CONVERSION OF D-TRYPTOPHAN TO L-TRYPTOPHAN BY CELL EXTRACTS OF A FLAVOBACTERIUM species*

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Martin and Durham (1962) reported that a bacterium, tentatively identified as belonging to the genus Flavobacterium, could utilize D-tryptophan as the sole source of carbon and energy for aerobic growth. Sequential induction studies suggested that this organism oxidatively degraded D-tryptophan via L-tryptophan, L-kynurenine, and anthranilic acid. The data suggested that the D- to L-isomeric conversion occurred by a mechanism other than direct racemization although Behrman and Cullen (1961) have reported the presence of a tryptophan racemase in a Pseudomonas species.

Results presented in this communication establish that the mechanism of D- to L-isomeric conversion by this species of Flavobacterium involves the initial oxidation of D-tryptophan to indolepyruvic acid followed by a transamination reaction.

MATERIALS AND METHODS

Cells were grown for 16-18 hours at 37°C on an inorganic salts medium (Durham, 1957) containing 0.07 per cent of the desired carbon source. The cells were harvested and washed twice with 0.01 M Tris (hydroxymethyl amino methane) buffer (pH 7.4). The packed cells were frozen in small pellets and ruptured in an X-press at dry ice temper-

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atures. The ruptured cell suspension was thawed and centrifuged at 20,000 X g for 30 minutes at 4°C. The supernatant solution was used for experimental purposes.

RESULTS AND DISCUSSION

Specific electron acceptors must be added to the system before extracts prepared from DL-tryptophan-grown cells could oxidize D-tryptophan. Phenazine methosulfate or 2,6-dichlorophenol-indophenol could function as electron acceptors and results indicated phenazine methosulfate was the most effective. The reaction could be followed manometrically by measuring oxygen uptake in the Warburg vessel. Figure 1 presents the results obtained when phenazine methosulfate was used as the electron acceptor. The oxidation of D-tryptophan resulted in an uptake of 0.64 μmoles oxygen/μmole of D-tryptophan (Fig. 1). This result is consistent with the concept that D-tryptophan is oxidized to indolepyruvic acid (theoretically the oxidation of D-tryptophan to indolepyruvic acid would necessitate the uptake of 0.5 mole oxygen/mole substrate). Chromatographic analysis (Kaper and Veldstra, 1958) of the deproteinized complete system containing D-tryptophan (Table I) revealed the presence of indolepyruvic acid. As shown in Table I and as observed by several authors (Kaper and Veldstra, 1958; Wildman et al., 1947) indolepyruvic acid is unstable and undergoes some spontaneous oxidation under experimental conditions. The D-tryptophan oxidase could not be demonstrated in L-tryptophan-grown cells.

Microorganisms have previously been reported to convert indolepyruvic acid to tryptophan. Aida et al. (1958) used dried cells of several bacterial species to demonstrate that indolepyruvic acid was readily converted to L-tryptophan via a transamination reaction. Glutamic and aspartic acids were the most efficient amino donors but a large number of L-amino acids could serve to some extent as sources of the amino group. Our studies with this species of Flavobacterium establish that glutamic and aspartic acids and glutamine will function as efficient amino donors in this micro-

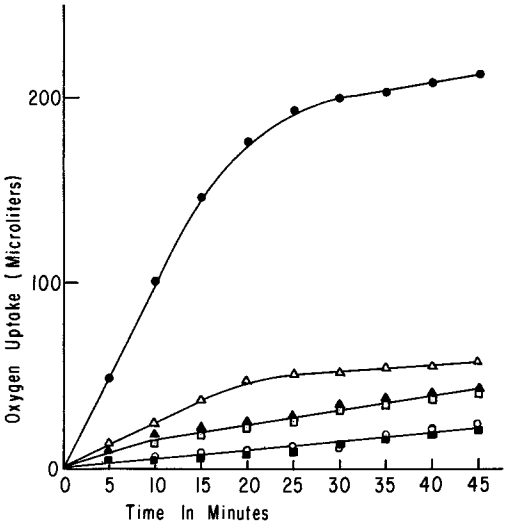

Figure 1. Oxidation of D-tryptophan by extracts of a DL-tryptophan-grown Flavobacterium species.

The main compartment of the Warburg flasks contained 100 μmoles of Tris-HCl buffer (pH 7.4) and 1.0 ml of extract from DL-tryptophan-grown cells (containing 15 mg of protein) in a total volume of 2.0 ml. The center well contained 0.2 ml of 20% KOH (w/v). After thermal equilibration at 37°C for 10 minutes, 10 μmoles of D-tryptophan and 0.2 ml of 1% (w/v) phenazine methosulfate were added from the side arms. The oxygen uptake (μliters) was recorded. ● =Complete system; ■ =phenazine methosulfate omitted; ▲ =D-tryptophan omitted; O=phenazine methosulfate and D-tryptophan omitted; □ =L-tryptophan replaces D-tryptophan; △ =indolepyruvic acid replaces D-tryptophan.

bial system. The data in Table I show the oxygen uptake values and compare a number of different systems for L-tryptophan production via the transamination reaction. L-Tryptophan was produced when the extract was incubated with D-tryptophan, phenazine methosulfate, and L-glutamic acid or when the cell extract was incubated with indolepyruvic acid and an amino donor (L-glutamic acid). When the same systems were incubated in the absence of added L-glutamic acid or other amino donors, a very small quantity of L-tryptophan was formed. This is probably due to transamination which occurs as the result of minute quantities of an amino donor in the cell

TABLE I

Formation of L-tryptophan by extracts of a DL-tryptophan-grown Flavobacterium species.

The main compartment of the Warburg flasks contained: 100 μmoles of Tris-HCl buffer (pH 7.4), 20 μmoles of NaCN, and 1.5 ml of extracts of DL-tryptophan-grown cells (containing 20 mg of protein) in a total volume of 2.4 ml. The center well contained 0.2 ml of 20% KOH (w/v). After thermal equilibration at 37°C, 10 μmoles of either D-tryptophan, L-tryptophan, or indolepyruvic acid and 0.2 ml of 1% (w/v) phenazine methosulfate were added from the side arms. Oxygen uptake was measured, then the flask contents were heated in a boiling water bath for 5 minutes and centrifuged. The supernatant solution was chromatographed employing the ascending technique with isopropanol-water-ammonium solution (sp gr 0.89) (8:1:1 v/v) as the solvent system. The presence of L-tryptophan was demonstrated by a bioautographic technique using Difco tryptophan assay medium plus 2.0% agar and Lactobacillus plantarum 17-5 as the assay organism. Growth was evaluated after 24 hours incubation. Rf values were: tryptophan, 0.24 and anthranilic acid, 0.57. Oxygen uptake values were corrected for endogenous.

Reaction Mixture	O_2 Uptake/Substrate (μmoles)	Growth and Relative Location on Chromatogram*
Complete system (D-tryptophan)	0.64	+++ (Tryptophan)
D-Tryptophan omitted	0.0	-
Phenazine methosulfate omitted	0.0	-
L-Glutamic acid omitted	0.64	+ (Tryptophan)
Complete system but cell extract boiled before incubation	0.0	-
Complete system (Indolepyruvic acid)	0.02	+++ (Tryptophan)
Indolepyruvic acid omitted	0.0	-
Phenazine methosulfate and sodium cyanide omitted	0.78	- (Anthranilic)
L-Glutamic acid omitted	0.01	+ (Tryptophan)
Phenazine methosulfate, sodium cyanide, and L-glutamic acid omitted	0.12	- (Anthranilic)
Complete system but cell extract boiled before incubation	0.03	-
Complete system (L-tryptophan)	0.0	+++ (Tryptophan)
Phenazine methosulfate and sodium cyanide omitted	0.98	- (Anthranilic)
Complete system but cell extract boiled before incubation	0.0	-

* +++=heavy growth; + =light growth; -=no growth.

extract. L-Tryptophan production was measured by the quantitative evaluation of growth using Lactobacillus plantarum 17-5 as the assay organism. The oxygen uptake data support the results obtained with the growth assay.

The formation of L-tryptophan in systems containing D-tryptophan could not be demonstrated unless an electron acceptor such as phenazine methosulfate was added. These data augment the earlier observations that direct racemization of D-tryptophan does not occur in this system and that the oxidation of D-tryptophan results in the formation of indolepyruvic acid which is transaminated to L-tryptophan.

In the absence of sodium cyanide or phenazine methosulfate the crude cell extract oxidized L-tryptophan to L-kynurenine which is rapidly hydrolyzed to anthranilic acid and alanine. Since L-tryptophan is not oxidized to L-kynurenine in the presence of cyanide or phenazine methosulfate, it is assumed that these compounds prevent cleavage of the indole nucleus probably by inhibiting the tryptophan peroxidase-oxidase enzyme. This finding accounts for the production of anthranilic acid in those systems lacking phenazine methosulfate and sodium cyanide.

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