PRODUCT INHIBITION OF ANTHRANILATE SYNTHETASE IN SALMONELLA TYPHIMURIUM

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Anthranilate synthetase (AS) catalyzes the first committed reaction in the biosynthesis of L-tryptophan. The catalytic activity of this enzyme in the Enterobacteriaceae (Ito & Yanofsky, 1966; Bauerle & Margolin, 1966; Egan & Gibson, 1966) is dependent upon its combination into a protein aggregate with anthranilate-5-phosphoribosyl-1-pyrophosphate phosphoribosyl transferase (PRT), the second enzyme in the tryptophan biosynthetic pathway. The PRT activity occurs without complex formation. The first two reactions in the biosynthesis of tryptophan can be represented as follows:

$$\text{Chorismate} \xrightarrow[\text{AS}]{\text{glutamine, } Mg^{2+}} \text{anthranilate}$$

$$\text{anthranilate} \xrightarrow[\text{PRT}]{\text{PRPP, } Mg^{2+}} \text{N-(5'-phosphoribosyl) anthranilate}$$

The AS activity is inhibited by L-tryptophan (Baker & Crawford, 1966; Bauerle & Margolin, 1966; Egan & Gibson, 1966); PRT activity is subject to inhibition by L-tryptophan when part of the AS-PRT complex, but not in its dissociated form (Egan & Gibson, 1966; Bauerle & Margolin, 1966). In this report we show the inhibition of AS by low concentrations of anthranilate. This is the first

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report in this pathway of product inhibition, a phenomenon which plays an important role in many enzyme catalyzed reactions (Walter & Frieden, 1963).

MATERIALS AND METHODS

Extracts containing wild type AS were obtained from a strain of Salmonella typhimurium carrying a mutation in trpC (trpC 20), the last gene in the tryptophan operon, which codes for the tryptophan synthetase A (α) component (Blume & Balbinder, 1966). A mutant form of AS was obtained from strain SO-207. This strain is prototrophic and carries an operator constitutive mutation (trpO 517), as well as a mutation in trpA (trpA 574), the gene coding for AS. It was isolated as a prototrophic revertant of strain SO-127 (genotype trpO 517 trpA 111). The mutant trpC 20 was grown overnight in Vogel & Bonner's (1956) minimal medium supplemented with L-tryptophan (2 µg/ml), to obtain cells fully derepressed for the enzymes of the tryptophan biosynthetic pathway. The L-tryptophan supplement was omitted for SO-207. The cells were harvested by centrifugation in a Sorvall refrigerated centrifuge, washed once and resuspended in 0.1 M potassium phosphate buffer, pH 7.8,and disrupted with a Bronwill probe sonifier. The cell debris was removed by centrifugation for one hour at 40,000 x g and aliquots of the crude supernatant were frozen at -15°. Each aliquot was thawed immediately prior to use; no loss of AS activity was noticeable under these conditions. The AS activity was measured at 37° as indicated in Figure 1, by following the increase in fluorescence on formation of anthranilate in a Turner fluorometer equipped with a temperature control door and a Rustrak recorder, in a modification of the procedure of Ito & Crawford (1965). The activation wavelength was 320 mµ (Corning filter #7-54 plus Wratten filter # 34A) and the emission wavelength was 410 mµ (Corning

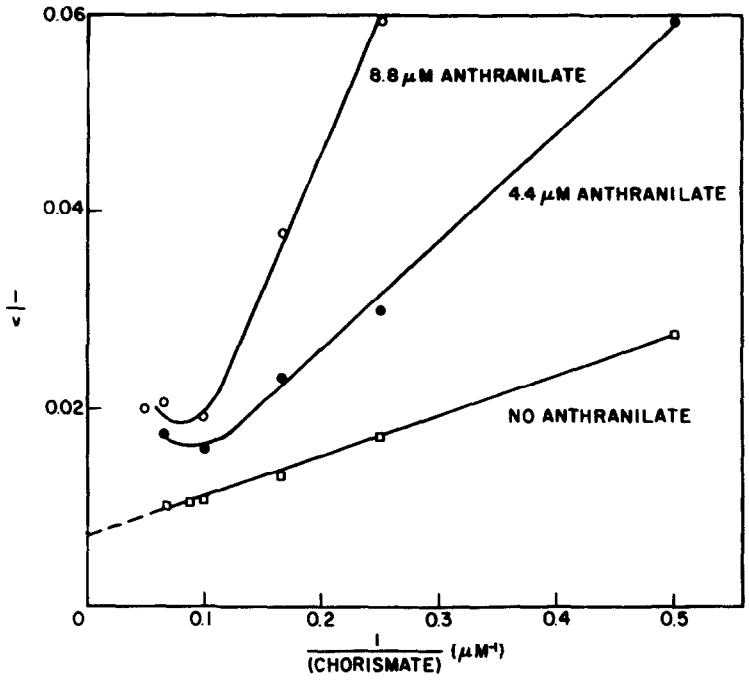

Fig. 1. Inhibition of anthranilate synthetase activity by anthranilate.

The reaction mixture (final volume, 1.0 ml) contained L-glutamine (10 μmoles), potassium phosphate buffer, pH 8.2 (50 μmoles), $MgSO_4$ (4 μmoles) and chorismate in concentrations varying within a range of 2 - 20 mμmoles/ml. The reaction was initiated by the addition of 0.1 unit of enzyme, one unit being defined as the amount of enzyme required to produce 0.1 μmole of anthranilate in twenty minutes. Anthranilate was added to the reaction mixture before addition of the enzyme. Anthranilate concentrations are shown in the plot as the changing fixed inhibitor. v is defined as units/ml extract. All assays were performed in duplicate and each experiment was performed at least twice. The results were highly reproducible.

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filter #5-58). Chorismate was prepared as the free acid by the procedure of Gibson (1968).

RESULTS AND DISCUSSION

The results of several experiments presented in Table 1 and Figure 1 lead to the following conclusions: (1) The AS activity of the AS-PRT enzyme aggregate can be inhibited by low concentrations of anthranilate as well as by tryptophan, but L-tryptophan is a more effective inhibitor than anthranilate. When both inhibitors are

TABLE 1

Inhibition (%) of wild type and mutant (S0-207) anthranilate synthetase by L-tryptophan and anthranilate.

Enzyme	Inhibitor concentration	Chorismate concentration 10 μM	6 μM	4 μM
	None	0	0	0
Wild type	4.4 μM anth.	27	34	54
	8.0 μM anth.	43	47	54
	5.0 μM trp.	55	70	74
	5.0 μM trp. plus 4.4 μM anth.	69	87	92
	None	0	0	0
S0-207	4.4 μM anth.	18	21	22
(trpO 517, trpA 574)	8.0 μM anth.	20	28	32
	0.25 μM trp.	65	67	72
	0.25 μM trp. plus 4.4 μM anth.	68	77	77

The figures in the table represent the percent inhibition of AS at the concentration of chorismate and inhibitor indicated. The reaction mixture is described in the legend to Figure 1, and the assay procedure in the text. Abbreviations: anth. = anthranilate; trp. = L-tryptophan.

present the effects are cumulative but not strictly additive (Table 1).

(2) Figure 1 indicates that at low substrate concentration the inhibition by anthranilate is competitive with chorismate. At high chorismate concentration the kinetics are complicated by substrate inhibition in the presence of anthranilate.

Table 1 also shows that the AS of the mutant S0-207 is hypersensitive to tryptophan inhibition and simultaneously more resistant to anthranilate inhibition. The concentration of L-tryptophan

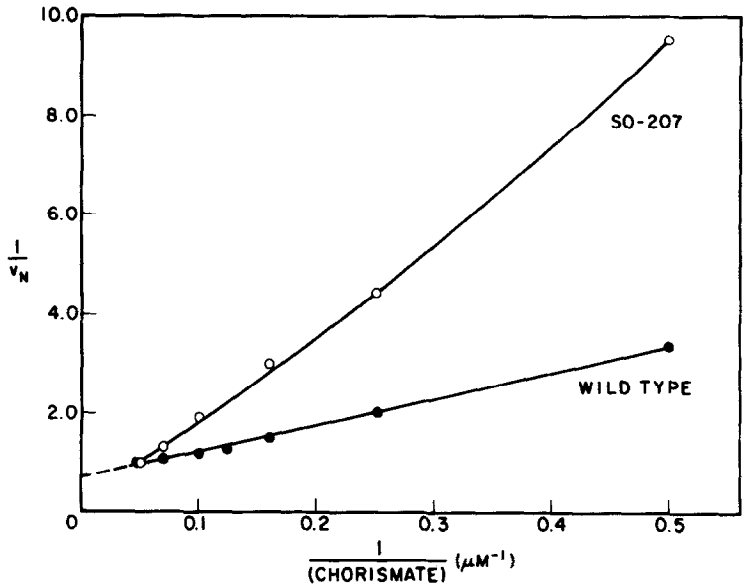

Fig. 2. Reciprocal plots of initial velocity versus chorismate concentration with AS from wild type and mutant SO-207.

v_N = all initial velocities normalized to V_{max} = 1
For details of the assay procedure see legend to Figure 1 and text.

(5 μM) inhibiting the wild type AS between 55-74% is completely inhibitory (100%) for the SO-207 enzyme. It takes twenty times less tryptophan (0.25 μM) to inhibit the mutant enzyme to the same extent as that of the wild type. The differences in response to anthranilate inhibition by both forms of AS are clear from the Table. A further difference between them is shown in Figure 2, which clearly indicates an alteration in the K_m for chorismate in the mutant enzyme. A single mutation in trpA (trpA 574) is responsible for the change in the properties of AS in the mutant.

These observations suggest conformational changes in the regulation of this enzyme. Although the exact mechanism awaits further investigation it is clear that product inhibition may play an important role.

Chorismate, the branch point intermediate in the aromatic pathway appears also to be a precursor for folic acid, vitamin K2 and ubiquinone (Cox & Gibson, 1964). The possibility that anthranilate does not inhibit AS but stimulates the activity of an enzyme present in our crude extracts for which chorismate is a substrate is excluded by the behaviour of the mutant SO-207. We did look for a stimulation by anthranilate of chorismate mutase, the first enzyme for tyrosine and phenylalanine synthesis past the branch point, and found none. We can also exclude the possibility that the inhibitions we observed resulted from the flow of anthranilate to later products in the tryptophan pathway both from the properties of SO-207 as well as the absence in our assays of phosphoribosyl pyrophosphate (PRPP), a substrate for PRT.

The physiological significance of product inhibition of AS is unclear. The control of the activity of this enzyme in the cell may depend on the combined effects of L-tryptophan and anthranilate. Also, anthranilate inhibition of AS may serve to avoid the drainage of chorismate, which is the common substrate for five pathways (Cox & Gibson, 1964) when PRPP is limiting. The requirement for PRT for AS activity may also relate to the potent product inhibition we have observed. That is, a rapid removal of anthranilate by PRT may be required to insure adequate synthesis of tryptophan. Alterations in the coupling between these enzymes, possibly brought about by tryptophan (Bauerle & Margolin, 1966), may accentuate the product inhibition of AS and thus may relate to the essentiality of the AS-PRT enzyme aggregate for AS activity.

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