Phytochemistry, Vol 27, No 4, pp 997–999, 1988
Printed in Great Britain

0031 9422/88 $3 00+0 00

METABOLISM OF PROPIONATE TO 3-HYDROXYPROPIONATE AND ACETATE IN THE LIMA BEAN *PHASEOLUS LIMENSIS*

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(*Received* 6 *July* 1987)

Key Word Index—*Phaseolus limensis*, Leguminosae, lima bean, propionate metabolism, vitamin B_{12}, 3-hydroxypropionate, acrylate

Abstract—No detectable levels of vitamin B_{12} were present in the leaves and stems of the lima bean, *Phaseolus limensis* [2-^{14}C]Propionate was metabolized to 3-hydroxypropionate, acetate and other organic acids in a time dependent manner. At no time period was radioactivity present in the fraction that corresponded to methylmalonate. [2,3-^{14}C]Acrylic acid was metabolized to 3-hydroxypropionate. These results are consistent with the major metabolic pathway of propionate being its conversion to acetate via acrylate and 3-hydroxypropionate rather than its metabolism to a methylmalonate derivative and then to succinate.

INTRODUCTION

The role of vitamin B_{12} and cobalamine in propionate metabolism in plants is not clear. Plants are generally considered to lack vitamin B_{12} and cobalamine related compounds In line with this, Stumpf *et al.* [1,2] reported that plant tissue metabolized propionate to 3-hydroxypropionate and then to acetate rather than to a methylmalonyl derivative and then to succinate, as occurs in vertebrates [3]. However, Laties and Hoelle [4] compared the metabolism of propionates labelled in the 1, 2 and 3 positions in aged potato tubers and reported data consistent with the participation of vitamin B_{12} dependent methylmalonyl-CoA mutase. Furthermore, Poston has reported the presence of methylmalonyl-CoA mutase and leucine 2,3-aminomutase in potato tubers [5] and leucine 2,3-aminomutase in bean seedlings [6]. Both of these enzymes are vitamin B_{12} dependent. Because of these apparent discrepancies in the occurrence of vitamin B_{12} and propionate metabolism in plants, we assayed the levels of vitamin B_{12} and determined the metabolic fate of propionate in the lima bean, *Phaseolus limensis* L.

RESULTS

No detectable levels (minimum level of detectability 0 04 pg/mg dry weight) of vitamin B_{12} were present in the leaves or stems of the lima bean, *P. limensis*. In contrast, the root tissue contained 46 ± 7 pg/mg dry weight of vitamin B_{12}

To study the metabolic fate of propionate in the lima bean, four- to five-day-old seedlings were injected with [2-^{14}C]propionate, incubated for varying periods of time, and then the organic acids were extracted and analysed by radio-HPLC. An example of the type of data obtained is presented in Fig. 1, and shows the profile of metabolites obtained after a 30 min incubation The major labelled organic acids were unreacted propionate and 3-hydroxypropionate. Lesser amounts of radioactivity were present in the fractions corresponding to citrate, acetate, and other organic acids. The effect of time on the accumulation of labelled metabolites is presented in Fig. 2, and shows that the major metabolite of propionate at all incubation times was 3-hydroxypropionate, with lesser amounts of radioactivity in other organic acids also present. At no time were appreciable amounts of radioactivity detected in the fraction corresponding to methylmalonic acid. The extraction conditions would convert CoA derivatives to their free acid form, and therefore it is not known whether the acids analysed were present in plant tissue in the free form or whether they were CoA derivatives. At no time point did high levels of radioactivity appear in the fraction corresponding to acetic acid The effect of time on the disappearance of [1-^{14}C]acetate injected into the stems of the plant indicated a relatively slow rate of disappearance, with only 52% of the recovered radioactivity from [1-^{14}C] acetate metabolized to other organic acids in 30 min This indicates that the relatively low levels of radioactivity in the acetic acid fraction from [2-^{14}C] propionate may be due to a slow conversion of 3-hydroxypropionate to acetate rather than the rapid removal of acetate formed.

Stumpf *et al.* [1, 2] suggested that acrylic acid was a likely intermediate in the conversion of propionate to 3-hydroxypropionate in plants. The efficient conversion of [2,3-^{14}C]acrylic acid to 3-hydroxypropionate (Fig 3) supports this suggestion

DISCUSSION

Vitamin B_{12} is required as a coenzyme in the metabolism of propionate to succinate The generally held view that plant tissue does not contain this vitamin was consistent with Stumpf *et al* [1, 2] observations that certain plants metabolized propionate to acetate rather than to succinate. Similar observations have been made in insects, where many species do not contain vitamin

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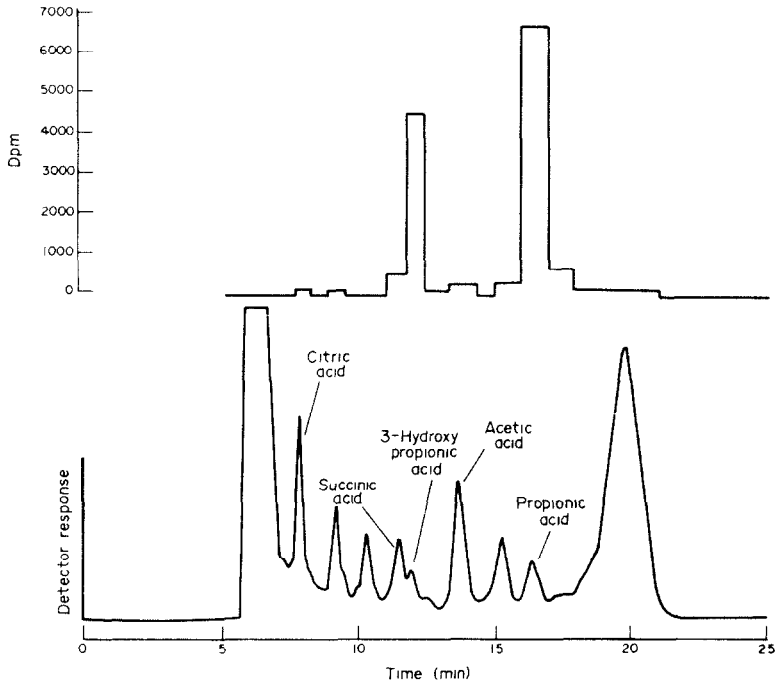

Fig 1 Radio-HPLC trace of the products of [2-^{14}C]propionate after a 30 min incubation in the lima bean The labelled substrate was injected into stems and after 30 min, the plant tissues were homogenized, organic acids extracted, the solvent reduced in volume, and radio-HPLC performed as described in Experimental

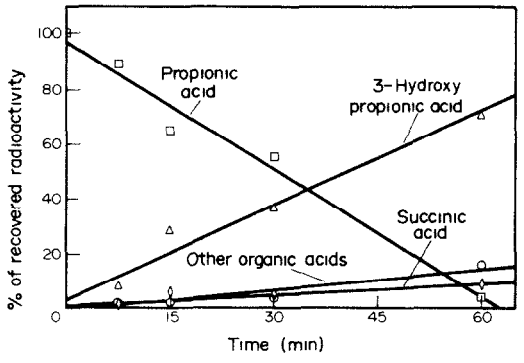

Fig 2 Effect of time on the metabolism of [2-^{14}C]propionate in the lima bean The labelled substrate was injected into stems and after the times indicated, the plant tissues were homogenized, the organic acids extracted, the solvent reduced in volume, and radio-HPLC performed as described in Experimental

B_{12} [7] and also convert propionate to acetate rather than to succinate [8–10] The reported presence of methylmalonyl-CoA mutase and leucine-2,3-aminomutase in several plants [5, 6], prompted further work on vitamin B_{12} levels and propionate metabolism The data reported here, in which *in vivo* studies show that the major route of propionate metabolism in the lima bean is the formation of 3-hydroxypropionate and then acetate, is consistent with the concept that many plants do not utilize vitamin B_{12} in propionate metabolism The reported presence of methylmalonyl-CoA mutase (which requires vitamin B_{12} as cofactor) in potato tubers may suggest that plants differ in their use of vitamin B_{12} or that different tissues in the same plant may use alternative pathways

EXPERIMENTAL

Radioactive substrates Na[2-^{14}C]propionate (27 mCi/mmol) was obtained from ICN Chemical and Isotope Division, Irvine, California [2,3-^{14}C] Acrylic acid (0 51 mCi/mmol) was purchased from Pathfinder Laboratories, St. Louis, Missouri Na[1-^{14}C]acetate (57 mCi/mmol) was purchased from Research Products International, Mount Pleasant, Illinois

Plants 4-to 5-day-old lima bean seedlings grown in planting mix were used for all experiments

Radioassay of vitamin B_{12} Between 2 and 3 g of plant tissue was used for each experiment Extraction and competitive binding radioassay was performed as described elsewhere [7] Separation of the free (unbound) vitamin B_{12} from the bound (Intrinsic Factor–B_{12} complex) was performed by a solid phase method in which purified swine intrinsic factor immobilized on microcrystalline cellulose particles was used instead of the dextran-coated charcoal method

In vivo *studies with labelled precursors* Labelled substrates in 0 5 to 1 μl H_2O were injected in separate experiments into the stems of lima beans (groups of two), and incubated for the time periods given in the text Incubations were stopped by homogenizing excised stems and leaves in 4 ml H_2O Six drops of 0 1M NaOH and 8 ml of MeCN were then added to ppt protein Precipitate and other debris were removed by centrifugation at 2000 *g* for 5 min The extract was evapd to 0 5–1 ml in a 60° sand bath under a stream of N_2 Extracts were then filtered (filter size 0 2 μ) and aliquots were analysed by HPLC

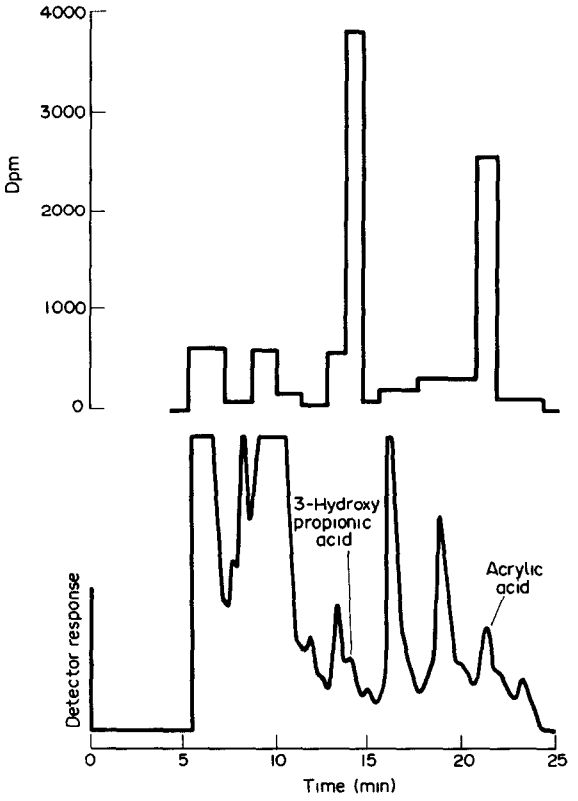

Fig. 3 Radio-HPLC trace of the products of [2,3-^{14}C]acrylic acid in a 15 min incubation in the lima bean. The labelled substrate was injected into stems and after 15 min, the tissue was homogenized, organic acids extracted, the solvent reduced in volume, and radio-HPLC performed as described in Experimental

HPLC. Organic acids were separated by HPLC on a Bio-Rad HPX-87 (9u) ion-exchange column using 0·005 M H_2SO_4 as eluent Organic acids were detected by a UV spectrometer at 210 nm The material corresponding to each component was collected in a scintillation vial Unlabelled standards were co-injected with each sample to allow detection by UV absorbance

Liquid scintillation counting 10 ml of Formula 963 (New England Nuclear Research Products, Boston, Massachusetts) was added to each sample and radioactivity was assayed by liquid scintillation counting at 80–85% efficiency

Acknowledgements—Supported in part by the Science and Education Administration of the U S Department of Agriculture under Grant 85-CRCR-1-1630 from the Competitive Research Grants Office A contribution of the Nevada Agriculture Experiment Station We thank Ken Kirkland for his technical assistance

REFERENCES

1 Giovanelli, J and Stumpf, P K (1958) *J Biol Chem* **231**, 411
2 Hatch, M D. and Stumpf, P K (1962) *Arch Biochem Biophys.* **236**, 526
3 Rosenberg, L E (1983) in *The Metabolic Basis of Inherited Disease*, 5th Edn (Stanbury, J B, Wyngaarden, J B, Frederickson, D S, Goldstein, J L and Brown, M S, eds) p 474 McGraw-Hill, New York
4. Laties, G G and Hoelle, C (1967) *Phytochemistry* **6**, 49
5 Poston, J M (1978) *Phytochemistry* **17**, 401
6. Poston, J M (1977) *Science* **195**, 301
7 Wakayama, E J, Dillwith, J W, Howard, R W and Blomquist, G J (1984) *Insect Biochem* **14**, 175
8 Halarnkar, P P, Heisler, C R and Blomquist, G J (1986) *Insect Biochem.* **16**, 455
9 Halarnkar, P P, Nelson, J H, Heisler, C R and Blomquist, G J (1985) *Arch. Biochem. Biophys* **96**, 193
10 Halarnkar, P P, Chambers, J D and Blomquist, G J (1986) *Comp. Biochem. Physiol* **84B**, 469