JOURNAL OF BACTERIOLOGY, May 1978, p. 668-670
0021-9193/78/0134-0668$02.00/0

Vol. 134, No. 2
Printed in U.S.A.

NOTES

Tetrahydrofolate Enzyme Levels in *Acetobacterium woodii* and Their Implication in the Synthesis of Acetate from CO_2

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Received for publication 5 December 1977

Acetate synthesis from CO_2 by *Acetobacterium woodii* may occur as in homoacetate-fermenting clostridia, as indicated by high levels of enzymes of the tetrahydrofolate pathway and by pyruvate-dependent formation of acetate from methyl-B_{12} and methyltetrahydrofolate.

Several species of bacteria synthesize acetate from CO_2 (18, 21). The pathway of this synthesis has been investigated in *Clostridium thermoaceticum* (2) and *C. formicoaceticum* (13). These clostridia perform a homoacetate fermentation of sugars, and neither organism produces or uses molecular hydrogen. Electrons generated during the fermentation are accepted by CO_2, which is reduced to acetate. The reduction of CO_2 to a methyl group occurs with formate, 10-formyl-H_4folate, 5,10-methenyl-H_4folate, 5,10-methylene-H_4folate, and 5-methyl-H_4folate as intermediates (2). The methyl group of 5-methyl-H_4folate is most likely transferred to a corrinoid and is subsequently carboxylated in a transcarboxylation reaction with pyruvate to yield acetate (17).

Acetobacterium woodii, which was recently described (3), performs homoacetate fermentation of fructose and, in addition, grows autotrophically, reducing CO_2 in the presence of H_2 to acetate. It is possible that *A. woodii* uses a pathway similar to that of saccharolytic clostridia for acetate synthesis. We wish to report the levels of enzymes of this pathway in extracts of *A. woodii*. Previously it has been demonstrated that such extracts are able to form acetate from CO_2 and H_2 (16).

A. woodii was mass-cultured, by the method of Balch et al. (3), either on fructose (N_2 atmosphere) or on an H_2-CO_2(80:20) gas mixture in basal medium supplemented with 2 g of Trypticase per liter of medium. Cells were harvested in the stationary phase with a continuous-flow Sharples centrifuge. Cells were suspended in 1 ml of *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.1) per g (wet weight) and stored at −20°C under an H_2 atmosphere until used.

Extracts of *A. woodii* were prepared by passing cell suspensions through a French pressure cell at 16,000 lb/in^2. A broken-cell suspension was centrifuged under an H_2 gas phase at 30,000 × *g* for 30 min at 5°C. The resulting supernatant solution was assayed at 30°C for formate dehydrogenase, formyl-H_4folate synthetase (EC 6.3.4.3), methylene-H_4folate dehydrogenase, and methenyl-H_4folate cyclohydrolase (EC 3.5.4.9) (modified by following the decrease in absorbancy at 350 nm) as described by O'Brien and Ljungdahl (13). Formation of acetate from [*methyl*-^{14}C]methylcobalamin and [*methyl*-^{14}C]methyl-H_4folate was investigated as described by Ghambeer et al. (7), except that the assays were performed at 30°C. Protein in extracts was estimated by measuring the turbidity at 400 nm of a sample in 20% trichloroacetic acid. Protein in whole cells was determined as described by Strickland (20). Bovine serum albumin was used as the standard. Corrinoids were extracted from whole cells by the method of Bernhauer et al. (4), converted to the dicyano form, and quantitated by absorbancy at 580 nm (molar extinction coefficient = 10.6×10^3). This method lacks sensitivity for determining low levels of corrinoids from whole cells.

H_4folate, 5,10-methenyl-H_4folate, and 5,10-methylene-H_4folate were prepared as described by O'Brien and Ljungdahl (13). [*methyl*-^{14}C]methylcobalamin was synthesized as described by Mervyn and Smith (12). [*methyl*-^{14}C]methyl-H_4folate was prepared as described by Blair and Saunders (5).

ATP, nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, bovine serum albumin, folic acid, coenzyme A, and

TABLE 1. *Levels of certain enzymes in crude extracts of some acetate-producing organisms*

Organism	Growth conditions	Formate dehydrogenase[a] units[b]	Formyl-H_4folate synthetase units	Methenyl-H_4folate cyclohydrolase units	Methylene-H_4folate dehydrogenase	
					Units	Electron acceptor[c]
A. woodii	$H_2 + CO_2$	0.11–0.17	9.01–12.7	0.59–1.07	0.59–1.27	NAD
A. woodii	Fructose + N_2	2.95–3.91	8.8–9.2	0.24–0.36	0.36–0.81	NAD
C. formicoaceticum[d]	Fructose + CO_2	0.15	8.7	0.15	8.1	NAD
C. thermoaceticum[e]	CO_2 + fructose, glucose, or xylose	2.63[f]	12.5	1.3	1.8	NADP

[a] Methyl viologen was used as the electron acceptor.
[b] Units are given as micromoles of substrate used or product formed per minute per milligram of protein.
[c] NAD, Nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate.
[d] O'Brien and Ljungdahl (13).
[e] Andreesen et al. (2).
[f] Andreesen and Ljungdahl (1).

cyanocobalamin were purchased from Sigma Chemical Co. *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid was purchased from Calbiochem. All other chemicals were of analytical grade and were from different sources.

The levels of formate dehydrogenase and the three H_4folate enzymes in extracts of *A. woodii* are presented in Table 1. The levels of these enzymes in *A. woodii* are comparable with those found in extracts of *C. thermoaceticum* and *C. formicoaceticum*. These values are 100 to 1000 times higher than those reported for *Saccharomyces cerevisiae* (9, 15) and *Escherichia coli* (6, 8, 19). The high levels of these enzymes indicate their probable importance in cell metabolism and suggest that *A. woodii* shares a common pathway with the two homoacetate-fermentative clostridia.

Table 2 shows the results of the synthesis of acetate from [*methyl*-^{14}C]methylcobalamin and [*methyl*-^{14}C]methyl-H_4folate by extracts of *A. woodii* grown on an H_2-CO_2 gas mixture. The conversion of these substrates to acetate in extracts of *A. woodii* is not as efficient as in extracts of *C. thermoaceticum*. Still, the acetate formation by extracts of *A. woodii* is dependent on the presence of pyruvate and coenzyme A. A requirement for pyruvate in these reactions has been demonstrated for *C. thermoaceticum* (14, 18) and for *C. formicoaceticum* (13). A requirement for coenzyme A for the incorporation of [*methyl*-^{14}C]methylcobalamin has been shown for *C. thermoaceticum* (14). Results of preliminary experiments with extracts of *A. woodii* prepared from fructose-grown cells also indicate a need for coenzyme A and pyruvate for the synthesis of acetate from [*methyl*-^{14}C]methylcobalamin in this assay system. The requirements for acetate formation, particularly that of pyruvate, are indicative of a similar reaction in *A. woodii* and in *C. thermoaceticum*.

TABLE 2. *Conversion of the methyl-^{14}C moiety of [methyl-^{14}C]methyl-H_4folate and [methyl-^{14}C]-methylcobalamin into acetate by cell extracts of A. woodii grown on H_2 plus CO_2*

methyl-^{14}C donor	Reaction system[a]	dpm of *methyl*-^{14}C added	dpm of acetate formed
CH_3-B_{12}	Complete incubation mixture	333,900	15,550
CH_3-B_{12}	– Pyruvate	333,900	1,100
CH_3-B_{12}	– Dithiothreitol	333,900	14,910
CH_3-B_{12}	– Ferrous ammonium sulfate	333,900	16,360
CH_3-B_{12}	– Coenzyme A	333,900	3,350
CH_3-H_4folate	Complete incubation mixture	49,700	1,340
CH_3-H_4folate	– Pyruvate	49,700	30

[a] The complete incubation mixture contained (in a volume of 1 ml) sodium pyruvate (30 μmol), dithiothreitol (10 μmol), ferrous ammonium sulfate (5 μmol), coenzyme A (3.3 μmol), [*methyl*-^{14}C]methylcobalamin (0.285 μmol) or [*methyl*-^{14}C]-methyl-H_4folate (0.85 μmol), and cell extract (15 mg of protein). The incubation was for 10 min under N_2 at 30°C. In the case of [*methyl*-^{14}C]methyl-H_4folate, the *dl* form was used, of which half was available as substrate.

Table 3 compares the levels of total corrinoids in the acetate-producing bacteria and in some non-homoacetate-fermentative organisms. There is evidence for the involvement of corrinoids in acetate synthesis in *C. thermoaceticum* (7, 10, 11). Whole cells of this bacterium, *C. formicoaceticum*, and *A. woodii* all contain levels of corrinoids about 100 times higher than those found in the non-homoacetate-fermentative organisms listed in the table.

The results presented here indicate similarities between *A. woodii* and the homoacetate-fermentative clostridia in that they possess high levels of H_4folate enzymes and high levels of corrinoid. Furthermore, extracts of all three bacteria catalyze pyruvate-dependent conversion of

TABLE 3. *Levels of corrinoids in whole cells of some homoacetate-fermentative bacteria and some non-homoacetate-fermentative bacteria*[a]

Bacterium	μmol of corrinoid per gram of whole-cell protein
Acetobacterium woodii (H_2 + CO_2)	0.64–0.94
A. woodii (Fructose + N_2)	1.21–1.36
Clostridium formicoaceticum	0.43–0.65
C. thermoaceticum	0.44–0.63
Pseudomonas putida	<0.01
Escherichia coli	<0.01
Bacillus alvei	0.02
C. sporogenes	0.02

[a] *P. putida* was a gift from I. C. Gunsalus; *E. coli* B was a gift from R. Gennis; *B. alvei* and *C. sporogenes* were gifts from R. DeMoss; *C. formicoaceticum* and *C. thermoaceticum* were grown, as described earlier (2, 13), in media containing 10^{-6} M selenite, 10^{-5} M molybdate, and 10^{-5} M tungstate.

[*methyl*-^{14}C]methylcobalamin to acetate. The results suggest that the synthesis of acetate in *A. woodii* is similar to that demonstrated in *C. thermoaceticum*. In autotrophically grown *A. woodii*, the reduction of CO_2 to acetate provides a system that is free from alternate carbon sources, in studies of acetate synthesis from CO_2.

This work was supported by the National Science Foundation (grant PCM 76-02652) at the University of Illinois, and by the Energy Research Development Administration [contract E (38-1)-888] and the Public Health Service (grant AM-12913 from the National Institute of Arthritis, Metabolism and Digestive Diseases) at the University of Georgia. R. S. Tanner is the recipient of a predoctoral fellowship from the National Science Foundation.

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