Preliminary Notes

PN 1248

Microbial dissimilation of D- and L-tryptophan in the presence of analogs

Certain tryptophan analogs have been reported to function in reactions with the tryptophan-activating enzymes[1,2] and to influence the synthesis and activity of tryptophan synthase[3], but little information is available regarding the influence of analogs on the microbial oxidation of the tryptophan isomers. A recent report from this laboratory established that a Flavobacterium sp. dissimilated D-tryptophan via a pathway involving L-tryptophan, L-kynurenine, and anthranilic acid[4].

This paper reports evidence that certain analogs influence oxidation of the tryptophan isomers by a resting-cell suspension of this organism. The cell suspension was prepared by growing the organism on an inorganic-salts medium[5] containing 0.1 % DL-tryptophan as the carbon and energy source. The cells were harvested, washed twice, and suspended to a standard density in 0.01 M potassium phosphate buffer (pH 7.2). All metabolites were dissolved in buffer and adjusted to pH 7.2. Oxidation studies were carried out in the Warburg apparatus at 37° with air as the gas phase.

Results presented in Fig. 1A demonstrate that the resting-cell suspension oxidizes L-tryptophan at a faster rate than D-tryptophan. DL-Tryptazan inhibits oxidation of both the D- and L-isomer. The degree of inhibition is a function of the substrate/inhibitor ratio suggesting that tryptazan competitively inhibits the oxidation of both

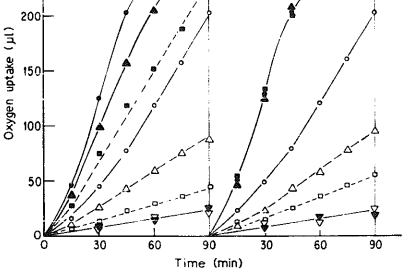

Fig. 1. Effect of analogs on oxidation of D- and L-tryptophan by a Flavobacterium sp. A = inhibitor–DL-tryptazan; B = inhibitor–DL-5-hydroxytryptophan. ●, L-tryptophan; ▲, L-tryptophan–inhibitor ratio of 1:1; ■, L-tryptophan–inhibitor ratio of 1:3; ○, D-tryptophan; △, D-tryptophan–inhibitor ratio of 1:1; □, D-tryptophan–inhibitor ratio of 1:3; ▽, endogenous; ▼, inhibitor control.

tryptophan isomers. Higher substrate/inhibitor ratios showed a more pronounced inhibition with both substrate systems. Controls indicated this organism was unable to oxidize the tryptazan molecule.

The addition of DL-5-hydroxytryptophan (Fig. 1B) in a substrate/inhibitor ratio of 1:1 and 1:3 did not significantly influence the microbial oxidation of L-tryptophan but drastically curtailed oxidation of D-tryptophan. However, when the ratio was decreased to 1:4 and 1:7 the oxidation of L-tryptophan was suppressed and, similar to the tryptazan inhibition, the inhibition by DL-5-hydroxytryptophan was dependent on the substrate/inhibitor ratio. Results obtained using DL-2-oxindolealanine as the antimetabolite indicated that this analog inhibited oxidation of D-tryptophan but not the L-isomer. Neither DL-5-hydroxytryptophan nor DL-2-oxindolealanine was oxidized by the resting-cell suspension as indicated by a lack of oxygen uptake in the inhibitor control vessels.

Tryptamine and indole-3-acetic acid were used as inhibitors lacking an asymmetric carbon on the side chain. Tryptamine in a maximum substrate/inhibitor ratio of 1:7 did not inhibit oxidation of either D- or L-tryptophan. Indole-3-acetic acid in a 1:7 ratio inhibits oxidation of D-tryptophan but does not influence oxidation of L-tryptophan. Indole-3-acetic acid inhibition also appears to be a function of the substrate/inhibitor ratio. Neither of these compounds was oxidized by the cell suspension.

These results indicate that, under the conditions of this experiment, tryptophan analogs may (A) inhibit the microbial oxidation of both D- and L-tryptophan (tryptazan, 5-hydroxytryptophan), (B) inhibit oxidation of D-tryptophan but not the L-isomer (indole-3-acetic acid, 2-oxindolealanine), or (C) exert no influence on the oxidation of either tryptophan isomer (tryptamine). The findings obtained while varying the substrate/inhibitor ratio suggested the inhibitors were competitive in nature. The results do not clearly define the site(s) of analog action. However, one may speculate that the primary site could be associated with the uptake system or with the internal enzymic oxidation mechanism. Studies are now in progress to elucidate these phenomena with the respective analog enantiomorphs.

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