The enzymic conversion of phenylalanine to tyrosine

Although the conversion of phenylalanine to tyrosine has been demonstrated *in vitro* with various kinds of tissue preparations[1,2,3], little progress has been made in elucidating the mechanism of the reaction. In 1952, the preparation of a soluble enzyme system from rat liver was described which catalyzed this reaction[4]. DPN^+ (diphosphopyridine nucleotide) and an alcohol or aldehyde were found to be required. Recently, MITOMA[5] reported that the cofactor actually involved was DPNH (reduced diphosphopyridine nucleotide). In addition, it was found that two crude protein fractions were required for full activity.

The conversion of phenylalanine to tyrosine has now been brought about by two enzymes, one isolated from rat liver extracts and another from sheep liver extracts, each of which has been purified about 25 fold. In this system, TPNH (reduced triphosphopyridine nucleotide) appears to be the specific coenzyme.

With the partially purified enzymes, the stoichiometry of the reaction has been determined.

TABLE I

STOICHIOMETRY OF THE REACTION

Experiment No.	ΔO_2	Δ *TPNH*	Δ *Phenylalanine*	Δ *Tyrosine*
1	— 1.40	— 1.21	—	+ 1.29
2	— 1.19	— 1.24	— 1.27	+ 1.30

In experiment 1, the system contained the following components: potassium phosphate buffer, pH 6.8, 150 μmoles; L-phenylalanine, 8.0 μmoles; TPNH, 8.0 μmoles; rat enzyme, 7.0 mg of protein; sheep enzyme, 5.0 mg of protein. Final volume, 1.70 ml. Incubated for 2 hours at 26.2° C. In experiment 2, the system contained the following components: potassium phosphate buffer, pH 6.8; ^{14}C-phenylalanine, 4.5 μmoles; TPNH, 4.0 μmoles; rat enzyme, 8.5 mg of protein; sheep enzyme, 4.0 mg of protein. Final volume, 1.40 ml. Incubated for 75 minutes at 26.2° C. In both experiments a control tube without phenylalanine was included and the values reported have been corrected for the small amount of TPNH oxidation which took place in the absence of phenylalanine. All values in the table are given in micromoles. Tyrosine was determined by the modification[6] of the method of THOMAS[7].

In experiment 2, phenylalanine was estimated by determining the counts in the phenylalanine spot after chromatographic separation from tyrosine.

As can be seen in Table I, essentially equimolecular amounts of oxygen, TPNH, and phenylalanine are utilized and an equivalent amount of tyrosine is formed. These results are consistent with the following formulation of the reaction:

$$TPNH + H^+ + O_2 + \text{phenylalanine} \longrightarrow TPN^+ + H_2O + \text{tyrosine}. \quad (1)$$

In contrast, MITOMA[5] has reported that two moles of DPNH were oxidized for each mole of tyrosine formed in his system.

That the oxidation of TPNH catalyzed by these enzymes is intimately linked to tyrosine formation is made probable by the finding of a phenylalanine dependence for this reaction. As can be seen in Fig. 1, experiment A, after an initial lag, the optical density decreases rapidly in the presence of phenylalanine, while in the control cuvette the same initial slow rate is maintained. At the arrows, glucose and an excess of glucose dehydrogenase were added and the optical density approached the original value, indicating that TPN^+ (triphosphopyridine nucleotide) was the final product of the reaction. In contrast to TPNH, the oxidation of DPNH (Fig. 1, experiment B) is barely stimulated by phenylalanine under these conditions.

Fig. 1 shows that a lag of about six minutes occurred before the rate of oxidation of TPNH in the presence of phenylalanine exceeded that of the control. It has been found that this lag period can be eliminated almost entirely by preincubation with both enzymes in the absence of phenylalanine. The data in Table II show that the presence of TPNH is required. It should be noted, however, that oxygen is not necessary. Although not shown in the table, it has recently been found that in contrast to aerobic preincubation, where both enzymes are required, an anaerobic preincubation with the sheep enzyme alone can eliminate the lag period. These findings suggest the possibility that the function of the TPNH in this system may be to reduce a cofactor. This reduced compound could, on oxidation, form the primary hydroxylating agent. Further studies are under way to determine more precisely the characteristics of this lag period and its possible significance for the mechanism of the reaction.

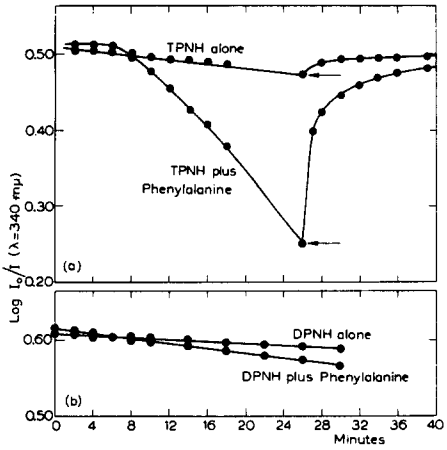

Fig. 1. Spectrophotometric demonstration of phenylalanine-dependent oxidation of TPNH compared with DPNH. A, potassium phosphate buffer, pH 6.8, 100 μmoles; TPNH, 0.08 μmole; rat enzyme, 1.5 mg of protein; sheep enzyme, 2.0 mg of protein. 4.0 μmoles of phenylalanine present where indicated. At the arrows, an excess of glucose dehydrogenase and 100 μmoles of glucose were added to both cuvettes. B, same conditions as in experiment A with DPNH instead of TPNH. Silica cells, d = 1.0 cm. Final volume made up to 1.0 ml with water.

TABLE II

THE EFFECT OF PREINCUBATION UNDER VARIOUS CONDITIONS

Conditions of preincubation	Δ *Optical density* $\times$ *1000/min*			
	0–3 min	*3–6 min*	*6–9 min*	*9–20 min*
No preincubation	0	3.0	9.0	15.2
Both enzymes, aerobically, in the presence of TPNH	12.0	17.0	16.0	16.1
Both enzymes, aerobically, in the absence of TPNH	0	3.3	8.3	14.3
Both enzymes, anaerobically, in the presence of TPNH	9.0	15.3	16.0	17.0

The reactions were carried out in 1.0 cm Beckman cuvettes, containing the following components: potassium phosphate buffer, pH 6.8, 100 μmoles; phenylalanine, 4.0 μmoles; TPNH, 0.08 μmole; rat enzyme, 1.6 mg of protein; sheep enzyme, 1.5 mg of protein. Final volume, 1.0 ml. Incubations carried out at room temperature. For the anaerobic preincubations, the enzymes were added to the main compartment of a Thunberg tube, and the buffer and TPNH were added to the side bulb. After a two-minute evacuation on a mechanical pump, the tube was closed and the contents of the side arm were tipped in. Each reported value has been corrected for any TPNH oxidation which occurred in the absence of phenylalanine. All preincubations were carried out for 15 minutes at room temperature.

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