Factors affecting the stereospecificity and catalytic efficiency of the tryptophan synthase-catalysed exchange of the pro-2*R* and pro-2*S* protons of glycine

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¹³C-NMR has been used to follow the tryptophan synthase (EC 4.2.1.20)-catalysed hydrogen–deuterium exchange of the pro-2*R* and pro-2*S* protons of [2-¹³C]glycine. The first- and second-order rate constants for exchange when the $\alpha_2\beta_2$ enzyme complex is or is not saturated with glycine have been determined at pH 7.0 and 7.8. At pH 7.8 the effects of binding the allosteric effector, DL- α -glycerol 3-phosphate, and of removing the α -subunits have been examined. The β -subunits preferentially catalyse the exchange of the pro-2*R* proton of glycine, but adding α -subunits decreases the stereospecificity of the exchange reactions. Likewise, binding

of DL- α -glycerol 3-phosphate to the $\alpha_2\beta_2$ enzyme complex causes a further decrease in the stereospecificity of this reaction. The stereospecificity of the second-order exchange reaction catalysed by the β -subunits is 136-fold larger than that of the $\alpha_2\beta_2$ enzyme complex in the presence of DL- α -glycerol 3-phosphate, while there is only a 5-fold decrease in the stereospecificity of the first-order exchange reaction under the same conditions. We discuss how these results relate to current theories which attempt to explain how the α -subunits and DL- α -glycerol 3-phosphate modify the catalytic properties of tryptophan synthase.

INTRODUCTION

Bacterial tryptophan synthase (EC 4.2.1.20) is an $\alpha_2\beta_2$ complex which catalyses the final two reactions in the biosynthesis of L-tryptophan [1–3]. The α -subunits catalyse the cleavage of indol-3-ylglycerol phosphate to indole and D-glyceraldehyde 3-phosphate. The β -subunits catalyse the formation of L-tryptophan from L-serine and indole. The isolated α -subunits exist as monomers and are known to only catalyse the α -reaction. The isolated β -subunits exist as dimers and they catalyse the deamination of L-serine to pyruvate and ammonia as well the β -reaction and a wide range of other reactions [3]. In the $\alpha_2\beta_2$ enzyme complex the α and β active sites are separated by 25–30 Å, and they are connected by a tunnel which allows the indole produced at the α active site to pass to the β active site without diffusing through the bulk solvent [3].

Large ligands such as DL- α -glycerol 3-phosphate which bind to the α -subunits can greatly inhibit the reaction of large nucleophiles such as indole with the aminoacylate intermediate at the β active site. The fact that DL- α -glycerol 3-phosphate obstructs the entrance to the tunnel connecting the α and β active sites is thought to be a major factor in this inhibition [4,5]. This will tend to mask allosteric effects on the β -subunit due to the DL- α -glycerol 3-phosphate binding to the α -subunits. However, amino acids such as glycine and L-serine do not pass through the tunnel but pass directly from the bulk solvent to the β active site [5]. Therefore, by studying the properties of such amino acids when they are bound at the β active site, the allosteric effects caused by DL- α -glycerol 3-phosphate binding to the α -subunits can be isolated and characterized. DL- α -Glycerol 3-phosphate can be considered as an analogue either of D-glyceraldehyde 3-phosphate or of indol-3-ylglycerol phosphate which binds to the indol-3-ylglycerol phosphate binding site on the α -subunit [2,3,6,7]. It has no effect on the catalytic properties of isolated β -subunits, but when it binds to the α -subunits of the $\alpha_2\beta_2$ enzyme complex it increases the rate of some catalytic steps while it decreases the rates of others. It has been suggested that DL- α -glycerol 3-phosphate binding to the α -subunits of the $\alpha_2\beta_2$ enzyme complex

either causes a conformational change at the active site of the β -subunits [8] or preferentially stabilizes a pre-existing $\alpha_2\beta_2$ enzyme conformation [7,8]. Likewise, removal of the α -subunits from the $\alpha_2\beta_2$ enzyme complex is thought to be associated with a conformational change which changes both the reaction specificity and the catalytic efficiency of the β -subunits [3,9,10].

Using an NMR kinetic technique we have shown that when tryptophan synthase binds glycine at pH 7.0 it preferentially catalyses the exchange of the pro-2*R* proton of glycine 380 times faster than that of the pro-2*S* proton [11]. As glycine is not a substrate of tryptophan synthase it is not metabolized by the enzyme and so it can be used to quantify how the α -subunits and allosteric effectors affect the stereospecificity and catalytic efficiency of the exchange of α -protons. However, most studies on tryptophan synthase have been carried out at pH 7.8 [2,3,7,12,13] and so in the present work we have studied the exchange of the α -protons of glycine at pH 7.8. We show that our earlier determinations of the exchange rates of the slowly exchanged pro-2*S* protons were incorrect because we assumed that the apparent binding constants for the exchange of the pro-2*R* and pro-2*S* protons of glycine were the same. In the present study the effect of glycine concentration on the tryptophan synthase-catalysed exchange rates of both the rapidly exchanged pro-2*R* proton and the slowly exchanged pro-2*S* proton of glycine have been determined. The stereospecificities of the first- and second-order hydrogen–deuterium exchange reactions catalysed by the $\alpha_2\beta_2$ complex at pH 7.0 and 7.8 have been quantified. We have also shown how the α -subunits and the allosteric effector DL- α -glycerol 3-phosphate affect the catalytic efficiency and stereospecificity of the tryptophan synthase-catalysed exchange of the α -protons of glycine.

MATERIALS AND METHODS

Materials

[2-¹³C]Glycine (99 atom %) and ²H₂O (99.8 atom %) were obtained from Aldrich Chemical Co., Gillingham, Dorset, U.K.

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All other chemicals used were obtained from Sigma Chemical Co., Poole, Dorset, U.K.

Isolation and assay of tryptophan synthase

The tryptophan synthase $\alpha_2\beta_2$ complex from *Salmonella typhimurium* was purified from the *Escherichia coli* strain CB149 that lacks the *trp* operon and harbours the high-copy plasmid pSTB7 containing the wild-type *trp A* and *trpB* genes from *S. typhimurium* (kindly provided by Dr. Edith Wilson Miles, National Institutes of Health, Bethesda, MD, U.S.A.), as described by Miles et al. [14].

Tryptophan synthase activity was determined by following the conversion of indole and serine to tryptophan using $\Delta\epsilon_{290} = 1850 \text{ M}^{-1}\cdot\text{cm}^{-1}$ [15,16], as described in [12]. The active site concentration (one active site/ β -subunit) of the $\alpha_2\beta_2$ enzyme complex was determined assuming that the pure enzyme had a maximum specific activity of $6.5 \mu\text{mol}/\text{min}$ per mg [12] and that the M_r values of the α - and β -subunits are 29000 and 43000 respectively [17].

Isolation and assay of the α - and β -subunits from intact $\alpha_2\beta_2$ tryptophan synthase

The α -subunit was isolated from the $\alpha_2\beta_2$ enzyme complex by acid precipitation of the β -subunit at pH 4.5 [12,18]. The β_2 -subunit was isolated from the $\alpha_2\beta_2$ enzyme complex by heat denaturation at 63°C of the α -subunit at pH 7.8 [12,19].

The concentrations of the α - and β -subunits were determined using ϵ_{280} values of $12760 \text{ M}^{-1}\cdot\text{cm}^{-1}$ [20] and $27950 \text{ M}^{-1}\cdot\text{cm}^{-1}$ [21] respectively. The serine deaminase activity of the β -subunits was followed spectrophotometrically by reducing the pyruvate produced from L-serine using NADH as described by Crawford and Ito [22]. The concentration of the functional active sites of the β -subunits was determined by assuming that the maximum specific activity for serine deamination by fully active β -subunits is $0.79 \pm 0.08 \mu\text{mol}/\text{min}$ per mg (see the Results and discussion section).

NMR samples

All solutions were made up in 99.8 atom % $^2\text{H}_2\text{O}$. Freeze-dried samples of the $\alpha_2\beta_2$ enzyme complex and samples of the α - and β -subunits were made up in solutions containing 99.8 atom % $^2\text{H}_2\text{O}$. For ^{13}C -NMR studies using $[2-^{13}\text{C}]\text{glycine}$ the enzyme-catalysed exchange reactions were initiated by adding 0.015–0.200 ml of enzyme in buffer to give a final volume of 1 ml. The accumulation of spectra occurred 4–7 min after addition of the enzyme. When the NMR observations were completed, the sample's temperature, pH and catalytic activity were determined. Except when stated otherwise, all samples were in 0.05 M potassium phosphate buffer. Sample temperatures were $27 \pm 1^\circ\text{C}$, and no loss of enzyme activity was detected during the experiments. pH values were determined at the end of the observations as described in [11].

NMR spectra

Spectra were recorded by using a Bruker WP80 wide-bore NMR spectrometer operating at 20.115 MHz for ^{13}C nuclei. Sample size was 1 ml in 10 mm-diameter sample tubes. The experimental conditions used were: 2000 time-domain data points; 1.27 s acquisition time, spectral width 40 p.p.m.; 0.8 Hz exponential weighting factor. The spin lattice relaxation times of $[2-^{13}\text{C}]\text{glycine}$ and $[2-^{13}\text{C}, ^2\text{H}_2]\text{glycine}$ were 4.5 s and 42 s respectively. When following the exchange of the rapidly exchanged pro-2R proton of $[2-^{13}\text{C}]\text{glycine}$, 1 W broadband proton decoupling and

a 41° pulse were used. Broadband proton decoupling was not used when following the exchange of the slowly exchanged pro-2S proton of $[2-^{13}\text{C}]\text{glycine}$; in this case a 14° pulse was used.

RESULTS AND DISCUSSION

Determination of the maximum specific activity of the β -subunits of tryptophan synthase from *S. typhimurium*

The concentration of fully active β -subunits was estimated by adding excess α -subunits and determining the amount of fully active $\alpha_2\beta_2$ enzyme complex formed by following the rate of tryptophan synthesis as described in the Materials and methods section. Using this procedure we estimated that our samples of freshly prepared β -subunits were 93.6 % fully active. The maximum specific activity for serine deamination was estimated to be $0.79 \pm 0.08 \mu\text{mol}/\text{min}$ per mg based on the amount of fully active β -subunits present in our samples of freshly prepared β -subunits. This value of the maximum specific activity for serine deamination by the β -subunits of tryptophan synthase from *S. typhimurium* is similar to the value of $0.695 \mu\text{mol}/\text{min}$ per mg obtained for β -subunits from the tryptophan synthase from *Escherichia coli* [12,22].

Kinetic analysis of the hydrogen–deuterium exchange reactions catalysed by tryptophan synthase

Scheme 1 represents the minimal kinetic scheme for the tryptophan synthase-catalysed exchange of the α -proton of amino acids, and eqn. (1) is the differential rate equation for hydrogen–deuterium exchange according to Scheme 1 [11].

$$+\frac{d[\text{SD}]}{dt} = \frac{k_a[\text{E}]_0([\text{SD}]_\infty - [\text{SD}])}{[\text{S}]_t + K + C[\text{SD}]} \quad (1)$$

For initial rate measurements the term $C[\text{SD}]$ is negligible and k_{obs} values can be determined using eqn. (2) [11].

$$+\frac{d[\text{SD}]}{dt} = \frac{k_a[\text{E}]_0[\text{SD}]_\infty}{[\text{S}]_t + K} \quad (2)$$

The exchange of the slowly exchanged pro-2S proton of glycine catalysed by the $\alpha_2\beta_2$ enzyme complex at pH 7.0 and pH 7.8, as well as the exchange of the pro-2R protons of glycine by the β -subunits at pH 7.0, were all followed by initial-rate measurements. The values of C obtained by analysing the exchange rates of the rapidly exchanged pro-2R protons of glycine at pH 7.8 (see the Materials and methods section) using the integrated form of

Scheme 1 Minimal kinetic scheme for the tryptophan synthase-catalysed exchange of the α -proton of amino acids

E, SH and SD are the free enzyme, the non-deuterated substrate and the deuterated substrate respectively. ESH and ESD are Schiff bases formed between pyridoxal 5'-phosphate and the α -amino group of the non-deuterated and deuterated amino acids respectively. EQ is a quinonoid intermediate resulting from the loss of either a proton from ESH or a deuteron from ESD.

Table 1 Kinetic parameters for the exchange of the α -protons of [2- ^{13}C]glycineAbbreviations: GP, DL- α -glycerol 3-phosphate; ND, not determined.

pH	Enzyme	GP(mM)	Pro-2R proton			Pro-2S proton			Stereospecificity	
			$k_a(\text{s}^{-1})$	$K(\text{M})$	$k_a/K(\text{M}^{-1}\cdot\text{s}^{-1})$	$k_a(\text{s}^{-1})$	$K(\text{M})$	$k_a/K(\text{M}^{-1}\cdot\text{s}^{-1})$	k_{ar}/k_{as}	$(k_a/K)_r/(k_a/K)_s$
7.0*	$\alpha_2\beta_2$	0	1.7	0.054	32.0	0.0069	0.130	0.053	250	600
7.0	β_2	0	0.13	0.190	0.68	ND	ND	ND	ND	ND
7.8	β_2	0	2.0	0.092	21.0	0.0025	0.350	0.0071	800	3000
7.8	$\alpha_2\beta_2$	0	3.2	0.054	59.0	0.011	0.041	0.27	290	220
7.8	$\alpha_2\beta_2$	50	1.8	0.024	75.0	0.011	0.0032	3.4	160	22

* Data from Malthouse et al. [12].

eqn. (1) [11] were all < 0.1 , and so $k_{\text{obs.}}\{k_{\text{obs.}} = k_a[E]_0/([S]_i + K)\}$ values were obtained using the integrated form of eqn. (2).

Determination of the hydrogen–deuterium exchange rates of the α -protons of glycine catalysed by the isolated β -subunits and the $\alpha_2\beta_2$ enzyme complex of tryptophan synthase at pH 7.0 and pH 7.8

The β -subunits catalysed the exchange of the α -protons of glycine at pH 7.0 and 7.8. Using the procedure of Malthouse et al. [11] we found that, like the $\alpha_2\beta_2$ enzyme complex, the β -subunits preferentially catalysed the exchange of the pro-2R proton of glycine. At pH 7.8 the first- and second-order rate constants for the exchange of the rapidly exchanged pro-2R protons of glycine were only 1.6- and 2.8-fold greater when catalysed by the $\alpha_2\beta_2$ enzyme complex than when catalysed by the β -subunits alone (Table 1). However, some of our preparations of tryptophan synthase contained a 15–25% molar excess of β -subunits over α -subunits. Therefore we added α -subunits (50% molar excess of α -subunits over β -subunits) to these enzyme preparations to ensure that we only measured exchange due to the $\alpha_2\beta_2$ enzyme complex. The enzyme preparations used in the earlier studies of the tryptophan synthase-catalysed exchange of the α -protons of glycine at pH 7.0 [11] had a 15% molar excess of β -subunits. However, at pH 7.0, the first- and second-order exchange rates of the rapidly exchanged pro-2R protons of glycine are at least 10-fold faster when catalysed by the $\alpha_2\beta_2$ enzyme complex than when catalysed by β -subunits (Table 1). Therefore at pH 7.0 the 15% molar excess of β -subunits does not make a significant contribution to the observed exchange rate for the $\alpha_2\beta_2$ enzyme complex, and so the previously reported [11] exchange rates of the rapidly exchanged pro-2R protons of glycine at pH 7.0 are correct. When determining the rate at which the $\alpha_2\beta_2$ enzyme complex catalysed the exchange of the α -protons of glycine at pH 7.8 (Table 1) a 50% molar excess of α -subunits over β -subunits was used to ensure that the β -subunits did not make a significant contribution to the observed exchange rate.

When tryptophan synthase was incubated with [2R- ^2H , ^{13}C]glycine at pH 7.8 in 99.98 atom% H_2O the pro-2R deuteron was rapidly exchanged for a proton, and when tryptophan synthase was incubated with [2S- ^2H , ^{13}C]glycine in 99.8 atom% H_2O the pro-2R proton was rapidly exchanged to give [^2H , ^{13}C]glycine. However, if tryptophan synthase was incubated with [2S- ^2H , ^{13}C]glycine in 99.98 atom% H_2O the pro-2S deuteron was not rapidly exchanged. Therefore there is no inversion of stereochemistry when [2R- ^2H , ^{13}C]glycine and [2S- ^2H , ^{13}C]glycine are incubated with tryptophan synthase.

Figure 1 Effect of the concentration of [2- ^{13}C]glycine on the hydrogen–deuterium exchange rate of the pro-2S proton of [2- ^{13}C]glycine catalysed by the $\alpha_2\beta_2$ enzyme complex of tryptophan synthase at pH 7.0

[2- ^{13}C]Glycine concentrations of 0.05–0.4 M and tryptophan synthase active site concentrations of 22.0–45.8 μM were used. All samples contained 0.05 M potassium phosphate buffer at pH 7.0. The continuous line was calculated using eqn. (2) and the fitted parameters $k_a = 0.0069 \pm 0.0001 \text{ s}^{-1}$, $K = 0.128 \pm 0.0032 \text{ M}$ and $k_a/K = 0.0528 \pm 0.0002 \text{ M}^{-1}\cdot\text{s}^{-1}$.

Determination of the degree of stereospecificity of the tryptophan synthase-catalysed exchange of the α -protons of glycine at pH 7.0 and pH 7.8

In our earlier studies [11] of the tryptophan synthase ($\alpha_2\beta_2$ complex)-catalysed exchange of the α -protons of glycine, we assumed that the apparent binding constants for the exchange of the pro-2R and pro-2S protons of glycine were the same. However, the apparent binding constant (K) is a complex assembly (see eqn. 4) of rate constants (k_1 – k_6 in Scheme 1). Therefore, as the values of these rate constants must be different for the slowly exchanged pro-2S protons and the rapidly exchanged pro-2R protons, then the apparent binding constants for these two processes must be different, and so our assumption was incorrect. In the present study we have used initial-rate methods to determine the substrate-concentration-dependence of $k_{\text{obs.}}/[E]$ for the exchange of the slowly exchanged pro-2S proton of glycine at pH 7.0 (Figure 1). With the $\alpha_2\beta_2$ enzyme

complex at pH 7.0 (Figure 1), the apparent binding constant ($K = 0.13$ M) for the slowly exchanged pro-2S proton is approx. 2.5-fold larger than that for the rapidly exchanged pro-2R proton (Table 1), and the value of k_a ($k_a = 0.0069$ s⁻¹; Table 1) is larger than our earlier estimate of k_a (0.0044 s⁻¹ [11]). Therefore we estimate that the first-order rate constant due to the tryptophan synthase-catalysed exchange of the pro-2R proton of glycine is approx. 250 times higher than that of the pro-2S of glycine at pH 7.0 (Table 1), which is lower than our earlier incorrect estimate of a 380-fold difference at this pH [11]. Due to weaker binding during the exchange of the slowly exchanged pro-2S proton at pH 7.0, the stereospecificity of the second-order reaction is 2.4-fold greater than that of the first-order reaction (Table 1).

As most studies to date on tryptophan synthase have been carried out at pH 7.8 [2,3,7,12,13], all of our current studies on the tryptophan synthase-catalysed exchange of the α -protons of glycine have been carried out at pH 7.8 (Table 1). With the $\alpha_2\beta_2$ enzyme complex, increasing the pH from 7.0 to 7.8 increases the k_a for the rapidly exchanged pro-2R proton 1.9-fold but it has no significant effect on the binding of glycine to the $\alpha_2\beta_2$ enzyme complex (Table 1). There is a 1.6-fold increase in the exchange rate (k_a) for the slowly exchanged pro-2S proton, and the apparent binding constant (K) is decreased by a factor of 3.2 (Table 1). Therefore, while increasing the pH from 7.0 to 7.8 decreases the stereospecificity of the second-order exchange rate (k_a/K) 2.7-fold, it does not have a significant effect on the stereospecificity of the first-order exchange rate (k_a) (Table 1).

Effect of α -subunits and 50 mM DL- α -glycerol 3-phosphate on the exchange rates and stereospecificity of the exchange of the α -protons of glycine catalysed by tryptophan synthase at pH 7.8

At pH 7.8 the stereospecificities of the first-order and second-order exchange reactions catalysed by the β -subunits were 2.8- and 13.6-fold larger respectively than those catalysed by the $\alpha_2\beta_2$ enzyme complex (Table 1). The α -subunits decrease the stereospecificity of the exchange of the pro-2R proton by preferentially promoting the exchange of the slowly exchanged pro-2S proton of glycine (Table 1).

DL- α -Glycerol 3-phosphate caused a 1.8-fold decrease in the stereospecificity of the first-order exchange rate and a 10-fold decrease in the stereospecificity of the second-order exchange rate (Table 1). The decrease in the stereospecificity of the first-order exchange rate was due to a decrease in the first-order exchange rate of the rapidly exchanged pro-2R proton of glycine (Table 1). The larger 10-fold decrease in the stereospecificity of the second-order exchange rate was mainly due to an increase in the second-order exchange rate of the slowly exchanged pro-2S proton of glycine (Table 1). The decreases in the first- (1.8-fold) and second- (1.3-fold) order exchange rates of the rapidly exchanged pro-2R proton due to DL- α -glycerol 3-phosphate (Table 1) are similar in magnitude to the decreases in $V_{\max}$ (3.3-fold) and $V_{\max}/K_m$ (1.4-fold) caused by DL- α -glycerol 3-phosphate being present during tryptophan synthesis [23]. The large increase in the second-order exchange rate of the slowly exchanged pro-2S proton of glycine results from DL- α -glycerol 3-phosphate causing a 12.8-fold decrease in the apparent binding constant (K) for the slowly exchanged pro-2S proton of glycine (Table 1). Spectrophotometric studies have shown that 50 mM DL- α -glycerol 3-phosphate decreases the apparent K_d for the binding of glycine, L-histidine and tryptophan [7,8]. By following changes in the absorbance at 410 nm due to the formation of an external aldimine with glycine, we have found that adding 50 mM DL- α -glycerol 3-phosphate decreases the apparent K_d for

glycine 3.6-fold from 7.2 ± 0.4 mM to 2.0 ± 0.2 mM at pH 7.8. A similar 4.75-fold decrease in the K_d value for glycine by 51.3 mM DL- α -glycerol 3-phosphate has been reported [8]. The apparent K_d values for glycine determined from the spectrophotometric studies with the $\alpha_2\beta_2$ enzyme complex are smaller than those determined from the exchange rates of its pro-2R and pro-2S protons (Table 1). Such differences are expected, since the apparent K_d obtained from the spectrophotometric studies (eqn. 3) consists of a different assembly of rate constants from the apparent K_d for the hydrogen-deuterium exchange studies (eqn. 4) [11]. Eqn. (3) can be derived by considering the equilibrium between E, ESH and EQ in Scheme 1, or from eqn. (4) by setting $k_5 = k_6 = k_7 = k_8 = 0$, as there was no hydrogen-deuterium exchange in the spectrophotometric studies.

$$K = \frac{k_2 k_4 [H]}{k_1 k_3 + k_1 k_4 [H]} \quad (3)$$

$$K = \frac{k_2 (k_4 k_6 [H] + k_2 k_4 [H] + k_2 k_5 [D] + k_3 k_5 [D])}{k_1 (k_2 k_3 + k_3 k_6 + k_2 k_4 [H] + k_4 k_6 [H] + k_2 k_5 [D] + k_3 k_5 [D])} \quad (4)$$

It has been proposed that the β -subunits of tryptophan synthase can exist in either an open or closed form [9]. According to this proposal the α -subunits stabilize the closed form of the β -subunits, which tends to exclude water from the active site and so produces a non-polar environment. In the absence of the α -subunits the open form of the β -subunits is favoured and this ensures solvent accessibility and a polar environment at the active site. It has been suggested that when α -subunit ligands such as DL- α -glycerol 3-phosphate bind to the α -subunits of the $\alpha_2\beta_2$ enzyme complex, they may cause a change in the β active site so that it is even more 'closed' than in the free $\alpha_2\beta_2$ enzyme complex [24]. We would expect a change from a polar to a non-polar environment, coupled with a decrease in solvent accessibility, to reduce the exchange rate of α -protons. The fact that the α -subunits and DL- α -glycerol 3-phosphate mainly increase exchange rates (Table 1) does not support the hypothesis [9,24] that they cause a decrease in the polarity and solvent accessibility of the β active site.

When the isolated β -subunits of tryptophan synthase catalyse the β -elimination of L-serine to give pyruvate and ammonia, a single suprafacial base is thought to catalyse the exchange of the α -proton of L-serine to the β -carbon of L-serine [25]. However, because the slowly exchanged pro-2S proton of glycine occupies the same position as the β -carbon of L-serine, it has been suggested that this base may be able to catalyse the exchange of both of the α -protons of glycine in the $\alpha_2\beta_2$ enzyme complex [11]. It has been proposed [10] that the α -subunits promote the removal of the β -hydroxy group of L-serine by producing a conformational change in the β -subunits which moves a catalytic group into the correct orientation to facilitate protonation of the hydroxy group. The 12.6-fold increase in the second-order exchange rate of the slowly exchanged pro-2S proton of glycine in the presence of α -subunits (Table 1) supports this proposal. DL- α -Glycerol 3-phosphate also produces a large (38-fold) increase in the second-order exchange rate of the slowly exchanged pro-2S proton of glycine. Therefore DL- α -glycerol 3-phosphate and the α -subunits cause conformational changes which appear to be functionally equivalent.

If the α -carboxylate group of glycine can occupy the binding site for the R-group of L-serine, then the pro-2S proton could occupy the same position as that occupied by the pro-2R proton when the α -carboxylate group of glycine is in its normal binding site. In this case the same base could catalyse the exchange of each proton with equal efficiency and the stereospecificity of

exchange should be determined by the relative populations of these two binding modes in first-order reactions [11] or by the relative populations of these two reaction modes in second-order reactions.

Adding both α -subunits and DL- α -glycerol 3-phosphate to the β -subunits causes a 5-fold decrease in the stereospecificity of the first-order exchange rate and a 136-fold decrease in the stereospecificity of the second-order exchange rate (Table 1). Consequently, while both the α -subunits and DL- α -glycerol 3-phosphate cause a large decrease in the stereospecificity of the free enzyme, the binding of glycine produces a considerable reduction in this decrease in stereospecificity. Therefore for this exchange reaction the allosteric effects due to the β -subunits binding α -subunits and the α -subunits of the $\alpha_2\beta_2$ enzyme complex binding DL- α -glycerol 3-phosphate are maximal with the free enzyme. We conclude that stereospecific exchange of the pro-2*R* proton of glycine is best achieved using the β -subunits of tryptophan synthase.

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REFERENCES

- 1 Yanofsky, C. and Crawford, I. P. (1972) *Enzymes* 3rd Ed. **3**, 1–31
- 2 Miles, E. W. (1979) *Adv. Enzymol. Relat. Areas Mol. Biol.* **49**, 127–186
- 3 Miles, E. W. (1991) *Adv. Enzymol. Relat. Areas Mol. Biol.* **64**, 93–172
- 4 Brzovic, P. S., Sawa, Y., Hyde, C. C., Miles, E. W. and Dunn, M. F. (1992) *J. Biol. Chem.* **267**, 13028–13038
- 5 Dunn, M. F., Aguilar, V., Brzovic, P., Drewe, W. F., Jr., Houben, K. F., Leja, C. A. and Roy, M. (1990) *Biochemistry* **29**, 8598–8607
- 6 Heyn, M. P. and Weischet, W. O. (1975) *Biochemistry* **14**, 2962–2968
- 7 Houben, K. F. and Dunn, M. F. (1990) *Biochemistry* **29**, 2421–2429
- 8 Dunn, M. F., Aguilar, V., Drewe, W. F., Jr., Houben, K., Robustelli, B. and Roy, M. (1987) *Indian J. Biochem. Biophys.* **24**, 44–51
- 9 Ahmed, S. A., Ruvinov, S. B., Kayastha, A. M. and Miles, E. W. (1991) *J. Biol. Chem.* **266**, 21548–21557
- 10 Yang, X. J. and Miles, E. W. (1992) *J. Biol. Chem.* **267**, 7520–7528
- 11 Malthouse, J. P. G., Milne, J. J. and Gariani, L. S. (1991) *Biochem. J.* **274**, 807–812
- 12 Miles, E. W., Bauerle, R. and Ahmed, S. A. (1987) *Methods Enzymol.* **142**, 398–414
- 13 Lu, Z., Nagata, S., McPhie, P. and Miles, E. W. (1993) *J. Biol. Chem.* **268**, 8727–8734
- 14 Miles, E. W., Kawasaki, H., Ahmed, S. A., Morita, H., Morita, H. and Nagata, N. (1989) *J. Biol. Chem.* **264**, 6280–6287
- 15 Faeder, E. J. and Hammes, G. G. (1970) *Biochemistry* **9**, 4043–4049
- 16 Higgins, W., Fairwell, T. and Miles, E. W. (1979) *Biochemistry* **18**, 4827–4835
- 17 Hyde, C. C., Ahmed, S. A., Padlan, E. A., Miles, E. W. and Davies, D. R. (1988) *J. Biol. Chem.* **363**, 17857–17871
- 18 Henning, U., Helinski, D. R., Chao, F. C. and Yanofsky, C. (1962) *J. Biol. Chem.* **237**, 1523–1530
- 19 Högberg-Raubaud, A. and Goldberg, M. E. (1977) *Biochemistry* **16**, 4014–4020
- 20 Adachi, O., Kohn, L. D. and Miles, E. W. (1974) *J. Biol. Chem.* **249**, 7756–7763
- 21 Hathaway, G. M. and Crawford, I. P. (1970) *Biochemistry* **9**, 1801–1808
- 22 Crawford, I. P. and Ito, J. (1964) *Proc. Natl. Acad. Sci. U.S.A.* **51**, 390–397
- 23 Miles, E. W. (1991) *J. Biol. Chem.* **266**, 10715–10718
- 24 Strambini, G. B., Cioni, P., Peracchi, A. and Mozzarelli, A. (1992) *Biochemistry* **31**, 7535–7542
- 25 Tsai, M. D., Schleicher, E., Potts, R., Skye, G. E. and Floss, H. G. (1978) *J. Biol. Chem.* **253**, 5344–5349