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Short Communication

Germination of Phaseolus vulgaris II. Stimulation of Axis Growth by DL-Fluorophenylalanines

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The initiation and continued elongation of excised embryonic axes from *Phaseolus vulgaris* L. (var. White Marrowfat) apparently depend on protein synthesis since puromycin, cycloheximide and actinomycin D strongly inhibit growth as well as amino acid incorporation into protein (9). In an attempt to extend these findings, I have tested the effects on axis growth of various amino acid analogues reported to inhibit growth in higher plants and/or bacteria (1, 2, 6, 7, 8). The analogues tested were fluorophenylalanine, β-phenylserine, β-2-thienylalanine, which are analogues of phenylalanine, and hydroxyproline, canavanine, ethionine, norleucine and 5-methyltryptophan.

The embryonic axes were excised from dry seeds and incubated for 11 hours (9). The initiation of axis elongation began after approximately 4.5 hours of incubation and the fresh weight increase was linear over the next 6.5 hours with a resultant increase of approximately 50 % in fresh weight. After incubation, axes were embedded in paraffin and sectioned, and the DNA was stained by the Feulgen method (4). *p*-Fluoro-DL-phenylalanine was recrystallized twice from water. Thin-layer chromatography in several solvent systems indicated the presence of only 1 compound, whether the spot was made visible by ninhydrin or by fluorescence quenching.

Of the compounds tested only the fluorophenylalanines and β-2-thienylalanine, analogues of phenylalanine, significantly affected the increase in axis fresh weight. DL-Fluorophenylalanine stimulated the fresh weight increase while β-2-thienylalanine had an inhibitory effect (table I). At 5×10^{-4} M, the concentration at which the majority of experiments were conducted, *p*-fluoro-DL-phenylalanine always increased the fresh weight gain in comparison with the controls. The stimulation varied from 20 to 40 %. The ortho and meta isomers also appeared to be stimulatory, but at 5×10^{-4} M showed only about 50 % of the activity of the para isomer.

The stimulation by *p*-fluoro-DL-phenylalanine was essentially reversed by L-phenylalanine, although the slightly inhibitory effect caused by 10^{-3} M L-phenylalanine alone makes these results less clear-cut (table II). Tyrosine had no effect on either the endogenous fresh weight increase or the *p*-fluorophenylalanine-stimulated increase.

A kinetic study of the effects of 5×10^{-4} M *p*-fluoro-DL-phenylalanine on growth showed that stimulation took place over the entire period of elongation, although there was no indication that initiation of elongation began earlier than usual.

Measurements of the intact axes indicated that the increased weight was due to an increased length. No mitotic figures were seen in either control axes or those treated with *p*-fluoro-DL-phenylalanine and presumably the effect of *p*-fluorophenylalanine was on cell elongation.

Since it has been reported that phenylalanine hydroxylase converts *p*-fluorophenylalanine to tyrosine and fluoride ion in rats (3), I tested the effects of varying concentrations of NaF on axis growth. No effects were noted between 5×10^{-7} M and 5×10^{-4} M, while at 5×10^{-3} M growth was inhibited by 90 %. It seems highly unlikely, therefore, that the stimulation of axis growth by fluorophenylalanine is due to the formation of fluoride ion.

The results of this study were unexpected since it was found that *p*-fluorophenylalanine stimulates axis growth by 20 to 40 % at concentrations which have been found to inhibit the growth of other tissues by 50 to 70 % (6). In addition, the other amino acid analogues tested, with the exception of β-2-thienylalanine, failed to inhibit growth.

The inhibitory effect of fluorophenylalanine on growth is generally attributed to reduced enzymatic activities resulting from the replacement of phenylalanine by fluorophenylalanine in proteins (7). In higher plants, however, phenylalanine is also a metabolic intermediate for other types of compounds, including phenolics, coumarins and flavonoids (5). Therefore, the effects of fluorophenylalanine on plant growth may be complicated by its effects on these metabolic conversions of phenylalanine. Watkins and Magrill have recently reported that chromatographs of extracts from plants fed ^{14}C-*p*-fluorophenylalanine showed new spots as compared with chromatographs of those fed ^{14}C-phenylalanine (10). They also suggested that biosynthesis of flavonoids from *p*-fluorophenylalanine is blocked when a hydroxylation is required at the 4′ position. These results suggest that the stimulation of axis growth by fluorophenylalanine may involve other metabolic effects in addition to replacement of phenylalanine in proteins.

Table I. *Effects of Fluorophenylalanine and β-2-Thienylalanine on Axis Fresh Weight Increase*

One hundred mg of tissue (20–25 axes) incubated in 50 ml Erlenmeyer flasks containing 2 ml of solution consisting of 0.01 M potassium phosphate buffer, pH 6.0, to which was added 50 units of penicillin G per ml and the appropriate test compound. The flasks, 3 per treatment, were shaken in a Dubnoff metabolic incubator at 26° for 11 hours.

Treatment	Concn	Fr wt increase Mg	% of Control
Expt 1			
Control		135 ± 8	
o-Fluoro-DL-phenylalanine	5×10^{-4} M	155 ± 1	115
m-Fluoro-DL-phenylalanine	5×10^{-4} M	151 ± 5	112
p-Fluoro-DL-phenylalanine	5×10^{-4} M	166 ± 8	123
Expt 2			
Control		144 ± 1	
p-Fluoro-DL-phenylalanine	10^{-4} M	164 ± 1	114
p-Fluoro-DL-phenylalanine	5×10^{-4} M	190 ± 2	132
p-Fluoro-DL-phenylalanine	10^{-3} M	180 ± 3	125
Expt 3			
Control		125 ± 7	
β-2-Thienylalanine	10^{-4} M	112 ± 8	90
β-2-Thienylalanine	10^{-3} M	96 ± 4	77

Table II. *Reversal of* p-*Fluoro-*DL-*phenylalanine Stimulation by* L-*Phenylalanine*

Conditions as described in table I.

Treatment	Fr wt increase Mg	% of Control
Control	112 ± 1.6	
5×10^{-4} M *p*-Fluoro-DL-phenylalanine	154 ± 6.9	138
5×10^{-4} M L-Phenylalanine	113 ± 9.1	101
5×10^{-4} M *p*-Fluoro-DL-phenylalanine + 5×10^{-4} M L-phenylalanine	136 ± 4.5	122
10^{-3} M L-Phenylalanine	100 ± 7.4	89
5×10^{-4} M *p*-Fluoro-DL-phenylalanine + 10^{-3} M L-phenylalanine	97 ± 8.0	87

The significance of the failure of the various amino acid analogues to inhibit growth, even though protein synthesis is apparently required, is not known at this time.

In Summary: *p*-, *o*- and *m*-Fluoro-DL-phenylalanine have been found to stimulate the growth of excised axes of *Phaseolus vulgaris*. At 5×10^{-4} M *p*-fluoro-DL-phenylalanine stimulated growth by 20 to 40 % and the other 2 isomers by 10 to 20 %. L-Phenylalanine (10^{-3} M) essentially reversed the stimulation by 5×10^{-4} M *p*-fluoro-DL-phenylalanine.

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Literature Cited

1. Bonner, J. 1949. Limiting factors and growth inhibitors in the growth of the *Avena* coleoptile. Am. J. Botany 36: 323–32.
2. Cleland, R. 1963. Hydroxyproline as an inhibitor of auxin-induced elongation. Nature 200: 908–09.
3. Dolan, G. and C. Godin. 1966. In vivo formation of tyrosine from *p*-fluorophenylalanine. Biochemistry 5: 922–25.
4. Gomori, G. 1952. Microscopic Histochemistry, Principles and Practice. University of Chicago Press, Chicago.
5. Neish, A. C. 1965. Coumarins, phenylpropanes and lignin. In: Plant Biochemistry. J. Bonner and J. E. Varner, eds. Academic Press, New York. p 581–617.
6. Noodén, L. D. and K. V. Thimann. 1966. Action of inhibitors of RNA and protein synthesis on cell enlargement. Plant Physiol. 41: 157–64.
7. Richmond, M. H. 1962. The effect of amino acid analogues on growth and protein synthesis in microorganisms. Bacteriol. Rev. 26: 398–420.
8. Schrank, A. R. 1956. Ethionine inhibition of elongation and geotropic curvature of *Avena* coleoptiles. Arch. Biochem. Biophys. 61: 348–55.
9. Walton, D. C. 1966. Germination of *Phaseolus vulgaris*. I. Resumption of axis growth. Plant Physiol. 41: 298–302.
10. Watkins, J. E. and D. S. Magrill. 1966. Accumulation of fluorinated analogues of intermediates on the path to flavonoids in plants fed DL-*p*-fluorophenylalanine. Fourth International Symposium on the Chemistry of Natural Products. Royal Institute of Technology, Stockholm. p 146–47.