764	0.01691	1.096	4.423	0.37	−46.58
298.15	7.26	0.09763	0.01691	1.113	4.490	0.37	−46.46
298.15	7.26	0.09763	0.01691	1.112	4.489	0.37	−47.56
				$\langle \Delta_r H_m(\text{cal}) \rangle = -(46.9 \pm 0.3)$ kJ mol^{-1}			

Table 3 (*Continued*)

Reaction (4): L-serine(aq) + 1-(indol-3-yl)glycerol 3-phosphate(aq) = L-tryptophan(aq) + D-glyceraldehyde 3-phosphate(aq) + H_2O(l)							
T (K)	pH	$m(Na_2HPO_4)$ (mol kg^{-1})	$m(H_3PO_4)$ (mol kg^{-1})	$10^3 \times m$(L-serine) (mol kg^{-1})	$10^3 \times m$(IGP) (mol kg^{-1})	I_m (mol kg^{-1})	$\Delta_r H_m$(cal)[a] (kJ mol^{-1})
298.15	7.57	0.08853	0.01533	1.285	2.012	0.36	−27.0
298.15	7.57	0.08853	0.01533	1.283	2.008	0.36	−29.2
298.15	7.57	0.08855	0.01534	1.278	2.001	0.36	−30.1
298.15	7.57	0.08579	0.01486	1.967	2.063	0.37	−25.7
298.15	7.57	0.08572	0.01485	1.989	2.087	0.37	−27.4
298.15	7.57	0.08588	0.01487	1.940	2.035	0.37	−27.6
$\langle \Delta_r H_m(\text{cal}) \rangle = -(27.8 \pm 1.3)$ kJ mol^{-1}							

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. Pyridoxal 5-phosphate, 1-(indol-3-yl)glycerol 3-phosphate, and DL-glyceraldehyde 3-phosphate are designated, respectively, as PLP, IGP, and DL-G3P. $\Delta_r H_m$(cal) is the calorimetrically determined molar enthalpy of reaction. The ionic strengths I_m are calculated (see Section 3.6). The $\alpha_2 \beta_2$ complex of tryptophan synthase was used to catalyze reactions (1), (3), and (4); the β_2 subunit was used for reaction (2). The respective mass fractions w of these enzymes in solution were, respectively, 0.0004, 0.0003, 0.0003, and 0.0006 for reactions (1), (2), (3), and (4). The uncertainties are equal to two estimated standard deviations of the mean.

[a] The fraction of 1-(indol-3-yl)glycerol 3-phosphate converted to indole was 0.128 in the first three experiments and 0.023 in the last two experiments. Therefore, the values of $\Delta_r H_m$(cal) include a correction for the formation of (indole + D-glyceraldehyde 3-phosphate) from 1-(indol-3-yl)glycerol 3-phosphate. A value of $\Delta_r H_m(\text{cal}) = -46.9$ kJ mol^{-1} for reaction (3) was used for the correction of the measured enthalpies.

and Woolley [31]. In the absence of data for pyruvate$^-$, we obtain $C^\circ_{p,2,m} = 115$ J K^{-1} mol^{-1} for pyruvic acid^0 by using the estimation method of Cabani et al. [34]. Then, with $\Delta_r C^\circ_{p,m}$ from Allred and Woolley [31] for the ionization of acetic acid, we estimate $\Delta_r C^\circ_{p,m} = -143$ J K^{-1} mol^{-1} for the reaction

$$\text{pyruvic acid}^0 = \text{pyruvate}^- + \text{H}^+(\text{aq}) \qquad (22)$$

This leads to $C^\circ_{p,2,m} = -28$ J K^{-1} mol^{-1} for pyruvate$^-$ and $\Delta_r C^\circ_{p,m} = -74$ J K^{-1} mol^{-1} for reaction (12).

3.6. Equilibrium model

The equilibrium model used for the calculation of equilibrium constants and standard molar enthalpies for the reference reactions has been described previously [28]. This model has been modified recently so that it now utilizes the Mathematica [35] computer code of Alberty and Krambeck (personal communication; in review) to solve the simultaneous non-linear equations that describe the various equilibria in these solutions. We have extended this Mathematica code so that the calculations include corrections for non-ideality and are made self-consistent [28] in regards to the ionic strength I_m. The non-ideality corrections are based on the extended Debye-Hückel equation in which the 'ion-size' parameter has been set at 1.6 kg$^{1/2}$ mol$^{-1/2}$. In the most recent implementation of the model, values of the standard transformed molar enthalpy of reaction $\Delta_r H'^\circ_m$ [36] are calculated by using the relationship

$$\Delta_r H'^\circ_m = RT^2 (\partial \ln K'/\partial T)_{P,I,pX \neq H} \qquad (23)$$

Specifically, values of K' are first calculated at temperatures at and near the reference temperature 298.15 K; $(\partial \ln K'/\partial T)_P$ is then calculated as a numerical derivative. Similarly, values of the changes in binding of the hydrogen ion $\Delta_r N(\text{H}^+)$ are calculated by using

$$\Delta_r N(\text{H}^+) = -(\partial \ln K'/\partial \text{pH})_{T,P,I,pX \neq H} \qquad (24)$$

We have used the equilibrium model to calculate $K = (1.2 \pm 0.2) \times 10^4$ for the reference reaction (13) at $T = 298.15$ K and $I_m = 0$. The following values of $\Delta_r H^\circ_m$, the standard molar en-

Table 4
Thermodynamic quantities for the aqueous proton dissociation reactions of substances pertinent to this study at $T = 298.15$ K and $I_m = 0$

Reaction	pK	$\Delta_r H^\circ_m$ (kJ mol^{-1})	$\Delta_r S^\circ_m$ (J K^{-1} mol^{-1})	$\Delta_r C^\circ_{p,m}$ (J K^{-1} mol^{-1})
$H_2PO_4^- = HPO_4^{2-} + H^+$	7.21	4.2	−124	−220
$NH_4^+ = NH_3 + H^+$	9.25	52.22	−2	7
D-G3P$^- =$ D-G3P$^{2-} + H^+$	6.6[a]	−2.0[a]	−133[a]	
IGP$^- =$ IGP$^{2-} + H^+$	6.6[a]	−2.0[a]	−133[a]	
PLP$^0 =$ PLP$^- + H^+$	4.05	16.4	−23	
PLP$^- =$ PLP$^{2-} + H^+$	6.46	−5.8	−143	
PLP$^{2-} =$ PLP$^{3-} + H^+$	8.89	23.1	−93	
Pyruvic acid$^0 =$ pyruvate$^- + H^+$	2.56	12.1	−8	−143[a]
L-serine$^+ =$ L-serine$^\pm + H^+$	2.19	5.0	−25	
L-serine$^\pm =$ L-serine$^- + H^+$	9.21	42.4	−34	
L-tryptophan$^+ =$ L-tryptophan$^\pm + H^+$	2.19	5	−25	
L-tryptophan$^\pm =$ L-tryptophan$^- + H^+$	9.60	43	−40	

The sources of the values of these quantities are given in the text (see Section 3.4). The substances D-glyceraldehyde 3-phosphate, 1-(indol-3-yl)glycerol 3-phosphate, and pyridoxal 5-phosphate are designated, respectively, as D-G3P, IGP, and PLP.
[a] Estimated (see Section 3.4).

thalpy for the reference reactions at $I_m = 0$ and $T = 298.15$ K, were also calculated: $\Delta_r H^\circ_m = -(74.3 \pm 0.7)$ kJ mol^{-1} for reaction (11); $\Delta_r H^\circ_m = -(12.2 \pm 0.6)$ kJ mol^{-1} for reaction (12); $\Delta_r H^\circ_m = -(46.9 \pm 1.2)$ kJ mol^{-1} for reaction (13); and $\Delta_r H^\circ_m = -(27.1 \pm 2.0)$ kJ mol^{-1} for reaction (14). Calculated values of the changes in binding of the hydrogen ion $\Delta_r N(H^+)$ in the calorimetry experiments were 0.0052, 0.0062, 0.000, and 0.0185 for the biochemical reactions (1), (2), (3), and (4), respectively. Thus, the buffer protonation corrections used to obtain values of $\Delta_r H^\circ_m$ from the measured values of $\Delta_r H_m$(cal) were relatively small (≤ 0.10 kJ mol^{-1}).

The uncertainties in the calculated values of the thermodynamic quantities for the reference reactions (11), (12), (13) and (14) have two components: the experimental uncertainties in the measured quantities (i.e. the apparent equilibrium constant for reaction (3) and $\Delta_r H_m$(cal) for reactions (1), (2), (3), and (4)) and possible errors in the quantities used in the equilibrium model. This latter component of uncertainty was examined by perturbing the various quantities in the model by their assumed possible errors. Specifically, the appropriate pKs (see Table 4) were perturbed by ± 0.1, the value of $\Delta_r H^\circ_m$ for the ionization of $H_2PO_4^-$ by ± 0.1 kJ mol^{-1}, and the 'ion-size' parameter used in the activity coefficient model by ± 0.3 kg$^{1/2}$ mol$^{-1/2}$. The effects on the calculated quantities due to these perturbations were then combined in quadrature with the experimental uncertainties to obtain the final uncertainties assigned to the thermodynamic quantities for the reference reactions (11), (12), (13), and (14). In all cases, the combined effects of the perturbation of the various quantities in the equilibrium model were much smaller than the experimental uncertainties. Because the experimental and the model uncertainties were combined in quadrature, the final uncertainties in the calculated values of the thermodynamic quantities are the same as the experimental uncertainties.

3.7. Thermochemical cycles

As mentioned in Section 1, it is possible to use thermochemical cycles to check the accuracy of the results. We do this in terms of the chemical reference reactions; namely, reaction (11) = {reaction (13) + reaction (14)} = {reaction (12) − reaction (15)}. Thus, by using $\Delta_r H^\circ_m = -(46.9 \pm 1.2)$ kJ mol^{-1} for reaction (13) and $\Delta_r H^\circ_m = -(27.1 \pm 2.0)$ kJ mol^{-1} for reaction (14), we calculate $\Delta_r H^\circ_m = -(74.0 \pm 2.4)$ kJ mol^{-1} for reac-

tion (11). All these values of $\Delta_r H^\circ_m$ refer to $T = 298.15$ K and $I_m = 0$. Similarly, we use $\Delta_r H^\circ_m = -(12.2 \pm 0.6)$ kJ mol^{-1} for reaction (12) together with $\Delta_r H^\circ_m = (62.0 \pm 2.3)$ kJ mol^{-1} for reaction (15) from the earlier study of Tewari and Goldberg [12] to calculate $\Delta_r H^\circ_m = -(74.2 \pm 2.4)$ kJ mol^{-1} for reaction (11). Both of the calculated values of $\Delta_r H^\circ_m$ are in excellent agreement with the measured result of $\Delta_r H^\circ_m = -(74.3 \pm 0.7)$ kJ mol^{-1}. This agreement lends a confidence to the correctness of the calorimetric measurements. These comparisons are summarized in Table 5.

3.8. Comparison with values in the literature

We now consider previously reported thermodynamic results on the reactions of interest in this study. Weischet and Kirschner [7] measured an apparent equilibrium constant for reaction (3). The procedure they used was to add tryptophan synthase to synthetic mixtures containing known molalities of indole, D-glyceraldehyde 3-phosphate, and 1-(indol-3-yl)glycerol 3-phosphate. The reaction was followed spectrophotometrically at $\lambda = 290$ nm. It was found that there was no change in the absorbance for a solution having concentrations of indole, D-glyceraldehyde 3-phosphate, and 1-(indol-3-yl)glycerol 3-phosphate equal to, respectively, 0.53 mol dm^{-3}, 0.47 mol dm^{-3}, and 2.00 mol dm^{-3}. This result corresponds to $K' = 8.03 \times 10^3$ for reaction (3). The reaction was carried out in Tris buffer ($c = 0.05$ mol dm^{-3}, pH = 7.8) at $T = 298.15$ K. From the stated concentrations of the buffer and substrates, we calculate that the ionic strength of the reaction mixture was 0.037 mol dm^{-3}. With the equilibrium model, we calculate K ($T = 298.15$ K, $I_m = 0$) $= 8.03 \times 10^3$ for reaction (13). Weischet and Kirschner [7] judged their results to be reliable within $\pm 0.1 \times K'$. Thus, their result is not in agreement with the

Table 5
Thermodynamic quantities for the chemical reference reactions in aqueous solution at $T = 298.15$ K, pressure $P = 0.1$ MPa, and $I_m = 0$

Reaction	K	$\Delta_r G^\circ_m$ (kJ mol^{-1})	$\Delta_r H^\circ_m$ (kJ mol^{-1})	$\Delta_r S^\circ_m$ (J K^{-1} mol^{-1})
(11) Indole^0 + L-serine$^\pm$ = L-tryptophan$^\pm$ + H_2O(l)	9.2×10^{12}	−74.0	−(74.3 ± 0.7)	−1.0
			−(79.7 ± 4.6)[a]	
(12) L-serine$^\pm$ = pyruvate$^-$ + NH_4^+	9.3×10^8	−51.2	−(12.2 ± 0.6)	130.5
			−(7.0 ± 0.4)[a]	
(13) Indole^0 + D-glyceraldehyde 3-phosphate^{2-} = 1-(indol-3-yl)glycerol 3-phosphate^{2-}	$(1.2 \pm 0.2) \times 10^4$	−(23.3 ± 0.5)	−(46.9 ± 1.2)	−(79 ± 5)
			−(33.8 ± 2.5)[a]	
(14) L-serine$^\pm$ + 1-(indol-3-yl)glycerol 3-phosphate^{2-} = L-tryptophan$^\pm$ + D-glyceraldehyde 3-phosphate^{2-} + H_2O(l)	7.6×10^8	−50.7	−(27.1 ± 2.0)	79
			−(26.0 ± 5.6)[a]	
(15) L-tryptophan$^\pm$ + H_2O(l) = indole^0 + pyruvate$^-$ + NH_4^+[b]	$(1.05 \pm 0.13) \times 10^{-4}$	22.7 ± 0.3	62.0 ± 2.3	132 ± 8
Values of $\Delta_r H^\circ_m$ obtained from thermochemical cycle calculations				
Reaction (11) = reaction (13) + reaction (14)			−(74.0 ± 2.4)[c]	
			−(59.8 ± 6.2)[d]	
Reaction (11) = reaction (12) − reaction (15)			−(74.2 ± 2.4)[e]	
			−(69.0 ± 2.3)[f]	

The standard state is the hypothetical ideal solution of unit molality. The values of K, $\Delta_r G^\circ_m$, and $\Delta_r S^\circ_m$ for reactions (11), (12), and (14) were calculated by using thermochemical cycles (see Section 3.7). The basis of the uncertainties is discussed in the text.
[a] Value calculated from the results of Wiesinger and Hinz [8].
[b] The results for this reaction are from Tewari and Goldberg [12].
[c] Value calculated from the results of this study.
[d] Value calculated from the results of Wiesinger and Hinz [8].
[e] Value calculated from the results of this study for reaction (12) and the result of Tewari and Goldberg [12] for reaction (15).
[f] Value calculated from the result of Wiesinger and Hinz [8] for reaction (12) and the result of Tewari and Goldberg [12] for reaction (15).

result of the present study, namely K ($T = 298.15$ K, $I_m = 0$) $= (1.2 \pm 0.2) \times 10^4$. Nevertheless, we judge the chromatographic method that was used in this study and that involves the separation of the 1-(indol-3-yl)glycerol 3-phosphate and the indole to be advantageous over the spectrophotometric method where these substrates are not separated.

Wiesinger and Hinz [8] reported values of $\Delta_r H_m$(cal) $= -(80.3 \pm 4.6)$, $-(7.3 \pm 0.4)$, $-(54.0 \pm 2.5)$, $-(13.4 \pm 5.6)$ kJ mol^{-1} for reactions (1), (2), (3), and (4), respectively. However, the enthalpies they reported for reactions (3) and (4) also included corrections for the hydration of the aldehyde form of D-glyceraldehyde 3-phosphate to its diol form. In the absence of these corrections, the values of $\Delta_r H_m$(cal) for reactions (3) and (4) are, respectively, $-(33.8 \pm 2.5)$ kJ mol^{-1} and $-(34 \pm 7)$ kJ mol^{-1} (H. Wiesinger, personal communication). These values, which will be used in the discussion that follows, pertain to the equilibrium mixture of the aldehyde and diol forms of D-glyceraldehyde 3-phosphate. All of the results of Wiesinger and Hinz [8] pertain to $T = 298.15$ K except for the result for reaction (2) which pertains to $T = 308.15$ K. Excluding only one individual experiment for reaction (1), the buffer was sodium diphosphate ($c = 0.1$ mol dm^{-3}, pH = 7.5). On the reasonable assumption that the concentrations of the substrates were negligible in comparison to the concentration of the sodium diphosphate buffer, the ionic strength to which these calorimetric enthalpies pertain is 0.78 mol dm^{-3}. With the equilibrium model, these calorimetric enthalpies lead to values of $\Delta_r H_m^\circ = -(79.7 \pm 4.6)$, $-(7.0 \pm 0.4)$, $-(33.8 \pm 2.5)$, $-(33 \pm 7)$ kJ mol^{-1} at $T = 298.15$ K and $I_m = 0$ for the reference reactions (11), (12), (13), and (14), respectively. In performing these calculations, we used pKs of 6.69 and 9.42 and values of $\Delta_r H_m^\circ =$ 1.0 kJ mol^{-1} and 0.4 kJ mol^{-1} for the respective ionizations of $H_2P_2O_7^{2-}$ and $HP_2O_7^{3-}$ at $T =$ 298.15 K and $I_m = 0$ [30]. These calculated values of $\Delta_r H_m^\circ$ for the reference reactions can be compared with the values of $\Delta_r H_m^\circ$ that were obtained in this study and that are summarized in Table 5. The results for reactions (11) and (14) can be considered to be in agreement. However, the results for the reactions (12) and (13) differ from our results by amounts that are outside the assigned uncertainties.

We now use the values of $\Delta_r H_m^\circ$ for the chemical reference reactions that were calculated from the results of Wiesinger and Hinz [8] in the same thermochemical cycle checks that were used above. With the Wiesinger and Hinz results for reactions (13) and (14), we calculate $\Delta_r H_m^\circ = -(67 \pm 8)$ kJ mol^{-1} for reaction (11) at $T =$ 298.15 K and $I_m = 0$. Similarly, with the Wiesinger and Hinz result for reaction (12) and the result of Tewari and Goldberg [12] for reaction (15), we calculate $\Delta_r H_m^\circ = -(69.0 \pm 2.3)$ kJ mol^{-1} for reaction (11). Thus, these calculated results for $\Delta_r H_m^\circ$ for reaction (11) are in agreement with each other. However, they do not agree either with the value $\Delta_r H_m^\circ = -(79.6 \pm 4.6)$ kJ mol^{-1} obtained by Wiesinger and Hinz [8] for this reaction or with the value of $\Delta_r H_m^\circ = -(74.3 \pm 0.7)$ kJ mol^{-1} obtained in the present study.

3.9. Calculation of additional equilibrium constants

We now consider two thermochemical cycles that allows us to calculate the equilibrium constants that we were unable to measure in this study. The first cycle uses previously tabulated [12] standard partial molar entropies of several of the reactants and the following experimental results for L-serine(s) from the literature: a third law standard molar entropy $S_m^\circ = 149.16$ J K^{-1} mol^{-1} [37]; a saturation molality m(sat) = 4.02 mol kg^{-1} [38]; a mean ionic activity coefficient $\gamma_\pm = 0.603$ at the saturation molality [38]; and a standard molar enthalpy of solution $\Delta_{sol} H_m^\circ = 2.8$ kJ mol^{-1} [39,40]. These properties, which pertain to $T = 298.15$ K, lead to a standard partial molar entropy $S_{2,m}^\circ = 165.9$ J K^{-1} mol^{-1} for L-serine$^\pm$(aq) at $T = 298.15$ K. This value of $S_{2,m}^\circ$ is in accord with the values of $S_{2,m}^\circ$ for structurally similar amino acids [41]. With the previously tabulated [12] values of $S_{2,m}^\circ$ for indole(aq), L-tryptophan$^\pm$(aq), pyruvate$^-$(aq), NH_4^+(aq), and H_2O(l) we can now calculate values of $\Delta_r S_m^\circ$ for reactions (11) and (12). These values of $\Delta_r S_m^\circ$ are then combined with the respective values of $\Delta_r H_m^\circ$ obtained in this study to give values of $\Delta_r G_m^\circ$ and

the equilibrium constants for these two reactions. The second cycle uses the relationship $\Delta_r G^\circ_m(14) = \Delta_r G^\circ_m(11) - \Delta_r G^\circ_m(13)$ which leads to $\Delta_r G^\circ_m = -50.7$ kJ mol^{-1} and $K = 7.6 \times 10^8$ for reaction (14) at $T = 298.15$ K. These calculated values are given in Table 5 together with the experimental results obtained in this study. These calculated values are consistent with the experimentally established lower limits (see above) on the values of the apparent equilibrium constants for reactions (1), (2), and (4).

3.10. Apparent equilibrium constants under approximately physiological conditions

It is also desirable to have values of apparent equilibrium constants K' for these several reactions under approximately physiological conditions. Here, physiological conditions are taken to be [42]: $T = 311.15$ K, pH = 7.0, pMg = 3.0, and $I_m = 0.25$ mol kg^{-1}. With the thermodynamic quantities obtained in this study for the reference reactions and with the equilibrium model, the following values of the apparent equilibrium constant K' for reactions (1), (2), (3), (4), and (5), respectively, are calculated for these conditions: 2.6×10^{12}, 1.5×10^9, 5.5×10^3, 4.8×10^8, and 5.5×10^{-4}. The standard transformed Gibbs free energy changes $\Delta_r G'^\circ_m$ for these biochemical reactions are, respectively: (−74.0, −54.6, −22.3, −51.7, and 19.4) kJ mol^{-1}. In performing these calculations, we assumed that the neutral species and those species having charge numbers of −1 do not bind to Mg^{2+}(aq) and that there was a cancellation of effects in reactions (3) and (4) due to the possible binding of Mg^{2+} to the 1-(indol-3-yl)glycerol 3-phosphate and D-glyceraldehyde 3-phosphate species.

3.11. Summary

The thermodynamic quantities obtained in this study provide the essential information needed to calculate the position of equilibrium of the overall biochemical reactions (1) to (5) as a function of temperature, pH, and ionic strength [28,43]. This information must ultimately be integrated with thermodynamic results on the binding of the substrates to the enzymes [44,45] and, via Haldane-type relations, to the kinetic constants for the various reactions studied herein. One qualitative application of the results of this study gives some insight into the operation of the metabolic pathway leading to the synthesis of L-tryptophan. In this pathway, reaction (3) proceeds in the direction of formation of indole and D-glyceraldehyde 3-phosphate (see Fig. 1) even though the apparent equilibrium constant greatly favors the reverse direction, i.e. the formation of 1-(indol-3-yl)glycerol 3-phosphate. The reason for this is that the indole that is formed reacts with L-serine to form L-tryptophan and water. Since the apparent equilibrium constant for this final reaction in this metabolic pathway is so large ($K' = 1.1 \times 10^{11}$ at $T = 311.15$ K and pH = 7.0), the indole that is formed is removed. Thus, reaction (3) is made to operate the way it does in the metabolic pathway because of Le Châtelier's principle. Clearly kinetic factors can also play a role in what happens under actual physiological conditions.

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