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## Lack of hydroxylation-induced migration with 4-iodophenylalanine

Recent studies have shown that liver phenylalanine hydroxylase converts 4-chloro- and 4-bromophenylalanine to 3-chloro- and 3-bromotyrosine, respectively[1]. A similar migration of deuterium[2] and tritium[3] to the *meta* position has also been noted upon enzymatic hydroxylation of 4-deutero- and 4-tritiophenylalanine. This enzymatic hydroxylation-induced migration has been called the "NIH shift". Experiments with 4-fluorophenylalanine, on the other hand, revealed that this shift did not occur and that hydroxylation was accompanied by loss of halogen to give tyrosine as the sole product[1]. The recent synthesis of 4-[$^{125}$I]iodophenylalanine in our laboratory[4] afforded us the opportunity to ascertain whether this analogue would behave in a manner similar to that of its chloro and bromo congeners and undergo the "NIH shift" to give the biologically important amino acid, 3-iodotyrosine. No migration of the halogen was observed when 4-iodophenylalanine was subjected to the action of rat liver phenylalanine hydroxylase.

DL-4-Iodophenylalanine and DL-4-[$^{125}$I]iodophenylalanine were synthesized in our laboratory and reported elsewhere[4]. L-3-Iodotyrosine and DL-4-chlorophenylalanine were purchased from Aldrich Chemical Corp. L-3-Chlorotyrosine was generously given by Dr. Gordon Guroff, Laboratory of Clinical Biochemistry, National Heart Institute. 6,7-Dimethyltetrahydropteridine was obtained from Cal Biochem, Los Angeles, Calif. L-[$^{14}$C]Phenylalanine (uniformly labeled) and L-[$^{14}$C]tyrosine (uniformly labeled) were purchased from New England Nuclear Corp.

Phenylalanine hydroxylase was prepared according to KAUFMAN[5] from rat liver and through the first ammonium sulfate fractionation. Incubations were performed essentially as described by GUROFF and co-workers[1-3]. The mixture contained Tris-HCl buffer (pH 7.3), 100 $\mu$moles; 6,7-dimethyltetrahydropteridine, 0.9 $\mu$mole; 2-mercaptoethanol, 15 $\mu$moles; either DL-4-chlorophenylalanine, 2 $\mu$moles, or L-[$^{14}$C]phenylalanine, 1 $\mu$mole; and enzyme (1 mg protein) in a final volume of 1.2 ml. The mixtures were incubated for 2 h when halogenated substrates were used and for 30 min when [$^{14}$C]phenylalanine was substrate. The reaction was stopped by heating for 5 min at 100°. The protein was separated by centrifugation and the supernatant was chromatographed on Whatman 3MM paper. The chromatogram was developed in 2-propanol–conc. $NH_4OH$ (2:1, v/v). The products were detected on the chromatogram with

TABLE I

$R_F$ VALUES FOR PHENYLALANINE DERIVATIVES

Solvent system: 2-propanol–conc. $NH_4OH$ (2:1, v/v).

| *Amino acid* | $R_F$ |
|---|---|
| L-[$^{14}$C]Phenylalanine | 0.63 |
| L-Tyrosine | 0.45 |
| DL-4-[$^{125}$I]Iodophenylalanine | 0.75 |
| L-3-Iodotyrosine | 0.33 |
| DL-4-Chlorophenylalanine | 0.76 |
| L-3-Chlorotyrosine | 0.36 |

ninhydrin spray and autoradiography using Kodak X-ray film. The $R_F$ values of the standards and substrates are given in Table I.

Five separate experiments with four different enzyme preparations were performed using DL-4-[$^{125}$I]iodophenylalanine as the substrate. Duplicate and triplicate incubations were performed for each experiment. No 3-iodotyrosine was detected in any of the experiments by either ninhydrin or autoradiography. A small amount of tyrosine could be detected, however, similar to the results previously reported for 4-fluorophenylalanine[1]. Under identical conditions DL-4-chlorophenylalanine gave ninhydrin-detectable amounts of 3-chlorotyrosine. The phenylalanine hydroxylase activity of the enzyme preparations ranged around 13 nmoles of tyrosine produced per min per mg of protein. We conclude that under the conditions of our assay no hydroxylation-induced migration of iodine from the 4 position of phenylalanine occurs.

It is generally agreed that the formation of 3-iodotyrosine *in vivo* involves iodination of tyrosine[6]. The recent finding that 4-chloro- and 4-bromophenylalanine give rise to 3-chloro- and 3-bromotyrosine, however, suggested that hydroxylation of 4-iodophenylalanine may represent an alternate pathway to 3-iodotyrosine. In this regard, our studies have shown that 4-iodophenylalanine behaves differently from 4-chloro- or 4-bromophenylalanine upon incubation with phenylalanine hydroxylase and is not transformed into 3-iodotyrosine. Whether there are thyroidal enzymes that can effect this transformation remains to be determined.

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