*Acta Bot. Neerl.* 24(2), April 1975, p. 203–206.

# METABOLIC FATE OF EXOGENOUSLY SUPPLIED BRANCHED-CHAIN AMINO ACIDS IN SPIRODELA POLYRHIZA

A. C. BORSTLAP

Botanisch Laboratorium, Utrecht

SUMMARY

$^{14}$C-labelled L-leucine, L-valine, or L-isoleucine was supplied to *Spirodela polyrhiza* (L.) Schleiden via the growth medium. Most of the radioactivity was recovered from the protein hydrolysate and the free amino acids. In both fractions the radioactivity was found exclusively in the original compound, except for valine which was partly converted to leucine. This conversion also accounted for the accumulation of leucine after growth inhibition of the duckweed by L-valine.

## 1. INTRODUCTION

The pathways for the biosynthesis of the branched-chain amino acids have been well documented in bacteria and fungi (RODWELL 1969). Circumstantial evidence indicates that in higher plants these amino acids are synthesized in a similar way (MIFLIN 1973). If so, valine may in plants be converted directly to leucine, because transamination of valine yields α-ketoisovaleric acid, the first precursor in the leucine pathway. Other interconversions of the branched-chain amino acids seem less probable although antagonism between valine and leucine in the growth of oat embryos (HARRIS 1956) might be explained by conversion of either amino acid to isoleucine (MIFLIN & CAVE 1972).

Growth inhibition of *Spirodela polyrhiza* by L-valine has been ascribed to strong inhibition of acetohydroxyacid synthetase, the first common enzyme in the biosynthesis of valine, leucine and isoleucine. Two observations have been made which indicate that the production of leucine was not blocked by excess of valine. First, the growth of the duckweed was normal on a medium supplied with L-valine *plus* L-isoleucine (BORSTLAP 1970). Secondly, after growth inhibition by L-valine the level of free leucine in the duckweed even increased thirty-fold (BORSTLAP 1972). Both observations indicate that exogenous valine was used for the production of leucine. More direct proof will be presented here.

## 2. PLANTS AND METHODS

Axenic cultures of *S. polyrhiza* were grown as described earlier (BORSTLAP 1970).

## 2.1 Extraction of free amino acids

Lyophilized fronds were homogenized in 80% ethanol, the homogenate was evaporated to dryness, and demineralized water and choloroform were added to the residue; amino acids were separated from the water phase with a cation-exchange resin column (BORSTLAP 1972). In case of further fractionation of the water phase into an anionic and a neutral fraction, a column with anion-exchange resin (Amberlite IRA-400, $HCO_3^-$ form; 20 × 0.6 cm) was used after the cation-exchange resin column. Organic acids were eluted from the Amberlite column with 50 ml of 1 M $NH_4HCO_3$, and the eluate was evaporated to dryness on a steam bath.

## 2.2 Extraction and hydrolysis of protein

Lyophilized fronds were ground in 0.05 M $Na_2B_4O_7$ with a pestle and mortar. The homogenate was centrifuged at 1000 × *g* for 10 min, and the supernatant was collected. Cell debris was washed three times with 10 ml of 0.05 M $Na_2B_4O_7$ and the washings were added to the supernatant. The supernatant was quantitatively transferred in portions of 6 ml to a Corning tube (No. 9826, Corning Glass Works Co.). 1.5 ml of 50% (w/v) trichloroacetic acid was added to each 6 ml of supernatant and the protein precipitates were collected by centrifugation. The precipitate was washed twice with 5% trichloroacetic acid, then twice with redistilled acetone. The protein was hydrolyzed in 1 ml of 6 N HCl under nitrogen at 110°C for 16 hr. After addition of 2 μmoles of α-aminoisobutyric acid and 2 ml of demineralized water, the hydrolysate was separated from the humin and evaporated to dryness in another Corning tube at 100°C under a stream of dry, filtered air.

Table 1. Distribution of radioactivity from $^{14}C$-labelled leucine, valine and isoleucine respectively, supplied to *Spirodela* via the growth medium. The figures represent percentages of the totally recovered radioactivity. Experimental details are given in the text.

| | | $^{14}C$-labelled amino acid supplied | | |
|---|---|---|---|---|
| | | L-leucine | L-valine | L-isoleucine |
| *water phase* | | | | |
| neutral | | 4.5 | 2.6 | 2.4 |
| anionic | | 1.1 | 0.6 | 0.4 |
| cationic | leu | 4.9 | 1.4 | |
| | val | | 9.2 | |
| | ile | | | 7.3 |
| *$CHCl_3$ phase* | | 0.6 | 0.1 | 0.4 |
| *protein hydrolysate* | leu | 83.7 | 10.8 | |
| | val | | 72.4 | |
| | ile | | | 86.1 |
| *residue** | | 5.2 | 2.9 | 3.4 |

* radioactivity may be due to protein which was not extracted from the cell debris.

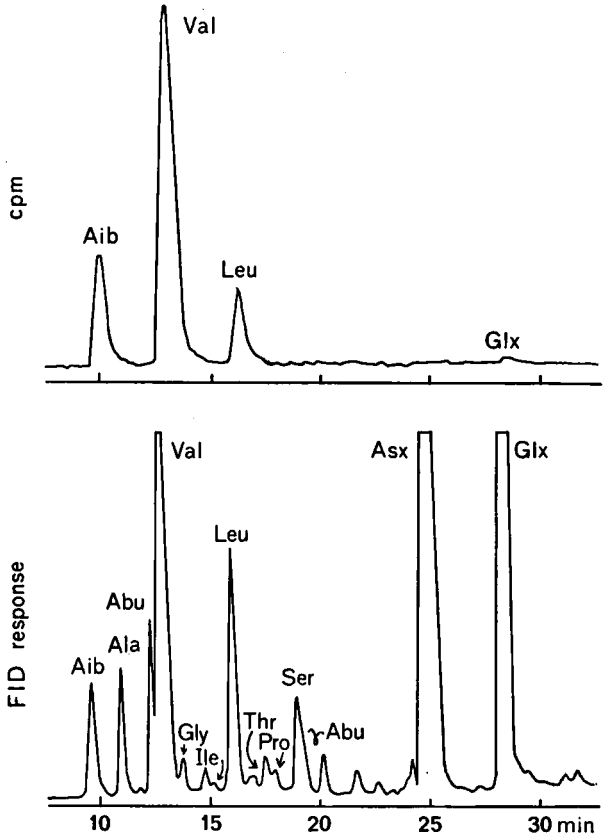


Fig. 1. Radio gas chromatogram of the free amino acids from a *Spirodela* culture after growth inhibition with 0.1 mM of L-(U-$^{14}$C) valine. α-Amino ($^{14}$C) isobutyric acid was added as an internal standard. Abbrevations for amino acids as suggested in *J. Biol. Chem.* 247:977–983 (1972). Upper curve: activity trace; lower curve: trace of mass detected by flame ionization detector (FID).

### 2.3 Radio gas chromatography of amino acids

The method for amino acid analysis by gas chromatography (BORSTLAP 1972) was modified as follows. Argon instead of nitrogen was used as carrier gas. At the column outlet a T-connexion, for the supply of purge gas, and a splitter were inserted. Via the splitter the column effluent was lead partly to the flame ionization detector, partly to the combustion furnace and proportional counter (Packard model 894). Organic compounds in the split carrier gas stream were combusted over CuO at 700° C, with water absorption in a $MgClO_4$ tube. Flow rate of argon through the column was set at 40 ml/min. Then purge gas (argon) was supplied to make the flow rate through the proportional counter 80 ml/min. Propane (8 ml/min) was used as quenching gas.

## 3. RESULTS AND DISCUSSION

A culture of about 70 fronds was placed on 10 ml of medium. 1 μCi of either L-(U-$^{14}$C) leucine, L-(U-$^{14}$C)valine or L-(U-$^{14}$C) isoleucine was added, and the

fronds were incubated for 5 hr at 27° C under illumination. The $^{14}$C-labelled amino acids (10 mCi/mmole) were obtained from The Radiochemical Centre, Amersham, U.K. After incubation the fronds were rinsed with tap water and lyophilized. Each culture was divided into two parts; free amino acids were extracted from one part, protein was extracted from the other. The results (*table 1*) show that the main part of the exogenously supplied amino acid was incorporated into protein. An appreciable part of the radioactivity from valine was recovered as leucine, both in the free amino acids and in the protein hydrolysate. Similar results have been obtained with *Escherichia coli* (ROBERTS et al. 1955) and with *Arthrobotrys conoides* (GUPTA & PRAMER 1970) where, after administration of L-($^{14}$C)valine, most of the radioactivity in the protein hydrolysate was found in valine and leucine. After feeding $^{14}$C-labelled leucine or isoleucine to *Spirodela* the radioactivity in the free amino acids and in the protein hydrolysate was found exclusively in the original compound. Apparently all three amino acids were catabolized to some extent, as radioactivity was found in the neutral (sugars) and in the anionic fraction (organic acids).

1 μCi of L-(U-$^{14}$C)valine was added aseptically to a *Spirodela* culture of 50 fronds. The volume of the medium was 100 ml, the final concentration of valine 0.1 mM. The culture was incubated for two days. Free amino acids were extracted and analysed by radio gas chromatography (*fig. 1*). If all leucine was formed from valine the ratio between the specific activities of leucine and valine should be 0.8, as only 4 of the 5 carbon atoms of the valine molecule find their way to the leucine molecule. The ratio calculated from the present results was 0.56, which means that 70 % of the leucine was formed from the supplied valine.

## REFERENCES

BORSTLAP, A. C. (1970): Antagonistic effects of branched-chain amino acids on the growth of Spirodela polyrhiza (L.) Schleiden. *Acta Bot. Neerl.* **19**: 211–215.

— (1972): Changes in the free amino acids of Spirodela polyrhiza (L.) Schleiden during growth inhibition by L-valine, L-isoleucine, or L-leucine. A gas chromatographic study. *Acta Bot. Neerl.* **21**: 404–416.

GUPTA, R. K. & D. PRAMER (1970): Metabolism of valine by the filamentous fungus Arthrobotrys conoides. *J. Bacteriol.* **103**: 131–139.

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

MIFLIN, B. J. (1973): Amino acid biosynthesis and its control in plants. In: *Biosynthesis and its control in plants* (B.V. MILBORROW, ed.), pp. 49–68, Academic Press, London and New York.

— & P. R. CAVE (1972): The control of leucine, isoleucine, and valine biosynthesis in a range of higher plants. *J. Exp. Bot.* **23**: 511–516.

ROBERTS, R. B., D. B. COWIE, P. H. ABELSON, E. T. BOLTON & R. J. BRITTEN (1955): *Studies of biosynthesis in Escherichia coli.* Carnegie Inst. Wash. Publ. No. 607.

RODWELL, V. W. (1969): Biosynthesis of amino acids and related compounds. In: *Metabolic Pathways* (D. M. GREENBERG, ed.), Vol. 3, pp. 317–373, Academic Press, New York and London.