3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Activity in Ochromonas malhamensis

A SYSTEM TO STUDY THE RELATIONSHIP BETWEEN ENZYME ACTIVITY AND RATE OF STEROID BIOSYNTHESIS

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ABSTRACT

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the key regulatory enzyme of the isoprenoid pathway, was found to be predominantly microscopic in Ochromonas malhamensis, a chrysophycean alga. Detection of HMG-CoA reductase requires the presence of 1% bovine serum albumin during cell homogenization, and the activity is stimulated by the presence of Triton X-100. The enzyme has a pH optimum of 8.0 and an absolute requirement for NADPH. When grown in 10 micromolar mevinolin, a competitive inhibitor of HMG-CoA reductase, O. malhamensis shows a 10- to 15-fold increase in HMG-CoA reductase activity (after washing) with little or no effect on cell growth rate. Cultures can be maintained in 10 micromolar mevinolin for months. O. malhamensis produces a large amount (1% dry weight) of poriferasterol, a product of the isoprenoid pathway. The addition of 10 micromolar mevinolin initially blocked poriferasterol biosynthesis by >90%; within 2 days the rate of synthesis returned to normal levels. Immediately after mevinolin was washed from the 2-day culture, there was a transient 2.5-fold increase in the rate of poriferasterol biosynthesis. The rate of poriferasterol biosynthesis and the level of HMG-CoA reductase activity both fell to control levels within hours.

The isoprenoid pathway in plants is the source of a variety of significant products, including plant hormones, low mol wt volatiles, vitamins A and D, β-carotene, steroids, and natural rubber. The pathway in mammalian tissue is tightly regulated at the entry point, where HMG-CoA is reduced to MVA. The enzyme that catalyzes this step, HMG-CoA reductase (mevalonate: NADPH oxidoreductase, EC 1.1.1.34) has been extensively studied in mammalian systems and is assumed to be the major rate-limiting enzyme in sterol biosynthesis (for review, see Refs. 5 and 21). In addition, HMG-CoA reductase provides nonsterol intermediates required for DNA replication and other cell functions (for review, see Ref. 14). Within the last 10 years, HMG-CoA reductase has been identified and studied in several higher plant systems (for review, see Ref. 15).

The objective of this research is to develop a system to study the biochemical and metabolic mechanisms that control the rate of synthesis of isoprenoid end products in plants. In mammalian tissue the isoprenoid pathway is regulated at the level of the gene and at the level of the enzyme. Steroids, for example, act in a negative-feedback loop to drastically lower the amount of mRNA available for the synthesis of HMG-CoA reductase (13). Other, shorter term, regulatory mechanisms control the rate of carbon flow by phosphorylating the enzyme to an inactive form via a protein kinase, and dephosphorylating the enzyme to an active form via an independent phosphatase (for review, Ref. 11). The approach used in this study was to increase the activity of HMG-CoA reductase in vivo with the use of the competitive inhibitor, mevinolin, and to determine the degree of enhancement of short-term end-product synthesis after release from mevinolin inhibition.

We chose to study HMG-CoA reductase and the control of sterol biosynthesis in a single-cell plant system, Ochromonas malhamensis. This chrysophycean unicellular alga produces a large amount of sterol (~1% dry weight), of which 98% is poriferasterol (16). The diversion of fixed carbon into one major product in this organism simplified considerably the analysis of 14C-labeled isoprenoid products. In addition, O. malhamensis lacks a cell wall, which facilitates the extraction and analysis of sterol content, as well as controlled cell homogenization and generation of organellar fractions.

MATERIALS AND METHODS

Materials. [1,2-14C]Sodium acetate, DL-[3-14C]HMG-CoA, [2-14C]mevalonic acid (DBED salt), and [5-3H]mevalonolactone were purchased from New England Nuclear Corporation. Whatman LK6D silica gel thin-layer plates were obtained from Pierce Chemical Company. β-Mercaptoethanol and the Bradford dye reagent were from Bio-Rad Laboratories. All other biochemicals were purchased from Sigma. Mevinolin was kindly provided by M. W. Alberts, Merck, Sharp & Dohme Research Laboratories, Rahway, N.J.

Cell Culture. Axenic cultures of Ochromonas malhamensis Pringsheim (strain 11532, American Type Culture Collection) were grown in 100-ml culture volumes in 250-ml-capacity Erlenmeyer shake flasks at 30°C under constant illumination from cool-white fluorescent lights at intensities below 50 μE·m−2·s−1. The growth medium of Betouhim-El et al. (6) was optimized for growth of O. malhamensis and contained the following modifications: NH4Cl was increased to 0.10% (w/v); 1 μg/100 ml vitamin B12; and 20 mM buffer (pH 6.5) were added; and the agar was omitted. The pH of the medium was adjusted to 6.5 before autoclaving. Cell number and size distribution were measured


2 Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; DBED, dibenzylethylenediamine; MVA, mevalonic acid; PVPP, polyvinylpolypyrrolidone.
with a Coulter counter model TA II. Cultures were monitored for contamination by both light microscopy examination and by plating on Difco nutrient agar with 0.4% glucose.

**Assays.** HMG-CoA reductase was assayed radiochemically. Analyses were carried out in duplicate or triplicate; the variation was typically less than 10%. Linearity of product formation with time was verified for each new experimental condition. Product formation was measured over 10 min intervals and remained linear for 60 to 90 min. An appropriate incubation time (generally 30 min) was chosen at which to assay the remaining samples from a given set of experiments. Each incubation mix contained (in 84 µl) 50 µM Tris (pH 8.0), 0.5% Triton X-100, 1 mM NADPH, 40 mM glucose 6-P, 0.8 unit glucose 6-P dehydrogenase, the enzyme suspension containing up to 0.5 mg of protein, and 250 µM [1^4C]HMG-CoA (2500 Cpm/nmol). The mixtures were incubated at 30°C for 30 min unless otherwise stated. The reaction was terminated by the addition of 10% of 6 N HCl.

After a 20-min incubation at room temperature to allow lactonization of the product, 60 µl of the mixture was applied to the preadsorbent area of one lane of a 20- by 20-cm silica thin layer plate (Whatman K6D). The chromatogram was developed in chloroform:acetone:water (30:15:1). Standard lactonized [1^4C] mevalonic acid was included on each plate, and its position was located by scraping 1 cm sections of that lane into scintillation vials and counting in a LKB RackBeta II liquid scintillation counter. The 1- to 2-cm area corresponding to the MVA-lactone for each sample was then quantitated as was the neighboring background area above and below the product. Control samples containing either no microsomal protein or heat inactivated (10 min at 100°C) microsomal protein produced no product above the plate background of about 50 dpm.

Protein was assayed by the method of Bradford (7), using bovine plasma gamma globulin as a standard.

**Cell Homogenization and Fractionation.** All steps of this procedure were carried out at 2 to 4°C. Cultures of *O. malhamensis*, grown to densities between 3 and 8 × 10^8 cells/ml, were harvested by centrifugation at 1,000g for 5 min. Pellets were resuspended in homogenization buffer similar to that used by Brooker and Russell (8), containing 10 mM Tris (pH 7.5), 0.03 M EDTA, 0.35 M sucrose, 10 mM β-mercaptoethanol (added fresh), and 2% (w/v) BSA. The suspension was centrifuged as described above, and the pellet was resuspended in homogenization buffer. The cells were broken by two passages through a Yeda press at 400 p.s.i. Unbroken cells were removed by centrifugation at 120g for 4 min. The supernatant was centrifuged at 3,000g for 10 min, and the resulting supernatant was centrifuged 145,000g for 1h. The microsomal (high-speed) pellet was resuspended into 0.1 M Tris buffer (pH 8.0) containing 10 mM DTT and was either assayed immediately or frozen in liquid N2 and stored at −80°C for up to 2 weeks. There was no detectable loss of activity upon freezing.

**Treatment of Cells with Mevinolin.** Mevinolin (2 mM) was converted to the sodium salt by incubation in 0.1 N NaOH at 50°C for up to 5 h. The solution was neutralized, filter sterilized (Millipore Acrodisc, 0.2 m) and stored at −20°C in small aliquots. Cultures were inoculated with various concentrations of mevinolin, and aliquots were removed at specified time points for measurement of cell density, microsomal HMG-CoA reductase, and rate of sterol biosynthesis. In some experiments, the effect of removing mevinolin from the cultures on the above parameters was examined. Cells were pelleted by centrifugation (1100g for 5 min at 25°C), resuspended in growth medium supplemented with 0.1% BSA, centrifuged, and resuspended, inoculated into shake flasks, and analyzed after various chase times.

[1^4C]Acetate Incorporation Experiments. For radiolabeling studies, 20-ml aliquots from appropriately treated experimental cultures were dispensed into 50-ml Erlenmeyer flasks and placed on a gyratory shaker maintained at 30°C. At a designated time after either the addition or removal of mevinolin (see "Results" for experimental details), 100 µl of sodium [1,2-^14C]acetate was injected into each flask. At selected time intervals after the addition of radioisotope, either 4- or 5-ml culture aliquots were withdrawn by pipette, vacuum filtered, and rinsed with 50 ml of medium. The filter paper was then plunged into a vial containing 5 ml of hot acetone and further extracted with vigorous agitation and 30 min of sonication in a sonic bath. After centrifugation, the residue was extracted with acetone, and the combined supernatants were reduced to near-dryness under a stream of N2. Diethyl ether (10 ml) and water (5 ml) were added. After thorough agitation, the ether phase was removed and the water phase was extracted twice more with ether. The ether extracts were combined and reduced to a suitable volume, and an aliquot was removed for liquid scintillation counting. To estimate the amount of radiolabeled sterol and sterol ester, 4 to 6 × 10^8 dpm of the ether extracts was spotted onto laminated silica gel TLC plates and developed in a 3:1 diethyl ether:petroleum ether (b.p., 40–60°C) solvent system. Poriferasterol and cholesteryl palmitate were spotted onto each plate to serve as standards. Quantitation of radiolabeled product was performed by scraping 1-cm sections of the lanes into scintillation vials and by counting in Ready-Solv (Beckman Laboratories, Inc.).

To determine the amount of radiolabeled sterol in the sterol ester fraction, the latter region was scraped off the TLC plate into a vial and saponified for 1 h in 0.2 ml of methanolic KOH at 70°C. After saponification, the sample was acidified with 1 N HCl and extracted with 5 volumes of diethyl ether. The combined ether extracts were reduced to a suitable volume, and free sterol was determined as previously described.

**RESULTS AND DISCUSSION**

**Properties of Microsomal HMG-CoA Reductase from *O. malhamensis*.** Little or no HMG-CoA reductase activity could be detected in either whole cell homogenates or in organellar fractions prepared by differential centrifugation when *O. malhamensis* was disrupted in several commonly used buffers (8, 17, 23). Of the compounds tested for their ability to stabilize HMG-CoA reductase activity during homogenization, only BSA at concentrations >1% (w/v) led to a consistent and pronounced increase in enzyme activity. BSA has been reported to enhance the HMG-CoA reductase activity of sweet potato root by 50%: it apparently prevents lactonization of HMG-CoA reductase caused by degradation of microsomal phospholipids (23). This might also be the case in *O. malhamensis*. In addition, the BSA might serve to prevent structural and functional changes in membranes or proteins induced by the free fatty acids (JH Golbeck, unpublished data) and/or chlorosulfonilipids (20) released during homogenization of *O. malhamensis*. Inclusion of 1% PVPP, 0.1 TIU/ml Aprotinin, 100 µl leupeptin, or 1 mM phenylmethylsulfonyl fluoride, either singly or in combination during homogenization of *O. malhamensis*, resulted in no improvement of recovered activity.

Subcellular fractions were prepared from *O. malhamensis* by stepwise centrifugation to localize the HMG-CoA reductase activity. The microsomal pellet contained 90% ± 7 (n = 12) of the HMG-CoA reductase activity with a specific activity of 5.8 ± 4.5 (n = 33) nmol MVA/mg protein-h; the remaining activity was located in the low-speed pellet. No HMG-CoA reductase activity was found in the final supernatant fraction. The enzyme is primarily microsomal in pea seedling (9) and radish seedling (4) but predominantly mitochondrial in uninected sweet potato root (22) and chloroplastic in *Nepeta cataria* leaves (12).

As in all other eukaryotic systems studied, microsomal HMG-CoA reductase from *O. malhamensis* has an absolute and specific requirement for NADPH. There was no detectable activity when an equivalent concentration of NADH was substituted for NADPH in the assay mixtures.
The effect of pH on HMG-CoA reductase is shown in Figure 1. The enzyme shows a sharp optimum at pH 8.0 with a large decline in activity above and below the peak. This pH optimum is somewhat higher than that reported for other plant systems which typically falls between 6.8 and 7.5 (15). Addition of 10 mM CaCl₂, 10 mM MgCl₂, or 10 mM EDTA had no effect on the specific activity of HMG-CoA reductase. BSA, although required during cell homogenization, had no effect when added to the assay mixture.

There was approximately 60% more activity measured in the presence of 0.5 to 1.0% Triton X-100 than in its absence. The stimulatory effect of Triton X-100 on microsomal HMG-CoA reductase was observed in freshly prepared enzyme suspensions and in suspensions that had been frozen and thawed before assay. Triton X-100 also enhances the activity of HMG-CoA reductase in sweet potato root (24).

No stimulation of reductase activity from freeze-thawed O. malhamensis microsome suspensions was observed after a preincubation at 30°C before assay. Thus, there is no evidence for reversible cold inactivation in this system, as is the case with soluble rat liver HMG-CoA reductase (18).

Effect of Mevinolin on Cell Growth. Mevinolin, a fungal metabolite similar in structure to HMG-CoA, has been shown to be a highly specific competitive inhibitor of HMG-CoA reductase from animal (1) and plant (2) tissues. Mammalian cells exposed to mevinolin are induced to synthesize additional HMG-CoA reductase to compensate for the enzyme inactivated by the inhibitor (1). This compound, as well as structurally similar compactin, has been exploited by investigators in a wide variety of systems to study aspects of the regulation of mammalian cholesterol biosynthesis (10), chloroplast biogenesis (19), plant growth (3, 11), and plant sterol and pigment accumulation (3).

Mevinolin was added to log phase cultures of O. malhamensis in concentrations ranging from 10 to 200 μM, and growth was monitored for a 3-d period (Fig. 2). Cultures grown in 10, 50, and 100 μM mevinolin exhibited logarithmic growth, but at progressively slower rates with increasing mevinolin concentration (Fig. 2). No lag period in growth was observed after mevinolin was added, and the size distribution of the cells grown in 10 to 100 μM mevinolin did not vary measurably from control cultures. Cultures could be passaged in the presence of 10 μM mevinolin for over 6 months with little or no detectable effect on growth rates. There was no growth of O. malhamensis in the presence of 200 μM mevinolin (Fig. 2).

Effect of Mevinolin on HMG-CoA Reductase Activity. HMG-CoA reductase activity in O. malhamensis, assayed after washing the cells free of inhibitor, increases in response to the presence of the inhibitor mevinolin (Table 1). Cultures grown for 2 to 3 d in the presence of 10 or 50 μM mevinolin showed the largest increase (10- to 16-fold) in microsomal HMG-CoA reductase activity relative to control cultures. Cells grown in 100 μM mevinolin showed a somewhat smaller increase in HMG-CoA reductase activity relative to control cultures (about 7-fold) and had pronounced slower growth rates than did the controls (Table 1; Fig. 2). The level of microsomal HMG-CoA reductase activity was also examined after prolonged culture in the presence of 10 μM mevinolin. After 23 and 100 d of subculture and growth in the continuous presence of 10 μM mevinolin, treated cultures had 3 to 4 times more microsomal HMG-CoA reductase activity after washing than did the control cultures.

Effect of Mevinolin on Poriferasterol Biosynthesis. The effect of mevinolin on the biosynthesis of poriferasterol was examined in log-phase cultures of O. malhamensis shortly after the introduction of 10 and 100 μM mevinolin and after 2 d of growth in 10 and 100 μM mevinolin. Total ¹⁴C incorporation into the ether-soluble fractions of treated cultures was reduced by about 35 to 55% compared with controls (data not shown). A significant proportion of this inhibition resulted from a dramatic reduction in the amount of poriferasterol labeling (Table II). The rate of [¹⁴C]acetate incorporation into poriferasterol was initially reduced >90% in the presence of 10 μM mevinolin; however, after 2 d of exposure to 10 μM mevinolin, [¹⁴C]acetate incorporation

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**Fig. 1.** Effect of pH on the specific activity of microsomal HMG-CoA reductase from O. malhamensis. Freshly prepared microsomes were suspended in 10 mM DTT and immediately delivered to tubes containing buffered solutions from pH 6.0 to 9.0. Cofactor and substrate were added and HMG-CoA reductase activity was determined radiochemically. The buffers used were 0.2 M KH₂PO₄ (pH 6.0–7.5) and 0.2 M Tris (pH 7.5–9.0).

**Fig. 2.** Effect of mevinolin on the rate of cell division of cultures of O. malhamensis. An inoculum of O. malhamensis was delivered to a series of shake flasks containing 0 μM (○), 10 μM (●), 50 μM (▲), 100 μM (□), 200 μM (▲) mevinolin in growth medium. At intervals aliquots of the cultures were removed, and cell density was determined with a Coulter counter. Values are the average of duplicate cultures.
Table 1. Effect of Mevinolin on Cell Growth and on the Specific Activity of HMG-CoA Reductase in O. malhamensis

An inoculum of O. malhamensis was delivered to shake flasks containing 0 \( \mu \text{g} \) (control), 10, 50, or 100 \( \mu \text{g} \) mevinolin in growth medium and incubated at 30°C. For long-term experiments, cells were diluted into fresh medium at 10 \( \mu \text{g} \) mevinolin every 3 to 4 d. Cell number was monitored at 24-h intervals throughout the exposure period. At the end of the indicated treatment period, cells were harvested and rinsed, microsomes were prepared, and HMG-CoA reductase was determined radiochemically.

<table>
<thead>
<tr>
<th>Mevinolin Treatment</th>
<th>Relative Rate of Cell Growth</th>
<th>Relative HMG-CoA Reductase Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 ( \mu \text{g} ) Mevinolin</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>10 ( \mu \text{g} ) Mevinolin (2-d exposure)</td>
<td>0.74</td>
<td>10.5</td>
</tr>
<tr>
<td>10 ( \mu \text{g} ) Mevinolin (3-d exposure)</td>
<td>0.90</td>
<td>16.3</td>
</tr>
<tr>
<td>23 ( \mu \text{g} ) Mevinolin (2-d exposure)</td>
<td>1.00</td>
<td>3.4</td>
</tr>
<tr>
<td>100 ( \mu \text{g} ) Mevinolin (3-d exposure)</td>
<td>1.15</td>
<td>4.0</td>
</tr>
<tr>
<td>50 ( \mu \text{g} ) Mevinolin (3-d exposure)</td>
<td>0.65</td>
<td>14.6</td>
</tr>
<tr>
<td>100 ( \mu \text{g} ) Mevinolin (3-d exposure)</td>
<td>0.44</td>
<td>6.2</td>
</tr>
<tr>
<td>50 ( \mu \text{g} ) Mevinolin (3-d exposure)</td>
<td>0.39</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* Relative rate of growth is defined as the specific growth rate (\( \mu \)) of the control culture divided by the specific growth rate of the treated culture. \( \mu = (1 - 1/n) / t \), where \( x = \) final cell density, \( x_0 = \) initial cell density, and \( t = \) elapsed time. * Relative reductase activity is defined as the specific activity of treated culture divided by the specific activity of the control culture.

Table 2. Effect of Mevinolin on [\( ^{14} \text{C} \)]Acetate Incorporation into Sterol

For the first two time points, cultures were exposed to mevinolin for 30 min prior to the addition of [\( ^{14} \text{C} \)]acetate. Aliquots were removed for sterol analysis at 1 and 2 h after the addition of radiosotope. The 2-d mevinolin treated culture was incubated with [\( ^{14} \text{C} \)]acetate for 1 h.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Free Sterol</th>
<th>Free Sterol</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>% of ether-soluble fraction</td>
<td>% of controls</td>
</tr>
<tr>
<td>1-h Exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23</td>
<td>93</td>
</tr>
<tr>
<td>10 ( \mu \text{g} ) mevinolin</td>
<td>4.6</td>
<td>93</td>
</tr>
<tr>
<td>100 ( \mu \text{g} ) mevinolin</td>
<td>2.4</td>
<td>6.0</td>
</tr>
<tr>
<td>2-h Exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>18</td>
</tr>
<tr>
<td>10 ( \mu \text{g} ) mevinolin</td>
<td>9.4</td>
<td>18</td>
</tr>
<tr>
<td>100 ( \mu \text{g} ) mevinolin</td>
<td>5.4</td>
<td>14</td>
</tr>
<tr>
<td>2-d Exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34</td>
<td>103</td>
</tr>
<tr>
<td>10 ( \mu \text{g} ) mevinolin</td>
<td>35</td>
<td>103</td>
</tr>
</tbody>
</table>

into poriferasterol returned to control levels. These experiments suggest that mevinolin addition blocks poriferasterol biosynthesis by inhibiting HMG-CoA reductase, but within a short time, HMG-CoA reductase activity and poriferasterol biosynthesis returns to near normal levels. One likely mechanism is the increased synthesis of enzyme to overcome the continued presence of the inhibitor.

Unlike free poriferasterol, the amount of [\( ^{14} \text{C} \)] associated with the steryl ester fraction was not significantly different from controls (data not shown). Analysis of the saponification products of the isolated steryl ester fractions revealed that virtually none of the radiolabel was associated with poriferasterol and must therefore be attributed to the fatty acid portion of the molecule.

Relationship Between HMG-CoA Reductase Levels and Poriferasterol Biosynthesis. The major objective of this work is the development of a system to study the relationship between HMG-CoA reductase activity and the rate of steroid biosynthesis. Since HMG-CoA reductase activity could be increased transiently by growth of O. malhamensis in mevinolin and washing, one strategy is to correlate HMG-CoA reductase activity and short-term carbon flow into sterol biosynthesis immediately after mevinolin removal.

A culture exposed to 10 \( \mu \text{g} \) mevinolin for 48 h was rinsed and resuspended in mevinolin-free medium; a similarly grown culture containing no inhibitor was identically processed and served as a control. Two consecutive [\( ^{14} \text{C} \)]acetate time-course incorporation experiments were conducted with 20-ml aliquots removed from the original cultures 30 min after the rinse (0.5, 1, 2, and 4 h time points), and 10 h after the rinse (10.5 h time point). HMG-CoA reductase activity was determined at the beginning of each incorporation experiment, i.e., 0.5 and 10 h. Initially, the HMG-CoA reductase activity in mevinolin-treated cultures was 13-fold greater than in the control cultures (Table III). After removal of the inhibitor, the HMG-CoA reductase activity fell to control values within hours. Immediately after mevinolin was removed by washing, the rate of [\( ^{14} \text{C} \)]acetate incorporation into poriferasterol was 2.5-fold greater per cell than in the control. However, this ratio declined steadily with time, so that by the beginning of the 10-h incorporation experiment and thereafter (data not shown), no significant difference in [\( ^{14} \text{C} \)]sterol/cell was evident. These results were in temporal, though not quantitative, agreement with the enzyme data: the rate of carbon flow is clearly related to enzyme activity although the magnitude of the increase in the rate of poriferasterol biosynthesis was lower than the absolute increase in HMG-CoA reductase activity.

This quantitative discrepancy could be due to the presence of residual mevinolin following washes or to insufficient in vivo levels of HMG-CoA. Alternatively, a rapid mechanism for inactivating HMG-CoA reductase in vivo may be present such as the phosphorylation-dephosphorylation system operative in mammalian systems (5). The possibility also exists that HMG-CoA reductase may not be the rate determining step when it is present at high levels. It is well known (10) that no intermediates accumulate in the pathway from mevalonic acids to steroids, implying that none of the intermediate enzymic steps are rate limiting under normal circumstances. However, when HMG-CoA reductase levels are elevated, as in this study, other secondary control points may become rate limiting and the rate of carbon flow to steroids may not be accurately reflected in the levels of HMG-CoA reductase. The ability to raise HMG-CoA reductase activity in a plant system therefore offers the possibility for studying secondary control points within the steroid pathway under conditions where HMG-CoA reductase may not be the rate-limiting enzyme. Indeed, deregulation of selected secondary control points may be desirable for shunting carbon to a small number of significant products such as \( \beta \)-carotene, vitamins, and steroids.

This research demonstrates that an important enzyme in the isoprenoid pathway operates under strict metabolic control in a unicellular plant system. The transient increase in poriferasterol biosynthesis indicates that in the short term, carbon flow through the isoprenoid pathway in O. malhamensis is related to the level of HMG-CoA reductase. Therefore, the potential exists for de-regulating this enzymic step so that increased rates of synthesis of isoprenoid products can be achieved. Further research must also take into account secondary metabolic controls exerted at other points on the pathway and the availability of carbon in the form of HMG-CoA at the entry point to the pathway.
HMG-CoA REDUCTASE IN OCHROMonas MALHAMENSIS

Table III. Relationship between HMG-CoA Reductase Level and Incorporation of [14C]Acetate into Poriiferasterol after Mevinolin Removal

A culture that had been passaged in 10 μM mevinolin for 2 d was rinsed with mevinolin-free medium and divided into two aliquots. After 0.5 h, the first sample was assayed for HMG-CoA reductase 0.5, 10, and 24 h after the rinse. After 10 h, the second sample was assayed for HMG-CoA reductase 10.5 h after the rinse. Aliquots removed from the culture at these times were utilized for [14C]Acetate incorporation experiments to determine the relative amount of carbon flow into poriferasterol compared with identically processed control cultures.

<table>
<thead>
<tr>
<th>Time after Mevinolin Removal (h)</th>
<th>HMG-CoA Reductase Relative to Control</th>
<th>[14C]Acetate Incorporation</th>
<th>[14C]Poriferasterol/Cell Relative to Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>10</td>
<td>2.5</td>
<td>0.9</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1.4</td>
<td></td>
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LITERATURE CITED


