Inhibition of Tryptophan Synthase by (1-Fluorovinyl)glycine†

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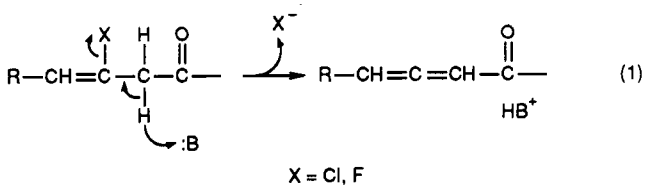
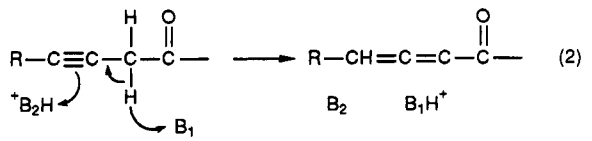
ABSTRACT: Tryptophan synthase ($\alpha_2\beta_2$ complex) from *Salmonella typhimurium* catalyzes the formation of tryptophan from serine and indole. The enzyme is inactivated by (1-fluorovinyl)glycine. Concomitant with enzyme inactivation, the absorbance at 485 nm increases, indicating covalent modification of pyridoxal 5′-phosphate. It is proposed that inactivation involves elimination of HF to form an allene, which reacts with a nucleophile at the active site. The inactivation reaction involves an α,β-elimination, as does the formation of tryptophan from indole and serine. The inactivation occurs with $k_{in} > 1.3\ s^{-1}$, which is very close to k_{cat} (6.4 s^{-1}) for the formation of tryptophan from indole and serine. The inactive enzyme ($\alpha_2\beta_2$) regains activity with $k_{off} = 0.005\ min^{-1}$. Aminoacetone is formed during reactivation, and pyridoxal 5′-phosphate is regenerated. Tryptophan synthase also catalyzes the dehydration of serine, or 3-fluoroalanine, to pyruvate in the absence of indole. This reaction involves an α,β-elimination and the intermediate formation of an aminoacrylate adduct with pyridoxal 5′-phosphate, as does the formation of tryptophan. Pyruvate formation proceeds at less than 5% the rate of tryptophan formation. With [2-^{2}H]serine an isotope effect ($^DV_{max} = 1.5$) is observed. We propose that pyruvate formation is limited by the rate of hydration of the aminoacrylate intermediate and the rate of the abstraction of the serine α-hydrogen.

We have proposed (Abeles & Maycock, 1976) a principal of suicide inactivation which involves the reaction



(1)

The elimination of HX leads to the formation of a conjugated allene which can react with a nucleophile at the active site of an enzyme. A conjugated allene can also be obtained from an acetylenic compound, such as the classic suicide inactivator of eq 2 (Bloch, 1971; Morisaki & Bloch, 1972):

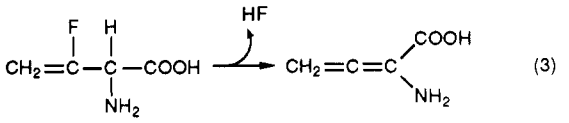


(2)

Halovinyl inactivators (eq 1) have two advantages over acetylenic inactivators (eq 2): (1) The halovinyl inactivator does not require protonation of the γ-position. The acetylenic inactivator requires protonation of the γ-position, and a proton donor may not be available at the active site of some enzymes. (2) Halovinyl analogs of amino acids can be synthesized. The synthesis of β,γ-acetylenic amino acids has, so far, not been achieved.

Inactivators containing a halovinyl group have been used to inactivate several enzymes. Plasma amine oxidase is inactivated by 2-chloroallylamine (Abeles & Maycock, 1976), and (1-fluorovinyl)glycine inactivates alanine racemase (Thornberry et al., 1991). We now examine the effect of (1-fluorovinyl)glycine on tryptophan synthase. Tryptophan synthase (EC 4.2.1.20) from bacteria is an $\alpha_2\beta_2$ complex which catalyzes the synthesis of L-tryptophan from L-serine and 3-indolyl-D-glycerol 3′-phosphate (Miles, 1979, 1986, 1991; Yanofsky & Crawford, 1972; Miles et al., 1987). The α subunits catalyze the cleavage of 3-indolyl-D-glycerol 3′-phosphate to D-glyceraldehyde 3-phosphate and indole. The β subunits catalyze the condensation of indole with L-serine to form L-tryptophan. Pyridoxal 5′-phosphate (PLP) is employed as a cofactor in this reaction (Scheme I).

The reaction of the tryptophan synthase ($\alpha_2\beta_2$) complex with L-serine (Scheme II) involves formation of an enzyme–substrate Schiff base **1**, abstraction of the α-proton of L-serine to produce a quinonoid intermediate **2** and loss of hydroxide ion to form the aminoacrylate intermediate **3**. Indole then adds to the aminoacrylate to give tryptophan as the product **4**. In the case of (1-fluorovinyl)glycine (eq 3), loss of HF from (1-fluorovinyl)glycine is expected to form an allene, a reactive electrophile potentially capable of covalently inactivating the enzyme.



(3)

Tryptophan synthase was chosen as a target enzyme because of its unique properties. Tryptophan synthase ($\alpha_2\beta_2$) not only catalyzes the substitution of the β-OH of serine by indole but also catalyzes the slow dehydration of serine to pyruvate (Miles, 1991). The substitution reaction and the dehydration involve a common intermediate, the pyridoxal adduct of aminoacrylate (compound **3**, Scheme II). Tryptophan synthase is "β-replacement specific"; other enzymes, for instance cysteine β-lyase (Kredich et al., 1973), catalyze both reactions at a similar rate. With tryptophan synthase some mechanism exists whereby the rate of dehydration of serine to pyruvate is greatly decreased in the absence of indole. A number of possibilities can be envisioned, and have been proposed (Miles, 1986), whereby the rate of serine dehydration is reduced in the absence of indole. One means of accomplishing this is by reducing the

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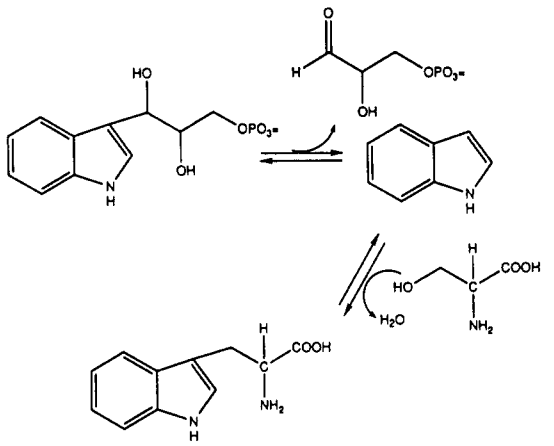
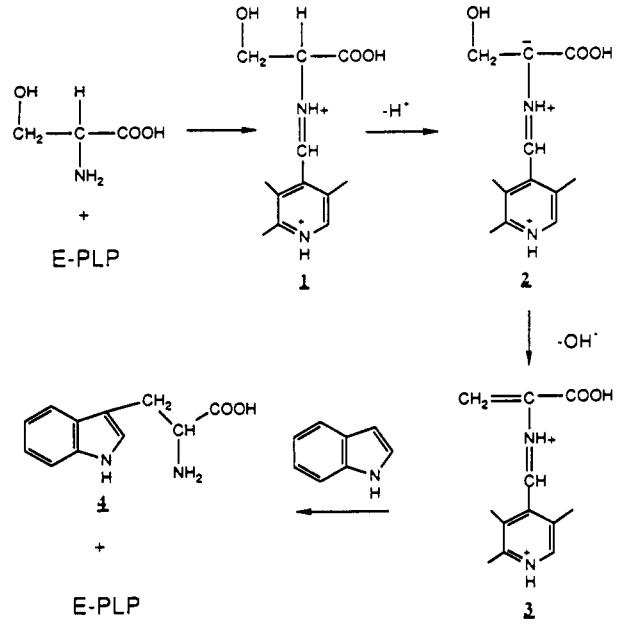
Scheme I: Catalytic Reaction of Tryptophan Synthase



Scheme II: Reaction Mechanism of Tryptophan Synthase



rate of any of the steps leading to aminoacrylate. If a step leading to the formation of aminoacrylate is slow in the absence of indole, then the addition of indole must bring about a conformational change in either the substrate or the enzyme. Another possibility is that the hydrolysis of the aminoacrylate–pyridoxal adduct proceeds slowly, while the reaction of this intermediate with indole is fast. This mechanism requires no indole-induced conformational change. It is also possible that both mechanisms are involved. The reaction of tryptophan synthase with (1-fluorovinyl)glycine offers an opportunity to examine an elimination reaction which does not involve the aminoacrylate.

EXPERIMENTAL PROCEDURES

Materials. E. coli strain CB149 harboring a high-copy plasmid (pSTB7) carrying wild-type *Salmonella typhimurium trpA* and *trpB* genes, were a gift from E. W. Miles. The cells were cultivated and the $\alpha_2\beta_2$ and β_2 enzymes isolated as previously described (Miles et al., 1989, 1987). The holo-β_2 subunit was isolated from the holo-$\alpha_2\beta_2$ complex by heat denaturation of the α subunit (Miles et al., 1987).

Enzyme Assays. The α activity was assayed by coupling to glyceraldehyde-3-phosphate dehydrogenase. The β reaction was assayed by means of the difference in absorption between indole and L-tryptophan at 290 nm ($\Delta\epsilon$ = 1.85 × 10^3 M^{-1} cm^{-1}) (Miles et al., 1987). The tryptophan-formation assay solution contains 20 mM L-serine, 0.1 mM indole, 180 mM NaCl, 0.05 mM PLP, and 100 mM Tris-HCl, pH 7.8. Pyruvate formation was measured with lactate dehydrogenase. All kinetic measurements were performed at 25 °C.

The concentration of the purified enzyme was determined at 278 nm using $A^{1\%}$ = 6.0 for $\alpha_2\beta_2$ and $A^{1\%}$ = 6.5 for β_2 (Miles et al., 1987). 3-Indolyl-D-glycerol 3′-phosphate (Kawasaki et al., 1987), DL-[2-^{2}H]serine (Miles & McPhie, 1974), and 1-aminoacetone (Hepworth, 1973) were synthesized as described previously. DL-(1-Fluorovinyl)glycine was prepared as described elsewhere (Thornberry et al., 1987) except that tetrabutylammonium bromide was substituted for tetraethylammonium bromide in the preparation of the intermediate 2-fluoroacrolein. All experiments were performed with racemic (1-fluorovinyl)glycine.

Inhibition Kinetics. Data were fit to the pseudo-first-order kinetic model of eqs 4a and 4b, where A_t is the absorbance at 485 nm, A_0 and A_f are respectively the initial and final absorbance at 485 nm, and k_{obs} is the apparent pseudo-first-order rate constant.

$$E + I \rightarrow EI \tag{4a}$$

$$A_t = A_f - (A_f - A_0)\exp(-k_{obs}t) \tag{4b}$$

The bimolecular rate constant was calculated from the inactivation rate constants observed at various inhibitor concentrations under pseudo-first-order conditions in the presence of serine. According to eq 5, k_{in}/K_I was determined from the plot of k_{obs} as a function of [I].

$$k_{obs} = k_{in}[I]/[K_I(1 + [S]/K_d)] \tag{5}$$

Reactivation Kinetics. DL-(1-Fluorovinyl)glycine (0.1 mM) was incubated with 7 μM $\alpha_2\beta_2$ complex and then diluted 100-fold into tryptophan-forming assay solutions. Enzyme activity was monitored at 290 nm. The data were fit to eq 6 (Brady & Abeles, 1990) where A_t is the absorbance at 290 nm, V_0 is the initial rate, V_f is the final steady-state rate, and k_{off} is the dissociation rate constant.

$$A_t = V_f t + (V_o - V_f)[1 - \exp(-k_{off}t)]/k_{off} \tag{6}$$

Identification of 1-Aminoacetone. This product was derivatized with ethyl acetoacetate to form 2,4-dimethyl-3-(ethoxycarbonyl)pyrrole. The pyrrole was identified through its mobility in an HPLC procedure (Thornberry et al., 1991) and quantified colorimetrically by means of Erhlich's reagent (Urata & Granick, 1963).

The $\alpha_2\beta_2$ complex (0.01 μmol) and DL-(1-fluorovinyl)glycine (0.03 μmol) were incubated in 60 μL of 100 mM Tris-HCl buffer at pH 7.8 and 23 °C for 5 min and then diluted with 500 μL of additional buffer. After 4 h at 23 °C, the solution was deproteinized by ultrafiltration. The filtrate was treated with 100 μL of a mixture containing 3 M sodium acetate (pH 4.6), ethanol, acetonitrile, and ethyl acetoacetate (5:1:1:1 v/v/v/v). The reaction mixture was incubated at 23 °C for 1 h in a sealed tube. The sample was then applied to a Waters μ-bondpack C18 reverse-phase HPLC column (10-μm particle size, 3.9 mm × 30 cm). The column was eluted with 25 mL of 10 mM potassium phosphate, pH 7.0, containing 10–15% acetonitrile in linear gradient. The effluent was monitored at 270 nm. At a flow rate of 1 mL/min, the pyrrole derivative of aminoacetone chromatographed with a retention time of 21 min.

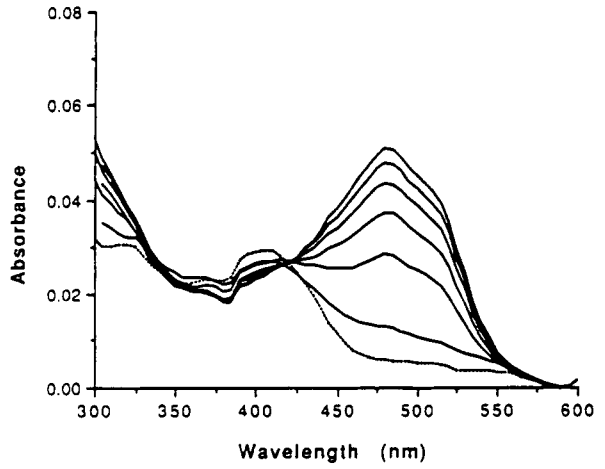


FIGURE 1: Electronic spectrum of enzyme–inhibitor complex. The reaction mixture contained 20 μM DL-(1-fluorovinyl)glycine, 5 μM $\alpha_2\beta_2$, and 100 mM Tris-HCl at pH 7.8 and 25 °C. The inactivation process was followed by recording the spectra at different times (0.1, 1.5, 4, 8, 14, and 25 min) following addition of (1-fluorovinyl)glycine.

The stoichiometry of aminoacetone release from the enzyme–inhibitor complex was determined colorimetrically. Enzyme–inhibitor complex was denatured with 1% trichloroacetic acid, and protein was separated by centrifugation. The pyrrole-forming reaction was carried out at 100 °C for 1 h in a sealed tube, and the pyrrole was determined with Ehrlich's reagent.

Exchange of α-Hydrogen with Solvent. The $\alpha_2\beta_2$-catalyzed hydrogen exchange from H_2O to C-2 of L-serine was measured using [^{3}H]H_2O (63 mCi/mL). The reaction mixtures contained 20 mM L-serine, $\alpha_2\beta_2$ complex (19.5 μM), 100 mM Tris-HCl, and 0.05 mM PLP at pH 7.8 and 25 °C. Reactions were stopped at intervals of 5–60 min by application to 1-mL columns of Dowex 50-2-400 (H^+ form). The columns were washed with water, and [^{3}H]serine was then eluted with 2 N HCl. The radioactive eluate was lyophilized, and [^{3}H]serine was determined by scintillation counting. The purity of [^{3}H]-serine (>95%) was determined by thin-layer chromatography on cellulose with two solvent systems [butanol, acetic acid, and water (4:1:1 v/v/v) or 1-propanol and concentrated NH_4-OH (7:3 v/v)].

Serine Formation from 3-Fluoroalanine. Enzyme ($\alpha_2\beta_2$, 16.5 μM) and DL-3-fluoroalanine (25 mM) were incubated in a solution containing 0.05 mM PLP, 180 mM NaCl, and 100 mM Tris-HCl at 25 °C and pH 7.8. Aliquots of 0.1 mL were withdrawn at intervals and quenched with 1% trichloroacetic acid. After centrifugation the supernatant fluid was adjusted to pH 7.8 with NaOH and assayed for serine by the periodate method (Frisell et al., 1954).

RESULTS

Kinetic Mechanism. When (1-fluorovinyl)glycine was added to a reaction mixture containing indole, serine, and tryptophan synthase ($\alpha_2\beta_2$), the rate of tryptophan formation, as measured by the change in absorbance at 290 nm, gradually decreased. When (1-fluorovinyl)glycine was added to tryptophan synthase ($\alpha_2\beta_2$) (in the absence of substrates), the absorbance at 485 nm increased (Figure 1) as the enzyme suffered inactivation. The absorbance at 485 nm changed with a bimolecular rate constant $k_{in}/K_i = 4.9 \times 10^4$ M^{-1} min^{-1}. Since the loss of catalytic activity is fast, the rate of inactivation was determined in the presence of serine, which reduces the rate of inactivation. The inactivation of tryptophan synthase ($\alpha_2\beta_2$) by (1-fluorovinyl)glycine (in the presence of serine as a competitive inhibitor) is a pseudo-first-order process. As

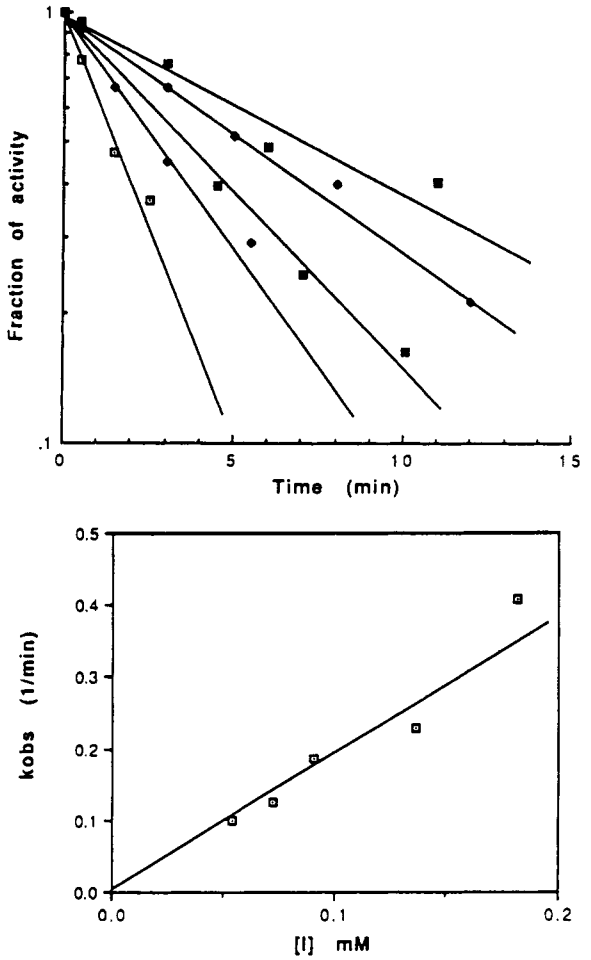


FIGURE 2: Inactivation kinetics. (Top) In the presence of 0.182 mM L-serine [as a competitive inhibitor of (1-fluorovinyl)glycine], the $\alpha_2\beta_2$ enzyme (2.86 μM) and different concentrations of (1-fluorovinyl)glycine (54–182 μM) were incubated in 100 mM Tris-HCl at pH 7.8 and 25 °C. Aliquots were diluted 100-fold into tryptophan-forming assay solutions at indicated times. (Bottom) Replot of the observed inactivation rate constants versus concentration of (1-fluorovinyl)glycine.

shown in Figure 2, k_{obs} is a linear function of [I] at inhibitor concentrations up to at least 0.18 mM. Taking the K_d of serine to be 11 μM, the second-order rate constant for the inactivation is 4.1×10^4 M^{-1} min^{-1}. The absorbance change and inactivation thus occur synchronously. The K_d of serine was determined by a published procedure (Lane & Kirschner, 1983).

Titration of Tryptophan Synthase with (1-Fluorovinyl)-glycine. When the $\alpha_2\beta_2$ complex was incubated with various amounts of DL-(1-fluorovinyl)glycine at pH 7.8, the remaining activity was proportional to the molar ratio of (1-fluorovinyl)-glycine to β subunit. As is apparent in Figure 3, 1.9 ± 0.2 molecules of DL-(1-fluorovinyl)glycine were required to inactivate each β monomer. Presumably, 1.0 equiv of the L-isomer is consumed for each β monomer inactivated.

Fluoride Release. Upon incubation of (1-fluorovinyl)-glycine with tryptophan synthase ($\alpha_2\beta_2$) for 1 h and removal of the protein by ultrafiltration, only one new fluorine species (which was not observed in the control sample) was detected by means of ^{19}F NMR spectroscopy. The new species was identified as inorganic fluoride by its chemical shift of −121.4 ppm.

Recovery of Catalytic Activity and Identification of 1-Aminoacetone. Enzyme ($\alpha_2\beta_2$), inactivated with (1-fluorovinyl)glycine, was diluted 100-fold into the tryptophan-

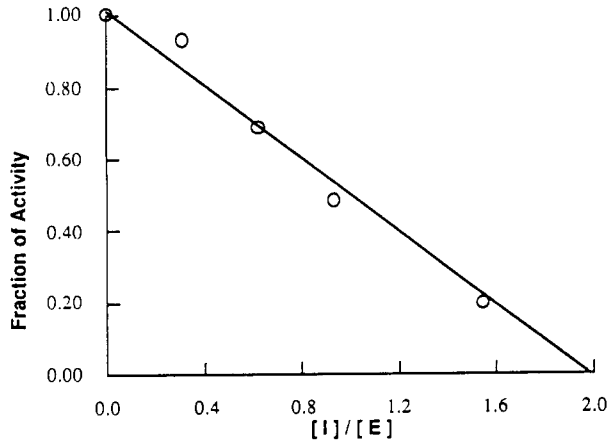


FIGURE 3: Titration of tryptophan synthase ($\alpha_2\beta_2$) with DL-(1-fluorovinyl)glycine. Enzyme ($\alpha_2\beta_2$, 6.8 μM) and DL-(1-fluorovinyl)-glycine (16–28 μM) were incubated for 5 min in 100 mM Tris-HCl at pH 7.8 and 23 °C, and aliquots were assayed for enzyme activity.

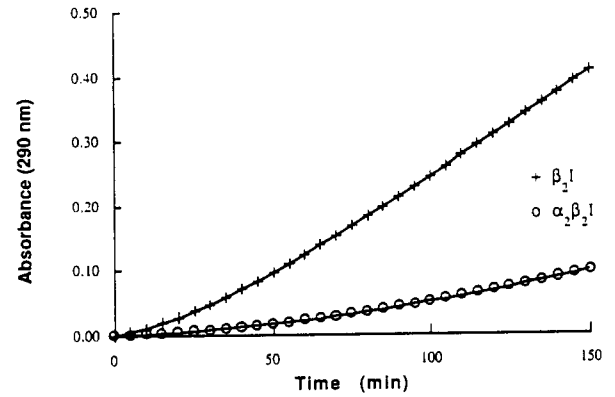


FIGURE 4: Reactivation of enzyme–inhibitor complex upon dilution into substrate solution. DL-(1-Fluorovinyl)glycine (0.1 mM) was incubated with 7 μM $\alpha_2\beta_2$, and then diluted 100-fold into tryptophan-forming assay solutions.

forming assay mixture. Enzyme activity gradually recovered in a first-order process (k_{off} = 0.005 min^{-1}). A similar experiment was done with the β_2 subunit. In that case, k_{off} = 0.02 min^{-1} (Figure 4). Experiments were carried out to determine the structure of the compound released during the reactivation process. When the $\alpha_2\beta_2$ enzyme inhibitor complex (0.02 mM) was maintained for 4 h in 100 mM Tris-HCl buffer at 7.8 and 23 °C, 60% of the catalytic activity was regained. The reaction mixture was then deproteinized by ultrafiltration. In the protein-free solution 1-aminoacetone was identified by colorimetric analysis and HPLC. In another experiment the enzyme–inhibitor complex was immediately denatured with trichloroacetic acid. The supernatant fluid was analyzed for 1-aminoacetone. The stoichiometry of 1-aminoacetone released upon acid denaturation of the enzyme–inhibitor complex was 0.73 mol/mol of β subunit.

PLP Release upon Denaturation of Enzyme–Inhibitor Complex. To assess the nature of the coenzyme–inhibitor linkage, (1-fluorovinyl)glycine-inhibited $\alpha_2\beta_2$ complex was denatured in 6 M urea. The reaction mixture contained 21 μM enzyme or enzyme–inhibitor complex in 180 mM NaCl and 100 mM Tris-HCl at pH 7.8, 6 M urea. After 1 h at 23 °C, the electronic spectra were recorded. The spectra indicated that PLP was quantitatively liberated upon denaturation of the enzyme–inhibitor complex. Thus, PLP is not irreversibly modified through interaction with (1-fluorovinyl)glycine.

Reactions of Noninhibiting Substrates. Tryptophan synthase $\alpha_2\beta_2$, as well as the β_2 subunit, catalyzes the dehydration

Table I: Relative Rates of Reactions Catalyzed by Tryptophan Synthase[a]

reaction (with $\alpha_2\beta_2$)	relative rate
L-serine + indole → L-tryptophan	100
L-serine → [^{3}H]serine	0.2 ± 0.2
DL-fluoroalanine + indole → L-tryptophan	88
L-serine → pyruvate + NH_4^+	1.7 ± 0.5
DL-fluoroalanine → serine	0.3 ± 0.2
DL-fluoroalanine → pyruvate + NH_4^+	3.0 ± 0.7

[a] All the values are represented as a percent of the rate of tryptophan synthesis from L-serine and indole as substrates. When present, L-serine was 20 mM (saturated), DL-fluoroalanine was 25 mM (saturated), and indole was 0.1 mM. Serine ^{3}H-exchange was measured in ^3H_2O. See Experimental Procedures for details.

Table II: Relative Rates of Tryptophan and Pyruvate Formation[a]

reaction (with β_2)	relative rate
L-serine + indole → L-tryptophan	100[b]
L-serine → pyruvate + NH_4^+	100
DL-fluoroalanine → pyruvate + NH_4^+	280

[a] All the values are represented as a percent of the rate of tryptophan synthesis from L-serine and indole as substrates. When present, L-serine was 20 mM (saturated), DL-fluoroalanine was 25 mM (saturated), and indole was 0.1 mM. See Experimental Procedures for details. [b] The absolute rate is at least 40 times slower than the rate of tryptophan formation catalyzed by $\alpha_2\beta_2$ under the same condition.

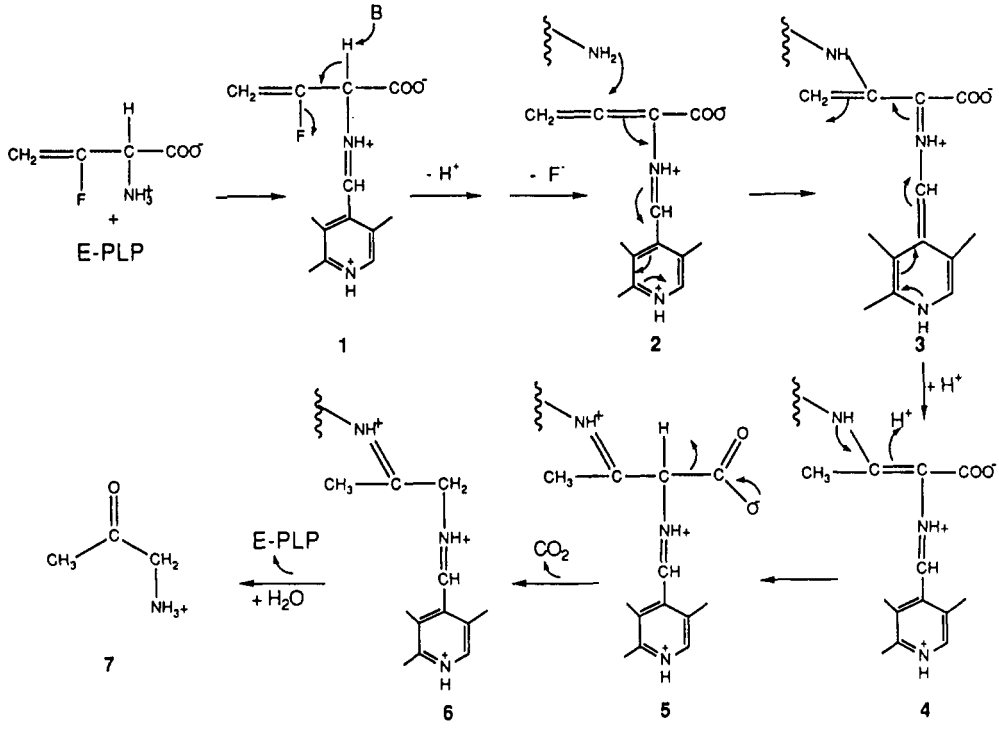
Table III: Deuterium Isotope Effect on Kinetic Constants of Tryptophan Synthase ($\alpha_2\beta_2$)

reaction	K_m (μM)	k_{cat} (s^{-1})	k_H/k_D
DL-[α-^{1}H]serine → pyruvate + NH_4^+	45	0.032	1.5 ± 0.1
DL-[α-^{2}H]serine → pyruvate + NH_4^+	59	0.022	1.5 ± 0.1

of serine and the dehydrofluorination of fluoroalanine to pyruvate in the absence of indole. The rate of the dehydration is given in Tables I and II, relative to the rate of tryptophan formation from serine and indole (the normal catalytic reaction). Formation of pyruvate catalyzed by $\alpha_2\beta_2$ proceeds at less than 5% the rate of the normal catalytic process. The rate of ^{3}H incorporation from [^{3}H]H_2O into serine was found to be less than 0.2% that of the catalytic process. This observation indicates that rapid reversible proton abstraction, followed by a slow elimination of the leaving group, does not occur. Possibly, the hydrolysis of the aminoacrylate pyridoxal 5′-phosphate adduct is slow and the adduct is hydrated and converted to serine. To evaluate the rate of this reaction we determined the rate of conversion of β-fluoroalanine to serine. The rate of the reaction was found to be slow (Table I). The deuterium isotope effect on the dehydration reaction was also determined (Table III). There is an isotope effect of 1.9 ± 0.1 on V_m/K_m and an isotope effect on V_{max} of 1.5 ± 0.1.

Effect of (1-Fluorovinyl)glycine on the α Reaction. Binding of serine to the $\alpha_2\beta_2$ complex can stimulate the α reaction (Anderson et al., 1991). It has been proposed that formation of the aminoacrylate on the β subunit leads to a change in protein conformation that is transmitted to the α subunit to enhance the rate of 3-indolyl-D-glycerol 3′-phosphate cleavage 150-fold. The effect of (1-fluorovinyl)glycine on the α reaction was thus examined. We found that, although (1-fluorovinyl)-glycine inhibited the β reaction, it had no effect on the α reaction. The (1-fluorovinyl)glycine-inhibited $\alpha_2\beta_2$ complex catalyzes the α reaction at the same rate as does native $\alpha_2\beta_2$, in the absence of serine. Thus the reaction of (1-fluorovinyl)-glycine with $\alpha_2\beta_2$ complex cannot bring about the change in protein conformation needed to activate the α reaction.

Scheme III: Mechanism of Inhibition of Tryptophan Synthase by (1-Fluorovinyl)glycine



[a] E-PLP = enzyme-bound pyridoxal phosphate; B = a base at the active site.

DISCUSSION

(1-Fluorovinyl)glycine inactivates tryptophan synthase. A plausible mechanism[1] of inactivation is proposed in Scheme III. A similar mechanism has been proposed for inactivation of alanine racemase by (1-fluorovinyl)glycine (Thornberry et al., 1991). Proton abstraction from the α-position of (1-fluorovinyl)glycine is followed by fluoride elimination to give a reactive allene **2**, which is converted to the transient adduct **4**. Adduct **4** tautomerizes to adduct **5**, which decarboxylates to **6**. Decarboxylation occurs, since the two positively charged imines provide effective electron sinks. A similar decarboxylation was previously observed in the inactivation of γ-cystathionase by β,β,β-trifluoroalanine (Silverman & Abeles, 1977). Adduct **6** can slowly release 1-aminoacetone and regenerate the active enzyme. The finding of nearly 1 equiv of 1-aminoacetone and the recovery of PLP upon decomposition of the enzyme–inhibitor complex, as well as F^- release, is consistent with the proposed mechanism. The change of the absorbance maximum from 420 nm (characteristic of pyridoxal Schiff base) to 485 nm during the inhibition indicates covalent modification of the coenzyme. The inactivation rate constant agrees with the rate constant for the change in absorbance at 485 nm. We attribute the absorbance of the final enzyme–inhibitor complex (that is, maximum absorbance at 485 nm) to the conjugated structure **4** (Scheme II), which is formed by the attack of the lysine residue at the active site on the allenic center. Faraci and Walsh (1989) have suggested that compounds resembling structure **4** exhibit absorbance maxima in the 460–490-nm range.

At 4 mM (1-fluorovinyl)glycine, the enzyme is not saturated; therefore $K_i > 2$ mM. Since $k_{in}/K_i = 4.9 \times 10^4$ M^{-1} min^{-1}, $k_{in} > 1.6$ s^{-1} and is comparable to k_{cat} of 6.4 s^{-1} for tryptophan synthesis with serine and indole as substrates. Therefore, an elimination reaction in the absence of indole proceeds rapidly. Surprisingly, the elimination of HF- from (1-fluorovinyl)-glycine is fast. This fast rate could be due to the enhancement of acidity of the α-proton by the fluorovinyl group. This elimination reaction, unlike the conversion of serine to pyruvate, does not involve the intermediate formation of the aminoacrylate adduct. The conversion of 2-amino-3-butenoic acid to α-ketobutyrate, a reaction similar in some respects to the reaction of (1-fluorovinyl)glycine, probably proceeds through an aminoacrylate adduct and is slow (Miles, 1978). We conclude that the "β-replacement specificity" of tryptophan synthase, is due, at least in part, to the slow hydrolysis of the aminoacrylate intermediate.[2]

Conversion of serine to pyruvate and NH_4^+ proceeds with a small deuterium isotope effect ($^DV_{max} = 1.5$) (Table III). The intrinsic isotope effect is not known, but it is very likely larger than 1.5. The isotope effect on the formation of the aminoacrylate intermediate from serine catalyzed by the β_2 subunit is 4.0 (Miles & McPhie, 1974). It is therefore likely that in the conversion of serine to pyruvate proton abstraction is only partly rate determining. If α-hydrogen abstraction were as fast as it is in the reaction of serine and indole it would be ~50-fold faster than k_{cat} for pyruvate formation from serine. An isotope effect on the conversion of serine to pyruvate would not be observed. Therefore, the rate of α-hydrogen abstraction is decreased in the absence of indole. "β-Replacement specificity" is due to the slow hydration of the aminoacrylate adduct as well as slow α-proton abstraction in the absence of indole.

[1] Alternatively, addition of the nucleophilic group of the enzyme to a tautomerized fluoroolefin would lead to elimination of fluoride and afford the same adduct.

[2] The departure of the leaving group (-OH or -F) might be partially rate determining rather than hydrolysis of the aminoacrylate adduct. This seems unlikely since the very little solvent $^3H^+$ is incorporated into unreacted serine. The rates of conversion serine and fluoroalanine to pyruvate or tryptophan do not differ greatly.

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REFERENCES

Abeles, R. H., & Maycock, A. L. (1976) *Acc. Chem. Res. 9*, 313–319.

Anderson, K. S., Miles, E. W., & Johnson, K. A. (1991) *J. Biol. Chem. 266*, 8020–8033.

Bloch, K. (1971) *Enzymes (3rd Ed.) 5*, 441–464.

Brady, K., & Abeles, R. H. (1990) *Biochemistry 29*, 7608–7617.

Faraci, W. S., & Walsh, C. T. (1989) *Biochemistry 28*, 431–437.

Frisell, W. R., Meech, L. A., & Mackenzie, C. G. (1954) *J. Biol. Chem. 207*, 709–716.

Hepworth, J. D. (1973) *Org. Synth. Collect. Vol. 5*, 27–29.

Kawasaki, H., Bauerle, R., Zon, G., Ahmed, S. A., & Miles, E. W. (1987) *J. Biol. Chem. 262*, 10678–10683.

Kredich, N. M., Foote, L. J., & Keenan, B. S. (1973) *J. Biol. Chem. 248*, 6187–6196.

Lane, A. N., & Kirschner, K. (1983) *Eur. J. Biochem. 120*, 561–570.

Miles, E. W. (1975) *Biochem. Biophys. Res. Commun. 66*, 94–102.

Miles, E. W. (1986) in *Vitamin B_6 Pyridoxal Phosphate* (Dolphin, Ed.) Vol. 1, Part B, pp 253–310, Wiley, New York.

Miles, E. W. (1991) *Adv. Enzymol. Relat. Areas Mol. Biol. 64*, 93–172.

Miles, E. W., & McPhie, P. (1974) *J. Biol. Chem. 249*, 2852–2857.

Miles, E. W., Bauerle, R., & Ahmed, S. A. (1987) *Methods Enzymol. 142*, 398–414.

Miles, E. W., Kawasaki, H., Ahmed, S. A., Morita, H., Morita, H., & Nagata, S. (1989) *J. Biol. Chem. 264*, 6280–6287.

Morisaki, M., & Bloch, K. (1972) *Biochemistry 11*, 309–314.

Patchett, A. A., Taub, D., Weissberger, B., Valiant, M. E., Gadebusch, H., Thornberry, N. A., & Bull, H. G. (1988) *Antimicrob. Agents Chemother. 32*, 319–323.

Silverman, R. B., & Abeles, R. (1977) *Biochemistry 16*, 5515.

Thornberry, N. A., Bull, H. G., Taub, D., Greenlee, W. J., Patchett, A. A., & Cordes, E. H. (1987) *J. Am. Chem. Soc. 109*, 7543–7544.

Thornberry, N. A., Bull, H. G., Taub, D., Wilson, K. E. Gimenez-Gallego, G., Rosegay, A., Soderman, D. D., & Patchett, A. A. (1991) *J. Biol. Chem. 266*, 21657–21665.

Urata, G., & Granick, S. (1963) *J. Biol. Chem. 238*, 811–820.

Yanofsky, C., & Crawford, I. P. (1972) Tryptophan Synthase, in *The Enzymes (3rd Ed.)* (Boyer, P. D., Ed.) Vol. 7, pp 1–31, Academic Press, Orlando, FL.