PN 10058

End-product regulation of the general aromatic pathway in *Escherichia coli* W

3-Deoxy-D-*arabino*-heptulosonic acid 7-phosphate synthetase is the first enzyme of a pathway leading in *Escherichia coli* to the aromatic amino acids. In such pathways early intermediates are shared and regulatory mechanisms must overcome the difficulty that excess of one end product should not inhibit the synthesis of others. The discovery in *E. coli* of two threonine deaminases[1] and of multiple aspartate kinases (E.C. 2.7.2.4)[2], each regulated by a specific end product, suggested a means of selective feedback control based on end-product inhibition and repression. Other experiments suggested a similar mechanism for control of the aromatic pathway[3]. SMITH *et al.*[4] have reported the existence of two DAHP synthetases in cells of *E. coli* starved of end products. One enzyme was allosterically inhibited by L-tyrosine and the other by L-phenylalanine. They found that these two amino acids together in low concentration *in vitro* inhibited DAHP synthetase by at least 95%. Our independent and simultaneous investigations, using different techniques, led to similar conclusions, but with reservations about the general applicability of the results. These reservations arose from a theoretical consideration of general aromatic biosynthesis.

If DAHP synthetase consists solely of the above isoenzymes the potential is that aromatic synthesis could be completely, or almost completely, halted by an

TABLE I

REPRESSIBILITY AND END-PRODUCT INHIBITION OF DAHP SYNTHETASE ACTIVITY IN *E. coli* W AND MUTANT EXTRACTS

Cells were grown on minimal medium[7] supplemented as shown. Strain 19-2 is a tryptophan auxotroph, 83-3A an aromatic auxotroph requiring all aromatic end products, or shikimic acid. Limiting L-tryptophan was 18 μM, limiting shikimic acid was 30 μg/ml. Extracts were prepared by sonication and dialysis. DAHP synthetase (see text) was measured essentially by the method of SRINIVASAN AND SPRINSON[8]. Per cent contribution to DAHP synthetase activity by the L-phenylalanine-sensitive isoenzyme is given by the % inhibition Phe. Per cent contribution of L-tyrosine-sensitive isoenzyme is (inhibition Phe + Tyr) *minus* inhibition Phe. Per cent residual activity is 100 *minus* (inhibition Phe + Tyr). Try is not an inhibitor.

Strain	*Supplement*	*Phase of growth at harvest*	*Relative DAHP synthetase activity*	*% inhibition*		*% residual activity*
				Phe	*Phe + Tyr*	
W	nil	Log	6.0	78	94	6
W	Tyr (1 mM)	Log	5.0	96	96	4
W	Phe (1 mM)	Log	1.6	40	88	12
W	Try (1 mM)	Log	1.7	74	97	3
W	Phe + Tyr	Log	2.0	76	81	19
W	Phe + Tyr + Try	Log	1.0	90	97	3
19-2	Try (limiting)	Stationary	1.8	44	82	18
83-3A	Phe + Tyr + Try (limiting) + POB (1 μM) + PAB (1 μM)	Stationary	1.6	72	72	28
83-3A	Shikimic acid (limiting)	Stationary	17.0	21	98	2

Abbreviations: DAHP, 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate; PAB, *p*-aminobenzoic acid; POB, *p*-hydroxybenzoic acid.

excess of phenylalanine plus tyrosine. It therefore became important that any scheme for aromatic regulation should account for continued tryptophan biosynthesis (10–20% of the total aromatic amino acid requirement) in the presence of phenylalanine plus tyrosine. Although PAB acid and *p*-hydroxybenzoic acid and at least one other factor are synthesized by the same path, these represent less than 1% of the total aromatic requirement and at present will not concern us.

In these studies we have regarded as "normal" the metabolism of wild-type cells growing exponentially under aerobic conditions in minimal medium, but have also considered it important to establish the effect of supplements to this medium on the enzymic constitution of the cell.

The influence of the environment on relative DAHP synthetase activity and the ratio of the component isoenzymes is given in Table I. While the derepression that followed end-product starvation greatly favoured the tyrosine-sensitive DAHP synthetase, this isoenzyme was a minor component of normal cells and the phenylalanine-sensitive isoenzyme predominated.

Extracts with low DAHP synthetase activity were frequently found to be incompletely inhibited by a mixture of L-phenylalanine and L-tyrosine. When L-phenylalanine plus L-tyrosine were added to the medium, but L-tryptophan was omitted, there was an enhancement of this residual activity. The effect was also enhanced when appropriate mutants were starved of L-tryptophan (Table I). In contrast to this, the presence of L-tryptophan in the medium repressed the non-inhibitable activity.

L-Tryptophan was not an inhibitor and the residual activity was not inhibited when L-tryptophan was added with L-phenylalanine and L-tyrosine. Chorismic acid[5], the last of the shared aromatic intermediates, was not an inhibitor, either singly, or with L-phenylalanine and L-tyrosine.

These results demonstrate the existence of a third DAHP synthetase, repressible by L-tryptophan, but not inhibited by any of the end products, by chorismic acid, or by any combination of these. The nature of this isoenzyme is being investigated. Partial purification has been achieved by ammonium sulphate fractionation.

The absence of coordinate repression in this pathway was also demonstrated. While the ratio of fully derepressed to fully repressed DAHP synthetase activity was at least 17:1 (Table I), the same extracts showed less than a 2-fold variation in the level of dehydroquinase (dehydroshikimate hydratase), a later enzyme of the shared aromatic sequence.

Under conditions where DAHP synthetase is potentially 95–100% inhibited by phenylalanine plus tyrosine it is still necessary for the cell to synthesize tryptophan. It is not yet clear whether the activity of the non-inhibitable enzyme is sufficient to account for this synthesis in the presence of excess phenylalanine and tyrosine. If it is insufficient, our results would mean that the full potential of end-product inhibition is not realised, or (and), that tryptophan is preferentially synthesized before phenylalanine and tyrosine. The latter possibility is also suggested by the work of DAVIS[6]. If DAHP synthetase is completely inhibited, preferential synthesis cannot explain tryptophan synthesis when phenylalanine and tyrosine are provided from the exogenous environment.

TABLE II

END-PRODUCT INHIBITION OF ACCUMULATION OF INTERMEDIATES BY AUXOTROPHS OF *E. coli* W

Cells were harvested in stationary phase, washed twice (0.9% NaCl) and suspended in minimal medium.[7] Concns. of supplements as in Table I except Q_i which was L-phenylalanine (10 μg/ml), L-tyrosine (10 μg/ml), L-tryptophan (5 μg/ml), PAB (0.1 μg/ml), POB (0.1 μg/ml), growth being limited by all 3 amino acids. Strain 83-24 is an aromatic auxotroph, other strains see Table I. End-products (1 mM) were added when linear accumulations were well established, aliquots of cells were taken at this time for preparation of extracts and measurement of DAHP synthetase activity and inhibition.

Strain	*Supplement*	*Intermediate accumulated*	*% inhibition of accumulation by*				*% inhibition of DAHP synthetase*
			Tyr	*Phe*	*Try*	*Phe + Tyr*	*Phe + Tyr*
83-3A	Shikimic acid (Limiting)	DAHP*	56	0	—	64	98
83-24	Q_i	Shikimic acid	67	0	0	78	—
19-2	L-tryptophan (Limiting)	Anthranilic acid	58	48	100	73	92

* With the dephosphorylated form.

Experiments with whole cells showed that normal growth was not slowed by the addition of phenylalanine plus tyrosine in the medium during log phase. Other whole cell results, but with auxotrophs, showed that the accumulations of DAHP, shikimic acid and anthranilic acid were only partly inhibited by the addition of these two end products. Extracts of these cells demonstrated a higher potential for inhibition than was realised when the end products were provided exogenously (Table II). This implies the presence of an accessibility barrier to the sites of inhibition which must be considered as part of the control mechanism.

We wish to thank P. MORGAN, M.Sc., who participated in preliminary experiments, also J. SCHIAVONE and L. MASON-JONES, for technical assistance. Dr. C. BALLOU kindly provided some of the erythrose 4-phosphate used in this work. This investigation was supported in part by U.S. Public Health Service Research Grant AM04632 from the National Institutes of Arthritis and Metabolic Diseases and a grant from the Victorian Anti-Cancer Council.

Department of Microbiology,
University of Melbourne,
Parkville, Victoria (Australia)

K. D. BROWN
C. H. DOY

[1] H. E. UMBARGER, B. BROWN, *J. Bacteriol.*, 73 (1957) 105.
[2] E. R. STADTMAN, G. N. COHEN, G. LE BRAS AND H. DE ROBICHON-SZULMAJSTER, *J. Biol. Chem.*, 236 (1961) 2033.
[3] C. H. DOY AND B. D. DAVIS, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 1.
[4] L. C. SMITH, J. M. RAVEL, S. R. LAX AND W. SHIVE, *J. Biol. Chem.*, 237 (1962) 3566.
[5] M. I. GIBSON AND F. GIBSON, *Biochim. Biophys. Acta*, 65 (1962) 160.
[6] B. D. DAVIS, *J. Bacteriol.*, 64 (1952) 729.
[7] B. D. DAVIS AND E. S. MINGIOLI, *J. Bacteriol.*, 60 (1950) 17.
[8] P. R. SRINIVASAN AND D. B. SPRINSON, *J. Biol. Chem.*, 234 (1959) 716.

Received May 24th, 1963