# FORMATION OF meta-TYROSINE FROM L-PHENYLALANINE BY BEEF ADRENAL MEDULLA. A NEW BIOSYNTHETIC ROUTE TO CATECHOLAMINES*

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Received May 24, 1971

Summary

Incubation of L-phenylalanine-$^{14}C$ with beef adrenal medulla homogenate in the presence of a pteridine co-factor and a DOPA decarboxylase inhibitor gave rise to three radioactive products which were identified with an amino acid analyzer as tyrosine, m-tyrosine and DOPA. The formation of m-tyrosine was completely prevented by the tyrosine hydroxylase inhibitors α-methyltyrosine and 3-iodotyrosine. This demonstration of the conversion of phenylalanine to m-tyrosine, combined with our previously reported demonstration of the formation of L-DOPA from L-m-tyrosine, provides a new pathway for the biosynthesis of catecholamines in beef adrenals.

In mammals, phenylalanine is in large part metabolized by conversion to tyrosine in the liver, followed by transamination and subsequent rearrangement and cleavage to two four-carbon units (1). In addition, in brain and adrenals, phenylalanine is transformed into the catecholamines by way of tyrosine and 3,4-dihydroxyphenylalanine (DOPA) (2). We have recently reported that L-m-tyrosine gives rise to L-DOPA in rat liver and beef adrenals in vitro (3). We now report evidence that when DOPA is being formed from L-phenylalanine in a beef adrenal system in vitro, besides tyrosine, m-tyrosine is also present in the system.

* Supported by grants from the Medical Research Council of Canada
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## Materials and Methods

Compounds were purchased as follows: L-phenylalanine-$^{14}$C, uniformly labelled, 384 mCi/mmol (New England Nuclear), DL-DOPA-2-$^{14}$C and L-tyrosine-$^{14}$C (U) hydrochloride (Nuclear Chicago), 6,7-dimethyl-5,6,7,8-tetrahydropterine·HCl·1½$H_2O$ ($DMPH_4$) (Calbiochem), NADPH and α-methyl-DL-tyrosine (Sigma), and 3-iodo-L-tyrosine (Aldrich). DL-m-Tyrosine-2-$^{14}$C was a product synthesized in our laboratory by Dr. S. Sharma. 3-Bromo-4-hydroxybenzyloxyamino phosphate (NSD-1055) was a gift from Smith and Nephew Research, Gilson Park, Harlow, U.K. Female Sprague-Dawley rats (250-300 g) killed by decapitation and fresh beef adrenals obtained locally were used. Protein was determined by the method of Lowry et al. (4) using bovine serum albumin as standard.

Radioactive products were identified by co-chromatography with authentic labelled compounds on the 50 cm AA-15 resin column of a Beckman model 120B amino acid analyzer coupled at the outlet of the column to a Nuclear Chicago model 4526 flow cell scintillation system. The order of emergence of the pertinent amino acids (Fig. 1) was consistent with that observed for the unlabelled compounds with the analyzer incorporating the ninhydrin system. The amount of radioactivity corresponding to each product (Fig. 2) was obtained by collecting the effluent from the column in 0.5-ml fractions, adding 10 ml of a toluene solution containing 0.4% 2,5-diphenyloxazole and 10% (v/v) Bio Solv BBS 3 (Beckman Instruments), and counting with a Beckman model LS-133 scintillation counter. A >95% recovery of radioactivity was obtained with this procedure in a control experiment.

## Results

Beef adrenal medulla was homogenized in two volumes of 0.32 M sucrose in a Servall omni mixer for one minute. The mixture was centrifuged at 1000 x g for 10 minutes, and aliquots were incubated at 37°C with L-phenylalanine-$^{14}$C (U) in the presence of the DOPA decarboxylase inhibitor NSD-1055 and a pteridine co-factor as described in Fig. 2. Analysis indicated the appearance of three radioactive products which were identified as tyrosine, m-tyrosine and DOPA by co-chromatography with authentic labelled compounds using an amino acid analyzer coupled to a flow cell system for scintillation counting. The chromatographic data are illustrated in Fig. 1.

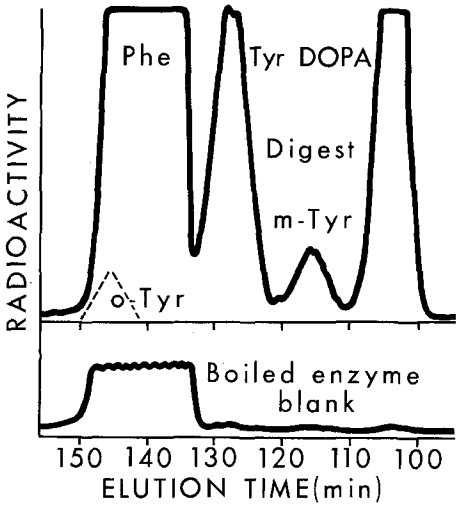


Fig. 1. Chromatographic separation of the products from the incubation of L-phenylalanine-$^{14}$C (U) with beef adrenal medulla. 2-ml of a supernatant from a 30-min digest (Fig. 2) was placed on a 50 x 0.9 cm column of AA-15 resin of a Beckman amino acid analyzer which was eluted with 0.2 N sodium citrate, pH 4.25, at 57° at a flow rate of 34 ml/h. The column effluent was directed into the flow cell of a scintillation counter. The dotted line indicates the time of elution of o-tyrosine with this system.

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The amounts of radioactivity present in each metabolite in the incubation mixture during the first two hours were then

determined by collecting the column effluent in fractions and counting the radioactivity of each with a scintillation counter. The results appear in Fig. 2. It is seen that initially, m-tyrosine was being formed at a rate of about 15% of that of tyrosine, that the amounts of the two levelled off after 1 hour, and that the amount of DOPA which accummulated remained constant after 90 minutes at a level equal to twice that of tyrosine. A control incubation using boiled enzyme showed that non-enzymatic hydroxylation of the substrate could not account for more than

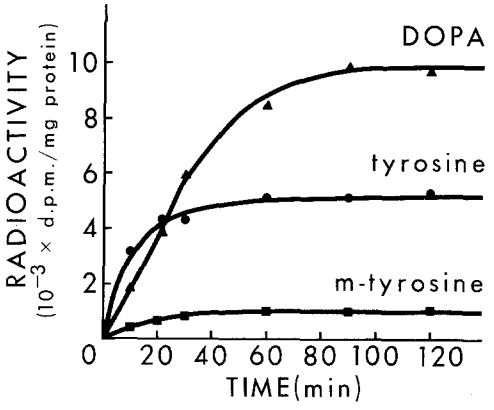


Fig. 2. Hydroxylation of L-phenylalanine-$^{14}$C by beef adrenal medulla. Digests contained 1 μCi of L-phenylalanine-$^{14}$C (U), 2 μmol each of $DMPH_4$ and NSD-1055, 100 μmol of mercaptoethanol, 0.5 ml of enzyme preparation, and 0.2 ml of N sodium citrate, pH 6.0, in 2.0 ml. Incubations were carried out in open flasks in a shaking water bath at 37°C, and terminated by the addition of 0.5 ml of 35% sulfosalicylic acid containing 0.5 μmol each of DOPA, m-tyrosine and tyrosine. 0.5 ml of 0.2 N sodium citrate, pH 2.2, was added, the mixture was centrifuged, and 2 ml of the supernatant was chromatographed with the amino acid analyzer as described in Fig. 1. The effluent from the column was collected with a fraction collector and analyzed for radioactivity as described in Materials and Methods.

5% of the m-tyrosine formed nor for more than 1% of the other products formed. Moreover, an incubation with DL-DOPA-2-$^{14}$C (0.1 μCi) as substrate using active enzyme yielded no m-tyrosine, indicating that the labelled m-tyrosine appearing in the digests containing L-phenylalanine-$^{14}$C could not have come from DOPA.

The same three metabolites of L-phenylalanine were found when the enzyme source was the 40% ammonium sulfate precipitate from the soluble fraction of the beef adrenal medulla homogenate.

In Table I are shown the results for 30-minute incubations of modifying the standard incubation mixture used. It is seen that the addition of NADPH or ferrous ion had no effect on the reactions. Substantial amounts of m-tyrosine and DOPA still accummulated even in the absence of the decarboxylase inhibitor. Very little hydroxylation took place in the absence of the reduced pteridine co-factor except for the formation of a small amount of tyrosine. With the addition of the tyrosine hydroxylase inhibitors α-methyltyrosine or 3-iodotyrosine at a concentration

Table I

Effect of various substances on the hydroxylation of L-phenylalanine-$^{14}$C by beef adrenal medulla homogenate[a]

| Modification to digest | Radioactivity (d.p.m./mg protein) accounted for as | | |
|---|---|---|---|
| | m-Tyrosine | Tyrosine | DOPA |
| - | 864 | 4370 | 5981 |
| +NADPH[b] | 823 | 4312 | 5925 |
| +$Fe^{++}$[c] | 831 | 4409 | 6203 |
| -NSD-1055 | 580 | 4080 | 3170 |
| -$DMPH_4$ | 34 | 335 | 13 |
| +α-Methyl-DL-tyrosine[b] | 12 | 29 | 6 |
| +3-Iodo-L-tyrosine[b] | 14 | 61 | 11 |

a Incubation mixture as in Fig. 2, for 30 minutes.
b 2 μmol.
c 0.5 μmol $FeSO_4$.

of $10^{-3}$ M, the hydroxylation of phenylalanine was completely prevented.

Similar experiments were carried out using rat liver homogenate. Under conditions where phenylalanine hydroxylase was shown to be active, no conversion of L-phenylalanine-$^{14}$C to m-tyrosine nor to DOPA could be detected. Our method would have been able to detect the conversion of as little as 0.2% of the phenylalanine.

## Discussion

Phenylalanine is transformed into tyrosine by phenylalanine hydroxylase of liver (5,6), and into tyrosine and DOPA by tyrosine hydroxylase of brain (7,8) and adrenals (7-9). We have now found and reported here that in a beef adrenal system carrying out this conversion of L-phenylalanine into tyrosine and DOPA, m-tyrosine is also present. The system was shown not to be active with boiled enzyme (Fig. 1), nor to convert DOPA into m-tyrosine. Therefore, neither the well known non-enzymatic hydroxylation of aromatic acids (10-13) nor the dehydroxylation of DOPA, which can give rise to tyrosine (14), can account for the appearance of m-tyrosine in our experiments. Our conclusion is that the labelled m-tyrosine was formed directly from L-phenylalanine. We have recently reported that the same system is capable of transforming L-m-tyrosine into L-DOPA (3). We are therefore suggesting that in the experiments reported here, labelled DOPA was formed from m-tyrosine as well as tyrosine. The course of appearance of the three metabolites (Fig. 2) is consistent with this suggestion. Our results provide an explanation for the appearance of m-tyramine (15,16) and m-hydroxyphenyl acids (17) in human urine, as well as a new biosynthetic route to the catecholamines. The possibility that

phenylalanine could give rise to m-tyrosine has been considered before but was not explored in depth (7,8).

Our observation that no m-tyrosine was formed from L-phenylalanine in rat liver agrees with the results of experiments using partially purified phenylalanine hydroxylase (18), though others have reported the formation of m-tyramine from phenylalanine by rat liver (19).

The two hydroxylation reactions of phenylalanine observed here were inhibited both by α-methyltyrosine and 3-iodotyrosine. These are specific inhibitors of tyrosine hydroxylase (8). The logical inference is that both reactions were catalyzed by the same enzyme. If this is accepted, it removes the apparent incongruity (8) that tyrosine hydroxylase oxidizes tyrosine at the meta-position and phenylalanine exclusively at the para-position

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