NONSPECIFICITY OF TYROSINE TRANSAMINASE: AN EXPLANATION FOR THE SIMULTANEOUS INDUCTION OF TYROSINE, PHENYLALANINE, AND TRYPTOPHAN TRANSAMINASE ACTIVITIES IN RAT LIVER

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Tyrosine-α-ketoglutarate transaminase activity of rat liver increases up to eight-fold after administration of tyrosine or hydrocortisone intraperitoneally (Lin and Knox, 1957). Tryptophan-α-ketoglutarate transaminase activity of rat liver has been reported to be a separate enzyme (Lin, Civen and Knox, 1958) which also increases several fold after administration of tryptophan or hydrocortisone intraperitoneally (Civen and Knox, 1959). Phenylalanine-α-ketoglutarate transaminase activity has been noted to increase following the administration of phenylalanine (Lin, cited by Auerbach and Waisman, 1958). We have observed that after the injection of tyrosine and hydrocortisone, not only tyrosine transaminase, but also phenylalanine and tryptophan transaminase activities are increased simultaneously. In further studies on the nature of this steroid-mediated enzyme induction, the characteristics of tyrosine transaminase purified from liver of induced rats have been re-examined. It has been found that phenylalanine and tryptophan transaminase activities with α-ketoglutarate could not be separated from tyrosine transaminase activity during a 500-fold purification of tyrosine transaminase, or after various treatments of the purified enzyme. It is concluded that the substrate specificity of tyrosine transaminase is broader than previously suspected, and further, that the apparent simultaneous induction of tyrosine, phenylalanine and tryptophan trans-

aminase activities can be largely accounted for by the increase in a single nonspecific tyrosine transaminase.

Adult male rats were injected intraperitoneally with a mixture of 600 mg L-tyrosine and 30 mg hydrocortisone (Solucortef) per kg to induce transaminase activity, and sacrificed 4-5 hours later. Tyrosine transaminase was purified from liver homogenates by methods similar to those described by Kenney (1959, 1962): ammonium sulfate fractionation, selective heat denaturation, adsorption and elution from calcium phosphate gel, chromatography on DEAE-cellulose, and a second calcium phosphate gel fractionation. These steps gave a purification of 500-fold over the activity of crude homogenates. Assays of transaminase activity with α-ketoglutarate were performed by the spectrophotometric method of Lin, Pitt, Given and Knox (1958), modified in the tryptophan transaminase assay by the addition of the glutathione-dependent indolylpyruvic acid tautomerase purified free of tryptophan transaminase activity from rat liver (Spencer and Knox, 1962).

The relative tyrosine, phenylalanine, and tryptophan transaminase activities with α-ketoglutarate in liver homogenates of uninduced and induced rats, and at various stages of purification of tyrosine transaminase, are shown in Table I. In liver homogenates from uninduced animals, the transamination activities of phenylalanine and tryptophan relative to that of tyrosine were 18% and 3%, respectively. Five hours after administration of tyrosine and hydrocortisone intraperitoneally, there was a 4.8-fold increase in tyrosine, 3.1-fold increase in phenylalanine, and 3.6-fold increase in tryptophan transaminase activity. These increases could be due to the induction of three separate enzymes as previously supposed, or to the induction of a single nonspecific transaminase with activity towards all three amino acid substrates.

Table I shows that phenylalanine and tryptophan transaminase activities are purified along with tyrosine transaminase activity. With 500-fold purified tyrosine transaminase, phenylalanine was transaminated at 8% and

TABLE I

TYROSINE, PHENYLALANINE, AND TRYPTOPHAN TRANSAMINASE ACTIVITIES

WITH INDUCTION AND PURIFICATION OF TYROSINE TRANSAMINASE

	Tyrosine Transaminase Specific Activity[1]	Phenylalanine Transaminase		Tryptophan Transaminase	
		Specific Activity[1]	Activity Relative to Tyrosine[2]	Specific Activity[1]	Activity Relative to Tyrosine[3]
			%		%
Enzyme Induction					
Uninduced rats[4]	.47	.08	18	.014	2.9
Induced rats[4]	2.24	.26	12	.049	2.2
Enzyme Purification from Induced Animals					
Crude homogenate	1.92	.27	14	.054	2.8
Ammonium sulfate fraction	5.3	.39	7.4	.104	2.0
Gel eluate I	72	6.0	8.3	1.15	1.6
DEAE eluate a	264	18.7	7.1	4.2	1.6
DEAE eluate b	370	31.2	8.4	6.7	1.8
Gel eluate II	1000	78.0	7.8	15.7	1.6
Above, aged 1 week	530	42.0	7.8	9.0	1.7
Enzyme Treatments[5]					
Heat 64°, 20 minutes	-58%	-50%		-60%	
Ultracentrifuge[6]	-44%	-48%		-50%	

[1] Specific activity = μmoles keto acid formed/hour/mg protein.

[2] $\frac{\text{Specific activity phenylalanine transaminase}}{\text{Specific activity tyrosine transaminase}} \times 100$

[3] $\frac{\text{Specific activity tryptophan transaminase}}{\text{Specific activity tyrosine transaminase}} \times 100$

[4] Average values of separate determination on 9000 x g liver homogenates from 5 uninduced and 5 induced rats

[5] Enzyme purified 80-fold was used in these studies.

[6] Fractional activity left in the upper compartment of a Yphantis-Waugh separation cell after the equivalent of 26 minutes at 59, 780 rpm.

tryptophan at 1.7% the rate of tyrosine. These ratios of activity remained essentially the same at each step of purification after ammonium sulfate fractionation. Furthermore, each fraction eluted from DEAE-cellulose, although varying in tyrosine transaminase activity, maintained the same relative ratios of activity for the other two amino acids. Storage of the 500-fold purified enzyme for one week resulted in a 47% loss of tyrosine transaminase activity. The other transaminase activities were reduced proportionately. Heating the purified enzyme at 64° C for 20 minutes resulted in an approximately equal per cent decrease in all three activities. In the ultracentrifuge, the same fraction of each activity was left in the upper compartment of a separation cell (Yphantis and Waugh, 1956) after the equivalent of 26 minutes at 59, 780 rpm.

These results indicate that phenylalanine and tryptophan transaminase activities could not be separated from tyrosine transaminase activity on purification of tyrosine transaminase from induced animals, or after various treatments of the purified enzyme. The Michaelis constants for tyrosine, tryptophan and phenylalanine were respectively 1.5×10^{-3}, 3×10^{-2}, and 8×10^{-2}. Further evidence (to be reported elsewhere) of the non-specificity of tyrosine transaminase is that a number of analogues of phenylalanine and tryptophan are also transaminated by purified tyrosine transaminase. As might be expected, phenylalanine, tryptophan, and their analogues also act as inhibitors of tyrosine transamination (Jacoby and La Du, 1962).

In the uninduced animal the higher ratios of phenylalanine and tryptophan transaminase activities to tyrosine than with purified tyrosine transaminase indicate that there are other transaminases in crude liver homogenates active with these substrates. However, it can be calculated from the relative activities of phenylalanine and tryptophan with purified tyrosine transaminase that 79% of the increase in phenylalanine and 85% of the increase in tryptophan transaminase activities in induced animals can be accounted for by the nonspecificity of tyrosine transaminase. Previous

studies of the substrate specificity of tyrosine transaminase (Kenney,1959) presumably failed to detect activity with phenylalanine and tryptophan because the substrate concentrations employed were too low and the method of product detection not sufficiently sensitive. It is interesting that several other enzymes of amino acid metabolism, phenylalanine hydroxylase (Freedland, et al.,1961; Renson, et al.,1962), and DOPA decarboxylase (Lovenberg, et al.,1962) initially thought to be specific for a single substrate, have recently also been shown to act on both phenylalanine and tryptophan derivatives.

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