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EFFECT OF GIBBERELLIC ACID ON MEVALONATE ACTIVATION IN GERMINATING *CORYLUS AVELLANA* SEEDS

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Key Word Index—*Corylus avellana*; Betulaceae; hazel seeds; mevalonic acid kinase; MVA-decarboxylation; gibberellic acid.

Abstract—Giberellic acid (GA) induced germination of hazel seeds is accompanied by early increases in the specific and total activities of MVA kinase in the embryonic axes. This is followed by an increase in the activity of decarboxylation of MVA by the whole axes. The activity of MVA kinase in the cotyledons is not affected by GA treatment although increased uptake of MVA results in increased decarboxylation by cotyledon slices. The effects of cofactors and inhibitors on the activities of MVA kinase and MVA decarboxylation in a cell free extract of hazel cotyledons are described.

INTRODUCTION

GIBBERELLIC ACID induced germination of hazel seeds is accompanied by the synthesis of large amounts of sterols in the embryonic axes.[1] The total sterol content of dormant and germinating cotyledons and dormant embryonic axes does not change over the same period, although exogenously supplied [2-^{14}C]MVA is readily incorporated into squalene by these tissues.[1]

The present report relates these observations to changes in the capacity of the tissues to activate mevalonate. This work forms part of an extensive programme of research into the importance of lipid changes in hormone-induced seed germination.[2–5]

RESULTS AND DISCUSSION

The germination rate of the seeds used for the MVA kinase assays is shown in Fig. 1. Little change occurred in the specific and total activities of MVA kinase in the germinating cotyledons (Fig. 2), both increasing slightly in dormant and germinating tissue. These increases were probably associated with the rehydration of the tissue. The decrease in the total activity of MVA kinase in the 14-day-old germinating cotyledons may have been associated with senescence of the tissue.

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† *Abbreviations*: MVA—DL-mevalonic acid; MVA-P—mevalonic acid phosphate; MVA-PP—mevalonic acid pyrophosphate; IPP—isopentenyl pyrophosphate; HMGCoA—β-hydroxy β methyl glutaryl CoA; *p*CMB—*p*-chloromercuribenzoate. GSH—reduced glutathione; NEM—N-ethyl maleimide.

[1] SHEWRY, P. R. and STOBART, A. K. (1974) *Phytochemistry*, **13**, 347.
[2] SHEWRY, P. R., PINFIELD, N. J. and STOBART, A. K. (1972) *Phytochemistry* **11**, 2149.
[3] SHEWRY, P. R. and STOBART, A. K. (1973) *J. Exp. Bot.* in press.
[4] SHEWRY, P. R., PINFIELD, N. J. and STOBART, A. K. (1973) *J. Exp. Bot.* in press.
[5] STOBART, A. K. and PINFIELD, N. J. (1970) *New Phytologist* **69**, 939.

In the embryonic axes, however, the specific and total activities of MVA kinase increased within 48 hr of GA treatment (Fig. 3), before any visible signs of germination or weight changes. The total activity continued to increase over the duration of the experiment whereas the specific activity fell after 8 days. The initial rise in activity may have been due to activation of pre-existing enzyme or hormone-stimulated enzyme synthesis.

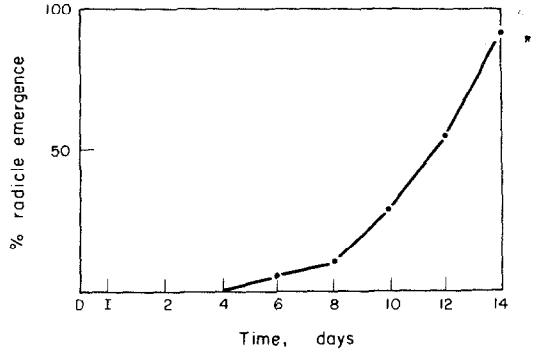

FIG. 1. THE GERMINATION RATE OF GA TREATED *Corylus avellana* SEEDS USED IN THE MVA KINASE ASSAYS. No germination was recorded in the H_2O treated seeds.

The decrease in the specific activity after 8 days was probably due to the synthesis of large amounts of soluble protein in the expanding axes. When expressed on a fresh or dry weight basis the activity rises at 2 days and falls below the H_2O control at 8 days.

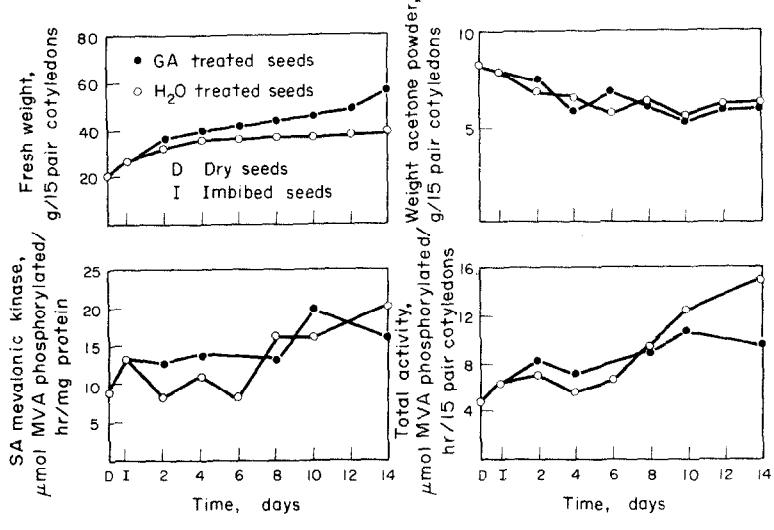

FIG. 2. THE FRESH WEIGHT, WEIGHT OF ACETONE POWDER AND MVA KINASE ACTIVITY IN *Corylus avellana* COTYLEDONS.

Figure 4 shows the decarboxylation of [1-^{14}C]MVA by the cotyledons. The greater activity of the GA treated cotyledons was due to greater permeability of the tissue to the isotope. The results of a replicate experiment showed that slices of GA treated tissue took up more isotope but decarboxylated a similar percentage of isotope taken up as slices of

H_2O treated tissue. GA affects the H_2O permeability of epidermal tissue of *Allium*.[6] The increased fresh weight of GA-treated hazel cotyledons (Figs. 2 and 4) provides further evidence for an effect on permeability. A GA-stimulated increase in the decarboxylation of [1-^{14}C]MVA occurred in the embryonic axes (Fig. 5), but this was not apparent until after the increase in MVA kinase activity.

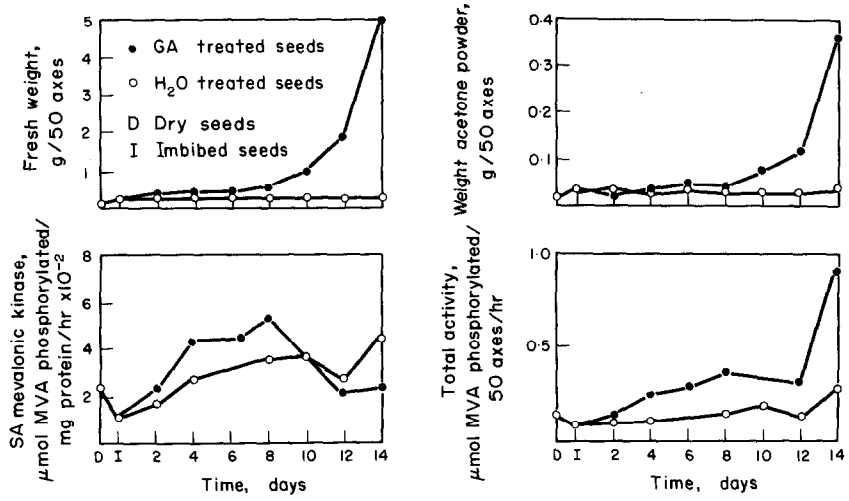

FIG. 3. THE FRESH WEIGHT, WEIGHT OF ACETONE POWDER AND MVA KINASE ACTIVITY IN EMBRYONIC AXES OF *Corylus avellana* SEEDS.

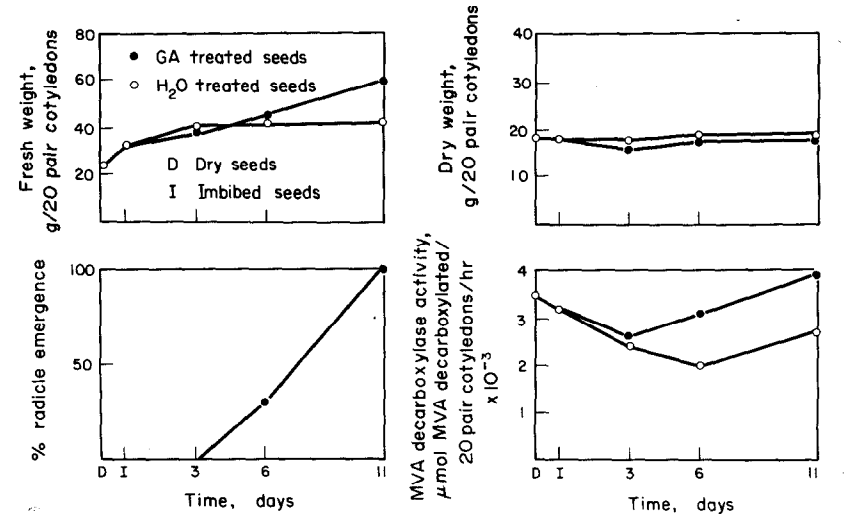

FIG. 4. THE FRESH WEIGHT, DRY WEIGHT, GERMINATION AND ACTIVITY OF [1-^{14}C]MVA DECARBOXYLATION IN COTYLEDONS OF *Corylus avellana* SEEDS.

The decarboxylation results are the average of those determined using two 1 g samples of cotyledon slices.

The demonstration of MVA kinase and [1-^{14}C]MVA decarboxylation activities in the dormant and germinating cotyledons and dormant embryonic axes is consistent with the

[6] HEINRICH, G. (1964) *Protoplasma* **58**, 402.

ready incorporation of [2-^{14}C]MVA into terpenoids by these tissues.[1] The total amount of sterols in the tissues did not increase suggesting that control is exerted by low activity of HMGCoA reductase or limited availability of substrate for this enzyme. HMGCoA reductase is important in the regulation of terpenoid synthesis in *Hevea* latex,[7] yeast[8] and animals.[9,10] The enzymes catalysing the activation of mevalonate in the cotyledons and dormant embryonic axes may have remained from the period of active terpenoid synthesis during seed development.

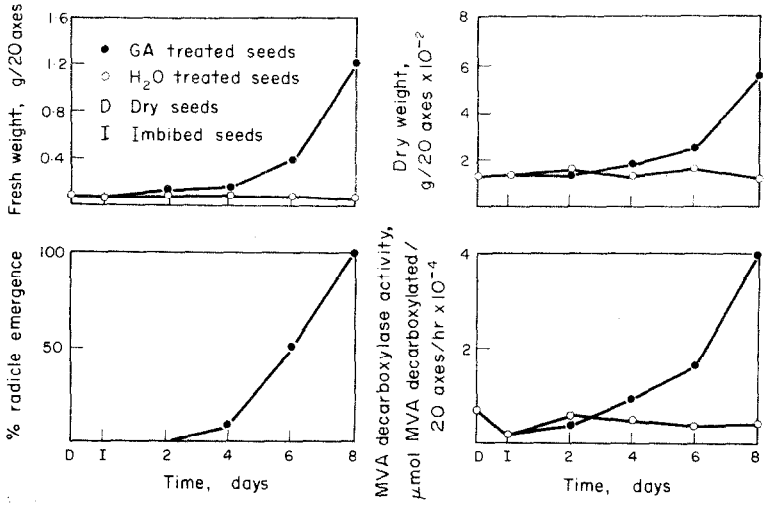

FIG. 5. THE FRESH WEIGHT, DRY WEIGHT, GERMINATION AND ACTIVITY OF [1-^{14}C]MVA DECARBOXYLATION IN EMBRYONIC AXES OF *Corylus avellana* SEEDS.
Results are the average of two replicate samples.

The increased activities of MVA kinase and [1-^{14}C]MVA decarboxylation in the germinating embryonic axes agree with the increasing total sterol content and incorporation of [2-^{14}C]MVA into sterols.[1] Green and Baisted[11] have reported changes in the activities of enzymes of the isoprenoid pathway during the early stages of pea germination. Their results were, however, not comparable to those reported here as the time scale was much shorter and the seedlings were not divided into embryonic axes and cotyledons.

Table 1 shows the effects of ATP, Mn^{2+}, Mg^{2+} and inhibitors on the activity of MVA kinase and [1-^{14}C]MVA decarboxylation in a cell-free extract of hazel cotyledons. It is apparent that the overall conversion of MVA to IPP was limited by 5-phospho-MVA kinase or 5-pyrophospho-MVA anhydrodecarboxylase. 5-Pyrophospho-MVA anhydrodecarboxylase is the limiting enzyme in this sequence in pea seeds.[11] The activity of the limiting enzyme relative to that of MVA kinase was much higher than in the pea system. The MVA kinase of hazel seeds closely resembled those of castor bean[12] and *Hevea* latex.[13] The kinetics of MVA kinase and [1-^{14}C]MVA decarboxylation were similar in many respects, both being activated more by a mixture of Mn^{2+} and Mg^{2+} than by either ion and

[7] HEPPER, C. M. and AUDLEY, B. G. (1969) *Biochem. J.* **114**, 379.
[8] KAWAGUCHI, A. (1970) *J. Biochem.* **67**, 219.
[9] SIPERSTEIN, M. D. (1970) *Current Topics in Cellular Regulation* **2**, 65.
[10] SIPERSTEIN, M. D., GYDE, A. M. and MORRIS, H. P. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 315.
[11] GREEN, T. R. and BAISTED, D. J. (1972) *Biochem. J.* **130**, 983.
[12] SHEWRY, P. R. and STOBART, A. K. (1973) *Plant Science Letters* **1**, 473.
[13] WILLIAMSON, I. P. and KEKWICK, R. G. O. (1965) *Biochem. J.* **96**, 862.

having an ATP optimum of 5 μmol/ml. Both were inhibited by GSH, cysteine and NEM and stimulated by mercaptoethanol. They differed, however, in the Mn^{2+}-optimum and in the effect of iodacetamide which had little effect on the kinase but inhibited decarboxylation. This suggests that the limiting enzyme differed from MVA kinase in some of its kinetic properties. It is probable, however, that the 'apparent' kinetic properties of the limiting enzyme were affected by the supply of substrate by MVA kinase.

TABLE 1. THE EFFECTS OF COFACTORS AND INHIBITORS ON THE ACTIVITIVES OF MVA KINASE AND [1-^{14}C]MVA DECARBOXYLATION IN A CELL FREE EXTRACT OF HAZEL COTYLEDONS

Alteration to basic reaction mixture	MVA kinase	Sp. act.* [1-^{14}C]MVA decarboxylation
No alterations	1316·0	320·6
No ions	2·6	3·0
0 μmol Mn^{2+}, 2·5 μmol Mg^{2+}	23·7	21·6
0 μmol Mn^{2+}, 5·0 μmol Mg^{2+}	234·3	59·7
0 μmol Mn^{2+}, 25 μmol Mg^{2+}	215·4	17·0
0 μmol Mg^{2+}, 2·5 μmol Mn^{2+}	1015·0	225·8
0 μmol Mg^{2+}, 5·0 μmol Mn^{2+}	1009·0	300·1
0 μmol Mg^{2+}, 25 μmol Mn^{2+}	124·3	171·8
0 μmol ATP	114·0	4·3
1 μmol ATP	533·3	71·3
5 μmol ATP	764·9	343·1
10 μmol ATP	98·2	11·3
10 μmol GSH	48·6	1·7
50 μmol GSH	0	1·5
10 μmol mercaptoethanol	1678·0	373·8
50 μmol mercaptoethanol	2053·0	346·0
10 μmol cysteine	97·4	3·2
50 μmol cysteine	34·5	0·7
10 μmol iodoacetamide	1299·0	223·2
50 μmol iodoacetamide	900·5	240·6
10 μmol NEM	874·9	175·5
50 μmol NEM	598·7	19·8

* Expressed as μmol MVA reacted/mg protein/hr.

The basic reaction mixture was 5 μmol $MgSO_4$, 5 μmol $MnCl_2$, 5 μmol ATP, enzyme and 0·073 μmol of either [1-^{14}C]MVA or [2-^{14}C]MVA in 1 ml 0·05 M Tris buffer, pH 7·2. Reaction mixtures were incubated at 25° for 30 min.

EXPERIMENTAL

Fruits of *Corylus avellana* L. (Kent Cob Nuts) were purchased from R. Gould, Mereworth, Kent. After deshelling and sterilizing the seeds were imbibed in H_2O and placed in the dark in Petri dishes containing 20 ml of either H_2O or 3×10^{-4} M GA_3. The hormone breaks the dormancy of the seed.[14]

MVA kinase (E.C. 2.7.1.36) was assayed in buffered extracts of acetone powders[15] of samples of 50 embryonic axes or 15 pairs of cotyledons. The reaction mixtures contained 6 μmol ATP, 4 μmol $MgSO_4$, 1 μCi (*ca.* 0·1 μmol) [2-^{14}C]MVA and enzyme in 1 ml 0·04 M phosphate buffer, pH 7·0. After incubating for 60 min at 37° the reaction was terminated by boiling. 20 μl aliquots of the protein-free supernatant were chromatographed on Whatman 1 paper in isobutyric acid–aq. NH_3–H_2O (22:1:10). Areas of the chromatogram containing MVA-P and MVA-PP were removed, sprayed with N NaOH and counted.[16]

[14] FRANKLAND, B. and WAREING, P. F. (1966) *J. Exp. Botany* **17**, 596.

[15] LOOMIS, W. D. (1959) *Plant Physiol.* **34**, 541.

[16] THOMAS, D. R. and STOBART, A. K. (1970) *Phytochemistry* **9**, 1443.

Samples of 20 embryonic axes or 1 g cotyledon slices were assayed for [1-^{14}C]MVA decarboxylation. The tissue was incubated at 25° for 60 min in 2·1 ml 0·1 M phosphate buffer, pH 7·2, containing 0·5 μCi (0·073 μmol) [1-^{14}C]MVA. $^{14}CO_2$ was collected in 2-phenylethylamine. Residual isotope was extracted by boiling the tissue with 3 × 20 ml 80% EtOH.

All radioactive samples were counted by liquid scintillation in toluene-based scintillant (5 g PPO and 0·3 g POPOP/l toluene). Protein was determined with Folins reagent.[17] Kinetic studies were made on the 40000 *g* supernatant of a 0·05 M, pH 7·2 Tris extract of dry hazel cotyledons.

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[17] LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951) *J. Biol. Chem.* **193**, 265.