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Influence of Corrinoid Antagonists on Methanogen Metabolism

WILLIAM KENEALY AND J. G. ZEIKUS*

Department of Bacteriology, University of Wisconsin, Madison, Wisconsin 53706

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Iodopropane inhibited cell growth and methane production when *Methanobacterium thermoautotrophicum*, *Methanobacterium formicicum*, and *Methanosarcina barkeri* were cultured on H_2-CO_2. Iodopropane (40 μM) inhibited methanogenesis (30%) and growth (80%) when *M. barkeri* was cultured mixotrophically on H_2-CO_2-methanol. The addition of acetate to the medium prevented the observed iodopropane-dependent inhibition of growth. The concentrations of iodopropane that caused 50% inhibition of growth of *M. barkeri* on either H_2-CO_2, H_2-CO_2-methanol, methanol, and acetate were 112 ± 6, 24 ± 2, 63 ± 11, and 4 ± 1 μM, respectively. Acetate prevented the iodopropane-dependent inhibition of one-carbon metabolism. Cultivation of *M. barkeri* on H_2-CO_2-methanol in bright light also inhibited growth and methanogenesis to a greater extent in the absence than in the presence of acetate in the medium. Acetate was the only organic compound examined that prevented iodopropane-dependent inhibition of one-carbon metabolism in *M. barkeri*. The effect of iodopropane and acetate on the metabolic fates of methanol and carbon dioxide was determined with ^{14}C tracers when *M. barkeri* was grown mixotrophically on H_2-CO_2-methanol. The addition of iodopropane decreased the contribution of methanol to methane and cell carbon while increasing the contribution of CO_2 to cell carbon. Regardless of iodopropane, acetate addition decreased the contribution of methanol and CO_2 to cell carbon without decreasing their contribution to methane. The corrinoid antagonists, light and iodopropane, appeared most specific for methanogen metabolic reactions involved in acetate synthesis from one-carbon compounds and acetate catabolism.

Methanogens are nutritionally diverse and display autotrophic, heterotrophic, or methylotrophic growth modes (33). H_2-CO_2, carbon monoxide, methanol, methylamines, formate, and acetate are the known carbon and energy sources of methanogenic bacteria (5, 12, 15, 25, 26, 28, 32). *Methanosarcina barkeri* is the most metabolically versatile methanogen (25, 26) and grows either unitrophically on all of these substrates, except for formate, or mixotrophically on combinations of substrates (e.g., H_2-CO_2 and methanol).

The metabolism of some of these substrates to methane has been under investigation since the 1950s, but only more recently with pure cultures. The discovery of coenzyme M (CoM) in pure cultures (16, 23) has advanced the knowledge of the biochemistry methanogens, but has challenged the previous role suggested for vitamin B_{12} as the methyl donor in methanogenesis (20, 30, 31). The exact pathways by which methanogen carbon and energy sources are converted to methane and cell carbon have not yet been determined (33). Autotrophy and methylotrophy in methanogens appear unique because neither the reductive pentose phosphate, serine, and hexulose phosphate pathways nor the reductive tricarboxylic acid cycle functions in these organisms (6, 8, 25, 27, 32).

Methanobacterium thermoautotrophicum and *M. barkeri* lack a complete tricarboxylic acid cycle when grown on H_2-CO_2 (8, 27), but both organisms use portions of it to obtain α-ketoglutarate for glutamate synthesis. *M. barkeri* employs the citrate synthase, aconitase, and isocitrate dehydrogenase reactions to synthesize α-ketoglutarate from acetyl coenzyme A (CoA) and oxaloacetate (27). *M. thermoautotrophicum* uses the malate dehydrogenase, fumarase, fumarate reductase, and α-ketoglutarate synthase reactions to synthesize α-ketoglutarate from oxaloacetate and CO_2 (8, 34). Both organisms obtain oxaloacetate from successive carboxylations of acetyl CoA (8, 27). Acetate is a significant precursor of cell carbon in methanogens; as much as 50 to 70% of the cell carbon of *Methanobacterium ruminantium* (4), *M. barkeri* (25, 26), and *M. thermoautotrophicum* (8) is derived

from acetate during growth on H_2–CO_2. The biochemical mechanism of two-carbon synthesis from one-carbon precursors remains to be elucidated in methanogens.

Recently, methanogens (13) were shown to contain corrinoids at levels equal to or greater than those reported in *Clostridium thermoaceticum* and *Acetobacterium woodii* (22). In these homoacetate-fermenting bacteria corrinoids function as methyl carriers during acetate synthesis via reductive carboxylation (9, 14, 18). *M. barkeri* contains more corrinoids than do the other methanogens examined, but the exact levels depend on the specific carbon and energy sources used during growth (13). Initial attempts to demonstrate a "homoacetate" pathway in methanogens were not conclusive (7).

Both light and iodopropane are inhibitors of corrinoid-dependent metabolism (9, 24, 30, 31). Iodopropane binds to corrinoid enzymes and inactivates them (3, 30, 31). Iodopropane inhibition of corrinoid functions requires dark reducing conditions (3, 24). The exposure of alkyl corrinoids to light causes photocleavage of the cobalt alkyl bond (9, 24, 30). Iodopropane inhibited methane formation in cell extracts of the mixed culture, *Methanobacterium omelianskii* (29, 30), and inhibited acetate synthesis in cell extracts of *C. thermoaceticum* (9).

The role of corrinoids in methanogen methyl transfer reactions is unclear. Methanogen cell extracts (pure cultures) display high activities for CoM methylation using methyl B_{12} as the methyl donor (10, 19, 23). A function for this activity has not been proposed. The final step in methane production from H_2–CO_2 involves CoM and not vitamin B_{12} because the purified catabolic methyl reductase from methanogens is specific for methyl CoM and not methyl B_{12} (11; R. S. Wolfe, personal communication).

The purpose of this paper is to ascertain whether corrinoids have a significant function in methanogen metabolism. These investigations examine the influence of corrinoid antagonists on methane production and growth of different methanogen species and during cultivation of *M. barkeri* on different carbon and energy sources.

MATERIALS AND METHODS

Chemicals and gases. All chemicals used were of reagent grade. Iodopropane was purchased from Eastman Kodak Co., Rochester, N.Y. [^{14}C]sodium carbonate (>50 mCi/mmol), [^{14}C]methanol (10 to 50 mCi/mmol), and [*U*-^{14}C]acetate (40 to 60 mCi/mmol) were purchased from Amersham Searle, Arlington Heights, Il. H_2–CO_2 (80:20, vol/vol, premixed) and N_2–CO_2 (95:5, vol/vol, premixed) were purchased from Matheson Scientific, Inc., Joliet, Il.

Organisms and cultivation. *M. barkeri* strain MS and *Methanobacterium formicicum* strain MF were routinely cultivated in phosphate-buffered basal medium (PBBM) which contained the following (per liter of double-distilled water): KH_2PO_4, 1.5 g; $K_2HPO_4 \cdot 3H_2O$, 2.9 g; NH_4Cl, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; NaCl, 0.9 g; vitamin solution (29), 10 ml; trace mineral solution (see below), 10 ml; and resazurin (0.2%), 1 ml. The trace mineral solution contained the following (per liter of distilled water): nitrilotriacetic acid, 15 g; $FeSO_2 \cdot 7H_2O$, 0.1 g; $MnCl_2 \cdot 4H_2O$, 0.1 g; $CoCl_2 \cdot 6H_2O$, 0.17 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnCl_2$, 0.1 g; $NiSO_4 \cdot 6H_2O$, 0.026 g; $CuCl_2$, 0.02 g; H_3BO_3, 0.01 g; $NaMoO_4 \cdot 2H_2O$, 0.01 g; NaCl, 1.0 g; and Na_2SeO_3, 0.016 g. The medium was prepared without the phosphates, brought to pH 7.2 with NaOH, boiled, sparged with N_2–CO_2, dispensed into 158-ml Wheaton serum vials sealed with flanged rubber stoppers (1), and autoclaved at 103 kN/m^2 for 20 min. Phosphates, sulfide (0.01%), methanol, H_2–CO_2 (80:20), and acetate were added after autoclaving.

M. barkeri was cultivated with acetate as the energy source on the Trypticase-yeast extract medium described by Mah et al. (15) with a yeast extract concentration of 0.2% and acetate concentration of 1%. The medium was gassed with N_2–CO_2 (95:5), dispensed into 158-ml serum vials, and autoclaved for 20 min. *M. thermoautotrophicum* strain ΔH was cultivated in a low-phosphate basal medium (LPBM) which contained the following (per liter of distilled water): KH_2PO_4, 0.3 g; $Na_2HPO_4 \cdot 7H_2O$, 2.1 g; NH_4Cl, 1.0 g; $MgCl_2 \cdot 6H_2O$, 0.2 g; trace mineral solution (see above), 10 ml; $FeSO_4$ (2.5%), 25 μl; and resazurin (0.2%), 1 ml. The medium was prepared as for PBBM, except it was gassed with H_2–CO_2 (80:20).

Inhibition experiments with iodopropane and light. A saturated solution of iodopropane was made by filter-sterilizing iodopropane in the culture medium. The stock solution was protected from light, and the concentration was calculated to be 18 mM from the density (1.75 g/ml at 20°C) and solubility (1 part in 575 parts water) of iodopropane. After addition of iodopropane to inoculated cultures the experiments were protected from light in a dark incubator maintained at the optimum growth temperature for each organism. Control cultures were inoculated to determine the growth in the absence of iodopropane. When controls reached the mid to late exponential phase of growth (i.e., 90% of maximal growth), the experimental cultures were removed from the incubator and analyzed for growth and methane production. All individual experiments were performed in duplicate (or more) serum vials, and the results were averaged. All the experiments were repeated several times.

In the experiments with light as an inhibitor, cultures were incubated 15 cm from two incandescent and fluorescent bulbs at an average light intensity of 105 microeinsteins per m^2 per s, 0.1 W/m^2, and 10 lux as determined by a Licor Model LI 185 quantum/radiometer/photometer. Dark controls were wrapped in aluminum foil and incubated at the same location. A fan was used to maintain constant temperature around the vials during incubation.

Analysis of growth, gases, and radioactivity. Growth of *M. barkeri* was determined by filtration of

the serum vial contents through 0.4 μM polycarbonate filters (Bio-Rad Laboratories, Richmond, Calif.) as described by Weimer and Zeikus (25). Growth of *M. formicicum* and *M. thermoautotrophicum* was determined by optical density at 660 nm that was corrected with a medium blank by using a Spectronic 20. Optical density was converted to dry weight by using the conversion factor of 550 mg of cells (dry weight) per liter of 1.0 optical density unit (660 nm).

Incorporation of ^{14}C label into cells was determined after filtration and washing by placing dried filters into 6-ml glass scintillation vials that contained 0.2 ml of TS-2 tissue solubilizer (Research Products International, Elk Grove Village, Il.). The samples were solubilized overnight, and the radioactivity was determined with Packard Instagel (5 ml) added as a cocktail. Samples were counted in a Packard Prias Pld Tricarb scintillation counter immediately after mixing the contents of the scintillation vial to prevent settling.

The methane content of experimental cultures was determined with a Varian aerograph 600D gas chromatograph as described by Zeikus et al. (35). Gas samples were removed with a pressure lock syringe (Mininert syringe valve; Supelco Inc., Bellefonte, Pa.). The radioactivity of $^{14}CO_2$ and $^{14}CH_4$ were determined by gas chromatography-gas proportional counting as described previously (17). Other radioactive measurements used a Packard Prias Pld Tricarb scintillation counter and Packard Instagel as a cocktail.

RESULTS

Iodopropane-dependent inhibition of methanogen H_2-CO_2 metabolism. The effect of iodopropane concentrations on H_2-CO_2 metabolism of *M. thermoautotrophicum*, *M. formicicum*, and *M. barkeri* is shown in Table 1. Precautions were taken not to expose cultures to light during incubation. The influence of iodopropane on growth and methanogenesis was lessened when *M. thermoautotrophicum* was cultured without these precautions and propane was detected in the gas phase of light-incubated cultures. The formation of propane depended on cells and light because it was not detected in controls or in cultures incubated in the dark.

Inhibition of growth and methanogenesis by iodopropane was concentration dependent in all three species. The quantitative effect of iodopropane inhibition of methanogen metabolism was decreased by acetate. Inhibition of methanogen growth by iodopropane concentrations (i.e., 80 μM) was nearly completely relieved by acetate in *M. barkeri* and *M. formicicum* cultures. At high iodopropane concentrations (i.e., 400 μM), acetate significantly relieved inhibition of growth and methanogenesis of *M. barkeri*, but not *M. thermoautotrophicum*.

Effect of iodopropane and light on *M. barkeri* metabolism. The effects of corrinoid antagonists were further examined when *M. barkeri* was grown on H_2-CO_2, H_2-CO_2-methanol, methanol, or acetate as the energy source. Growth and methane production as a function of iodopropane concentration for *M. barkeri* grown on H_2-CO_2-methanol is shown in Fig. 1. The iodopropane inhibition of growth and methane production demonstrated an extended plateau region at concentrations of >100 μM. The initial kinetics of iodopropane inhibition significantly influenced growth more than methanogenesis; for example, a 30% inhibition of methane production correlated with an 80% inhibition of growth. A decrease in the molar cell yield (i.e. grams [dry weight] of cells per mole of methane) was detected with increasing iodopropane concentrations. When 20 mM acetate was present in the medium with either 40, 80, or 400 μM iodopropane the inhibition of growth observed was 0, 10, or 18%, respectively, and the inhibition

TABLE 1. *Effect of iodopropane on methanogen H_2-CO_2 metabolism*[a]

Species	Acetate	Growth (mg [dry wt] increase) at iodopropane concn (μM):			Methane formation (total μmol) at iodopropane concn (μM):		
		0	80	400	0	80	400
M. barkeri	−	2.2 ± 0.5	1.9 ± 0.2	0.5 ± 0.3	618 ± 81	519 ± 43	178 ± 91
	+	2.5 ± 0.5	2.2 ± 0.4	1.8 ± 0.2	737 ± 101	689 ± 27	545 ± 90
M. thermoautotrophicum	−	4.0 ± 0.6	1.5 ± 0.8	0.0 ± 0.0	1888 ± 160	1125 ± 232	57 ± 37
	+	4.5 ± 0.6	2.5 ± 0.3	0.2 ± 0.4	1806 ± 142	1511 ± 281	291 ± 450
M. formicicum	−	1.1 ± 0.3	0.3 ± 0.1	—[b]	845 ± 134	358 ± 44	—
	+	1.3 ± 0.2	1.6 ± 0.2	—	964 ± 185	474 ± 35	—

[a] *M. barkeri*, *M. formicicum*, and *M. thermoautotrophicum* were grown in 158-ml serum bottles which contained 22 ml of medium and 2 atm (ca. 203 kPa) H_2-CO_2 (80:20). The cultures were incubated in the dark without shaking. The inoculum size was 0.4 to 0.6 mg of cells per ml. *M. barkeri* and *M. formicicum* were incubated in PBBM at 37°C for 11 and 7 days, respectively. *M. thermoautotrophicum* was incubated in LPBM at 65°C for 3 days. Acetate was included where indicated at a final concentration of 20 mM. The results are expressed as mean values of triplicate cultures.

[b] —, Not tested.

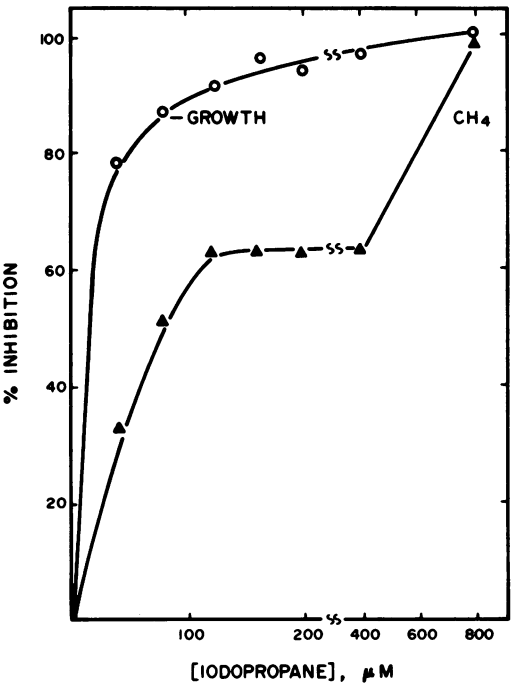

FIG. 1. *Effect of iodopropane on inhibition of one-carbon metabolism in M. barkeri. M. barkeri was grown in 158-ml serum vials that contained 22 ml of PBBM, 100 mM methanol, and 2 atm H_2-CO_2 (80:20). Cultures were incubated in the dark for 5 days at 37°C. The values are expressed as the mean values of triplicate cultures; 0% inhibition represents 6.1 mg of cell dry weight increase or 1,310 μmol of methane for each experiment.*

of methanogenesis observed was 0, 10, or 29%, respectively.

Determination of inhibition versus iodopropane concentration curves enabled a comparison of the affects of iodopropane on the metabolism of H_2-CO_2, H_2-CO_2-methanol, methanol, or acetate. The concentrations of iodopropane which caused 50% inhibition of growth and methane production for these substrates are given in Table 2. Growth and methane production were the least sensitive to iodopropane inhibition with H_2-CO_2-grown cells and most sensitive with acetate-grown cells. The widest difference between iodopropane concentrations required to inhibit growth and methanogenesis was observed with H_2-CO_2-methanol-grown cells. In parallel experiments, cell growth on H_2-CO_2, H_2-CO_2-methanol, or methanol was not inhibited when 20 mM acetate was present in the medium with 80 μM iodopropane.

The specificity of acetate in preventing the inhibition by iodopropane of the one-carbon metabolism of *M. barkeri* is shown in Table 3. The organic compounds tested were selected on the basis of similarity to acetate as possible precursors of acetate or as possible products of iodopropane-sensitive reactions. It is not known whether the compounds tested (other than acetate) are significantly incorporated by *M. barkeri*. Aside from acetate none of these organic compounds (which included yeast extract) reversed the metabolic inhibition by iodopropane.

The effect of bright light on H_2-CO_2-methanol metabolism by *M. barkeri* is shown in Table 4. Growth and methanogenesis were inhibited in the presence of continuous illumination. The

TABLE 2. *Iodopropane concentrations causing 50% inhibition of M. barkeri metabolism*[a]

Energy source	Iodopropane concn (μM) required to inhibit growth	Iodopropane concn (μM) required to inhibit CH_4 production
H_2-CO_2	112 ± 6	232 ± 67
H_2-CO_2-methanol	24 ± 2	164 ± 62
Methanol	63 ± 11	101 ± 9
Acetate	4 ± 1	14 ± 5

[a] *M. barkeri* was grown on one-carbon compounds in 158-ml serum bottles that contained 22 ml of PBBM and 2 atm H_2-CO_2 (80:20), 100 mM methanol, or both, as indicated. *M. barkeri* was grown on acetate in complex medium that contained 1% acetate. Cultures were incubated in the dark at 37°C for 11, 5, 10, and 10 days for H_2-CO_2, H_2-CO_2-methanol, methanol, and acetate, respectively. Data represent the mean values of duplicate inhibition level determinations calculated from inhibition versus iodopropane concentration curves.

TABLE 3. *Effect of organic additions on iodopropane-dependent inhibition of M. barkeri metabolism*[a]

Additions[b]	Growth (mg [dry wt])	Methane formation (total μmol)
Control (with iodopropane)	0.8	195
Control (no iodopropane)	5.7	895
Acetate	5.8	663
Formate	0.5	206
Pyruvate	0.7	251
Succinate	0.4	121
Yeast extract	0.6	213

[a] *M. barkeri* was grown in 158-ml serum vials that contained 22 ml of PBBM 100 mM methanol, 400 μM iodopropane, and 2 atm H_2-CO_2 (80:20). All additions, except yeast extract (0.5%), were at 20 mM. The vials were incubated in the dark at 37°C for 5 days. The results are expressed as the mean value of duplicate vials. Inoculum accounted for 0.5 mg of the cells present. Controls lacked organic additions.

[b] Other compounds tested that also had no effect were: cysteine, isocitrate, glycine, aspartate, methionine, glutamate, alanine, malate, citrate, glycollate, glyoxylate, glucose, ethanol, and butyrate.

presence of acetate increased growth and methanogenesis. Succinate was added as a control to insure that acetate was not scavenging a free radical or acting in some non-physiological manner. Small changes in the method of incubation under light (i.e., bottles standing upright or incubated on their sides) produced different effects. Hence, differential effects of light intensities were not investigated.

TABLE 4. *Effect of light on the H_2–CO_2–methanol metabolism of M. barkeri*[a]

Conditions	Growth (mg [dry wt] increase)	Methane formation (total μmol)
Dark	7.0	1,496
Light	4.3	1,047
Light plus acetate	6.5	1,273
Light plus succinate	2.7	903

[a] *M. barkeri* was grown in 158-ml serum bottles that contained 22 ml of PBBM, 100 mM methanol, 2 atm H_2–CO_2 (80:20), and 20 mM acetate or succinate where indicated. The cultures were incubated in bright light (105 microeinsteins per m^2 per s 0.1 W/m^2, and 10 lux) for 6 days at 37°C. The bottles were incubated in an upright position under the lights with the intensity measured at the top of the bottles.

Effects of iodopropane on the metabolic fates of CO_2 and methanol. The effects of acetate and iodopropane on the one-carbon metabolism of *M. barkeri* were investigated by using ^{14}C tracers (Table 5). The percentage of the methane derived from methanol when acetate and iodopropane were absent was about 90%, with the remainder coming from carbon dioxide. The amount of methane derived from CO_2 and methanol did not change when acetate was present, and acetate was not significantly metabolized to methane. A trend was observed for increased contribution of CO_2 to methane with increasing iodopropane. The methanol contribution to methane decreased with increasing iodopropane. When acetate and iodopropane were both added, this same trend was observed, but it was diminished in magnitude.

The percent of the cells derived from the various ^{14}C labels was not greatly influenced by iodopropane. CO_2 contributed to 65% and methanol contributed to 35% of the cell carbon in the absence of acetate. Approximately 50% of the cell carbon was derived from acetate when it was added to the medium. The contribution of acetate to cell carbon did not change with iodopro-

TABLE 5. *Effect of acetate and iodopropane on one-carbon metabolism of M. barkeri*[a]

Additions to growth medium	^{14}C label	SR of CH_4 (dpm/μmol)	Methane derived from label (%)[b]	SR of cells (dpm/μmol of carbon)[c]	Cells derived from label (%)[d]
None	CO_2	822	9.03	3,735	65.0
	CH_3OH	2,417	90.97	590	35.0
20 mM acetate	CO_2	824	8.51	1,975	36.0
	CH_3OH	2,349	90.87	195	13.5
	Acetate[e]	10	0.62	230	50.8
80 μM iodopropane	CO_2	2,493	20.49	1,930	62.0
	CH_3OH	2,060	79.51	250	38.0
80 μM iodopropane	CO_2	1,093	11.08	1,810	38.7
20 mM acetate	CH_3OH	2,364	88.47	100	7.9
	Acetate[e]	8	0.45	300	53.4
400 μM iodopropane	CO_2	5,045	35.32	3,295	79.0
	CH_3OH	1,887	64.68	180	21.0
400 μM iodopropane	CO_2	1,751	15.21	1,880	37.0
20 mM acetate	CH_3OH	2,549	84.32	180	13.3
	Acetate[e]	9	0.47	300	49.8

[a] *M. barkeri* was grown in 158-ml serum vials that contained 22 ml of PBBM, 100 mM methanol, and 2 atm H_2–CO_2 (80:20). Cultures were incubated in the dark for 5 days at 37°C. Acetate was present where indicated at a final concentration of 20 mM. The specific radioactivities (SR) of methanol and acetate were 2,680 and 1,670 dpm/μmol, respectively. The specific radioactivity of CO_2 was determined for each individual vial with an average specific radioactivity of 10,870 ± 1,600 dpm/μmol of CO_2. The results are expressed as mean values of duplicate vials.

[b] Calculated as: $100 \times \dfrac{\text{SR of methane}}{\text{SR of substrate}} \Big/ \Sigma \dfrac{\text{SR of methane}}{\text{SR of substrate}}$

[c] Values represent: $\dfrac{\text{dpm}}{\text{mg of cells}} \times \dfrac{\text{mg of cells}}{0.45\text{ mg of carbon}} \times \dfrac{0.012\text{ mg of carbon}}{\mu\text{mol of carbon}}$

[d] Calculated as: $100 \times \dfrac{\text{SR of cells}}{\text{SR of substrate}} \Big/ \Sigma \dfrac{\text{SR of cells}}{\text{SR of substrate}}$

[e] Uniformly labeled.

pane even though the presence of acetate allowed greater growth. The percent contribution of both methanol and CO_2 to cell carbon decreased when acetate was included. When these results are compared with those found under conditions without acetate, it is apparent that acetate replaces carbon from both CO_2 and methanol.

DISCUSSION

These data clearly indicate that growth and methanogenesis have metabolic steps which are very sensitive to iodopropane and light. The high levels of corrinoids previously demonstrated in methanogens (13, 20) indicated that corrinoids fulfilled important metabolic functions. The present studies suggest corrinoid involvement in acetate synthesis from one-carbon compounds and acetate catabolism. The specific biochemical reaction(s) that iodopropane inhibits must be interpreted with caution and needs documentation at the biochemical level with detailed ^{14}C tracer and enzymological studies. In addition to inhibiting corrinoid dependent reactions, iodopropane and light may interact with a variety of other metabolic steps, especially at high concentrations. For example, propyl CoM should be formed by cells in the presence of reducing conditions, iodopropane, and CoM. Most notably, *M. ruminantium* requires CoM as a growth factor, but is unable to utilize propyl CoM as a vitamin (2), and propyl CoM is not metabolized by cell extracts of *M. thermoautotrophicum* (11).

Growth of all three methanogenic species examined here on H_2–CO_2 was inhibited by iodopropane. The addition of acetate to the cultures significantly prevented growth inhibition by this corrinoid antagonist. Acetate also reversed the light-dependent inhibition of the growth of *M. barkeri*. The data clearly indicate that the observed growth inhibition was an anabolic effect, because *M. formicicum* and *M. thermoautotrophicum* do not significantly catabolize acetate (32, 35), and *M. barkeri* requires an extended adaptation period on acetate before its catabolic consumption (26). These findings suggest the hypothesis that methanogens synthesize acetate or acetyl CoA from one-carbon compounds via corrinoid-dependent reactions.

The biochemical reactions that account for the synthesis of a two-carbon compound from CO_2 or CH_3OH (or both) in methanogens have not been described. The involvement of corrinoids in bacterial acetate synthesis from CO_2 has only been demonstrated in *C. thermoaceticum* (9, 14). The speculative scheme shown in Fig. 2 is presented to indicate how the data presented here are consistent with the hypothesized involvement of corrinoids in methanogen anabolism. This speculative scheme suggests that anabolic and catabolic reactions of methanogen one-carbon metabolism are unified by common intermediates (33); however, the data at hand are also consistent with totally separate pathways for methane formation and cell carbon synthesis. The ^{14}C tracer studies clearly demonstrated that acetate addition to cells grown on H_2–CO_2–methanol greatly diminished the contribution of CO_2 and methanol to cells but iodopropane addition did not significantly alter the distribution of label incorporated. Hence, acetate appears as a direct precursor to cell carbon, and its synthesis from one-carbon compounds is iodopropane sensitive. In addition, the relief of growth inhibition by corrinoid antagonists appeared absolutely specific for acetate.

At present (28, 33), one-carbon metabolism in methanogens is thought to proceed via one-carbon carriers (i.e., as indicated by X on Fig. 2).

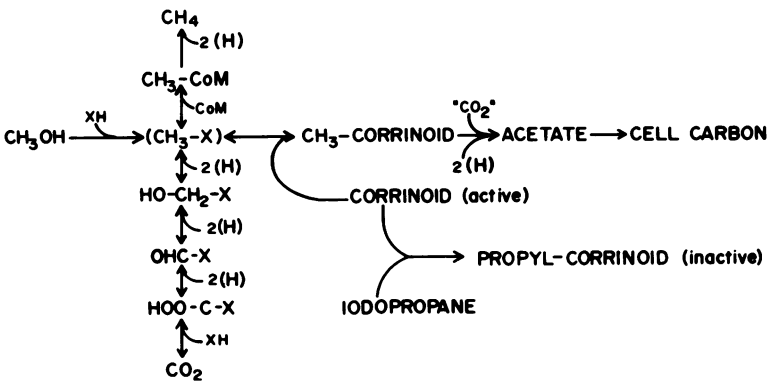

FIG. 2. *Speculative scheme for iodopropane inhibition of cell carbon synthesis in methanogens. X represents an unknown carrier. More than one carrier may exist at any bound one-carbon oxidation state. X-COOH may represent coenzyme YFC (6), a unique carboxylated pteridine (G. Vogels, personal communications).*

The individual carriers involved at the different carbon oxidation states remain to be characterized. Nonetheless, at the methyl level both CH_3-CoM and CH_3-B_{12} are converted to methane in cell extracts (16, 23) and a methyl transferase activity readily converts CH_3-B_{12} and CoM (16, 19). The data here indicate that cell carbon synthesis from one-carbon compounds is more sensitive to iodopropane inhibition than is methanogenesis. This inhibition is consistent with inactivation of acetate synthesis via corrinoid propylation in the absence of significant inhibition of methanogenesis. This result is to be expected if the terminal methyl reductase of methanogenesis from one-carbon compounds involves CoM and not CH_3-B_{12} (11).

Corrinoid antagonists also notably inhibited methanogen catabolism. Most notably, acetate catabolism of *M. barkeri* was exceptionally sensitive to low concentrations of iodopropane. This result provides evidence suggestive of an important role of corrinoids in acetate catabolism. In addition, methanol conversion to methane was more sensitive to iodopropane inhibition than was H_2-CO_2 metabolism. The previous studies of Stadtman and Blaylock (20, 21) suggested a major function for corrinoids in methanogenesis from methanol and acetate by *M. barkeri*. In view of the discussion above, the specific biochemical functions of corrinoids warrant further attention in all methanogenic species.

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