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Utilization of Acetate by *Methanomonas methanooxidans*

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Methanomonas methanooxidans incorporates both carbon atoms of acetate into the glutamate and aspartate families of amino acids during growth on methane; carbon dioxide is also evolved from both carbon atoms of acetate. The distribution of carboxyl-labeled acetate incorporated into covalently bound glutamate is consistent with the operation of the tricarboxylic acid cycle in this species, and the presence of α-ketoglutarate dehydrogenase was demonstrated in cell-free extracts.

Obligate methylotrophs have been divided into two main groups on the basis of their gross morphology, the structural arrangement of intracytoplasmic membranes, and their resting forms (4, 11). Type I organisms include the proposed genera *Methylomonas*, *Methylococcus*, and *Methylobacter*, and type II organisms are exemplified by the genera *Methylosinus* and *Methylocystis* (11). This division now has strong biochemical support on the basis of the different pathways of incorporation of C_1 compounds into cellular constituents and also the specific deficiencies of certain enzymes of general metabolism (1, 4). The major differences are summarized in Table 1.

One of the earliest characterized methylotrophs that showed a dependence on methane for growth was named *Methanomonas methanooxidans* (10). Our ultrastructural studies showed that this strain possessed type II intracytoplasmic membranes (9). The experiments of Quayle (4) demonstrated that the serine pathway of formaldehyde incorporation was responsible for synthesis of cellular constituents, and R. Whittenbury (personal communication) typed this strain to the *Methylosinus* genus. One of the biochemical characteristics of the type II group of methylotrophs is the presence of α-ketoglutarate dehydrogenase in extracts of cells (1), unlike type I organisms (1, 3; R. Patel, D. S. Hoare, and B. F. Taylor, Bacteriol. Proc., p. 128, 1969). The physiological significance of the presence or absence of this enzyme during growth has only been investigated for *M. capsulatus* (type I) by Patel et al. (3); they demonstrated conclusively that the tricarboxylic acid cycle was inoperative in *M. capsulatus*, in that [^{14}C]acetate did not yield $^{14}CO_2$ although it was incorporated into the glutamate family of amino acids. [^{14}C]acetate was not incorporated into amino acids derived from aspartate in this species. We have performed similar experiments with *M. methanooxidans*, and the results indicate that the tricarboxylic acid cycle is functional during growth on methane in the presence of acetate, giving further support to the conclusions drawn from the enzyme assays for type II organisms (1), as well as for the assignment of *M. methanooxidans* to the genus *Methylosinus*.

Initial attempts (A. M. Wadzinski and D. W. Ribbons, Bacteriol. Proc., p. 166, 1972) to assay enzymes of the tricarboxylic acid cycle in extracts of *M. methanooxidans* failed to reveal fumarase (5) and α-ketoglutarate dehydrogenase (6), other enzymes of the cycle being found with activities of similar magnitude reported for other methylotrophs (1). Extracts of cells harvested from stirred fermentors (instead of shake cultures) during log phase, however, gave specific activities (micromoles/minute per milligram of protein) of 10 to 20 and 0.6 to 1.0 for fumarase and α-ketoglutarate dehydrogenase, respectively, the latter being 10-fold less than for other type II organisms (1).

When acetate was incorporated into the growth medium, the cell yield was greatly enhanced for the same amount of methane supplied. Utilization of acetate for cellular synthesis was confirmed by showing that both [1-^{14}C]- and [2-^{14}C]acetate were incorporated into hot acid-stable products, the former radiochemical species contributing about ⅓ of the values of the latter (A. M. Wadzinski, Ph.D. thesis, Univ. of Miami, Coral Gables, Fla., 1973). [2-^{14}C]acetate contributed approximately 15% of the cell carbon, ¼ of which appeared in the hot acid-insoluble fraction. Examination of the 24-h HCl hydrolysates of hot trichloroacetic acid-insoluble precipitates showed that both

TABLE 1. *Major biochemical differences between methylotrophs possessing type I membranes and type II membranes*[a]

Property	Type I:	Type II:
Carbon assimilation pathway	Hexose-phosphate	Serine
α-Ketoglutarate dehydrogenase	−	+
Complete tricarboxylic acid cycle	−	+
Glucose 6-phosphate dehydrogenase	+	−
6-Phosphogluconate dehydrogenase	+	−

[a] Consult references 1, 3, 4, and 7 for details.

TABLE 2. *Decarboxylation of glutamate isolated from Methanomonas methanooxidans after growth on methane in the presence of [1-^{14}C]acetate*[a]

Decarboxylation method	Counts/min		
	[^{14}C]-Glutamate supplied	$^{14}CO_2$ released	Residue ^{14}C[b]
Chloramine T[c]	2,995	905	2,063
	4,110	1,522	2,800
	4,110	1,632	2,825
Glutamate[d]	2,780	714	1,960
	2,780	716	1,959

[a] *M. methanooxidans* was grown on methane in the presence of 5 mM [1-^{14}C]acetate (5 μCi/mmol) into stationary phase. Cells were harvested, washed, and fractionated (8). Glutamate was isolated from 24-h HCl hydrolysates of hot trichloroacetic acid-insoluble residues by high-voltage electrophoresis, eluted with water, concentrated, and subjected to decarboxylation. Glutamate concentration was determined by the ninhydrin method (12).

[b] Nonvolatile ^{14}C in solution (γ-aminobutyrate).

[c] Specific activity of [^{14}C]glutamate (1,580 counts/min per μmol).

[d] Glutamate decarboxylase (EC 4.1.1.15) from *Escherichia coli* ATCC 11246; specific activity of [^{14}C]glutamate (1,445 counts/min per μmol).

carbons of acetate were incorporated into amino acids of glutamate and aspartate families, as well as those derived from pyruvate, namely, serine, glycine, alanine, valine, leucine, phenylalanine, and tryptophan. $^{14}CO_2$ was also released from both radiochemical species of [^{14}C]acetate, and [1-^{14}C]acetate gave approximately twice as much labeled CO_2 as did [2-^{14}C]acetate.

Covalently bound glutamate was isolated by paper electrophoresis (2) of the HCl hydrolysates of hot trichloroacetic acid-insoluble precipitates of *M. methanooxidans* grown in the presence of [1-^{14}C]acetate. The glutamate was decarboxylated with chloramine T and glutamate decarboxylase (3) to determine the distribution of radioactivity in the carbon skeleton. The results (Table 2) show that about 33% of the isotope is contained in C1 of glutamate; this is the proportion expected for the synthesis of glutamate for [1-^{14}C]acetate by reactions of the tricarboxylic acid cycle, assuming that citrate synthesis and dehydration occur in the usual stereochemical sense. These results are in direct contrast to those obtained for *M. capsulatus*. Thus, it is suggested that the tricarboxylic acid cycle is functionally operative during growth of *M. methanooxidans* in the presence of methane and acetate, lending further evidence for the same conclusion drawn from enzyme assays (1).

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