METABOLISM OF MEVALONIC ACID TO PHOSPHORYLATED DERIVATIVES IN Chlorella

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SUMMARY.- The incorporation of mevalonic acid into phosphorylated derivatives was studied for the first time in Chlorophyceae algae. Cell-free extracts and cell suspensions of Chlorella fusca were able to phosphorylate mevalonic acid. The total mevalonate phosphorylation showed a clear maximum at pH 8.2. The time course of phosphorylated derivatives formation was studied. A decrease in the amount of pyro phosphomevalonate formed was observed during prolonged incubation times. Mevalonate-activating enzymes were inhibited by supplementation of high concentrations of Mg^{2+} or Mn^{2+}. At any condition assayed more pyrophosphomevalonate than phosphomevalonate was observed.

Mevalonic acid (MVA)* have been shown to be the precursor of the isoprene unit from which different terpenoid compounds are formed in animals, plants and yeasts (1). In higher plants, mevalonate kinase (EC 2.7.1.36) and phosphomevalonate kinase (EC 2.7.4.2) have been partially purified from several sources (2-8) and demonstrated in other plant preparations (9, 10).

The enzymes of MVA metabolism in algae have received little attention. Only Cooper and Benedict (11) have examined some characteristics of mevalonate kinase in Euglena gracilis. However, it is well known that algae contain a variety of sterols and carotenoids (12, 13). In Chlorophyceae, Chlorella fusca synthesizes mainly Δ^5 sterols (14). On the other hand, the quantitative pattern of carotenoids is similar to that found in higher plants (15, 16).

*Abbreviations: MVA, mevalonic acid; MVAP, phosphomevalonic acid; MVAPP, pyrophosphomevalonic acid; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA.

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It was of interest therefore to examine the metabolism of MVA in the alga Chlorella fusca. This paper describes the incorporation of MVA into phosphorylated derivatives, as the first steps in the biosynthesis of sterols and carotenoids in C. fusca. The conditions, rate and requirements of these reactions have been investigated for the first time in Chlorophyceae algae.

MATERIALS AND METHODS

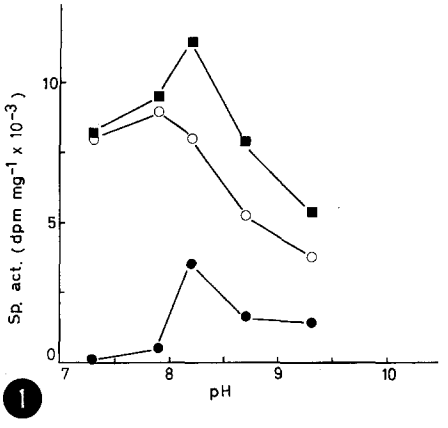
All the experiments were carried out with Chlorella fusca Sihira et Kraus, strain 211-15 from Pringsheim culture collection at Göttingen. The algae were cultured autotrophically as described by Vega et al. (17) with 8 mM nitrate as nitrogen source. Growth was determined by measuring absorbance changes at 660 nm. The cells were harvested at the logarithmic phase by low speed centrifugation and washed twice with isotonic saline solution. [2-^{14}C]MVA was supplied as the lactone by the Radiochemical Centre, Amersham. The potassium salt was prepared as previously described (18).

Cellular suspensions were obtained by suspending the cellular pellet in 0.1 M Tris-HCl buffer at the adequate pH, containing 0.01 M β-mercaptoethanol. Cell-free extracts were obtained by ultrasonic treatment for five periods of 1 min. All procedures were performed at 4º. The broken cell suspension was centrifuged at 38000 g for 60 min. Protein content of the enzyme preparations was determined by the method of Lowry et al. (19).

Enzymatic reactions were carried out incubating the extracts at 20º. Unles otherwise stated, the reaction system contained 12 µmoles of ATP, 6 µmoles of $MgCl_2$, 42.5 nmoles of [2-^{14}C]MVA, 150 µmoles of Tris-HCl buffer, pH 8.2, and enzyme preparation in a final volume of 1.5 ml. Reactions were stopped by heating the reaction tubes at 90º for 5 min. Precipitated protein was centrifuged off at 2000 g for 5 min. Identification of MVAP and MVAPP was previously described (20).

RESULTS

Cell-free extracts from C. fusca phosphorylated MVA to MVAP and MVAPP by mevalonate kinase and phosphomevalonate kinase. Formation of both phosphorylated derivatives was studied over the range of pH 7.3-9.3 by using 0.1 M Tris-HCl buffer. As shown in Fig. 1 a clear difference has been obtained in the pH-activity profiles for MVAP and MVAPP formation. The MVAPP formation was high and quite similar at pH 7.3-8.2 whereas the MVAP formation was lower and sharply increased from 7.9 to 8.2 pH values. The total MVA



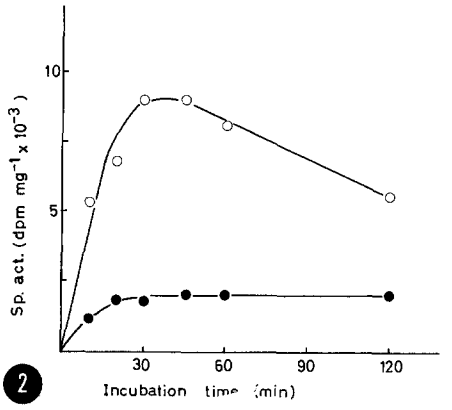


Fig. 1.- Effect of pH on the MVA phosphorylation by cell-free extracts from C. fusca. ●, MVAP; ○, MVAPP; ■, MVAP+MVAPP.

Fig. 2.- MVA phosphorylation by cell-free extracts from C. fusca at different incubation times. ●, MVAP; ○, MVAPP.

phosphorylation showed a clear maximum at pH 8.2. At any pH value the amount of MVAPP observed was higher than of MVAP.

Al pH 8.2 a little amount of an unidentified compound with a Rf value of 0.48-0.52 in the solvent system employed was observed. This compound was detected when 0.1 M Tris-HCl buffer was used. With 0.05 M and 0.01 M buffer, no formation of this compound was observed. The presence of this product in reactions carried out with [1-^{14}C]MVA indicated that it is not isopentenyl pyrophosphate nor other related metabolite, because of ^{14}C of [1-^{14}C]MVA was lost as $^{14}CO_2$ in the decarboxylation of MVAPP by pyrophosphomevalonate decarboxylase (EC 4.1.1.33). Moreover, the formation of 3-hydroxy-3-methylglutaryl-CoA seems to be impossible because of the well known irreversibility of reaction catalyzed by HMG-CoA reductase (EC 1.1.1.34). On the other hand, in reactions carried out with boiled cell-free extracts, the presence of this unidentified compound was also detected. These results suggest the formation in these conditions of a non-enzymatic derivative of MVA that can interfere as an artifact in the study of metabolism of this acid.

The mevalonate phosphorylation is quite similar at 20º and 37º. No difference in the amount of MVAP and MVAPP formed was observed in incubations carried out at these temperatures.

Disruption of cellular membranes, that certainly occurs in frozen-thawed Chlorella as shown by the leakage of soluble substances from the cells, produced an inactivation of mevalonate-activating enzymes. No MVA phosphorylation was observed in experiments carried out with these extracts.

Phosphorylation of [2-^{14}C]MVA by cell-free extracts from C. fusca was investigated in reactions carried out for 10 min to 2 hr. As can be seen in Fig. 2 the MVAP formation reached its maximal value within 30 min becoming practically level afterwards. Fig. 2 also shows that MVAPP formation was maximal at 30-45 min and decreased at increasing incubation time. In any case, more MVAPP than MVAP was formed.

The cell-free extracts from C. fusca were able to phosphorylate MVA without additions of metal ions. The results given in Table 1 show that omitting divalent cations sharply reduced the formation of MVAPP, while the amount of MVAP formed is only slightly lower. Supplementation of Mg^{2+} or Mn^{2+} strongly increased the MVA phosphorylation. The data presented in Table 1 also show that when Mg^{2+} or Mn^{2+} concentration increased a clear inhibition in the MVAPP formation was observed, inhibition that is obvious but lower in the MVAP formation at the same ion concentrations.

The cells of C. fusca suspended in 0.1 M Tris-HCl buffer, pH 8.2, incorporated MVA to phosphorylated derivatives when they were incubated with [2-^{14}C]MVA for 1 hr (Table 2). The ratio of MVAPP to MVAP formation in the cell suspensions was quite similar to that obtained working with cell-free extracts.

DISCUSSION

In this paper the characteristics of mevalonate-activating enzymes in Chlorophyceae algae have been examined for the first time.

Table 1

Effect of different concentrations of Mg^{2+} and Mn^{2+} on the MVA phosphorylation by cell-free extracts from C. fusca.

Ion	Concentration (mM)	Specific activity (dpm $mg^{-1} \times 10^{-3}$)	
		MVAP	MVAPP
-	-	1.0	2.0
Mg^{2+}	0.8	2.0	15.0
	4.0	2.0	14.0
	8.0	1.5	10.0
Mn^{2+}	0.8	2.0	16.5
	4.0	2.0	13.5
	8.0	1.2	11.5

Table 2

MVA incorporation to phosphorylated derivatives by different enzyme preparations from C. fusca.

Enzyme preparation	Specific activity (dpm $mg^{-1} \times 10^{-3}$)	
	MVAP	MVAPP
Cell suspensions	1.2	9.6
Cell-free extracts	1.6	14.4

In all the conditions assayed, more MVAPP than MVAP was observed, in spite of the reaction catalyzed by phosphomevalonate kinase is freely reversible (21). The amount of MVAPP formed or accumulated is regulated by the equilibrium constant of this reaction, particularly when the rate of forward reaction is low (22). In Chlorella the ratio of MVAPP to MVAP seems to be higher than described (1), suggesting

that the pyrophosphomevalonate decarboxylase is one rate-limiting step in synthesis of isoprenoids in these algae.

The maximal MVA phosphorylation occurs in Chlorella at pH higher than reported in other sources. In pumpkin fruit (2), Pinus radiata seedlings (23) and orange (9) mevalonate phosphorylation was maximal at pH 5.5-6.5, whereas in Pinus pinaster and Agave americana (6) we have found an optimum pH about 7.5-8.0, similar to that reported in Hevea latex (3) and animals. We have also reported the separation by Sephadex G-100 of two fractions with mevalonate kinase activity from P. pinaster and A. americana, both fractions being active at pH 7.9 (24). On the other hand, the optimum pH of the phospho-mevalonate kinase from pig liver (21) and Hevea latex (7) is 7.0-7.5.

The time course of mevalonate phosphorylation in cell-free extracts from C. fusca shows a clear decrease in the amount of MVAPP observed when the incubation time is prolonged for 1-2 hr. This decrease can be produced by a phosphatase similar to that reported in other plant sources (6, 9, 10). The presence of this phosphatase activity would affect the mevalonate-activating enzymes assay, through removal ATP necessary for MVA phosphorylation and through degradation of the phosphorylated products of MVA metabolism (25).

The mevalonate-activating enzymes from Chlorella are activated by divalent ions such as Mg^{2+} and Mn^{2+}. However, like with the enzymes from other sources, a clear inhibition in the MVA phosphorylation is found at high Mg^{2+} or Mn^{2+} concentration. The extent of activation and inhibition by different metal ion concentrations varies according to the source of the enzyme preparations.

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