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MEVALONATE DECARBOXYLATION IN LEMON GRASS LEAVES

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Key Word Index—*Cymbopogon citratus*; Graminae; lemon grass; isoprenoid biosynthesis; mevalonate-5-pyrophosphate decarboxylase.

Abstract—The activity of mevalonate-5-pyrophosphate (MVAPP) decarboxylase was assayed in the extracts of green leaves of lemon grass. The enzyme was found to be exclusively cytosolic, had a pH optimum of 6.0 and had a specific requirement for ATP; Mg^{2+} was required and Mn^{2+} could replace it partially. The phenolic compounds, *p*-coumaric acid, protocatechuic acid, ferulic acid and phloroglucinol carboxylic acid inhibited the activity.

INTRODUCTION

The last step in the biosynthesis of isopentenyl pyrophosphate from mevalonate involves the decarboxylation and dehydration of mevalonate-5-pyrophosphate, a reaction catalysed by the enzyme mevalonate pyrophosphate decarboxylase [ATP: 5-diphosphomevalonate carboxylase (dehydrating) EC 4.1.1.33]. This enzyme has been purified partially from yeast [1] and to homogeneity from rat liver [2] and from pig liver [3]. In plants, the enzyme is known to be present in the latex of the rubber plant (*Hevea brasiliensis*) [4] and in orange juice vesicles [5]. However, studies on this enzyme in plants have been confined to identification of isopentenyl pyrophosphate as a product formed in the incubation mixtures capable of synthesizing terpenyl pyrophosphates [6, 7], carotenes [8] and rubber [9].

In the present paper we report for the first time in plant leaves the presence of mevalonate pyrophosphate decarboxylase in green leaves of lemon grass (*Cymbopogon citratus*) and describe some of its properties.

RESULTS

Mevalonate-metabolizing enzymes in plants

Three enzymes are involved in the conversion of mevalonate to isopentenyl pyrophosphate, each step requiring ATP, producing mevalonate-5-phosphate (MVAP), mevalonate-5-pyrophosphate (MVAPP) and isopentenyl pyrophosphate (IPP). Combination of the three steps results in the loss of C-1 by decarboxylation of mevalonate. Initially various plant materials were tested for their ability to decarboxylate mevalonate. This activity, measured as release of $^{14}CO_2$ from [1-^{14}C]mevalonic acid was found in seedlings of green gram, ground nut, peas and rice and in green leaves of lemon grass (Table 1). Lemon grass was selected for further study as leaf tissues have been studied least.

Properties of mevalonate-5-pyrophosphate decarboxylase

MVAPP decarboxylase from lemon grass was found to be exclusively cytosolic. The enzyme activity was linear up to a concentration of 1 mg protein/ml and also for 40 min of incubation time. Maximum activity was obtained at pH 6.0. The enzyme had a specific requirement for ATP and Mg^{2+}; Mn^{2+} was able to substitute for Mg^{2+} but could give only 50% of activity. A small activity was supported by Fe^{2+} but was not concentration dependent. A K_m value of 10 μM for MVAPP was calculated from the double reciprocal plot (Table 2) which is the same as that of rat liver but was 2.5 higher than the *Hevea brasiliensis* enzyme.

MVAPP decarboxylase was found to exhibit seasonal variation in its activity. The enzyme showed maximum activity in the hot summer months in Bangalore from March to June with a specific activity of 50–60 nmoles/hr per mg protein. With the onset of monsoon there is a drastic decrease in the activity and in the cold winter months the activity could not be detected (Fig. 1).

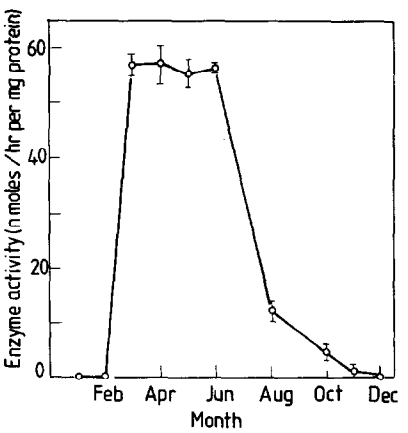

Fig. 1. Seasonal variation in the activity of MVAPP decarboxylase. Assays were performed as described in the Experimental. The values given are means ± s.d. of independent analysis of three sets in each month.

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Table 1. Release of $^{14}CO_2$ from [1-^{14}C]mevalonate*

Plant	Days after germination	nmoles of CO_2 released/hr/mg protein
Green gram (*Vigna radiata*) seedlings	15	5.7
Cow-pea (*Vigna catjang*) seedlings	15	nil
Peas (*Pisum sativum*) seedlings	15	3.8
Lemon grass (*Cymbopogon citratus*) leaves	60	0.6
Rubber plant (*Ficus elastica*) leaves	60	nil
Ground nut (*Arachis hypogaea*) seedlings	15	1.0
Ground nut (*Arachis hypogaea*) leaves	60	0.3
Rice (*Oryza sativa*) seedlings	15	0.8

*The assay was carried out as described in the Experimental.

Table 2. Nucleotide specificity of MVAPP decarboxylase*

Substrates	Apparent K_m (mM)	V_{max} (nmoles/hr/mg protein)
ATP	1.66	67
GTP	0.30	11
CTP	0.47	8

*The assays were carried out as described in the Experimental. The reaction mixture contained phosphate buffer (pH 6.8, 100 mM), $MgCl_2$ (12.5 mM), [1-^{14}C]MVAPP (15 μM, 300 cpm/nmole), enzyme protein (0.3 mg), and varying concentrations of ATP, GTP or CTP. V_{max} and apparent K_m were calculated from the double reciprocal plot.

Inhibition of phenolic compounds

MVAPP decarboxylase from green leaves of lemon grass was inhibited by a variety of phenyl and phenolic compounds, particularly by protocatechuic acid, *p*-coumaric acid and cinnamic acid. Ferulic acid was a very potent inhibitor of MVAPP decarboxylase and at a concentration of 10 mM it completely abolished the enzyme activity. Phloroglucinol and resorcinol caused marginal inhibition whereas phloroglucinol carboxylic acid and resorcyclic acid inhibited the enzyme activity by about 86% and 69% respectively (Table 3).

DISCUSSION

Treatment of the plant tissue with polyclar AT is essential for the removal of phenolics [10]. However,

Table 3. Effect of phenyl and phenolic compounds on MVAPP decarboxylase*

Compound	K_I (mM)	Enzyme activity at 10 mM (nmoles/hr/mg protein)	Inhibition (%)
Control	—	52	—
Resorcinol	—	51	2
Phloroglucinol	—	45	14
Resorcylic acid	0.20	16	69
Phloroglucinol carboxylic acid	0.16	7.2	86
Protocatechuic acid	6.33	19	64
p-Coumaric acid	2.30	9	83
Cinnamic acid	1.01	7	87
Ferulic acid	0.03	0	100
Isoferulic acid	—	49	6

*The assay was carried out using the standard assay mixture. The buffer extract of the acetone powder was subjected to ammonium sulphate fractionation and the 30–60% fraction was used as the enzyme source. Varying concentrations of phenolic compounds (0.5–10 mM) were used as neutral solutions. The apparent K_I was calculated using the Dixon plot. The values of enzyme activity and inhibition at 10 mM concentration are also given.

when the tissue is homogenized with polyclar AT a certain amount is released as soluble PVP and this interferes with the studies on the effects of phenolic compounds. This soluble PVP was removed by ammonium sulphate fractionation and the 30–60% fraction was used as the enzyme source for all studies.

EXPERIMENTAL

Materials. Lemon grass was grown in pots in the Institute nursery. Other plant materials were obtained from the nursery or the market.

3*RS*-1[1-^{14}C]Mevalonic acid lactone (6 mCi/mmol) was purchased from the Radiochemical Centre, Amersham, Bucks, U.K. ATP (disodium salt), dithiothreitol (DTT), polyclar AT, 2-mercaptoethanol, resorcinol (1,3-dihydroxybenzene), protocatechuic acid (3,4-dihydroxybenzoic acid), *p*-hydroxyphenylpropionic acid and *p*-coumaric acid (4-hydroxycinnamic acid) were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Cinnamic acid (3-phenyl-2-propenoic acid) and phloroglucinol (1,3,5-trihydroxybenzene) were obtained from Koch-Light laboratories, Colnbrook, Bucks, U.K. All other chemicals used were of analytical reagent grade.

Preparation of enzyme extract. The plant tissue (about 10-day-old green leaves) was homogenized in 4 vols of medium containing 50 mM KPi buffer (pH 6.8), 30 mM EDTA (pH 6.8) and 10 mM β-mercaptoethanol with 10% polyclar AT (w/w). The homogenate was filtered through four layers of cheese cloth and the filtrate centrifuged at 800 *g* for 10 min. The supernatant was again treated for 2 hr with 5% polyclar AT (w/v) at 0°. It was then centrifuged at 8000 *g* for 15 min and the supernatant was centrifuged at 105 000 *g* for 65 min. The 105 000 *g* supernatant was treated with 5 vols of Me_2CO (at $-20°$) and the ppt was dried with Et_2O. The buffer extract of Me_2CO powder was used as the source of the enzyme.

Preparation of (3R)-[1-^{14}C]*mevalonate-5-pyrophosphate.* [1-^{14}C]MVAP and [1-^{14}C]MVAPP were synthesized from 3*R*-[1-^{14}C]MVA as described before [11] using the lemon grass enzyme system.

Assay of mevalonate pyrophosphate decarboxylase. The assay was carried out in stoppered Warburg flasks at 37° without shaking as described by Shama Bhat and Ramasarma [11]. The reaction mixture consisted of ATP (10 mM), $MgCl_2$ (12.5 mM), potassium phosphate buffer (100 mM, pH 6.8), enzyme protein (0.2 mg) and [1-^{14}C]MVAPP (20 μM, 300 cpm/nmole).

In all experiments blanks were maintained where perchloric acid was added before the addition of the substrate. These blanks always gave 50–60 cpm. In all experiments the blank values were subtracted from the experimental values.

Release of $^{14}CO_2$ *from* [1-^{14}C]*mevalonate.* The reaction was carried out in stoppered Warburg flasks at 37° without shaking. The buffer extract of the acetone powder was used as the source of the enzymes and the reaction mixture consisted of potassium phosphate buffer (pH 6.8, 50 mM), ATP (10 mM), $MgCl_2$ (10 mM) DTT (12.5 mM), enzyme protein (0.3 mg) and [1-^{14}C]MVA (sp. radioactivity 300 cpm/nmole, 50 nmoles). Other conditions were similar to the MVAPP decarboxylase assay.

Protein estimation. Protein was determined by the method of Bradford *et al.* [12] using bovine serum albumin as the standard.

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