CONVERSION OF TRYPTOPHAN TO 5-HYDROXYTRYPTOPHAN BY PHENYLALANINE HYDROXYLASE

by

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Two recent reports on the hydroxylation of trytophan to 5-hydroxyindole products represent the first demonstrations of this reaction in cell-free extracts. The characteristics of the two reported systems appear to be quite different. Cooper et al (1961) using a particulate fraction obtained from rat intestinal mucosa showed that the reaction required ascorbate and cupric ion, but not oxygen or a pyridine nucleotide. The system of Freedland et al (1961) employed a 20,000 x g supernatant fraction obtained from rat liver homogenates. However, requirements for oxygen and DPN were clearly demonstrated We have investigated the liver tryptophan hydroxylating system further and have obtained data showing that it is, in fact, catalyzed by the same system which converts phenylalanine to tyrosine, i.e./phenylalanine hydroxylase (Udenfriend et al. 1952; Mitoma, 1956; Kaufman, 1957 and 1959).

Tyrosine formation was assayed by the method of Udenfriend and Cooper (1952b) and total 5-hydroxyindoles by the nitrosonaphthol reaction of Udenfriend et al (1955). The phenylalanine hydroxylating system has been shown to require two enzymes (Enz. I and II), (Mitoma, 1956) and a coenzyme (Kaufman, 1959). The second enzyme is thought to function by regenerating the coenzyme. The naturally occurring coenzyme is a pteridine derivative, although several chemically synthesized pteridines are also active in the enzymatic reaction (Kaufman, 1959). 2-Amino-4-hydroxy-6,7-dimethyltetrahydropteridine kindly supplied by Dr. W. Bridges and Mr. Funk of the National

Institutes of Health, was used in the present studies. Both Enzyme I (thru the $CaPO_4$ gel stage) and Enzyme II (thru the first ammonium sulfate step) were prepared from rat liver and sheep liver respectively, as described by Kaufman (1957) and Kaufman et al (1959). The incubation conditions were essentially those described by Kaufman (1959) except that the enzymatic reactions were carried out at 37°. With tryptophan, a final substrate concentration of 0.01 M was employed. The conditions for the hydroxylation of tryptophan by high speed supernatant fractions of rat liver were the same as reported by Freedland et al (1961) but β-phenylisopropylhydrazine was included in all incubation mixtures.

Our preliminary experiments showed that the system necessary for the hydroxylation of tryptophan resided in the 78,000 x g supernatant fraction of rat liver. Fractionation of the supernatant enzyme with ammonium sulfate as described by Mitoma (1956) showed that the two ammonium sulfate fractions required for the conversion of phenylanine to tyrosine were also necessary for the hydroxylation of tryptophan. The purified phenylalanine hydroxylase system was then tested comparing phenylalanine and tryptophan as substrates. As shown in Table I, with the purified system, similar requirements were seen for both phenylalanine and tryptophan hydroxylation. Enzyme I, Enzyme II, the cofactor, and TPNH were necessary for the hydroxylation of both amino acids. Azide inhibited both reactions; L-phenylalanine but not D-phenylalanine inhibited tryptophan hydroxylase. Preliminary studies indicate that the L-phenylalanine inhibition is of a competitive nature. In addition the ratio of activity between the two substrates remained substantially the same throughout all the steps of enzyme purification.

The Km for tryptophan in the purified system was calculated to be 3×10^{-3}M. Under similar conditions the Km for phenylalanine is 1×10^{-5}M. The poor affinity towards tryptophan could account for the failure to detect this reaction in the early studies on phenylalanine hydroxylase.

The major product of the enzyme reaction with tryptophan was isolated by paper chromatography. In three different solvent systems: butanol, pyridine

TABLE I

Comparison of Phenylalanine and Tryptophan Hydroxylation

	Mμ moles per hour	
	Tyrosine	5-Hydroxyindoles
Complete system	1,400	50
Minus enzyme I	0	0
Minus enzyme II	180	5
Minus dimethyltetrahydropteridine	0	0
Minus TPNH	200	10
Azide, 10 μM/ml	0	0
L-phenylalanine 1.5 μM/ml	-	20
D-phenylalanine 1.5 μM/ml	-	44

The complete system contained in a final volume of 1 ml, potassium phosphate buffer pH 6.8, 100 μmoles; L-phenylalanine, 1.0 μmole or L-tryptophan, 10 μmoles; TPNH, 1.0 μmole; 2-amino-4-hydroxy-6,7-dimethyl-tetrahydropteridine 25 μg; rat liver enzyme, 2.4 mg protein; sheep liver enzyme 3.6 mg protein.

and water (1:1:1); butanol, acetic acid and water (120:30:50); iso-propanol, ammonia and water (20:1:2), its R_f values, and colorimetric and fluorescent characteristics were found to be similar to those of authentic 5-hydroxy-tryptophan.

Although 4, 6 and 7 hydroxyindoles do not give a positive nitroso-naphthol reaction and do not fluoresce in strong acid, it is still possible that small amounts of these compounds may also have been formed.

The question still remains as to the physiological significance of the system in the formation of 5-hydroxytryptamine (serotonin). Phenylalanine hydroxylase has been shown to occur only in mammalian liver although there is much evidence which suggests that serotonin is formed locally in the gut and the central nervous system. If the liver system is of prime importance in 5-hydroxyindole formation, a mechanism for the transport of 5-hydroxytryptophan would have to be involved. There is no evidence of circulating 5-hydroxtryptophan. However, there have been reports of a lowered excretion of 5-hydroxtryptophan metabolites in patients with phenylketonuria (Pare et al 1959), a disorder in

which liver phenylalanine hydroxylase is markedly diminished. These would tend to confirm the relationship between the two hydroxylations. Nevertheless, the very low affinity of tryptophan for this enzyme makes it doubtful whether it could serve as a major source of serotonin *in vivo*. It remains to be seen whether this enzyme or the intestinal enzyme of Cooper *et al.* (1961) is of greater importance in the production of the humoral agent, serotonin, in the intact animal.

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