THE FORMATION OF 3,4-DIHYDROXY-L-PHENYLALANINE FROM L-meta-TYROSINE BY RAT LIVER AND BEEF ADRENAL MEDULLA*

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Summary

Incubation of L-m-tyrosine with rat liver homogenate in the presence of a pteridine co-factor and a Dopa decarboxylase inhibitor gave rise to a new amino acid which was identified as 3,4-dihydroxyphenylalanine (DOPA) on the basis of its fluorescence spectrum, and chromatography. When L-m-tyrosine or L-tyrosine was incubated with a partially purified beef adrenal tyrosine hydroxylase preparation in the presence of the same additives, DOPA appeared in the medium in amounts greater than when no substrate was added. No DOPA was detected when the substrate was D-m-tyrosine. L-o-Tyrosine gave no new amino acid under the same conditions. The finding that partially purified rat liver phenylalanine hydroxylase and beef adrenal tyrosine hydroxylase preparations hydroxylate m-tyrosine is contrary to previous reports.

Catecholamines are formed in adrenals and brain as a result of the hydroxylation of phenylalanine in the para position to give tyrosine, followed by a second hydroxylation in the adjacent position to give 3,4-dihydroxyphenylalanine (DOPA), and subsequent decarboxylation to 3,4-dihydroxyphenylethylamine (DOPamine) (1). In liver, because of the absence of tyrosine hydroxylase, only the first of the above reactions takes place. Conversion of phenylalanine to DOPA by way of the same reactions in the alternative sequence, that is, beginning with the formation of 3-hydroxyphenylalanine (m-tyrosine), has never been demonstrated. However,

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in view of the demonstration that an increase in kidney DOPamine levels resulted from the administration of DL-m-tyrosine to rats (2) and man (3), that m-hydroxyphenylethylamine was formed from L-phenylalanine by rat liver homogenate (4), and that m-hydroxyphenyl acids of unknown origin are excreted by phenylketonuric and normal individuals (5), it seems that this alternative sequence might in fact be operative in animals.

We report here the demonstration of the conversion of L-m-tyrosine to L-DOPA by both a partially purified rat liver phenylalanine hydroxylase preparation and a partially purified beef adrenal tyrosine hydroxylase preparation.

Materials and Methods

Compounds were purchased as follows: L-phenylalanine and L-tyrosine (General Biochemicals), DL-DOPA (Mann), NADPH, tetrasodium salt, type II (Sigma), 6,7-dimethyl-5,6,7,8-tetrahydropterine·HCl·$1\frac{1}{2}$ H_2O ($DMPH_4$) (Calbiochem). 3-Bromo-4-hydroxybenzyloxyamine phosphate (NSD-1055) was a gift from Smith and Nephew Research, Gilson Park, Harlow, U.K. L-m-tyrosine, D-m-tyrosine and L-o-tyrosine were obtained by resolution of the racemic ethyl ester using chymotrypsin (6). Female Sprague-Dawley rats (250-300 g) killed by decapitation were used. Protein was determined by an automated biuret method (7).

Results

Rat liver was homogenized in two volumes of 0.9% sodium chloride with a glass homogenizer. The mixture was centrifuged at 22,000 x g for one hour, and aliquots were incubated at 37°C with L-m-tyrosine in the presence of the DOPA decarboxylase inhibitor NSD-1055 and a pteridine co-factor as described in Fig. 1. Analysis indicated the formation of a new amino acid which

Table I

Elution times of amino acids on the amino acid analyzer[a]

	0.2 N sodium citrate buffer, pH 4.25		
	50 cm AA-15 resin		15 cm Aminex A-5 resin[b]
	A: 68 ml/h	B: 34 ml/h	C: 34 ml/h
Phenylalanine	86	172	55
Tyrosine	79.5	158	51
o-Tyrosine	90.5	179	56
m-Tyrosine	72	145	46.5
DOPA	66	132	43.5

a System A used for determining tyrosine, A or B for DOPA, and A, B and C when o-tyrosine was used as substrate.
b Bio.Rad Laboratories, Richmond, Calif.

was indistinguishable from DOPA when chromatographed on the analyzer using the three systems described in Table I. The rate of formation of this DOPA is shown in Fig. 1. Also included for comparison purposes is the rate of formation of tyrosine from phenylalanine by the same preparation.

Similar incubations were then carried out using as enzyme source, beef adrenal medulla which had been homogenized in one volume of 0.25 M sucrose. The appearance of DOPA in these digests with added L-m-tyrosine and L-tyrosine is illustrated in Fig. 2. It is seen that in both cases, DOPA was present in amounts significantly greater than that which originated from endogenous substrate, and that it was being formed from m-tyrosine at about one-half the rate at which it was being formed from tyrosine. Tissues were then fractionated to give a partially purified rat liver phenylalanine hydroxylase, according to Kaufman (8), and

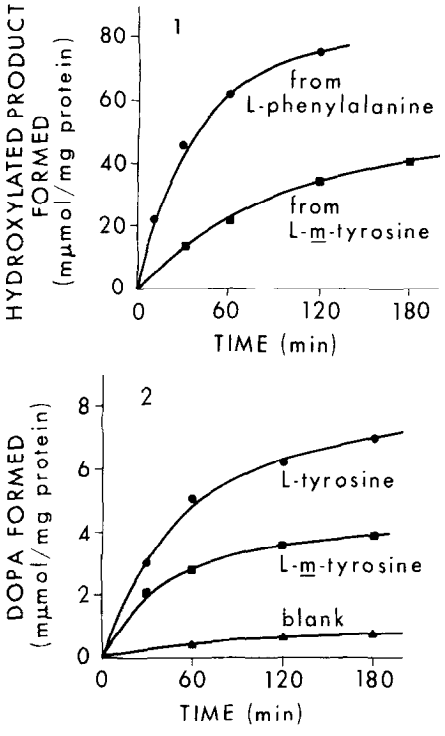

Fig. 1. Formation of DOPA from L-m-tyrosine, and tyrosine from L-phenylalanine by rat liver homogenate. Digests contained 2 μmol each of substrate, NADPH, $DMPH_4$ and NSD-1055, 100 μmol of mercaptoethanol, 0.5 ml of enzyme preparation, and 0.2 ml of N sodium phosphate, pH 7.0, in 2.0 ml. Incubations were carried out in open flasks in a shaking water bath at 37°, and terminated by the addition of 0.5 ml of 35% sulfosalicylic acid. The mixtures were centrifuged, 0.5 ml of 0.2 N sodium citrate, pH 2.2, was added to 2 ml of the supernatant, and 1 ml was analyzed with a Beckman model 120B amino acid analyzer (Table I).

Fig. 2. Formation of DOPA from L-m-tyrosine and L-tyrosine by beef adrenal medulla homogenate. Conditions as in Fig. 1 except that digests contained 2.5×10^{-4} M $FeSO_4$ and the buffer was sodium citrate, pH 6.0.

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a more purified beef adrenal medulla tyrosine hydroxylase, according to Nagatsu et al (9). The results of these and some of the other experiments appear in Table II, and are summarized as follows: when L-m-tyrosine was incubated with rat liver homogenate in the presence of NSD-1055, DOPA appeared in the medium. Twice as much DOPA was detected when $DMPH_4$ had also been added. No DOPA was detected in the absence of the decarboxylase

TABLE II

Formation of DOPA[a] from tyrosine

Substrate	Modification to digest	Rat liver[b]			Beef adrenal medulla[c]		
		Homogenate	Second ethanol fraction	First $(NH_4)_2SO_4$ fraction	Homogenate	Particles	40% $(NH_4)_2SO_4$ ppt. from sol. fraction
L-m-Tyrosine		42.1	86.6	193	2.8	3.3	17.4
L-m-Tyrosine	-NSD-1055	0	2.8	178	1.9	2.6	13.8
L-m-Tyrosine	$-DMPH_4$	21.7	8.9	0	0	0	0
L-m-Tyrosine	-NADPH	42.0	87.0	193	3.25	3.5	17.7
L-m-Tyrosine	boiled enzyme	0	0	0	0	0	0
-		0	0	0	0.5	0.7	0.6
L-m-Tyrosine	+D-m-Tyrosine[f]	41.0			3.0		
D-m-Tyrosine		0	0	0	0.55	0.8	0.5
L-o-Tyrosine		0			0.5		
L-Tyrosine		0	0	0	5.8	6.0	35.2
L-Tyrosine	$-DMPH_4$				0	0	0
L-Phenylalanine		49.7[g]	120[g]	293[g]			
L-Phenylalanine	$-DMPH_4$	28.7[g]	17.8[g]	0[g]			

a mμmol/3 h/mg protein; 37 °C.
b Digests contained ingredients as described in Fig. 1.
c Digests as for rat liver except that the buffer was sodium citrate, pH 6.0, and digest contained 2.5×10^{-4} M ferrous sulfate.
d Partially purified phenylalanine hydroxylase, as per Kaufman (8).
e Partially purified tyrosine hydroxylase, as per Nagatsu et al. (9).
f 2 μmol
g Tyrosine, mμmol/30 min.

inhibitor, or when the substrate was D-m-tyrosine or L-tyrosine. When either L-m-tyrosine or L-tyrosine was incubated with beef adrenal medulla homogenate or purified beef adrenal tyrosine hydroxylase in the presence of $DMPH_4$, DOPA appeared in the medium in amounts greater than when no substrate was present. The amount of DOPA detected when D-m-tyrosine was added as substrate was not greater than that detected in the absence of added substrate. In neither case did the formation of DOPA from L-m-tyrosine seem to have been affected by the presence of D-m-tyrosine, nor was a new amino acid detected when L-o-tyrosine was the substrate. No DOPA could be detected when L-m-tyrosine was incubated with rat brain homogenate.

That the product formed from L-m-tyrosine in the presence of rat liver homogenate (Fig. 1) was DOPA was confirmed by observation of its fluorescence spectrum and paper chromatography. After the addition of sulfosalicylic acid and pH 2.2 buffer to a 5-hour incubation, 2 ml was chromatographed on the analyzer using system B with the column effluent being directed into a fraction collector. Fractions were collected at 3-min intervals. m-Tyrosine was found in fractions 35-38, the new amino acid, in fractions 31-34. The fluorescence spectrum of an aliquot from fraction 33 (activation max. at 285 nm; emission max. at 320 nm) was identical with that of an authentic sample of DL-DOPA. Part of fraction 33 was extracted with N HCl-saturated n-butanol which was then chromatographed on paper using as solvent N HCl-saturated n-butanol-acetic acid-water (78:5:17) (10). The amino acid coincided with DOPA in this system which is reported to separate the 2,3-, 2,5-, and 3,4-isomers of dihydroxyphenylalanine.

Discussion

Based on the evidence presented above, our conclusion is

that L-m-tyrosine can be converted to L-DOPA both by rat liver and beef adrenals. Such a transformation has already been shown to be effected by a soluble cell-free preparation from Bacillus cereus (11) however, it has never been shown to take place in a mammalian system. DOPA, which gives rise to noradrenaline and adrenaline in beef adrenals (12), is the product of the hydroxylation of tyrosine (13,14) which comes from phenylalanine (13-16). m-Tyrosine has been rejected as an intermediate in the formation of DOPA from phenylalanine in beef adrenal (13,14) and dog brain (13), and this partly on the basis that it has been shown not to be a substrate for beef adrenal tyrosine hydroxylase (9). Our results indicate that L-m-tyrosine is, in fact, a substrate for the partially purified beef adrenal tyrosine hydroxylase preparation (9). Moreover, they show the L-m-tyrosine is a substrate for the rat liver phenylalanine hydroxylase preparation which is contrary to preceeding reports (8,17). They support the finding that m-tyrosine was converted to DOPamine in the rat (2) and, coupled with the demonstration of the hydroxylation of phenylalanine in the meta position (giving rise ultimately to m-hydroxyphenylethylamine (4)), revive the possibility that some DOPA is formed from phenylalanine by way of m-tyrosine. The latter induces us to suggest consideration of m-tyrosine for the treatment of parkinsonism, which is known to be partially relieved by L-DOPA (18). We have confirmed, using L-m-tyrosine, that m-tyrosine has the same awakening effect as DOPA on reserpine-treated mice (19). It is known that the depleting action of reserpine in the brain is partially protected by m-tyrosine (20).

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