PREPHENATE DEHYDROGENASE FROM NEUROSPORA: FEEDBACK ACTIVATION BY PHENYLALANINE

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Received July 7, 1969

Prephenate dehydrogenase, the first enzyme in the tyrosine branch of the aromatic pathway is subject to feedback inhibition by tyrosine and also to activation by phenylalanine. The arrangement of activated steps in aromatic amino acid biosynthesis suggests that control of the aromatic pathway is adapted to supply a greater flux of tyrosine than is required for vegetative growth. Mutation of the structural gene (pt) for chorismate mutase has no effect upon the control functions of prephenate dehydrogenase, indicating that these enzymes are not structurally associated in Neurospora.

Chorismate mutase, in Neurospora, is subject to feedback inhibition by phenylalanine and by tyrosine and to activation by tryptophan[1,2]. Further investigation of the regulatory role of this enzyme[3,4] has led to the discovery of another step in aromatic amino acid biosynthesis that is also controlled by feedback activation.

Materials and Methods Conidia, of wild-type Neurospora (Emerson A) and a strain carrying a mutation (pt MN64) in the structural gene for chorismate mutase[4], were inoculated into 8 l. of Vogel's minimal medium[5] and grown for 3 days at 25°C with forced aeration. The mycelia, harvested and washed with distilled water by vacuum filtration, were freeze dried and reduced to powders in a coffee mill. The powders, kept at -15°C prior to use, were extracted with 0.05 M iminazole-HCl (pH 6.8) at 4°C and cell debris removed by centrifugation. Nucleic acid was precipitated with 0.05 volumes (v/v) of 1 M manganous chloride and removed by centri-

fugation. The protein, precipitated between 40% and 60% saturation of ammonium sulphate, was collected by centrifugation, re-dissolved in column buffer and transferred to 0.05 M sodium phosphate, 5 mM sodium EDTA (pH 6.8) in a sephadex G-25 column. The protein solutions were kept at -15°C prior to use. Protein was estimated by the method of LOWRY et al.[7] using human γ globulin as a standard.

Prephenate dehydrogenase was assayed at 37°C by following the reduction of NAD (nicotine adenine dinucleotide) fluorimetrically (activation 354 nm, fluorescence 458 nm : uncorrected). The assay mixture contained, in order of addition, 50 µmoles sodium phosphate (pH 7.5), 0.13 µmoles NAD, protein and 0.15 µmoles sodium prephenate in a total volume of 1.5 ml. Phenylalanine and tyrosine did not cause any reduction of NAD or oxidation of NADH in the absence of prephenate.

Prephenate dehydratase was assayed by the method described by BAKER[6] except that 20 µmoles of sodium phosphate (pH 7.5) were used in place of tris-maleate.

Results and Discussion Prephenate dehydrogenase activity, at the first step in the tyrosine specific pathway (Fig. 1), is inhibited by tyrosine (Table 1) and is activated by phenylalanine. Phenylalanine also reverses the inhibition caused by tyrosine. Prephenate dehydratase activity, at the first step in the phenylalanine specific pathway, is inhibited by phenylalanine (Table 2). However, tyrosine appears to cause little, if any, stimulation of this enzyme.

Mutation of the structural gene (pt) of chorismate mutase does not result in any change in the properties of prephenate dehydrogenase (Table 1). This is consistent with the indication[6] that the enzymic activities are not associated in Neurospora, in

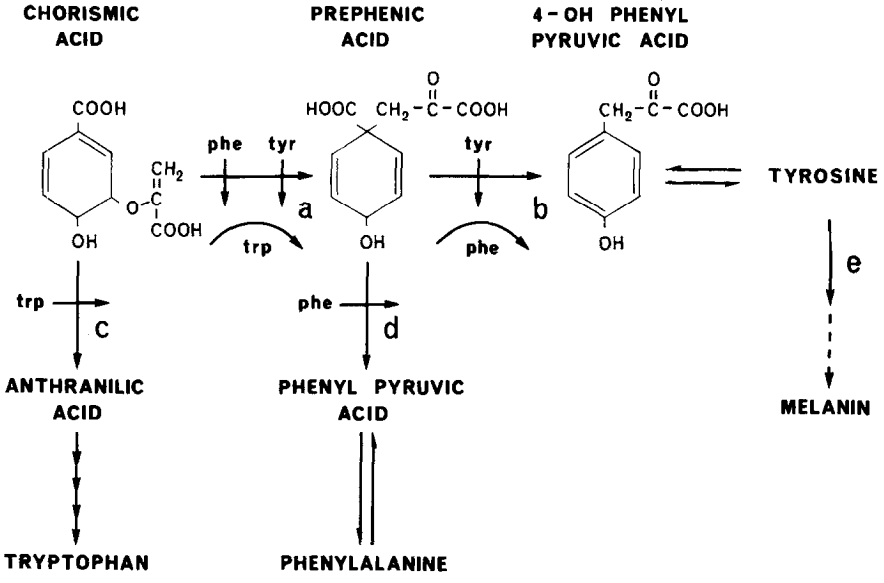

Figure 1: The pathway and control of aromatic amino acid biosynthesis from chorismic acid in Neurospora. Enzymes: a: chorismate mutase, b: prephenate dehydrogenase, c: anthranilate synthase, d: prephenate dehydratase, e: tyrosinase. Effectors: phe: phenylalanine, tyr: tyrosine, trp: tryptophan. Intersecting arrows indicate inhibition, curved arrows indicate activation.

Additions to assay mixture	mμmoles NAD reduced/mg protein/min	
	wild type	pt MN64
none	1.45	1.51
phenylalanine	25.4	22.7
tyrosine	0.04	0.03
phenylalanine + tyrosine	3.93	4.02
tryptophan	1.53	1.49

Table 1 Prephenate dehydrogenase from Neurospora. Amino acids were added to a final concentration of 0.05 mM.

contrast to Aerobacter aerogenes in which mutations affect both activities simultaneously[8].

In Neurospora, therefore, two enzymes involved in the syn-

thesis of tyrosine are subject to control by stimulation, each by the end product of the particular branch of the aromatic amino acid pathway that diverges from the tyrosine sequence immediately preceding the activated step. Tryptophan, which inhibits anthranilate synthase (Fig. 1) and therefore tryptophan synthesis, albeit with low efficiency at high substrate concentrations[1,4], is an activator of chorismate mutase [1-4]. Phenylalanine, which inhibits prephenate dehydratase and therefore phenylalanine biosynthesis, though with low efficiency in the presence of 0.25 mM substrate (Table 2), activates prephenate dehydrogenase. The combined effect of activation, of chorismate mutase and prephenate dehydrogenase, is stimulation of the biosynthesis of tyrosine. The production of aromatic amino acids is also controlled by feedback inhibition of chorismate synthesis at the first step in the common aromatic pathway where tyrosine, phenylalanine and tryptophan each inhibit a portion of the DAHP synthase activity[9].

The role of activation in the control of aromatic amino acid biosynthesis is not simple to evaluate. This is due, in part, to the difficulty of estimating the intracellular environment of the regulated enzymes, particularly with regard to the pools of free amino acids which, in the case of tryptophan at least[10],

Additions to assay mixture	mμmoles phenylpyruvic acid formed/mg protein/min
none	2.0
phenylalanine	0.5
tyrosine	2.1
phenylalanine + tyrosine	0.6

Table 2 Prephenate dehydratase from wild type Neurospora. Amino acids were added to a final concentration of 0.6 mM.

appear to be unevenly distributed in the cell. The activations could be considered to ameliorate the apparent inefficiency of feedback inhibition of anthranilate synthase by tryptophan and of prephenate dehydrogenase by phenylalanine. However, in addition to and not incompatible with this hypothesis, it is possible that the arrangement of activating controls ensures rapid synthesis of tyrosine in response to any increase in its utilisation. This correlates with a potential for a large increase in the rate of utilisation of tyrosine due to derepression of tyrosinase. Tyrosinase is repressed in vegetative cultures of Neurospora but is derepressed in the presence of certain exogenous substances and especially during differentiation in the sexual phase of the organism[11,12] when it catalyses the conversion of tyrosine to precursors of melanin, which is required for maturation of protoperithecia, perithecia and ascospores. Tyrosinase comprises 1-5% of the extractable cell protein in derepressed cells[13], implying a considerable demand for tyrosine. The activating controls that tend to prevent tyrosine from inhibiting its own synthesis from chorismate would appear to facilitate supply of the apparently large quantities of tyrosine required during sexual differentiation. This hypothesis suggests that Neurospora, in vegetative culture, has a potential for excess aromatic synthesis. This conclusion is supported by the fact that strains which carry the pt MN64 mutation grow at a normal rate on minimal medium even though cell free extracts contain only 5% of the chorismate mutase activity found in similar extracts of wild type cells[4].

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