ISOLATION OF TWO FRACTIONS WITH MEVALONATE KINASE ACTIVITY FROM *PINUS PINASTER* AND *AGAVE AMERICANA*

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1. Introduction

Mevalonic acid (MVA), the key precursor of isoprenoids in higher plants [1], is converted into isopenetenylpyrophosphate through mevalonate-5-phosphate (MVAP) and mevalonate-5-pyrophosphate (MVAPP). The first stage is catalyzed by mevalonate kinase (ATP-mevalonate 5-phosphotransferase) (EC 2.7.1.36). This enzyme has been partially purified from hog liver [2], yeast [3], *Cucurbita pepo* seedlings [4], *Hevea brasiliensis* latex [5] and other sources. In French-bean seedlings, Rogers et al. [6] demonstrated the presence of two isoenzymes of mevalonate kinase, one located in the chloroplasts and the other one outside them, with an optimum pH of 7.5 and 5.5, respectively.

The present paper reports the separation by Sephadex G-100 of two fractions with mevalonate kinase activity from *Pinus pinaster* seedlings and *Agave americana* leaves, both fractions being equally active at pH 7.9.

2. Materials and methods

Pinus pinaster seedlings 60 days old grown from seeds stratified at 5° for 6 weeks and *Agave americana* plants growing locally, were used.

[2-^{14}C]MVA was supplied as the lactone by the Radiochemical Centre, Amersham, England. The potassium salt was prepared by treating the lactone at 36° for 30 min with an excess of a KOH solution.

Acetone-dried powders were prepared as described previously [7]. The extracts were prepared with 0.1 M Tris-HCl buffer, pH 7.9, containing 0.01 M β-mercaptoethanol. All procedures in the preparation of extracts were done at 0–4°.

The extracts were centrifuged at 10 000 *g* for 30 min and the supernatant was fractionated with solid ammonium sulfate. The fraction obtained between 30–45% of saturation was dissolved in 0.1 M Tris-HCl buffer, pH 7.9, containing 0.01 M β-mercaptoethanol, and filtered upward through a Sephadex G-100 column (4.5 × 45 cm) previously equilibrated with the same buffer. Protein was eluted at a flow rate of 40 ml/hr. Fractions of 10 ml were collected.

The protein content was determined by the method of Lowry et al. [8] and from absorption at 280 nm.

The radiochemical incubation mixture for the enzyme assay contained Tris-HCl buffer, pH 7.9, 300 μmoles; β-mercaptoethanol, 30 μmoles; ATP-potassium salt, 24 μmoles; $MgCl_2$, 12 μmoles; [2-^{14}C]MVA, 150 nmoles (1 μCi) and 0.5–5.0 mg protein in a final volume of 3 ml. The reaction mixture was incubated aerobically at 37° for 30 min in the assays with *Pinus pinaster* extracts and 60 min when the enzyme preparations from *Agave americana* were used [7]. Reactions were stopped by heating the tubes to 90° for 2 min. The precipitate was centrifuged off at 2000 *g* for 5 min. Aliquots (25 μl) of supernatants were applied to Whatman No 1 paper strips and developed by the ascending technique in n-butanol–formic acid–water (77:13:10) [3]. Radioactive spots on the dried strips were detected and measured in a Nuclear-Chicago Actigraph III system. Measurement conditions were previously established.

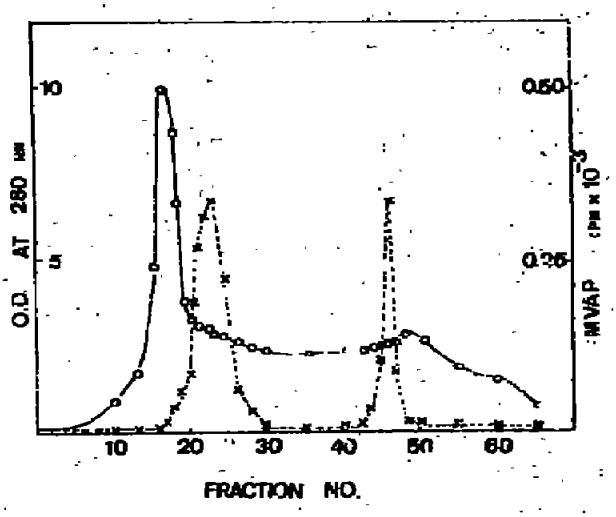

Fig. 1. Sephadex G-100 gel filtration of MVA kinase from *Pinus pinaster* seedlings. 30 mg of protein precipitated between 30–45% saturation ammonium sulfate was redissolved in 6 ml of 0.1 M Tris-HCl buffer, pH 7.9, containing 0.01 M β-mercaptoethanol and applied to a column (4.5 × 45 cm) of Sephadex G-100 previously equilibrated with the same buffer. Fractions of 10 ml were collected at a flow rate of 40 ml/hr. Temperature 4°. Absorbance at 280 nm (o——o——o); mevalonate kinase activity (×---×---×).

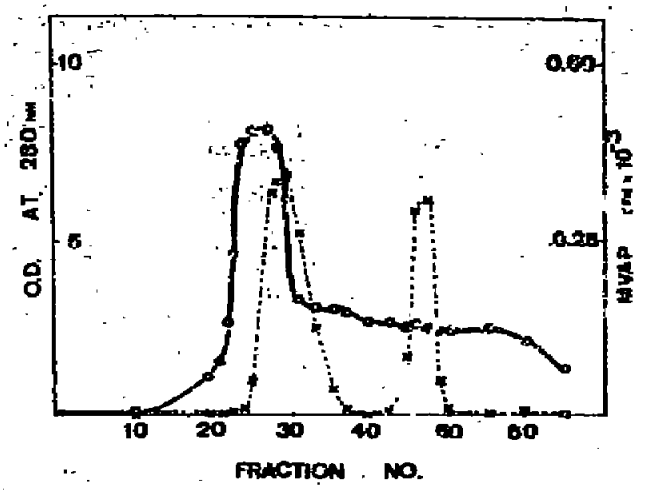

Fig. 2. Sephadex G-100 gel filtration of mevalonate kinase from *Agave americana* leaves. The experimental conditions are given in fig. 1. Absorbance at 280 nm (o——o——o); mevalonate kinase activity (×---×---×).

3. Results and discussion

The extracts from both *Pinus pinaster* and *Agave americana* acetone-dried powders redissolved in 0.1 M Tris-HCl buffer show a clear mevalonate kinase activity only when the pH is above 7.0. These results are in good agreement with those reported by Williamson et al. [5] in rubber latex whereas Polty et al. [9] reported a maximal MVA phosphorylation in the orange at pH 6.5 and Valenzuela et al. [10] an optimum pH of 6.0 for mevalonate kinase from *Pinus radiata* seedlings. In their work on mevalonate kinase activity of enzymatically active preparations from green leaves, etiolated leaves, etiolated cotyledons and chloroplasts from French-bean, Rogers et al. [11] concluded that the optimum pH of the chloroplastic mevalonate kinase activity was near pH 7.5 while that of the extrachloroplastic enzyme was about 5.5. Loomis et al. [4] found similar results in pumpkin seedlings, suggesting a different optimum pH according to the intracellular localization.

We have also investigated the incorporation of MVA by extracts from etiolated leaves of *Agave americana* and the phosphorylation is maximal at pH above 7.0.

The results obtained after fractionation of *Pinus pinaster* seedlings extracts are shown in fig. 1. Two peaks of enzyme activity were observed, one of them appearing near the main peak of protein elution, whereas the other one is clearly separated. The elution profile represented in fig. 1 has been repeated over 5 times with the same pattern of enzyme activity.

In *Agave americana* leaves the results are very similar (fig. 2). Two active fractions were also separated. As in *Pinus pinaster* seedlings both show enzymatic activities at pH 7.9, whereas Rogers et al. [6, 11] reported two activities of mevalonate kinase at different pH values. Further experiments are being carried out to elucidate whether one of the active fractions is exclusively located in the chloroplasts, in spite of both isoenzymes being equally active at pH above 7.0.

Acknowledgement

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