Mevalonate Kinase from Etiolated Cotyledons of French Beans

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From a comparison of the pH optima of mevalonate kinase activity in extracts of French-bean tissues, Rogers, Shah & Goodwin (1966) concluded that isoenzyme forms existed in the chloroplast and cytoplasm. The pH optimum of the chloroplast enzyme (pH 7.5) was similar to that of the *Hevea* enzyme (Williamson & Kekwick, 1965) and to those of enzymes from animal sources, but the pH optimum of the cytoplasmic enzyme (pH 5.5) was quite different, though similar to that of etiolated pumpkin cotyledons (Loomis & Battaile, 1963).

Preliminary studies with acetone-dried powder preparations of etiolated cotyledons of French beans (Rogers *et al.* 1966) demonstrated the presence of a phosphatase acting on ATP with an activity 3000 times that of the mevalonate kinase. This phosphatase activity also hydrolysed ADP and mevalonate 5-phosphate. Cotyledon mevalonate kinase was therefore purified, with a view to removing the phosphatase activity. The properties of the cotyledon enzyme were investigated preliminary to a study of the properties and development of the chloroplast enzyme.

Macerated cotyledons from 4–5-day-old dark-grown French-bean seedlings suspended in an equal volume of 0.08M-phosphate buffer, pH 7.0, were centrifuged at 105000g for 75 min. at 2°. The fraction from the scum-free supernatant soluble in 20%-satd. and precipitated by 45%-satd. ammonium sulphate was dissolved in and dialysed against the phosphate buffer, applied to a Sephadex G-200 column and eluted with the same buffer. The active fractions were concentrated, applied to a DEAE-Sephadex column equilibrated with the same buffer and eluted with potassium chloride increasing in concentration linearly to 0.5M; the mevalonate kinase was present in fractions containing 0.1–0.2M-potassium chloride. These combined fractions had a specific activity about 40 times that of the starting material, with a phosphatase activity only 25% of that of the mevalonate kinase.

These preparations had a pH optimum at 7.0 assayed by both spectrophotometric and radiochromatographic procedures (Williamson & Kekwick, 1965) and a K_m for mevalonate of 0.04 mM. The product of the reaction was chromatographically indistinguishable from authentic mevalonate 5-phosphate (Williamson & Kekwick, 1965), and was converted into mevalonate by calf intestinal alkaline phosphatase and into mevalonate 5-pyrophosphate by *Hevea* phosphomevalonate kinase (Skilleter, Williamson & Kekwick, 1966).

In contrast with the results of Rogers *et al.* (1966) the mevalonate kinase from etiolated French-bean cotyledons appears to resemble the *Hevea* enzyme in its kinetic properties more closely than that from pumpkin cotyledons.

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Effect of SKF7997 and SKF525 on Diterpene and Sterol Biosynthesis in *Gibberella fujikori* from [2-¹⁴C]Mevalonate

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The compounds SKF7997 [tris(2-diethylaminoethyl) phosphate] and SKF525 (2-diethylaminoethyl 2,2-diphenylvalerate), which were shown to inhibit phytosterol biosynthesis in *Nicotiana* (Reid, 1968a) and β -amyrin and phytosterol biosynthesis in *Pisum* (Reid, 1968b), were examined for their effect on shake cultures of *Gibberella fujikori* fed on [2-¹⁴C]mevalonate.

Four fractions were isolated: culture acids (A), culture neutrals (B), mycelium acids (C) and mycelium neutrals (D). Fractions (C) and (D) were derived by saponification of the lipids of the mycelium, which contained esters and glycerides; these were greatly increased in the presence of SKF525 and the formation of red pigment also increased with SKF525.

The total incorporations of the precursor were: control, 66.7%; with SKF7997, 60.9%; with SKF525, 54.3%. The largest incorporation was into fraction (A) (47.0%, 44.5% and 26.5% respectively) and fraction (D) (12.8%, 8.3% and 20.0%).

The major labelled components in fraction (D) were kaurene, kaurenol, epimanoyl oxide and material that chromatographed with the methyl sterols and sterols. Mycelia grown with SKF525 showed in addition the accumulation of radioactivity in kaurenol, enhanced accumulation in the sterol groups and in a fraction more polar than the sterols that could be resolved into a major component with the properties of a diol, and in 7-hydroxykaurenolide and 7,18-dihydroxykaurenolide as minor components. The control and

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