Note

Growth Inhibition by Phenylalanine
in *Euglena gracilis*Yuji ODA, Yoshihisa NAKANO
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Received January 7, 1982

When 10 mM L-phenylalanine was added to a culture of *Euglena gracilis* at the mid-exponential phase (10^6 cells/ml), the growth was inhibited and this inhibition was specifically relieved by 5 mM L-tryptophan.¹⁾ In the present paper, the effects of some other compounds on the growth inhibition by phenylalanine are reported.

E. gracilis SM-ZK, a streptomycin-bleached mutant derived from strain z, was grown in medium containing 30 mM L-glutamate as the sole carbon and nitrogen source.¹⁾ Harvested cells were extracted with 2.5% (w/v) trichloroacetic acid containing 2% (v/v) thioglycolate to prevent destruction of tryptophan.²⁾ The extract was used for the determination of the amino acid pool with a Hitachi KLA 5 amino acid analyzer.

Among aromatic amino acids and their derivatives examined, tyrosine and 4-hydroxyphenylpyruvate (both 10 mM) did not show significant inhibition of the growth of *E. gracilis*. Phenylpyruvate was inhibitory at 10 mM, but this inhibition was not restored by tryptophan; the effect of phenylpyruvate is probably due to the generalized

oxoacid toxicity reported in some organisms.³⁾

Such metabolites of tryptophan as tryptamine (5 mM), kynurenine (0.5 mM), nicotinate (0.05 mM), quinolinate (1 mM), indoleacetate (1 mM) and 5-hydroxytryptophan (5 mM) did not reverse the inhibition by phenylalanine. However, anthranilate and indole, intermediates of tryptophan synthesis, relieved the inhibition by phenylalanine effectively (Fig. 1). The effect of 5 mM tryptophan was equal to that of 0.2 mM anthranilate and only slightly greater than that of 0.2 mM indole, but the cell growth was depressed by the latter two compounds at higher than 0.2 mM. The reversing effect of indole was not elevated by the addition of serine which condenses with indole to form tryptophan.

Table I shows the contents of phenylalanine, tyrosine and tryptophan in *Euglena* cells whose growth was in-

FIG. 1. Growth Inhibition by Phenylalanine and Its Restoration by Tryptophan, Anthranilate or Indole in *E. gracilis*.

To the medium containing cells grown on 30 mM L-glutamate alone (○) 10 mM L-phenylalanine (●), 10 mM L-tyrosine (▲), and 10 mM L-phenylalanine plus 5 mM L-tryptophan (■), 0.2 mM anthranilate (△), or 0.2 mM indole (□) were added as indicated by an arrow.

TABLE I. AMINO ACID POOL IN CELLS OF *E. gracilis* INCUBATED WITH AROMATIC AMINO ACIDS
The amino acid pool in 3-day-old cells as shown in Fig. 1 was analyzed by the method described in the text.

Additions	Contents of amino acids (nmol/ 10^6 cells)		
	Phenylalanine	Tyrosine	Tryptophan
None	0.009	0.029	—*
10 mM Phenylalanine	2.49	0.044	—*
10 mM Phenylalanine plus 5 mM tryptophan	5.83	0.071	1.06
10 mM Phenylalanine plus 0.2 mM anthranilate	3.60	0.053	0.694
10 mM Phenylalanine plus 0.2 mM indole	3.11	0.049	0.250
10 mM Tyrosine	0.218	39.2	0.124

* Not detected.

hibited by phenylalanine and restored by tryptophan and related compounds. Tryptophan was hardly detected in the cells in the presence or absence of exogenous phenylalanine while the content of tryptophan increased following the reversal of growth inhibition. The results show that the growth inhibition by phenylalanine is caused by the shortage of intracellular tryptophan. The amount of exogenous tryptophan required to relieve the inhibition by phenylalanine was higher than that of anthranilate or indole, but this difference may be due to the lower rate of tryptophan uptake by *Euglena* cells. Although shikimate is involved in the common biosynthetic pathway of the three aromatic amino acids in *E. gracilis*,⁴ exogenous shikimate did not reverse the inhibition by phenylalanine. This compound may not be converted readily into tryptophan in *E. gracilis*. In the cultured plant tissue exogenous shikimate is not used for the syntheses of aromatic amino acids.⁵ Tyrosine, which had no inhibitory effect on the growth, was taken up by the cells more than phenylalanine (Table I).

In some microorganisms, growth inhibition by phenylalanine and its restoration by tyrosine and/or tryptophan have been reported and a possible mechanism has been proposed in terms of characteristic properties of 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase (EC 4.1.2.15),^{6,7} the initial step of the biosynthetic pathway of aromatic amino acids. DAHP synthases of *Thiobacillus neapolitanus*⁶ and a cyanobacterium, *Synechocystis* sp. 29108,⁷ are so sensitive to phenylalanine that the syntheses of tyrosine and tryptophan are inhibited severely by phenylalanine. Thus lowered production of both amino acids in the cells by exogenous phenylalanine limits protein synthesis and consequently growth. In *Agmenellum quadruplicatum*, however, feedback-inhibition of DAHP synthase does not explain the growth inhibition by phenylalanine, and accumulation of some toxic intermediate has been suggested as the possible cause.⁸ In a

recent report by Byng *et al.*,⁹ DAHP synthase in *E. gracilis* was shown to be feedback-inhibited by tyrosine but not by phenylalanine or tryptophan; excess tyrosine should cause a shortage of phenylalanine and tryptophan. However, the present experiments have shown that exogenous tyrosine does not inhibit the growth of *E. gracilis* in spite of the high level of tyrosine in the amino acid pool (Table I). A slight increase of the phenylalanine and tryptophan pool in the cells incubated with tyrosine should indicate that some intermediates in the shikimate pathway are converted into these two amino acids, suggesting that DAHP synthase is not strictly feedback-inhibited by tyrosine *in vivo*.

It appears that the growth inhibition by phenylalanine in *E. gracilis* cannot be explained in terms of characteristic properties of DAHP synthase. Results of the present experiments suggest that phenylalanine disturbs in some way the biosynthesis of tryptophan in this organism.

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