BACTERIAL METABOLISM OF MEVALONIC ACID

CONVERSION TO ACETOACETATE

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Although microorganisms played an important role in the discovery and isolation of mevalonic acid (Folkers et al., 1959), little is known of its metabolism in bacteria. Biosynthesis of mevalonic acid from acetyl-CoA by yeast and by mammalian tissues involves condensation with acetoacetyl-CoA to form hydroxymethylglutaryl-CoA (Ferguson and Rudney, 1959; Durr and Rudney, 1960) and reduction to mevalonic acid and free CoA by a NADPH-dependent hydroxymethylglutaryl-CoA reductase (Durr and Rudney, 1960; Brodie and Porter, 1960). A limited incorporation of isotope from mevalonic acid-2-C^{14} into acetoacetate catalyzed by yeast enzymes has been reported by Lynen (1959). In bacterial systems, Lynen and Grassl (1959) isolated (-)-mevalonic acid from the culture media of a Mycobacterium species metabolizing (±)-mevalonic acid.

Using the elective culture technique (Jawetz et al., 1958), four strains of microorganisms capable of growth upon D,L-mevalonic acid as sole carbon source were obtained. A nitrate-containing liquid medium at pH 7.0 (Jakoby, 1958) containing 0.05% neutralized mevalonic acid lactone (Nutritional Biochemicals Corp.) as the sole carbon source was inoculated with soil. After several transfers in liquid media, pure strains were isolated from solid media. The most rapidly growing culture, a Gram-positive, non-motile, non-acid fast filamentous rod, designated S_4, was selected for study.

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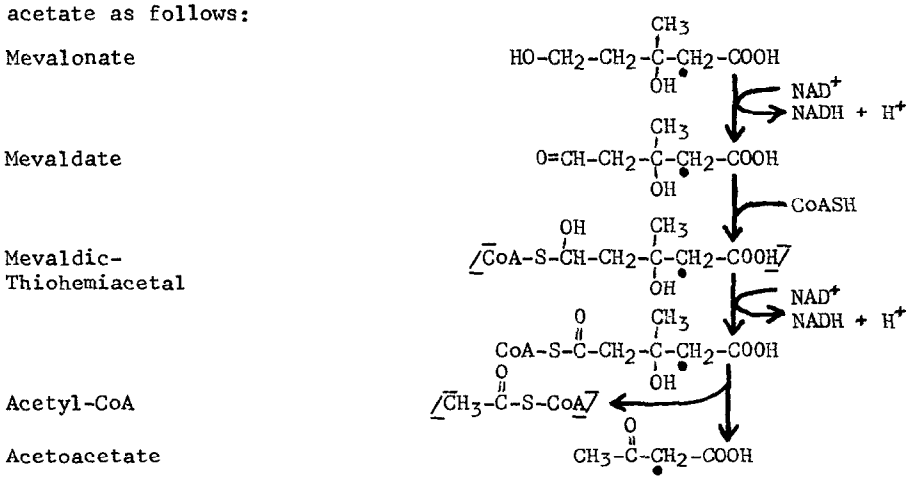
Cells grown on 0.4% mevalonate were subjected to 30 minutes treatment in a sonic oscillator. The clear supernatant liquid obtained after 15 minutes centrifugation at 25,000 x g was used as enzyme. This preparation catalyzed the NAD and CoA-dependent incorporation of isotope of mevalonic acid-2-C^{14} into acetoacetate (Table I) and also the formation of acetoacetate from hydroxymethylglutaryl-CoA alone or from mevalonate or mevaldate plus NAD and CoA (Table II).

These data are consistent with the conversion of mevalonate to acetoacetate as follows:



This sequence resembles closely the reverse of that proposed for biosynthesis of mevalonate in yeast and mammalian tissues. The major difference is that the bacterial system specifically requires NAD rather than NADP. The final step parallels the hydroxymethylglutaryl-CoA cleaving enzyme described by Bachhwat et al. (1955). Mevaldic thiohemiacetal, proposed by Durr and Rudney (1960) as intermediate in the conversion of hydroxymethylglutaryl-CoA to mevalonate, is proposed here to account for the observed labelling pattern and for the apparent absence of any requirement for high energy compounds which would be anticipated if mevaldyl-CoA were an intermediate.

Table I

Incorporation of Isotope from Mevalonate-2-C^{14} into Acetoacetate

Enzyme	Acetone Dinitrophenylhydrazone		
	Specific Activity	Total Activity	Incorporation
	c.p.m./μmole	c.p.m.	%
-	16	800	0.001
+	7.3×10^3	4.0×10^5	35
+	8.5×10^3	4.5×10^5	40

The following were incubated in a volume of 1.0 ml: Tris-HCl buffer, pH 8.0, 100 μmoles; CoA, 2.0 μmoles; NAD, 2.9 μmoles; potassium D,L-mevalonate-2-C^{14}, 0.75 μmoles (1.15×10^6 c.p.m.), and enzyme equivalent to 5.0 mg protein. Enzyme was omitted from control samples. After 3 hours incubation at 30°, tubes were chilled and deproteinized with 0.1 ml of cold 25% trichloracetic acid. After centrifugation, 54.5 μmoles of carrier acetone was added to the protein-free supernatant liquid. After treatment with aniline-HCl at pH 5 to decarboxylate acetoacetate to acetone (Barkulis and Lehninger, 1951), 10 ml of 0.5% 2,4-dinitrophenylhydrazine in 2 N HCl were added. Acetone dinitrophenylhydrazone precipitated immediately and was collected by filtration and washed thoroughly with water to remove traces of adsorbed mevalonic acid. The product gave the correct M.P. for and did not depress the M.P. of authentic acetone dinitrophenylhydrazone. It chromatographed as the authentic material (R_f = 0.82) in n-butanol:ethanol:0.5 N NH_4OH:: 70:10:20 and in n-heptane saturated with phenoxyethanol (Lynn et al., 1956). In the latter solvent, mevalonate, mevaldate, hydroxymethylglutarate and acidic dinitrophenylhydrazone remain at the origin. After chromatography in the phenoxyethanol:n-heptane solvent, the product was eluted, counted, and its concentration determined from its absorbancy at 358 mμ. Incorporation data are uncorrected for the fact that only one isomer of mevalonic acid is physiologically active.

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Table II

Precursors of Acetoacetate

Substrate		Enzyme	Total Aceto-acetate Found (μmoles)	Net Synthesis of Acetoacetate (μmoles)
Mevalonate	0.83	-	0.015	
	0.83	+	0.318	0.303
Mevaldate	8.85	-	0.204	
	8.85	+	0.700	0.496
β-Hydroxy-β-methyl-glutarate	9.00	-	0.025	
	9.00	+	0.026	0.001
Hydroxy-methylglu-taryl-CoA*	1.2	-	0.020	
	1.2	+	0.194	0.174

* Performed using 2.1 mg of the 25-50% ammonium sulfate precipitate prepared from the crude 25,000 x g supernatant liquid. Incubation time was one hour.

The following were incubated in a volume of 1.0 ml: Tris-HCl, pH 8, 100 μmoles; CoA, 1.85 μmoles; NAD, 2.6 μmoles, enzyme as indicated equivalent to 5.0 mg protein, and substrates as indicated. After incubation at 30° for three hours, tubes were chilled and deproteinized with 0.1 ml of cold 25% trichloracetic acid. After centrifugation, suitable aliquots of the protein-free supernatant liquid were analyzed for acetoacetate (Walker, 1954).

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