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SHORT COMMUNICATION

NICOTINIC ACID DECARBOXYLATION IN TOBACCO ROOTS*

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Abstract—An enzyme system which catalyzes the O_2 dependent release of $^{14}CO_2$ from nicotinic acid-7-^{14}C is present in tobacco roots The enzyme is not present in tobacco leaves and stems The nature of reaction catalyzed and its tissue distribution suggest that this enzyme may be involved in nicotine biosynthesis

INTRODUCTION

THE BIOSYNTHESIS of the pyridine alkaloid nicotine in various *Nicotiana* species has been studied intensively in several laboratories It has been shown that the pyridine ring of nicotinic acid[1] but not the carboxyl group,[2] is incorporated into nicotine Therefore, the carboxyl group of pyridine ring precursors must be released (presumably as CO_2) during the synthesis of nicotine This fact led us to examine crude extracts of *Nicotiana rustica* for nicotinic acid decarboxylase activity In this paper, we report the results of experiments on the release of $^{14}CO_2$ from nicotinic acid-7-^{14}C by a particulate fraction obtained from tobacco roots. A preliminary report of this work has appeared [3]

RESULTS

Intracellular Distribution

Centrifugation studies indicated that the enzymatic activity sedimented at 20,000 *g* The results of a typical experiment are shown in Table 1

TABLE 1 INTRACELLULAR DISTRIBUTION OF NICOTINIC ACID DECARBOXYLASE

Subcellular fraction	Specific activity $\mu\mu$moles/hr/mg protein
Crude homogenate	6 7
500 *g* supernatant	13 2
500 *g* pellet	2 4
20,000 *g* supernatant	2 7
20,000 *g* pellet	42 0

The complete reaction mixture contained 60 μmoles K_2HPO_4, pH 7 0, 80 nmmoles NA-7-^{14}C, (1 μc) and 2 ml of enzyme prep in a total volume of 3 0 ml

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[1] R F DAWSON, D R CHRISTMAN, A D'ADAMO, M S SOLT and A P WOLF, *J Am Chem Soc* **82,** 2628 (1960)

[2] R F DAWSON, D R CHRISTMAN and R C ANDERSON, *J Am Chem Soc* **75,** 5114 (1953)

[3] R K GHOLSON, J L. R CHANDLER, K S YANG and G R WALLER, *Fedn Proc* **23,** 528 (1964)

pH Optimum

The catalytic activity was determined as a function of pH in 0 01 M potassium phosphate buffer A broad optimum was observed from pH 6 0 to 7 0, however, the activity decreased rapidly above pH 7 5. All further studies were conducted at pH 7 0 in 0 1 M potassium phosphate buffer The release of $^{14}CO_2$ from nicotinic acid-7-^{14}C was linear with time for at least 4 hr under the assay conditions employed

TABLE 2 SUBSTRATE REQUIREMENT FOR NICOTINIC ACID DECARBOXYLASE

Experiment	Relative activity
Control	1 00
Evacuated (—O_2)	0 01
—Nicotinic acid, + nicotinic acid mononucleotide-7-^{14}C	0 44
—Nicotinic acid, + quinolinic acid -2,3,7,8-^{14}C	0 001

The complete reaction mixture contained 2 5 mg protein of the 20,000 *g* pellet, 80 nmoles NA-7-^{14}C (1 μc), and 60 μmoles potassium phosphate (pH 7 0) in a volume of 3 0 ml The reaction was incubated at 27° for 4 hr

Substrate Requirement

The data shown in Table 2 suggest that O_2 is required for the release of $^{14}CO_2$ from nicotinic acid If the Thunberg tube was evacuated with a water aspirator for 5 min, only 1 % of the control activity was observed Quinolinic acid-2, 3, 7, 8-^{14}C was not appreciably decarboxylated by this enzyme system The enzyme preparation released $^{14}CO_2$ from nicotinic acid-(7-^{14}C)-mononucleotide with about 40% of the efficiency of the nicotinic acid control. This activity may be due to prior hydrolysis of nicotinic acid mononucleotide to nicotinic acid during the relatively lengthy incubation time The formation of nicotinic acid from nicotinic acid mononucleotide has been observed in beef liver [4] In a separate experiment streptomycin sulfate at a final concentration of 0 0067 % had no significant effect on the release of $^{14}CO_2$ from nicotinic acid-7-^{14}C Control 2977 counts/min, plus streptomycin sulfate 2424 counts/min

Cofactors

No cofactors for the reaction were identified. Addition of ATP, $MgCl_2$, ornithine, NAD, NADH, NADP, and pyridoxal phosphate did not stimulate the release of $^{14}CO_2$ from nicotinic acid by this tobacco root preparation Ornithine was included because it has been shown to be a precursor of the pyrolidine ring of nicotine

K_m for Nicotinic Acid

The catalytic activity as a function of nicotinic acid concentration was determined. The apparent K_m value for nicotinic acid as determined from a Lineweaver–Burk plot was $1{\cdot}4 \times 10^{-6}$ M

[4] R K GHOLSON, I UEDA, N OGASAWARA and L M HENDERSON, *J Biol Chem* **239**, 1208 (1964)

TABLE 3 EFFECT OF SOME POTENTIAL COFACTORS ON NICOTINIC ACID DECARBOXYLASE ACTIVITY

Additions	$^{14}CO_2$ (counts/min)
1 None	4143
2 ATP 3×10^{-4}M, Mg 10^{-4} M	3992
3 2 + Ornithine 3×10^{-4} M	3840
4 3 + NADH 3×10^{-5} M	1266
5 3 + NAD 3×10^{-5} M	909
6 5 + Pyridoxal P 3×10^{-5} M	1055
7 3 + NADP 3×10^{-5} M	2874
8 Boiled Enzyme	255

Reaction system given in Table 2

Tissue Distribution

The enzymic activity in crude extracts prepared from leaves and stems was only 1–5% of that observed in preparations obtained from roots. Nicotinic acid decarboxylase activity was not observed in extracts of cotyledons of *Ricinus communis* seedlings

DISCUSSION

These experiments define a tobacco root enzyme system capable of releasing $^{14}CO_2$ from nicotinic acid-7-^{14}C. The enzyme system is located in a particulate fraction obtained after centrifugation at 20,000 *g* for 30 min. This fraction contains mitochondria but association of the activity with the mitochondria was not established

The role of this enzyme system in the metabolism of nicotinic acid remains to be determined. However, it is tempting to speculate that it may function as an early enzymatic step in the biosynthesis of nicotine. Experiments *in vivo* have demonstrated that the carboxyl group is lost when the pyridine ring of nicotinic acid is incorporated into nicotine [2] It has also been shown that nicotine biosynthesis takes place in the roots of tobacco plants rather than the leaves and stems [5] It, therefore, appears possible that the enzyme system described above, which releases the carboxyl group of nicotinic acid as CO_2 and which is present in tobacco roots, but is not found in tobacco leaves and stems or in castor seedlings, may be involved in nicotine biosynthesis However, these experiments provide no direct evidence as to the fate of the ring carbons of nicotinic acid and it is also possible that the observed release of CO_2 may be a result of a more complete degradation of this molecule.

Although it is possible that the observed conversion of nicotinic acid could be due to microorganisms contaminating the tobacco root preparation, this seems to be highly unlikely on the basis of the following considerations. Streptomycin sulfate did not materially affect the extent of the reaction. The reaction was linear with time for 4 hr, if microorganisms were responsible an increasing rate of $^{14}CO_2$ release with time would be expected as they multiplied. The apparent K_m for nicotinic acid is very low and in the range where microorganisms would be expected to use the compound as a vitamin rather than a substrate for growth. The reaction was only observed in tobacco root preparations and not in tobacco leaves or castor bean seedling cotyledons.

[5] R F DAWSON and M L SOLT, *Plant Physiol* **34**, 656 (1959)

EXPERIMENTAL

Tobacco plants (*Nicotina rustica*) were grown in a greenhouse in hydroponic medium [6] The roots were excised, thoroughly washed with deionized water, blotted, and weighed After cutting the roots into short segments, they were ground in a pre-chilled mortar with 2 vol of 0 01 M $KHPO_4$ buffer pH 7 0 The resulting brei, which usually contained 1 1 mg of protein/ml, was filtered through 5 layers of cheese cloth to remove fiberous material

The reaction was carried out in Thunberg tubes as previously described for quinolinate phosphoribosyl transferase [4] After incubation for the desired time, the reaction was terminated by addition of 0 2 ml of N KOH through the side-arm After evacuating the tube with an aspirator, 1 0 ml of 15% trichloroacetic acid was carefully admitted into the vessel to release the $^{14}CO_2$ to dissolve in 0 3 ml of 1 0 N NaOH in the upper bulb for 1 hr, then air was admitted into the vessel and the amount of $^{14}CO_2$ released was determined by liquid scintillation counting of an aliquot of NaOH Protein concentration was determined by the method of Lowry *et al* [7] Tobacco root mitochrondria (20,000 *g* pellet of Table 1) were prepared as described by Walker and Beevers [8]

[6] D R. HOAGLAND and D I ARNON, *California Agricultural Experiment Circular* 347 (1950)
[7] O H. LOWRY, N J ROSENBROUGH, A L FARR and R J RANDALL, *J Biol Chem* **193,** 265 (1951)
[8] D A. WALKER and H BEVERS, *Biochem J* **62,** 120 (1956)

Key Word Index—Nicotiana rustica, Solanaceae, nicotine, alkaloid, biosynthesis, nicotinic acid decarboxylase