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Antimetabolite Action of 5-Methyltryptophan in *Bacillus subtilis*

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The roles of anthranilate and histidine in the regulation of tryptophan synthesis were implicated by observations of antagonistic relationships with 5-methyltryptophan in *Bacillus subtilis*.

The structural analogue of L-tryptophan, 5-methyltryptophan (5-MTRP), inhibits the growth of *Escherichia coli* by means of its action as a false feedback inhibitor of anthranilate synthetase (3). 5-MTRP also behaves as a false feedback inhibitor of *Bacillus subtilis* anthranilate synthetase (R. A. Jensen and E. Nester, *unpublished data*). One would expect the intermediary metabolites of tryptophan, anthranilate, and indole to counter the inhibitory effects of the analogue, since they would bypass the site of antimetabolite action. Although indole overcomes 5-MTRP inhibition rather well, anthranilate is a poor antagonist (Table 1). In a liquid minimal medium (2) where the specific growth rate, k, was depressed 10-fold by 5-MTRP, the specific growth rate was increased sixfold by addition of indole compared to twofold by addition of anthranilate. L-Tryptophan, of course, overcame the inhibition almost completely. Although L-histidine alone did not significantly affect the specific growth rate, the addition of histidine to a culture containing 5-MTRP increased the growth rate fourfold. At the concentration of 5-MTRP indicated in Table 1, concentrations of histidine greater than 50 μg/ml did not increase the value of k further; less antagonism of 5-MTRP inhibition was observed at lower concentrations of histidine.

When a background of *B. subtilis* NP-40 [a prototrophic derivative of strain 168 (R. A. Jensen, Genetics, *in press*)] on a solid medium containing 5-MTRP was spotted with 0.01 ml of a solution of 2 mg anthranilic acid per ml, a halo of moderately heavy growth developed around the periphery of the spot. The lack of growth in the center of the spot suggested an optimal concentration of anthranilate for reversal of 5-MTRP inhibition. Figure 1 illustrates the result of an experiment in which inhibition caused by 50 μg of 5-MTRP per ml was followed as a function of variable anthranilate concentration. An anthranilate concentration of 50 μg/ml decreased the inhibition of growth rate from 85 to 69%, whereas 1 μg of anthranilate per ml decreased the inhibition to 20%. An even greater reversal of 5-MTRP inhibition was achieved when histidine was present; nearly total reversal of 5-MTRP inhibition occurred in the presence of the combination of 1 μg of anthranilic acid per ml plus 50 μg of histidine per ml.

TABLE 1. *Influence of metabolites upon 5-MTRP-resistance in Bacillus subtilis NP-40*

Growth supplement[a]	Specific growth rate[b]
None	0.41
Anthranilate	0.40
Indole	0.40
L-Tryptophan	0.39
L-Histidine	0.43
5-MTRP	0.04
5-MTRP + L-tryptophan	0.38
5-MTRP + indole	0.22
5-MTRP + anthranilate	0.09
5-MTRP + L-histidine	0.15

[a] Final concentration of each supplement was 50 μg/ml.

[b] The value of k was determined from the slopes of the linear portion of the growth curve plotted on semilogarithmic coordinates. Cultures (10 ml) were grown in a minimal medium at 37 C ± 0.5 C in a rotary action water bath shaker. Growth was estimated by monitoring optical density with a Klett colorimeter. Values are expressed as k(hr^{-1})

The basis of the decreased potency of anthranilate as an antimetabolite of 5-MTRP at higher concentrations of anthranilate is unknown. Possibly anthranilate inhibits the action of indoleglycerol 3-phosphate. (IGP) synthetase in *B. subtilis* as it is known to do in *E. coli* (1). The

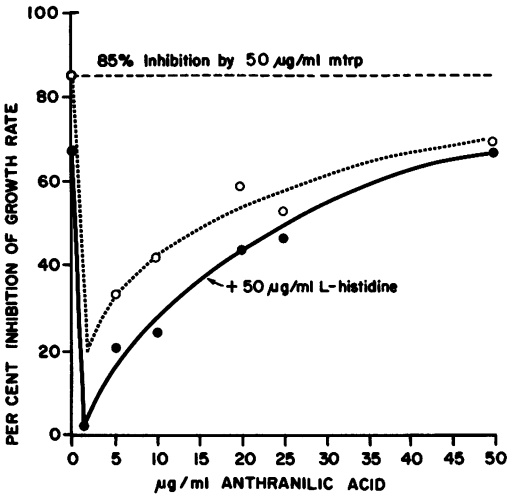

FIG. 1. *NP-40 grown in minimal glucose medium at 37 C was in the mid-exponential phase of growth; k was 0.43. The latter culture was diluted to a Klett reading of 25 (red filter) with a prewarmed minimal glucose medium. Portions (10 ml) were distributed into sidearm 125-ml flasks in a shaking water bath. One set of flasks contained 50 µg of 5-MTRP per ml plus the variable concentration of anthranilate indicated (○); the other set of flasks was identical except that 50 µg of histidine per ml was added (●). Values of k were determined from the linear portion of the growth curve obtained at 37 C. Per cent inhibition of the growth rate was determined by relating each k value to that of the appropriate control grown in the absence of 5-MTRP.*

molecular mechanism for the latter possibility could involve the macromolecular aggregates of tryptophan enzymes presumed to exist based on the acquisition of pleiotrophic mutants (5). However, high concentrations of anthranilic acid do not inhibit the growth of strain NP-40 in the absence of 5-MTRP. Regardless of the specific basis for the anthranilate concentration effect, its occurrence would appear to constitute a trivial explanation for the apparent inability of anthranilate to reverse 5-MTRP inhibition (4).

5-Phosphoribosyl-1-pyrophosphate (PRPP) is a multifunctional metabolite marking a metabolic branch point leading to both histidine and tryptophan. The observation that even at optimal concentrations of anthranilate, complete antimetabolite action requires the presence of both histidine and anthranilate may reflect the ability of histidine to spare PRPP, increasing the intracellular level as substrate available to phosphoribosyl transferase.

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