TRYPTOPHAN AS AN INHIBITOR OF 3-DEOXY-ARABINO-HEPTULOSONATE 7-PHOSPHATE SYNTHETASE.

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The present communication describes conditions whereby tryptophan has been shown to inhibit a portion of 3-deoxy-arabino-heptulosonate 7-phosphate synthetase (DAHP synthetase) activity in extracts of Neurospora crassa and Escherichia coli.

DAHP synthetase is the first enzymic function of the aromatic path of micro-organisms and the earliest of a number of feedback control points. In a series of publications relating to Escherichia coli W the necessity for at least three regulatory DAHP synthetases has been stressed (Brown and Doy, 1963; Doy and Brown, 1965; Brown and Doy, 1966). Phenylalanine and tyrosine were shown to be inhibitors of specific isoenzymes (Smith, Ravel, Lax and Shive, 1962; Doy and Brown, 1965) tryptophan was not and the third isoenzyme recognised was not inhibited (Brown and Doy, 1963; Doy and Brown, 1965). It should be emphasised that tryptophan appears to be an inhibitor of all these functions, but this is due to inhibition of the chemical assay for DAHP. All three aromatic amino acids participated in control by repression (Brown and Doy, 1966).

The above system seemed adequate to meet the requirements

of the cell with one possible exception. Thus, a situation of aromatic amino acid starvation followed by an excess might result in an excess of functional DAHP synthetase until repression of the non-inhibited isoenzyme took affect. It has always been realised that technical difficulties may have obscured the recognition of inhibition by tryptophan in crude extracts and that methods used to demonstrate the isoenzymes could have resulted in loss of this property.

In work with extracts of the wild type Neurospora crassa 74A repression could not be demonstrated unequivocally (Doy, unpublished results, see Neurospora Newsletter No.10) and therefor particular attention has been given to the nature of control of DAHP synthetase by feedback inhibition. Inhibition by phenylalanine and tyrosine has been demonstrated and shows homo- and hetero-molecular cooperation (Doy, 1966). For technical reasons, inhibition, or non-inhibition, by tryptophan alone is difficult to establish with certainty (see later). It was realised that tryptophan might require the presence of (an)other modifier(s).

Therefore tryptophan was tested in the presence of a high level of phenylalanine plus tyrosine using a large amount of dialysed crude extract. Under the conditions the amount of activity was sufficiently high to allow the chance of detecting additional inhibition. It was found that tryptophan inhibited strongly that portion remaining active in the presence of phenylalanine plus tyrosine (Table 1). The result takes into account effects of tryptophan other than on the enzymic reaction.

Assuming that inhibition by phenylalanine plus tyrosine is the same as in a control experiment using enzyme levels appropriate for measuring maximum velocity, the addition of

TABLE 1

The % inhibition of that portion of the DAHP synthetase of N. crassa not inhibited by 10^{-3}M phenylalanine plus 10^{-3}M tyrosine.

Tryptophan x10^{-5}	1	2	3	4	5	6	7	8	9	10	100
% inhibition	43	72	75	81	83	82	84	80	80	79.5	79.5

Extracts were prepared by grinding with glass and 0.1M KH_2PO_4 - NaOH buffer pH 6.4, centrifuging and dialysing the supernatant for 2x2 hr. against 0.025M of the same buffer. DAHP synthetase was assayed as described by Doy and Brown (1965) using 0.83 mg. protein and cyclohexanone extraction.

tryptophan advanced overall inhibition from 82% to 97%. Under the conditions it is likely that 82% is an overestimate. There is therefore a residual 3%, or more, that is not inhibited. It is impossible technically to ensure that all factors are saturated in these experiments and therefore under some biological conditions residual activity may be less than 3%.

What are probably the most reliable data for inhibition by tryptophan alone are given in Table 2. If accepted they indicate that tryptophan is both an activator and an inhibitor. In early work with extracts of E. coli (Morgan and Doy, unpublished results) it was frequently thought that tryptophan might stimulate slightly some extracts.

TABLE 2

Some effects of tryptophan on the DAHP synthetase of N. crassa.

Tryptophan x10^{-5}M	0.5	1	1.25	1.5	1.75	2	4	10	50	100
% activation or inhibition	+6	+4.5	+3.5	+2.3	+1.2	0	-8.5	-10	-9	-13

Conditions as for Table 1 except no phenylalanine and tyrosine and using about 0.12 mg. protein (different extract).

The effect of tryptophan in the presence of a mixture of phenylalanine and tyrosine also was tested using sonic extracts of E. coli W (wild type), its derived aromatic auxotroph 83-2 and the tryptophan auxotroph Aerobacter aerogenes NC3. 83-2 was grown for about 18 hours on balanced, but limiting, aromatic supplements (Brown and Doy, 1966). Using the conditions indicated in Table 1, and undialized 82-2 extract equivalent to 1.5 mg. protein, it was found that 10^{-4}M tryptophan inhibited 28% and 10^{-3}M tryptophan 32% of the activity not inhibited by phenylalanine plus tyrosine. The conditions of growth are probably the most favourable for showing isoenzymes (Brown and Doy, 1966). With strain W, ex minimal medium, it is just possible that there was a trace of inhibition. None was detected with NC3. Thus, under certain circumstances, tryptophan can inhibit a portion of the DAHP synthetase of E. coli. After dialysis, results with 83-2 were still positive, but less convincing, with W the possibility of inhibition was again noticed, with NC3 the result was again negative.

The experiments with A. aerogenes NC3 were done because of the hypothesis of Doy, 1966b, concerning the phenotypic expression of mutation in this organism. It was argued that all published data was consistent with the conclusion that tryptophan inhibited certain o-dihydric phenol formation because of an altered anthranilate synthetase retaining the allosteric site. The present negative findings with NC3 do not demand the rejection of the "altered anthranilate synthetase hypothesis". For rejection it is required to demonstrate firstly the possibility of inhibition by tryptophan of the DAHP synthetase of NC3 and secondly, that, under the conditions of o-dihydric phenol accumulation by this organism, the DAHP synthetase function is

completely inhibited by tryptophan. This implies a superimposed continuing saturation of inhibition-repression effects of tyrosine and phenylalanine restricted to tryptophan auxotrophs rather than polyaromatic or tyrosine auxotrophs (see Pittard, Gibson and Doy, 1962).

In the earliest studies on DAHP synthetase (Doy, unpublished results) work was done with the DAHP synthetase of A. aerogenes. This was abandoned in favour of E. coli because of difficulties associated with an identical chromogen, produced in the assay by compounds other than DAHP. Under some circumstances (use of PEP, R5-P and Mg^{++} at pH 7.4) this may have derived partly from the analogous octonate and under others entirely from a non-dialysable and presumed high molecular weight compound present in extracts. It was considered that the latter may have been associated with a deficiency in capsule synthesis that had been introduced deliberately by mutation. In these examples chromogen formation was inhibited by tryptophan, but, far as could be determined, not at the enzymic level. A high non-dialysable blank was again noticed in the work with NC3.

It is not clear whether the presently described inhibition by tryptophan requires a fourth portion of activity that is not inhibited by the aromatic amino acids. Assuming the biological possibility of conditions of saturating aromatic amino acids, but not the vitamins, the problem is whether the characteristics of the non-competitive type of inhibition result in a finite, or zero, velocity.

Wallace and Pittard (in press) have extended further the gene-isoenzyme relationships of the DAHP synthetase of E. coli K12 (Brown and Maas, 1966). They found no significant inhibition by tryptophan and in general terms confirm our earlier

conclusions. They do find two small peaks of activity not associated with inhibition by either phenylalanine, or tyrosine and recognise that this is not necessarily evidence for a fourth function. It will be interesting to discover whether any of the variously obtained non-inhibited portions of DAHP synthetase reported in the literature are inhibited by tryptophan in the presence of phenylalanine and tyrosine and whether association with (an)other isoenzyme(s) activates this property.

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