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Stereochemistry and Mechanism of Reactions Catalyzed by Tryptophanase and Tryptophan Synthetase

Sir:

The stereochemistry of some pyridoxal phosphate-catalyzed enzymatic β-replacement reactions of amino acids has recently been studied in several laboratories including this one. The synthesis of tryptophan from serine and indole or indole glycerol phosphate catalyzed by tryptophan synthetase (native or β_2 protein),[1,2] the formation of L-tyrosine from L-serine and phenol,[3] and the conversion of L-tyrosine to L-Dopa,[4] both catalyzed by tyrosine phenol-lyase, were all found to proceed with retention of configuration at C-3 of the amino acid side chain. We can now add to this list of examples another reaction, the synthesis of L-tryptophan from indole and L-serine catalyzed by tryptophanase. Using previously established methodology[1] and tryptophanase from *E. coli*, (2*S*,3*R*)- and (2*S*,3*S*)-serine-*U*-^{14}*C*-3-*t*[1] (T/^{14}C 7.9 and 5.3) gave samples of tryptophan (T/^{14}C 7.7 and 5.0), which were analyzed for their configurations at C-3 by feeding them to cultures of *Streptomyces griseus* ATCC 12648 to produce two samples of the antibiotic indolmycin of T/^{14}C 7.8 and 0.1, respectively. It had been previously established[1,5] that in the biosynthesis of indolmycin the *pro-R* hydrogen from C-3 of the tryptophan side chain is eliminated and the *pro-S* hydrogen is retained. Thus the above result shows that the tryptophanase-catalyzed synthesis of tryptophan from serine also occurs with retention of configuration at C-3.

We now wish to report on the steric course of the α,β-elimination reactions catalyzed by two enzymes, *E. coli* tryptophanase (Sigma Chemical Corp.) and tryptophan synthetase β_2 protein purified from *E. coli* mutant A2/F'A2. The deamination of L-serine to pyruvate and ammonia by either enzyme was examined using (2*S*,3*R*)- and (2*S*,3*S*)-serine-^{14}C-3-t and the deamination of L-tryptophan to indole, pyruvate, and ammonia by tryptophanase was studied with (2*S*,3*R*)- and (2*S*,3*S*)-tryptophan-*3*-^{14}*C*-*3*-*t*.[1] The reactions were carried out in D_2O, and the resulting pyruvate was trapped as lactate using an excess of lactate dehydrogenase and NADH. The lactate samples were isolated by paper chromatography (Whatman #3, 1-propanol:concentrated NH_4OH:H_2O 6:3:1) and oxidized to acetate.[6] The chirality of the methyl group in the acetate samples was then determined by the method of Cornforth et al.[7] and Arigoni et al.,[8] following Eggerer's procedure.[7] In this analysis procedure, which involves conversion to malate with malate synthetase followed by reaction with fumarase, (*R*)-acetate-*2*-d_1-*2*-t_1 gives rise to malate, which retains more than half of its tritium in the fumarase reaction, whereas the *S* isomer produces malate which retains less than half of its tritium in the fumarase reaction. The results of these experiments, which are summarized in Table I, show that in both deamination reactions catalyzed by tryptophanase the protonation at C-3 of the amino acid side chain occurs stereospecifically with retention of configuration. This finding parallels observations by Yang et al.[9] on the deamination of D-threonine by D-serine dehydratase and by Kapke[10] on the deamination of L-threonine by L-serine dehydratase, both of which also proceed with retention of configuration. In contrast, in the deamination of serine catalyzed by tryptophan synthetase β_2 protein, OH is replaced by hydrogen nonstereospecifically, suggesting as the most plausible explanation that the protonation at C-3 is in this case nonenzymic.[11] Although both types of enzymes operate through the same α-aminoacrylate–pyridoxal phosphate Schiff's base intermediate, these results point to a possible subtle difference between the enzymes primarily catalyzing α,β-eliminations and those primarily catalyzing β-replacement reactions: the presence in the former and absence in the latter of a base which can protonate C-3 of this common intermediate.

Finally, we determined the origin of the hydrogen at C-3 of the indole produced from tryptophan by tryptophanase. By carrying out the reaction with nonlabeled tryptophan in D_2O and with tryptophan-(*alanine-2-d*) in H_2O and D_2O and analyzing the indole for deuterium content at C-3 by FT-proton NMR it was established (Table II) that this hydrogen originated from C-2 of the amino acid side chain by a partially intramolecular transfer. These results suggest the following conclusions: (1) A single base on the enzyme catalyzes the proton abstraction from C-2 of the side chain, protonation of C-3 of the indole moiety, and, most likely, also protonation of C-3 of the aminoacrylate intermediate. (b) The proton transfer must be suprafacial. (c) The α,β-elimination of the indole is a syn elimination.

Table II. Intramolecularity of Hydrogen Transfer from the α-Carbon to C-3 Indole in the Tryptophanase-Catalyzed Decomposition of Tryptophan

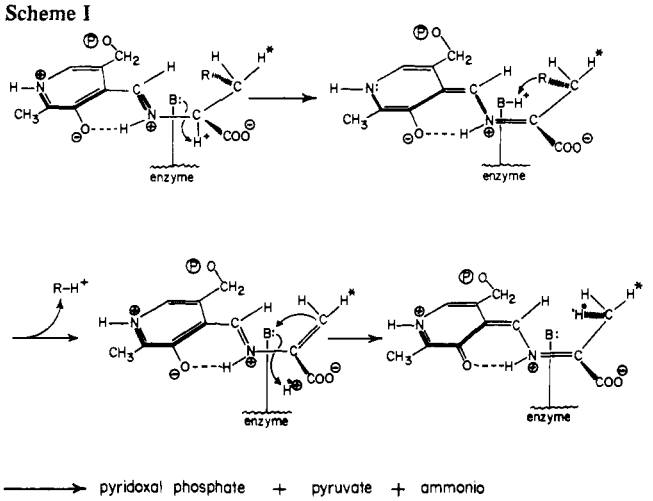
Label in tryptophan	Solvent	% H/% D at C-3 of indole	% intramolecular transfer
α-H	D_2O	63.5/36.5	63.5
α-D	H_2O	92.1/7.9	7.9
α-D	D_2O	0/100	n.a.

Table I. Stereochemistry of the Protonation at the β-Carbon Atom of Serine and Tryptophan in the Deamination Reaction Catalyzed by Tryptophanase or Tryptophan Synthetase β_2 Protein in D_2O

	Tryptophanase				Tryptophan synthetase β_2		Control	
	Serine-*3*-^{14}*C*-*3*-*t*		Tryptophan-*3*-^{14}*C*-*3*-*t*		Serine-*U*-^{14}*C*-*3*-*t*		Acetate-*2*-^{14}*C*-*2*-*d*, -*2*-*t*	
T/^{14}C of	3*R*	3*S*	3*R*	3*S*	3*R*	3*S*	2*R*	2*S*
Substrate	7.87	5.29	79.3	83.6	2.00	2.00	—	—
Lactate	8.11	5.73	76.0	79.8	2.09	2.04	—	—
Acetate	7.93	5.46	71.9[a]	74.3[a]	3.02	2.90	7.60	8.15
Malate	7.94	7.01	5.62	7.06	2.56	2.47	6.12	6.68
Fumarate	5.66	2.84	1.96	5.71	1.31	1.27	4.42	2.25
% T-retention of fumarase reaction	71.2	40.6	34.8	80.8	51.1	51.4	72.2	33.7

[a] T/^{14}C ratio readjusted by addition of ^{14}C reference compound.

Scheme I



pyridoxal phosphate + pyruvate + ammonia

A plausible mechanism which incorporates these features is shown in Scheme I. It appears that the enzyme obeys the minimal base number rule proposed by Hanson and Rose.[12]

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Bis(2,6-dimethylpyridine)chromium. A π-Heterocyclic Complex

Sir:

Heterocyclic compounds are known to act as 5- or 6-electron ligands to form π-complexes with transition metals. Thus pyrrole,[1] pyridine,[2] indole,[2a] carbazole,[2a] and thiophene[3] nuclei are reported to form π-complexes to metal carbonyl moieties; the iron atom in azaferrocene, which is isomorphous with ferrocene, carries both pyrrole and cyclopentadienyl as π-complexed ligands.[1,4] We wish to report the synthesis and structure of bis(2,6-dimethylpyridine)-chromium.

Table I. Crystallographic Data

	Form A	Form B
a (Å)	7.307 (1)	9.421 (1)
b (Å)	8.242 (2)	10.496 (2)
c (Å)	6.316 (2)	12.725 (2)
α (deg)	112.50 (3)	90
β (deg)	104.01 (2)	90
γ (deg)	102.46 (2)	90
Space group	$P\bar{1}$	$Pbcn$
No. of molecules per crystallographic asymmetric unit	1/2	1/2
Crystallographic site symmetry occupied by molecule	$\bar{1}(C_i)$	$2(C_2)$
Final $R(=\Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|)$	0.087	0.045

Bis(2,6-dimethylpyridine)chromium was prepared by cocondensing chromium atoms with the ligand at 77 K, the latter substance being present in excess; the apparatus employed is essentially that described by Timms.[5,6] The frozen matrix of ligand containing metal atoms undergoes the typical color change observed in other syntheses of this type (colorless → dark) as it warms to room temperature. The final reaction mixture is a red-brown solution in excess ligand containing dispersed unreacted metal. Excess ligand was removed in high vacuum, and the dark residue was sublimed to give a red-brown substance, as the sole product, in 2% yield (based upon chromium) which proved to be bis(2,6-dimethylpyridine)chromium (mp 79–80 °C). The product sublimes readily at 65 °C (10^{-3} Torr) and exhibits a molecular ion peak in the high resolution mass spectrum at 266.0877 mass units consistent with the expected molecular composition ($C_{14}H_{18}N_2{}^{52}Cr$ = 266.0875). The proton magnetic resonance spectrum of the substance shows a singlet at δ 2.38 and a complex multiplet centered at δ 4.40; the free ligand exhibits resonances at δ 2.52, assigned to CH_3, and δ 6.97 and 7.45 which are assigned to ring protons. The upfield shifts in the proton resonances are typical of those observed for analogous bisarenechromium compounds.[6] The proton magnetic resonance spectrum of bis(2,6-dimethylpyridine)chromium in toluene-d_8 is consistent with free rotation of the complexed rings down to −65 °C. The pure compound is unstable in air, decomposing to a nonvolatile green substance (Cr_2O_3?). Bis(2,6-dimethylpyridine)chromium is soluble in deoxygenated benzene, pyridine, tetrahydrofuran, hexane, acetone, and water; these solutions appear to be stable. The compound decomposes rapidly in carbon tetrachloride and carbon disulfide.

In order to obtain definitive proof of structure by x-ray crystal structure analysis, crystals were grown by vacuum thermal gradient sublimation. Crystals exhibiting two different morphologies were obtained: one form (A) grew as parallelepipeds, for the most part twinned, and proved to be triclinic; the other, plate-like form (B) was orthorhombic. X-Ray crystal structure studies have been carried out on both forms. In all cases, the crystal was mounted in an atmosphere of N_2 and then quickly transferred to a Syntex $P2_1$ diffractometer. During the course of all diffraction experiments[7] the data crystal was maintained in an environment of N_2 ca. −35 °C. Relevant crystal data are presented in Table I.

Both crystal structure determinations support the formulation of the molecule as the sandwich complex bis(2,6-dimethylpyridine)chromium. In crystal modification A (Figure 1), the molecule occupies a crystallographic inversion center, and thus the pyridine rings are strictly parallel. In