THE CONVERSION OF TRYPTOPHAN TO 2,3-DIHYDROXYBENZOIC ACID AND CATECHOL BY ASPERGILLUS NIGER

P. V. Subba Rao, K. Moore and G. H. N. Towers,
Department of Botany, The University of British Columbia,
Vancouver, Canada

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The importance of 2,3-dihydroxybenzoic acid as a growth factor for certain multiple aromatic auxotrophs of Aerobactor aerogenes and Escherichia coli has been pointed out recently (Young, et al., 1967). Using cell-free preparations from these two organisms, Young et al. (1967) demonstrated that chorismic acid is the precursor for 2,3-dihydroxybenzoic acid. Terui and his co-workers (1953, 1961) reported that Aspergillus niger can metabolize salicylic acid as well as anthranilic acid by a route involving the formation of 2,3-dihydroxybenzoic acid. The conversion of 2,3-dihydroxybenzoic acid to catechol by purified preparations from A. niger has been reported by Subba Rao et al. (In press). In the present communication we report the results of tracer studies to show the origin of 2,3-dihydroxybenzoic acid and catechol from tryptophan in A. niger.

EXPERIMENTAL

A strain of Aspergillus niger (UBC 814) was grown at 26° for 72 hr. in 500 ml flasks on a synthetic medium (Byrde et al., 1956) supplemented with 0.1% L-tryptophan. The mycelial felt was washed aceptically with distilled water and replaced with 0.01M phosphate buffer, pH 7 (50 ml) containing 4 μcuries of DL-tryptophan-C^{14} (uniformly labeled in the benzene ring) (Nuclear-Chicago). After 2 hr. incubation with gentle agitation at 26°, the reaction was arrested by acidification with 1N HCl and the mycelium was macerated in a mortar along with the medium. The extract was centrifuged and the supernatant extracted

thrice with equal volumes of peroxide-free ether. The organic layer was dried in vacuo after shaking with anhydrous Na_2SO_4. The residue, after dissolving in ethanol, was subjected to two-directional chromatography and radioautography (Ibrahim and Towers, 1959, 1960) on thin-layer cellulose plates and the radioactivity determined in a Nuclear-Chicago scintillation spectrometer. The radioactivity in $C^{14}O_2$ was measured after trapping in a solution of hyamine hydroxide.

To determine the 2,3-dihydroxybenzoic acid decarboxylase activity, acetone powder (1 g) prepared from the mycelia of A. niger was extracted with 20 ml of 0.05M phosphate buffer (pH7) and centrifuged at 20,000 xg for 20 min. A reaction mixture (2 ml) containing 0.5 ml enzyme, 1 ml citrate-phosphate buffer (pH 5.2) and 2 µmoles 2,3-dihydroxybenzoic acid was incubated for 20 min. at 30°. The reaction was stopped by the addition of 1 N HCl (1 ml) and the amount of 2,3-dihydroxybenzoic acid was determined spectrophotometrically at 320 mµ after extraction into ethylacetate.

RESULTS AND DISCUSSION

As shown in Table I, the radioactivity from the benzene ring of tryptophan-C^{14} was incorporated into anthranilic acid, 3-hydroxyanthranilic acid, 2,3-dihydroxybenzoic acid, catechol and CO_2. D-Glucose-C^{14} (uniformly labeled), acetate-2-C^{14}, DL-Shikimic acid-1,2-C^{14} or DL-phenylalanine-C^{14} (uniformly ring-labeled) failed to serve as precursors for 2,3-dihydroxybenzoic acid or catechol when supplied to mycelia grown either in the presence or absence of tryptophan.

2,3-Dihydroxybenzoic acid and catechol which accumulated in the culture filtrates of A. niger grown in the presence of L-tryptophan, L-kynurenine, anthranilic acid or 3-hydroxyanthranilic acid (0.1%) were isolated and identified by comparing with authentic samples. When the organism was grown on L-tryptophan, L-kynurenine or anthranilic acid, 3-hydroxyanthranilic acid was found to be an obligatory intermediate in the pathway. Neither 2,3-dihydroxybenzoic acid nor catechol were detected

TABLE I

Metabolism of DL-tryptophan-C^{14} by Aspergillus niger

Compounds in ether extract	Radioactivity (mμcuries)
Anthranilic acid	100
3-Hydroxyanthranilic acid	56
2,3-Dihydroxybenzoic acid	204
Catechol	25
Nonenzymic oxidation products * of 3-hydroxyanthranilic acid	32
Unknown	38

The amount of radioactivity in $C^{14}O_2$ was 137 mμcuries.

*3-Hydroxyanthranilic acid is unstable on chromatograms. The oxidation products move as a single spot with a characteristic position.

TABLE II

Induction of 2,3-dihydroxybenzoic acid decarboxylase in A. niger by tryptophan and its metabolites

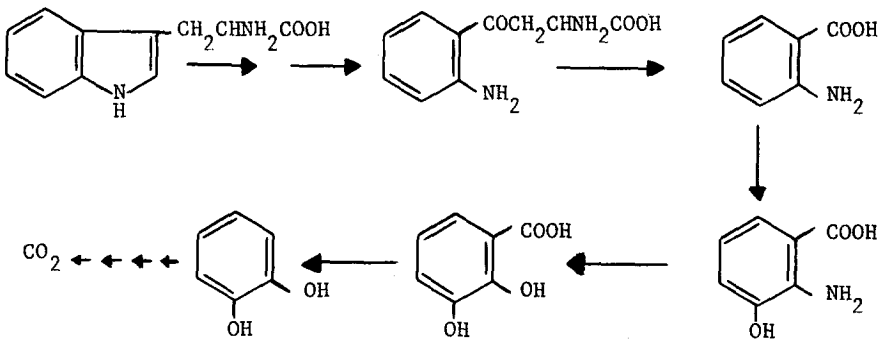
Inducer	units/gm acetone powder
L-Tryptophan	50
L-Kynurenine	41
Anthranilic acid	44
3-Hydroxyanthranilic acid	46
2,3-Dihydroxybenzoic acid	35
Catechol	0

A unit activity is defined as the amount of enzyme that catalyzes the disappearance of 1 μmole of 2,3-dihydroxybenzoic acid.

in culture filtrates when the organism was grown on phenylalanine, glucose, shikimic acid, or m-hydroxybenzoic acid. In the presence of salicylic acid, however, catechol could be identified as an intermediate, probably formed by direct oxidative decarboxylation (Yamamoto et al., 1965).

Soluble preparations obtained from the acetone powders of the mycelia grown in the presence of 0.1% tryptophan or its metabolites, catalyzed the decarboxylation of 2,3-dihydroxybenzoic acid to catechol (Table II). Phenylalanine, glucose, shikimic acid, salicylic acid and m-hydroxybenzoic acid were not effective in inducing the decarboxylase. Terui et al. (1956) reported, however, that salicylic acid was also converted to 2,3-dihydroxybenzoic acid and catechol by A. niger.

The results presented above are consistent with the following sequence of reactions for the formation of 2,3-dihydroxybenzoic acid and catechol in A. niger:



Pathway for the degradation of tryptophan by A. niger

This pathway for the conversion of anthranilic acid to catechol by A. niger is different from that reported for Pseudomonas which involves the direct conversion (Taniuchi et al., 1964). Also, it differs from the anthranilic acid-catechol pathway operating in the leaves of Tecoma stans (Nair and Vaidyanathan, 1964). While 2,3-dihydroxybenzoic acid in E. coli and A. aerogenes is derived from chorismic acid (Young

et al., 1967), its formation in A. niger appears to be the result of tryptophan degradation.

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