ANTAGONISTIC EFFECTS OF BRANCHED CHAIN AMINO ACIDS ON THE GROWTH OF SPIRODELA POLYRHIZA (L.) SCHLEIDEN

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SUMMARY

Strong inhibition of the growth of *S. polyrhiza* occurs when L-valine, L-isoleucine or L-leucine are added singly to the culture medium in a concentration of about 5×10^{-5} M or higher. Excellent growth takes place, however, when L-valine and L-isoleucine are supplied together, also in combination with L-leucine. When L-leucine and L-isoleucine are added simultaneously, growth was completely inhibited, just as with the combination L-valine and L-leucine. The results are explained in terms of end-product inhibition of a common enzymatic step in the synthesis of these branched chain amino acids.

1. INTRODUCTION

It is well known that, if supplied exogenously, several amino acids exert an inhibitory action on the growth of bacteria, fungi and higher plants. The toxicity of some amino acids can be reversed by certain others (STREET 1959). Antagonism between valine on the one hand and isoleucine, leucine or norleucine on the other in the growth of plant organ cultures has been reported, but the nature of the toxic and antagonistic effects remained obscure (HARRIS 1956; DAVID 1963).

Studies on amino acid metabolism in *Escherichia coli* have greatly elucidated the biochemical basis of the antagonism between valine and isoleucine (UMBARGER & BROWN 1955; LEAVITT & UMBARGER 1962). Exogenous valine prevents its own synthesis in the cell, but also that of isoleucine. The growth inhibiting effect due to the supply of valine therefore actually depends on a developing deficiency of isoleucine.

In higher plants the synthesis of valine, leucine and isoleucine seems to follow the same pathway as in bacteria and fungi (FOWDEN 1965). It might therefore be expected that the regulatory mechanism found for *E. coli* could also be visualized in higher plants if a suitable object is available. In a recent study MIFLIN (1969) observed slight inhibitions and stimulations of the growth of barley embryos in sterile cultures when branched chain amino acids were added to the medium.

The present paper reports part of an investigation on the metabolism of valine, leucine and isoleucine in *S. polyrhiza*. The results are briefly discussed.

2. PLANTS AND METHODS

Fronds of *S. polyrhiza* were grown on 100 ml nutrient solution in 300 ml erlenmeyer flasks, as described by LACOR (1968); the medium always contained 1% sucrose. After the addition of the amino acid(s) the solutions were sterilized for 30 minutes at 110°C. The medium was inoculated with a two frond colony from an axenic culture of *S. polyrhiza*. Growth took place at 27°C under continuous illumination (Philips TLF 55/40 W) with an intensity of 15 W m^{-2} at the level of the fronds. In each culture the fronds were counted every other day. The growth rate of the cultures was expressed as the multiplication rate, MR, defined as log Nt_2 – log Nt_1/t_2 – t_1, in which Nt_1 and Nt_2 denote the number of fronds on day t_1 and t_2 respectively. Dry weights of the cultures were determined after freeze drying at –20°C. L-valine was obtained from Merck, L-isoleucine from the British Drug Houses and D-valine, D-allo-isoleucine, L-leucine and D-leucine from Fluka.

3. RESULTS

The growth of *S. polyrhiza* can be completely inhibited by the L-isomers of each of the three branched chain amino acids. The D-isomers of valine and isoleucine did not inhibit at all, not even in a concentration of 10^{-3}M. D-leucine is also toxic, albeit less than the L-isomer (*table 1*). The relation between
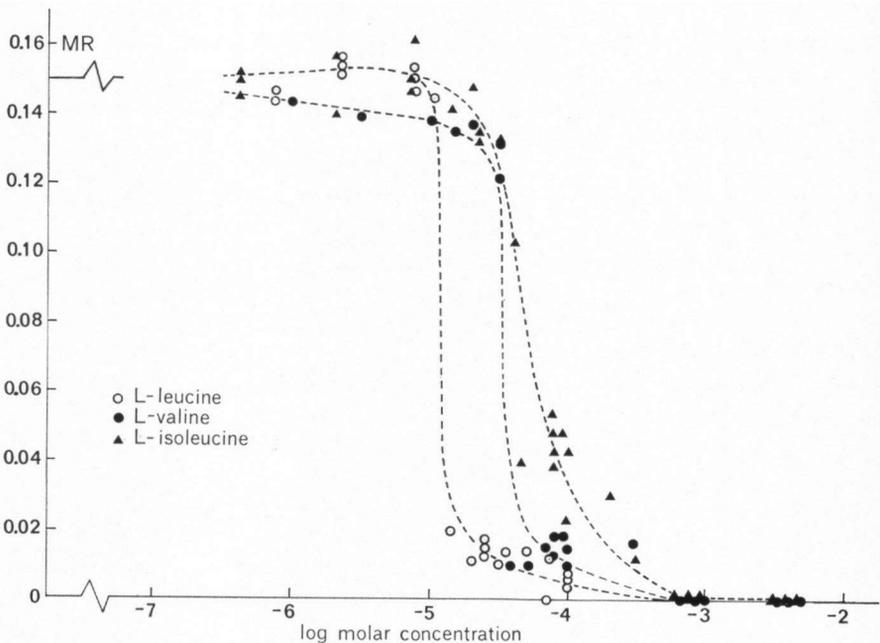

Fig. 1. Relation between the concentration of exogenously supplied L-valine, L-isoleucine or L-leucine and the multiplication rate (MR) of *S. polyhiza*.

Table 1. Effects of branched chain amino acids, supplied singly or together, on the growth
of *S. polyrhiza.*

number of replicates	addition	concentration (M)	number of fronds 10 days after inoculation	multiplication rate (MR)
14	None		62	0,149 ($\pm$0.009)
6	L-valine	8.5×10^{-5}	6	0.046*
2	D-valine	10^{-3}	50	0.140
2	L-isoleucine	7.6×10^{-5}	6	0.048*
2	D-allo-isoleucine	10^{-3}	54	0.143
2	L-leucine	7.6×10^{-5}	3	0.013*
3	D-leucine	10^{-4}	9	0.064
7	L-valine L-isoleucine	8.5×10^{-5} 7.6×10^{-5}	48	0.138
3	L-valine L-leucine	8.5×10^{-5} 7.6×10^{-5}	2	0.000
3	L-isoleucine L-leucine	7.6×10^{-5} 7.6×10^{-5}	4	0.034*
6	L-valine L-isoleucine L-leucine	8.5×10^{-5} 7.6×10^{-5} 7.6×10^{-5}	93	0.167

* Growth was restricted to the first 3 days after inoculation, so the figure does not represent
a real multiplication rate.

the concentration of the amino acids and the growth rate has a sigmoid character
as is shown in *fig. 1.* It also demonstrates that the sensitivity of *S. polyrhiza*
to the various branched chain amino acids is slightly different.

When L-valine and L-isoleucine were supplied together to the nutrient
medium an approximately normal growth rate of the culture could be observed.
A combination of L-valine and L-leucine, however, showed a complete arrest-
ment of the growth, just as was the case when L-isoleucine and L-leucine were
added together to the medium. Simultaneous addition of L-valine, L-isoleucine
and L-leucine, each in a concentration that is fully inhibitory if the acids were
supplied singly, produced excellent growth of the duckweed (*table 1*).

The antagonism between L-valine and L-isoleucine was further investigated
by supplying these compounds together in various concentration ratios.
The concentration range for L-valine was taken between 8.5×10^{-5} and
6.8×10^{-4} M, that for isoleucine between 7.6×10^{-5} and 3.0×10^{-4} M.
Thus it appeared that the growth rate was governed by the ratio of both amino
acids in the medium. A molar ratio valine/isoleucine of approximately 4 emer-
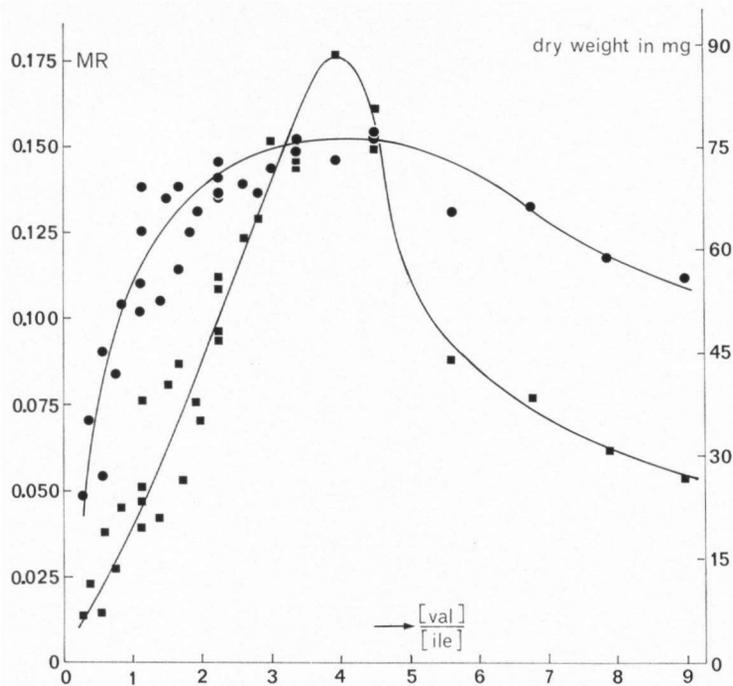ged as the optimal one for growth (*fig. 2*).

Fig. 2. Antagonism between L-valine and L-isoleucine as revealed in growth experiments with *S. polyrhiza*. Multiplication rate (MR) —●— and dry weight —■— of the cultures are plotted versus the molar ratio valine / isoleucine in the medium. Dry weights were determined 11 days after inoculation.

4. DISCUSSION

The interactions found between the various branched chain amino acids in *S. polyrhiza* are essentially the same as reported for *Bacillus anthracis* by GLADSTONE (1939). They are also in agreement with the work of MIFLIN (1969) with barley embryos. Inhibitions and stimulations found in the present work, however, are much more distinct and are obtained with at least a tenfold lower concentrations.

In bacteria and fungi the biosyntheses of valine, leucine and isoleucine have a number of enzymatic steps in common (FOWDEN 1965). Scattered evidence that this biosynthetic pattern also exists in higher plants has been provided by work with *Phaseolus radiatus* seedlings (SATYANARAYANA & RADHAKRISHNAN 1962), maize (OAKS 1965) and a variety of other seedlings (WIXOM & HUDSON 1961). Acetohydroxyacid synthetase is the first common enzyme in the biosynthesis of valine, leucine and isoleucine. LEAVITT & UMBARGER (1962) showed that growth inhibition of *E. coli* K-12 by valine results from a typical end-product inhibition of the acetohydroxyacid synthetase which catalyses the

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formation of both acetolactate, the precursor of valine and leucine, and acetohydroxybutyrate, the precursor of isoleucine.

The present results can be fully explained if a biosynthetic pattern and a regulatory mechanism in *S. polyrhiza* are assumed to be similar to those found in *E. coli*. Exogenous supply of one of the amino acids: valine, leucine or isoleucine, gives rise to end-product inhibition, probably of the acetohydroxy-acid synthetase, resulting in a deficiency of both of the others. The fact, however, that good growth can be obtained with the combination valine plus isoleucine indicates that in this case leucine can be formed in the plants. Experimental evidence for this view will be published elsewhere.

REFERENCES

DAVID, S. B. (1963): Effects of certain amino acids on the growth of excised roots of Phaseolus mungo L. In: *Plant tissue and organ culture – a symposium* (P. MAHESHWARI & N. S. RANGA SWAMY). International Society of Plant Morphologists, University of Delhi.

FOWDEN, L (1965): Origins of the amino acids. In: *Plant Biochemistry* (J. BONNER & J. E. VARNER). New York, London. p. 361–390.

GLADSTONE, G. P. (1939): Inter-relationships between amino acids in the nutrition of B. anthracis. *Brit. J. Exp. Pathol.* 20: 189–200.

HARRIS, G. P. (1956): Amino acids as sources of nitrogen for the growth of isolated oat embryos. *New Phytol.* 55: 253–268.

LACOR, M. A. A. M. (1968): Flowering in Spirodela polyrhiza (L.) Schleiden. *Acta Bot. Neerl.* 17: 357–359.

LEAVITT, R. I. & H. E. UMBARGER (1962): Isoleucine and valine metabolism in E. coli. XI. Valine inhibition of the growth of E. coli K-12. *J. Bacteriol.* 83: 624–630.

MIFLIN, B. J. (1969): The inhibitory effects of various amino acids on the growth of barley seedlings. *J. Exp. Bot.* 20: 810–819.

OAKS, A. (1965): The effect of leucine on the biosynthesis of leucine in maize root tips. *Plant Physiol.* 40: 149–155.

SATYANARAYANA, T. & A. N. RADHAKRISHNAN (1962): Biosynthesis of valine and isoleucine in plants, *Biochim. Biophys. Acta* 56: 197–199.

STREET, H. E. (1959): Special problems raised by organ and tissue culture. Correlations between organs of higher plants as a consequence of specific metabolic requirements. In: *Encyclopedia of Plant Physiology* (W. RUHLAND). Berlin, Göttingen, Heidelberg, Band 11: 153–178.

UMBARGER, H. E. & B. BROWN (1955): Isoleucine and valine metabolism in E. coli. V. Antagonism between isoleucine and valine. *J. Bacteriol.* 70: 241–248.

WIXOM, R. L. & R. J. HUDSON (1961): Studies in valine biosynthesis: II. Enzymatic dehydration of α, β-dihydroxyisovaleric acid by plant extracts. *Plant Physiol.* 36: 598–604.