Conversion of Mevalonic Acid to $\gamma,\gamma$-Dimethylallyl Pyrophosphate by Mycoplasma

CARL V. HENRIKSON AND PAUL F. SMITH

Department of Microbiology, School of Medicine, University of South Dakota, Vermillion, South Dakota

Received for publication 2 May 1966

ABSTRACT

HENRIKSON, CARL V. (University of South Dakota, Vermillion), AND PAUL F. SMITH. Conversion of mevalonic acid to $\gamma,\gamma$-dimethylallyl pyrophosphate by Mycoplasma. J. Bacteriol. 92:701–706. 1966.—Three representative strains of Mycoplasma, M. laidlawii strain B, Mycoplasma sp. avian strain J, and M. hominis type 2 strain O7, were examined for the presence or absence of enzymes associated with the biosynthetic pathway from mevalonic acid to $\gamma,\gamma$-dimethylallyl pyrophosphate. M. laidlawii served as a control organism, since it is capable of de novo biosynthesis of carotenoids. All four enzymes, namely, adenosine triphosphate (ATP)-mevalonate 5-phosphotransferase (EC 2.7.1.36), ATP-5-phosphomevalonate phosphotransferase (EC 2.7.4.2), ATP-5-phosphomevalonate carboxy-lyase (EC 4.1.1.33), and isopentenylpyrophosphate $\Delta^2,\Delta^3$-isomerase (EC 5.3.3.2), were demonstrated in this organism. Mycoplasma sp. avian strain J, which contains all enzymes necessary for the biosynthesis of mevalonic acid, lacks the first three of the above enzymes but contains isopentenyl pyrophosphate $\Delta^2,\Delta^3$-isomerase. M. hominis, which lacks the enzymes necessary for the biosynthesis of mevalonic acid, also is deficient in the enzymes involved in its conversion to $\gamma,\gamma$-dimethylallyl pyrophosphate.

The Mycoplasma behave as two nutritional types on the basis of lipid requirements. Mycoplasma sp. avian strain J and M. hominis type 2 strain O7 require sterols for growth, whereas M. laidlawii strain B synthesizes carotenoids (10). Carotenoids appear to have a functional role similar to that of sterols, as evidenced by the sparing action of cholesterol on carotenoid synthesis (9) and the capacity of carotenoid intermediates or exogenous carotenoids to substitute for sterol. Further evidence for analogous functions of sterol and carotenol in Mycoplasma is the reversal by cholesterol of growth inhibition due to inhibition of carotenoid synthesis (12). Biosynthesis of mevalonic acid in Mycoplasma proceeds by the condensation of 1 mole of acetyl coenzyme A (CoA) with 1 mole of acetoacetyl CoA to form $\beta$-hydroxyl-$\beta$-methylglutaryl CoA (HMG-CoA), followed by reduction by a pyridine nucleotide-linked reductase to form mevalonic acid (11). All the organisms are capable of acetate activation and acetoacetyl CoA formation (11, 15). M. laidlawii and Mycoplasma sp. strain J are capable of incorporation of acetate-2-C$^{14}$ into mevalonic acid (11). No acetate-2-C$^{14}$ incorporation occurs with M. hominis, owing to the lack of 3-hydroxy-3-methylglutaryl CoA acetoacetyl CoA-lyase (EC 4.1.3.5) and mevalonate-nicotinamide adenine dinucleotide phosphate-(NADP) oxidoreductase (EC 1.1.1.34), thus explaining its growth requirement for sterol (11). Nutritional experiments showed that none of the biosynthetic precursors of polyprenes supported growth of M. hominis. Isopentenyl pyrophosphate partially replaced the sterol growth requirement of Mycoplasma sp. strain J, indicating an enzymatic block between mevalonic acid and isopentenyl pyrophosphate (Henrickson and Smith, J. Gen. Microbiol., in press).

The present study was undertaken to examine these representative strains of Mycoplasma for enzymes in the biosynthetic pathway from mevalonic acid to $\gamma,\gamma$-dimethylallyl pyrophosphate.

MATERIALS AND METHODS

 Cultures. Three representative strains were examined, namely, M. laidlawii strain B, Mycoplasma sp. avian strain J, and M. hominis type 2 strain O7. M. laidlawii was grown in a medium of the following composition: 2% tryptose (Difco), 0.5% sodium chloride, 0.5% sodium acetate, and 0.5% glucose; the pH was 7.8. Sterol-requiring strains were grown in the medium described by Morton, Smith, and Leberman (7) supplemented with 1% PPLO Serum Fraction (Difco). The organisms were grown and harvested as reported previously (8). Thallium acetate
(1:4,000 w/v) was used as a selective inhibitor of bacterial growth.

**Enzymatic methods.** The organisms suspended in 0.2 M potassium phosphate buffer (pH 7.5) were disrupted in a 10-ka Raytheon sonic oscillator for 15 min. The protein concentration of the disrupted cells was determined by the trichloroacetic acid method of Stadtman, Novelli, and Lipmann (13), with bovine serum albumin as a standard.

Adenosine triphosphate (ATP)-mevalonate 5-phosphotransferase (EC 2.7.1.36) activity was determined by a modification of the procedure of Witting and Porter (16). Iodoacetamide (0.005 M) was employed as a selective inhibitor for isopentenylpyrophosphate Δ⁴, Δ⁵-isomerase (EC 5.3.3.2) to permit accumulation of the phosphorylated mevalonic acids (6). Controls containing heat-inactivated enzyme protein (10 min at 100 C) were employed with each experiment. At the termination of incubation, the enzyme proteins were inactivated by heating at 70 C for 3 to 5 min. Coagulated protein was removed by centrifugation, and supernatant solutions were extracted four times with ethyl alcohol-ether (1:1, v/v). After solvent removal under a stream of nitrogen, the aqueous solutions were concentrated by lyophilization. Chromatographic separation by the ascending method was achieved on Whatman no. 1 filter paper with i-butanol-formic acid-water (40:10:16, v/v) as developing solvent (5). The dried chromatograms were cut into 0.5-cm strips and counted in a Packard TriCarb liquid scintillation counter with 2,5-diphenyloxazole (PPO) and 1,4-bis[2-(5-phenyloxazolyl)] benzene (POPOP) as scintillators.

Detection of ATP-5-phosphomevalonate phosphotransferase (EC 2.7.4.2) activity was achieved on in an identical manner except for use of mevalonate-5'-phosphate (15 μmoles), doubling of enzyme protein, and the addition of 10 μc of ATP²³ labeled in the terminal position.

Both mevalonic acid-1-C¹⁴ and mevalonic acid-2-C¹⁴ served as substrates for determination of decarboxylation of mevalonic-5'-pyrophosphate. The reaction mixtures were incubated in screw-cap tubes to eliminate loss of carbon dioxide. After heat inactivation (70 C for 5 min), one-half of each reaction mixture was extracted and treated as described above. The remainder of the reaction mixtures, after acidification (pH 4), were bubbled with nitrogen for 30 min in a closed system, trapping the evolving carbon dioxide in saturated barium hydroxide. A small quantity of barium carbonate was added as a carrier, and the precipitates were washed, weighed, and counted in a Tracerlab automatic proportional counter. Counts were corrected for self-absorption by extrapolation to infinite thinness.

Determination of ATP-5'-pyrophosphomevalonate carboxylase (EC 4.1.1.12) activity was performed with either mevalonate-5'-pyrophosphate-1-C¹⁴ or mevalonate-5'-pyrophosphate-2-C¹⁴ as substrates were carried out in a similar way.

Isopentenylpyrophosphate Δ⁴, Δ⁵-isomerase was assayed by measuring the disappearance of substrate, i.e., isopentenyl pyrophosphate, according to a modification of the method described by Agranoff et al. (1). Samples taken at various time intervals were treated with 0.5 ml of trichloroacetic acid, which inactivated the enzyme and hydrolyzed γ,γ-dimethylallyl pyrophosphate. After removal of γ,γ-dimethylallyl alcohol by extraction with diethyl ether (three times), samples of the aqueous layer which still contained residual isopentenyl pyrophosphate were transferred to planchets and counted in a Tracerlab proportional counter. Isopentenylpyrophosphate Δ⁴,Δ⁵-isomerase also was assayed by measuring the disappearance of isopentenyl pyrophosphate by gas chromatography. After inactivation (70 C for 3 to 5 min), the residual isopentenyl pyrophosphate-1-C¹⁴ was extracted with ethyl alcohol-ether (1:1, v/v), the solvents were evaporated under a stream of nitrogen, and the aqueous solutions were concentrated by lyophilization. The phosphorylated compounds were converted to their alcohols forms enzymatically by use of orthophosphoric monooester phosphohydrolase (EC 3.1.3.1) in a reaction mixture of (3): 0.02 M magnesium acetate; 0.2 ml of 0.04 M tris(hydroxymethyl)amino- methane-maleate buffer (Tris-maleate buffer), pH 10; and 8 mg of orthophosphoric monooester phosphohydrolase in a total volume of 1.5 ml. After the reaction mixtures were incubated for 30 min at 37 C, the enzyme protein was heat-inactivated (70 C for 3 to 5 min). The supernatant solutions were extracted four times with 1-ml quantities of diethyl ether, and ether extracts were dried under a stream of nitrogen. The residues were dissolved in 0.1 ml of tetrahydrofuran and 0.05 ml was subjected to gas-liquid chromatography in a Beckman model GC 2A instrument equipped with a Thermotrac temperature programmer, a thermistor detector, and matched 6-ft (1.8-meter) glass and packed columns of 20% W (carrier) on Chromosorb W 42/60 (Beckman no. 70402) under the following conditions: column temperature, 50 C; gas, helium; flow rate, 85 ml/min; current, 300 ma; sensitivity, 50; chart speed, 0.5 inch/min. Concentrations of isopentenol were determined by measurement of areas under the peaks by planimetry relative to areas under peaks of known control amounts of isopentenol.

**Materials.** The following compounds were synthesized in our laboratory from mevalonic acid-1-C¹⁴ and mevalonic acid-2-C¹⁴ with enzymes isolated from dried yeast as described by Bloch et al. (3): mevalonic-5'-phosphate-1-C¹⁴, mevalonic-5'-phosphate-2-C¹⁴, mevalonic-5'-pyrophosphate-1-C¹⁴, mevalonic-5'-pyrophosphate-2-C¹⁴, and isopentenyl pyrophosphate-1-C¹⁴. The yeast ATP-mevalonate 5-phosphotransferase reaction was carried out by the method of Tchen (14). All reagents were increased 10-fold, and 4 mg of enzyme protein was used per test. The reaction mixture was incubated at 30 C for 40 min in a total volume of 1.0 ml. The yeast ATP-5-phosphotransferase reaction was performed by the method of Bloch et al. (3). The reaction mixture contained the following components: mevalonic acid (carrier), 5 μmoles; mevalonic acid-1-C¹⁴ or mevalonic acid-2-C¹⁴, 2 μmoles containing 0.5 μc/μ mole; manganese chloride, 5 μmoles; phosphate buffer, 50 μmoles (pH 6.7); ATP, 10 μmoles; 0.03 M nitro-
MEVALONIC ACID CONVERSION BY MYCOPLASMA

Vol. 92, 1966

potassium fluoride; ATP-mevalonate 5-phosphotransferase, 4 mg; ATP-5-phosphothevalonate phosphotransferase, 4 mg. The reaction mixture was incubated at 30 C for 40 min in a total volume of 1.0 ml. The ATP-5-phosphothevalonate carboxy-lyase reaction also was conducted by the method of Bloch et al. (3). A control consisting of heat-inactivated enzyme protein was employed for each of the reactions. All reactions were stopped by heating at 70 C for 3 to 5 min. The phosphorylated intermediates were extracted with ethyl alcohol-ether (1:1, v/v). The solvents were removed under a stream of nitrogen, and the aqeous solutions were concentrated by lyophilization. Samples were chromatographed as described by Chaykin et al. (5), and detection of reaction products was accomplished by counting 0.5-cm strips in a Packard TriCarb liquid scintillation counter. For large quantities of substrate, the contents were increased 10-fold.

Adenine triphosphate labeled with P32 in the terminal phosphate position was obtained from Schwarz Bio Research, Inc., Orangeburg, N.Y. Mevalonic acid-1-C14 and mevalonic acid-2-C14 were obtained as the lactones from New England Nuclear Corp., Boston, Mass. After the benzene was evaporated, the residual lactones were hydrolyzed with potassium hydroxide, followed by adjustment of pH to 8.0 by the addition of acid (2).

DL-Mevalonic acid, DL-mevalonic-5'-phosphate (Tris cycloammonium salts), isopentenyl pyrophosphate (Tris cycloammonium salt), and DL-mevalonic acid-5'-pyrophosphate (dibrucine salt-6H2O) were obtained from Mann Research Laboratories, Inc., New York, N.Y. Orthophosphoric monoester phosphohydrolase was obtained from Worthington Biochemical Corp., Freehold, N.J. Adenine-5'-triphosphate (disodium salt; ATP) was purchased from the Sigma Chemical Co., St. Louis, Mo. All other chemicals were commercial preparations of reagent grade.

RESULTS AND DISCUSSION

The biosynthetic pathway of terpenoids in Mycoplasma is believed to be identical to that of yeast. Mevalonic acid is converted to mevalonic-5'-phosphate by ATP-mevalonate 5-phosphotransferase. ATP-5-phosphothevalonate phosphotransferase catalyzes the next reaction in which mevalonic-5'-phosphate is phosphorylated to form mevalonic-5'-pyrophosphate. Mevalonic-5'-pyrophosphate is decarboxylated by ATP-5'-pyrophosphate thevalonate carboxy-lyase to form isopentenyl pyrophosphate. Isopentenyl pyrophosphate is converted to γ,γ-dimethylallyl pyrophosphate by isopentenylpyrophosphate \( \Delta^3,\Delta^2 \)-isomerase.

ATP-mevalonate 5-phosphotransferase. Chromatographic data presented in Table 1 demonstrate the conversion of mevalonic acid-2-C14 to isopentenyl pyrophosphate-1-C14 by \( M. \) laidlawii. Hence, it would appear that ATP-mevalonate 5-phosphotransferase, ATP-5-phosphothevalonate phosphotransferase, and ATP-5-pyrophosphate thevalonate carboxy-lyase are present in this organism. Mycoplasma sp. strain J and \( M. \) hominis exhibited no ATP-mevalonate 5-phosphotransferase activity, as evidenced by the nonappearance of any phosphorylated intermediates. In the presence of 0.005 m iodoacetamide, which is a specific inhibitor of isopentenylpyrophosphate \( \Delta^3,\Delta^2 \)-isomerase (6), an accumulation of mevalonic-5'-phosphate and mevalonic-5'-pyrophosphate was noted in the \( M. \) laidlawii system (Table 2). No phosphorylated mevalonic acids accumulated with \( M. \) hominis sp. strain J or \( M. \) hominis.

ATP-5-phosphothevalonate phosphotransferase. Mevalonic-5'-pyrophosphate (\( R_p \) 0.35) and isopentenyl pyrophosphate (\( R_p \) 0.61) were detected only in \( M. \) laidlawii when cell-free extracts were incubated with mevalonic-5'-phosphate and ATP. The radioactive (P32) spots on the chromatograms corresponded with the phosphate spots detected with a modified Hanes-Isherwood reagent developed under ultraviolet irradiation (4).

ATP-5-pyrophosphate thevalonate carboxy-lyase. The formation of isopentenyl pyrophosphate from mevalonic acid results in the removal of carbon atom 1 of mevalonic acid by decarboxylation. Hence, mevalonic acid-1-C14 should give rise to carbon dioxide-C14, whereas the mevalonic acid-2-C14 would produce unlabeled carbon dioxide. ATP-5-pyrophosphate thevalonate carboxy-lyase activity was demonstrated only in \( M. \) laidlawii by employing mevalonic acid-1-C14 as the substrate, based upon liberation of labeled carbon dioxide. No activity was detected in \( M. \) hominis sp. strain J or \( M. \) hominis. When mevalonic acid-2-C14 was employed as a substrate, no label appeared in the trapped carbon dioxide (Table 3). Since the absence of ATP-mevalonate 5-phosphotransferase and ATP-phosphothevalonate phosphotransferase in \( M. \) hominis prevents the formation of mevalonic-5'-pyrophosphate-1-C14, further experiments were performed with mevalonic-5'-pyrophosphate-1-C14 as the reaction substrate. With mevalonic-5'-pyrophosphate-1-C14 as the substrate, ATP-5-pyrophosphate thevalonate carboxy-lyase activity was demonstrated in \( M. \) laidlawii by the liberation of labeled carbon dioxide. No labeled carbon dioxide was demonstrated with \( M. \) hominis sp. strain J and \( M. \) hominis. The use of mevalonic-5'-pyrophosphate-2-C14 as substrate failed to produce labeled carbon dioxide, as expected (Table 4).

Isopentenylpyrophosphate \( \Delta^3,\Delta^2 \)-isomerase. Isopentenylpyrophosphate \( \Delta^3,\Delta^2 \)-isomerase was measured as the rate of disappearance of isopentenyl pyrophosphate-1-C14, as illustrated in
TABLE 1. Incorporation of mevalonic acid-2-C\textsuperscript{14} into isopentenyl pyrophosphate by Mycoplasma\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_P ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Known</td>
</tr>
<tr>
<td>Mevalonic acid</td>
<td>0.80</td>
</tr>
<tr>
<td>Mevalonic-5'-phosphate</td>
<td>0.66-0.68</td>
</tr>
<tr>
<td>Mevalonic-5'-pyrophosphate</td>
<td>0.37-0.40</td>
</tr>
<tr>
<td>Isopentenyl pyrophosphate</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction conditions: each tube contained 20 \( \mu \)moles of adenosine triphosphate, 30 \( \mu \)moles of manganese chloride, 30 \( \mu \)moles of glutathione, 500 \( \mu \)moles of phosphate buffer (pH 7.5), 8 \( \mu \)moles of mevalonic acid, 2 \( \mu \)moles of mevalonic acid-2-C\textsuperscript{14} (0.5 \( \mu \)c/\( \mu \)mole), and enzyme protein from 2.5 liters of sonically treated cells (approximately 20 mg). Reactions were carried out at 37°C for 3 hr in a total volume of 5.0 ml.

TABLE 2. Incorporation of mevalonic acid-2-C\textsuperscript{14} into mevalonic-5'-phosphate, mevalonic-5'-pyrophosphate, and isopentenyl pyrophosphate by Mycoplasma in the presence of iodoacetamide\textsuperscript{a}

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R_P ) values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Known</td>
</tr>
<tr>
<td>Mevalonic acid</td>
<td>0.80</td>
</tr>
<tr>
<td>Mevalonic-5'-phosphate</td>
<td>0.66-0.68</td>
</tr>
<tr>
<td>Mevalonic-5'-pyrophosphate</td>
<td>0.37-0.40</td>
</tr>
<tr>
<td>Isopentenyl pyrophosphate</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction conditions: same as Table 1 except for the addition of 0.005 M iodoacetamide.

TABLE 3. Liberation of \( ^{14} \text{O}_2 \) from mevalonic acid-1-C\textsuperscript{14} and -2-C\textsuperscript{14} by Mycoplasma\textsuperscript{a}

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>( ^{14} \text{O}_2 ) liberation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mevalonic acid-2-C\textsuperscript{14}</td>
<td>Mevalonic acid-1-C\textsuperscript{14}</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>count/ min</td>
<td>count/ min</td>
</tr>
<tr>
<td>M. laidlawii strain B.</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mycoplasma sp. strain J.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. hominis strain O7.</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction conditions: 20 \( \mu \)moles of adenosine triphosphate, 30 \( \mu \)moles of magnesium chloride, 30 \( \mu \)moles of glutathione, 500 \( \mu \)moles of phosphate buffer (pH 7.5), 10 \( \mu \)moles of mevalonic acid-2-C\textsuperscript{14} (0.5 \( \mu \)c/\( \mu \)mole) or 10 \( \mu \)moles of mevalonic acid-1-C\textsuperscript{14} (0.5 \( \mu \)c/\( \mu \)mole), and enzyme protein from 2.5 liters of sonically treated cells (approximately 20 mg). Reaction was carried out at 37°C for 3 hr in a total volume of 5.0 ml.

TABLE 4. Liberation of \( ^{14} \text{O}_2 \) from mevalonic-5'-pyrophosphate-1-C\textsuperscript{14} and -2-C\textsuperscript{14} by Mycoplasma\textsuperscript{a}

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>( ^{14} \text{O}_2 ) liberation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mevalonic-5'-pyrophosphate-2-C\textsuperscript{14}</td>
<td>Mevalonic-5'-pyrophosphate-1-C\textsuperscript{14}</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td></td>
<td>count/ min</td>
<td>count/ min</td>
</tr>
<tr>
<td>M. laidlawii strain B.</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Mycoplasma sp. strain J.</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>M. hominis strain O7.</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Reaction conditions: each tube contained 20 \( \mu \)moles of adenosine triphosphate, 30 \( \mu \)moles of manganese chloride, 30 \( \mu \)moles of magnesium chloride, 30 \( \mu \)moles of glutathione, 500 \( \mu \)moles of phosphate buffer (pH 7.5), 0.03 M potassium fluoride, mevalonic-5'-pyrophosphate-1-C\textsuperscript{14} synthesized from 20 \( \mu \)moles of mevalonic acid-1-C\textsuperscript{14} (0.5 \( \mu \)c/\( \mu \)mole) or mevalonic-5'-pyrophosphate-2-C\textsuperscript{14} synthesized from 20 \( \mu \)moles of mevalonic acid-2-C\textsuperscript{14} (0.5 \( \mu \)c/\( \mu \)mole), and 30 mg of enzyme protein.
Fig. 1. Isopentenyl pyrophosphate isomerase activity of Mycoplasma. (A) M. laidlawii strain B. (B) Myco-
plasma sp. strain J. (C) M. hominis strain O7. Reaction mixtures: each tube contained 15 μmoles of glutathione,
2 μmoles of magnesium chloride, 2 μmoles of Tris buffer (pH 8.0), isopentenyl pyrophosphate-1-C14 (IPP)
synthesized with yeast enzymes from 20 μmoles of mevalonic acid-2-C14 (0.5 μc/μmole); 0.03 M potassium
fluoride, and 15 mg of enzyme protein. The tubes containing 0.005 μM iodoacetamide (IAA) lacked glutathione.
Control tubes contained heat-inactivated enzyme.

Fig. 1. No disappearance was expected in the tubes which contained iodoacetamide, which is a
specific inhibitor of isopentenylpyrophosphate Δ^3,Δ^2-isomerase. Figures 1a and 1b demonstrate
that isopentenylpyrophosphate Δ^3,Δ^2-isomerase activity was present in M. laidlawii and Myco-
plasma sp. strain J. No activity was demonstrated in M. hominis (Fig. 1c). By employing similar
reaction mixtures, confirmation of the presence of isopentenylpyrophosphate Δ^3,Δ^2-isomerase
was demonstrated in M. laidlawii and Myco-
plasma sp. strain J by measurement of the disappear-
ance of isopentenyl pyrophosphate gas chromatographically by quantitating residual
isopentenol (Table 5). No γ,γ-dimethylallyl
alcohol would be expected to accumulate, since the
succeeding steps in the biosynthetic pathway
were not inhibited. The lack of inhibition by
iodoacetamide in this experiment can be attrib-
uted to the lower concentration (0.0025 μM versus
0.005 μM) of inhibitor and the higher concentration
of enzyme proteins (20 mg versus 15 mg). Even
so, some inhibition is noted with Mycoplasma sp.
strain J.

These results indicate that all four enzymes re-
quired to transform mevalonic acid to γ,γ-di-
methylallyl pyrophosphate are contained in M.
laidlawii but are absent from M. hominis. Al-
though Mycoplasma sp. strain J is capable of
synthesis of mevalonic acid (11), it is deficient in
the three enzymes involved in the formation of
isopentenyl pyrophosphate: ATP-mevalonate 5-
phosphotransferase, ATP-5-phosphomevalonate
phosphotransferase, and ATP-5-phospho-
mevalonate carboxy-lyase. However, this organism
appears to possess isopentenylpyrophosphate
Δ^3,Δ^2-isomerase. This evidence is compatible with
the results of nutritional experiments in which
growth was not supported by mevalonic acid,
mevalonic-5'-phosphate, or mevalonic-5'-py-
ophosphate when these compounds were
substituted for the sterol requirement of M.
hominis and Mycoplasma sp. strain J (Henrikson
and Smith, in press). The presence of isopentenyl-
pyrophosphate Δ^3,Δ^2-isomerase in Mycoplasma
sp. strain J explains the significant growth re-
ponse when isopentenyl pyrophosphate was
substituted for sterol. The ability of isopentenyl
pyrophosphate to support growth of this organ-
ism in lieu of sterol indicates that all other en-
zymes of the carotenoid biosynthetic pathway
probably are present. This possibility is cur-
rently under investigation. On the other hand, the
absence of isopentenylpyrophosphate Δ^3,Δ^2-
isomerase in M. hominis explains the inability of
proteins. The reaction was carried out at 37 C in a
total volume of 2.0 ml with samples being removed at zero-time and at 5, 10, and 30 min for assay purposes.
TABLE 5. Isopentenyl pyrophosphate isomerase activity of Mycoplasma

<table>
<thead>
<tr>
<th>Organism</th>
<th>Isopentenyl pyrophosphate-1-(^{14}C) utilized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>(\mu)oles</td>
</tr>
<tr>
<td><em>M. laidlawii</em> strain B</td>
<td>0</td>
</tr>
<tr>
<td><em>Mycoplasma</em> sp. strain J</td>
<td>0</td>
</tr>
<tr>
<td><em>M. hominis</em> strain O7</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^{a}\) Reaction conditions: each tube contained the same components as described in Fig. 1 except for use of 20 mg of enzyme protein and 0.0025 M iodoacetamide. The reaction mixtures were heat-inactivated (70 C for 3 to 5 min) after incubation at 37 C for 30 min.

isopentenyl pyrophosphate to support growth when substituted for sterol.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service research grant AI 04410-05 and graduate training grant 5 T1 AI 232-03 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED