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Sodium Dependence of Acetate Formation by the Acetogenic Bacterium *Acetobacterium woodii*

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Growth of *Acetobacterium woodii* on fructose was stimulated by Na^+; this stimulation was paralleled by a shift of the acetate-fructose ratio from 2.1 to 2.7. Growth on H_2-CO_2 or on methanol plus CO_2 was strictly dependent on the presence of sodium ions in the medium. Acetate formation from formaldehyde plus H_2-CO by resting cells required Na^+, but from methanol plus H_2-CO did not. This is analogous to H_2-CO_2 reduction to methane by *Methanosarcina barkeri*, which involves a sodium pump (V. Müller, C. Winner, and G. Gottschalk, Eur. J. Biochem. 178:519–525, 1988). This suggests that the reduction of methylenetetrahydrofolate to methyltetrahydrofolate is the Na^+-requiring reaction. A sodium gradient (Na^+_{out}/Na^+_{in} = 32, ΔpNa = −91 mV) was built up when resting cells of *A. woodii* were incubated under H_2-CO_2. Acetogenesis was inhibited when the ΔpNa was dissipated by monensin.

Many acetogenic and methanogenic bacteria grow and hence are able to gain energy by the reduction of CO_2 with H_2 to acetate and methane, respectively. Methanogens require sodium ions for growth and methane formation (22), and evidence has been provided that methylene-tetrahydromethanopterin reduction is coupled to the extrusion of Na^+ (20). Thus, a primary sodium pump is operative, and the transmembrane electrochemical sodium gradient established can be utilized by the organisms for their energy expenditures, e.g., for solute transport (13), pH regulation under acidic conditions (24), or ATP synthesis via a secondary proton gradient (20). The energy metabolism of acetogenic bacteria fermenting H_2 plus CO_2 to acetate is still obscure. As is apparent from Fig. 1, 1 ATP is converted to ADP + P_i in the formyl-tetrahydrofolate synthetase reaction, and 1 ATP is formed in the final step of acetogenesis. The ATP balance from substrate level phosphorylation is therefore zero. Clearly, additional mechanisms for energy conservation must be present. In this context, the discovery of an Na^+/H^+ antiporter in *Clostridium thermoaceticum* by Terraciano et al. (26) was important. Because of this and the analogy of the two methylene-group reduction reactions methylenetetrahydromethanopterin + H_2 → methyltetrahydromethanopterin ($\Delta G^{\circ\prime}$ = −20 kJ/reaction [15]) and methylenetetrahydrofolate + H_2 → methyltetrahydrofolate ($\Delta G^{\circ\prime}$ = −57.3 kJ/reaction [8, 27]), it was hypothesized than an Na^+ gradient would be also generated in acetogens (10). Such a gradient would allow these organisms to produce additional ATP during acetogenesis from H_2 plus CO_2. As a typical acetogen, we studied *Acetobacterium woodii* (1), and we show here that it requires Na^+ for acetogenesis and that it generates an Na^+ gradient across the cytoplasmic membrane.

(Preliminary results of this study were presented at the Annual Meeting of the Vereinigung für Allgemeine and Angewandte Mikrobiologie [R. Heise, V. Müller, and G. Gottschalk, Forum Mikrobiol. 12:54, 1989].)

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MATERIALS AND METHODS

Strains and growth of organisms. *Acetobacterium woodii* (DSM 1030) was obtained from the German Collection of Microorganisms (DSM), Braunschweig, Federal Republic of Germany. The medium was prepared according to the anaerobic techniques of Hungate (12) as modified by Bryant (4). The medium contained (in grams per liter): KH_2PO_4, 0.2; NH_4Cl, 0.25; NaCl, 1.16; $MgSO_4 \cdot 7H_2O$, 1.45; $CaCl_2$, 0.11; KCl, 0.50; yeast extract, 2.0; plus trace element solution SL 9 and selenite-tungstate solution (28), 1 ml/liter each; and vitamin solution DSM 141, 20 ml/liter. The vitamin solution contained (in milligrams per liter): biotin, 2.0; folic acid, 2.0; pyridoxine hydrochloride, 10.0; thiamine hydrochloride, 5.0; riboflavine hydrochloride, 5.0; nicotinic acid, 5.0; DL-calcium panthothenate, 5.0; vitamin B_{12}, 0.1; *p*-aminobenzoic acid, 5.0; and lipoic acid, 5.0. The medium was gassed with either N_2-CO_2 (80:20, vol/vol) or H_2-CO_2 (80:20, vol/vol). The pH was adjusted to 7.0 to 7.1 with solid $KHCO_3$ (6 g/liter). The sterilized medium was reduced just before inoculation with $Na_2S \cdot 9H_2O$ (0.15 g/liter) and cysteine hydrochloride (0.3 g/liter). Energy sources were fructose (20 mM), methanol (12 mM), or H_2-CO_2. Growth experiments were done in either 500-ml (H_2-CO_2 as substrate) or 250-ml (fructose or methanol as substrate) bottles with 50 ml of medium. Growth was followed by measuring the A_{600}.

Preparation of cell suspensions. For resting-cell experiments with methanol, the organisms were grown on 12 mM methanol. In all other cases, they were grown on 20 mM fructose. Cultures were harvested at the end of the exponential growth phase by centrifugation (23,500 × *g*, 20 min, 4°C) under anaerobic conditions and washed twice with 20 mM imidazole–HCl buffer, pH 6.7, containing 50 mM KCl, 5 mM $MgSO_4$, and 6 mM dithiothreitol (DTT). The cells were resuspended in the same buffer to a final protein concentration of 15 to 20 mg/ml (growth on methanol) or 25 to 30 mg/ml (growth on fructose) under an atmosphere of nitrogen. This suspension was used immediately for the experiments. The protein concentration of the cell suspension was determined by the method of Schmidt et al. (23). All manipulations were done under strictly anaerobic conditions in an anaerobic chamber (M. D. H. GmbH, Rüsselsheim, Federal Republic of Germany).

Experiments with cell suspensions. The experiments with

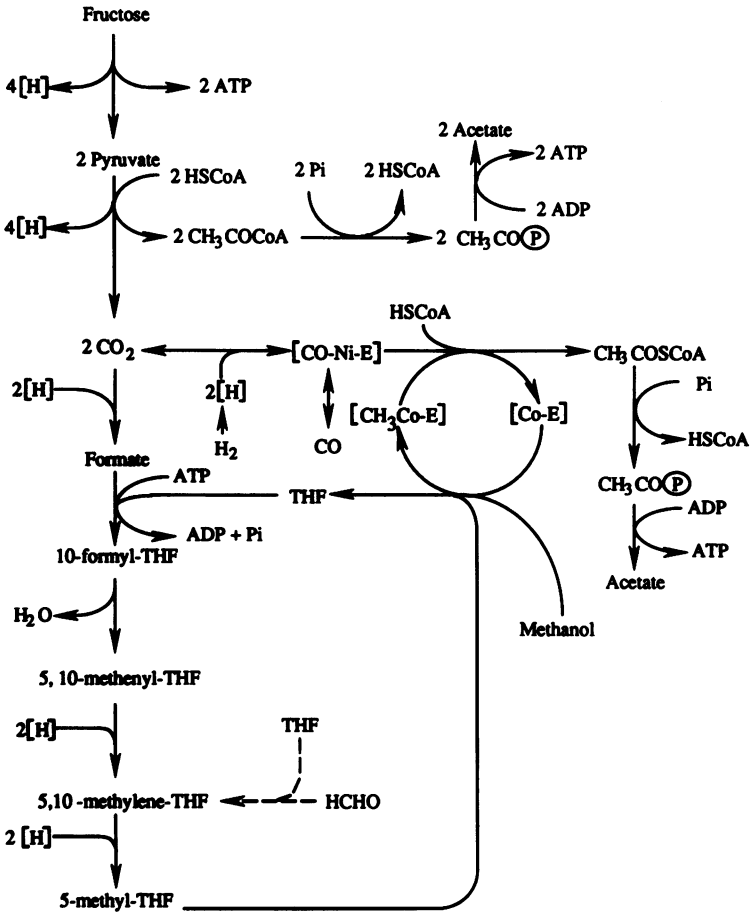

FIG. 1. Tentative scheme for the fermentation of fructose, methanol, and H_2-CO_2 by *A. woodii*. Abbreviations: HSCoA, coenzyme A; $CH_3CO(P)$, acetylphosphate; $CH_3COSCoA$, acetyl-CoA; CH_3Co-E, methylcorrinoid protein; CO-Ni-E, enzyme-bound CO; THF, tetrahydrofolic acid.

cell suspensions were performed in 115-ml bottles; they contained, in a final volume of 10 ml, 50 mM imidazole buffer, 20 mM KCl, 5 mM $MgSO_4$, 6 mM DTT, and cells as given in the figure legends. The buffer was adjusted to pH 7 by titration with HCl for all experiments without CO_2 in the gas phase and by gassing with N_2-CO_2 (80:20, vol/vol) for all those experiments which contained CO_2 in the gas phase. NaCl, fructose, methanol, or formaldehyde was added as indicated for each experiment. Incubations were done at 30°C on a rotary shaker (70 rpm) under an atmosphere of N_2-CO_2 (80:20, vol/vol; 111 kPa), H_2-CO_2 (80:20, vol/vol; 111 kPa), or H_2-CO (92:8, vol/vol; 121 kPa). To determine H_2 formation from CO, the experiments were performed in 58-ml bottles under an atmosphere of N_2 (111 kPa). Additions were made as indicated. Monensin was added as an ethanolic solution; the control received only the solvent. The formaldehyde solution was prepared by the method of Blaut et al. (3). The experiments were started by the injection of 0.3 to 0.5 ml of the concentrated cell suspension. At the times indicated, samples (0.4 ml) were withdrawn by syringe and centrifuged, and the supernatant was analyzed for acetate and fructose by enzymatic assays (2, 6). Rates of acetate formation were calculated from sampling periods of at least 45 min. H_2 was measured by gas chromatography (17). The external sodium concentration was determined with a sodium electrode (Orion Research AG, Küsnacht, Switzerland) connected to an ion meter (Orion Research AG).

Measurements of Na^+ movements. To determine the substrate-dependent movement of Na^+ across the membrane, cell suspensions in the above imidazole buffer plus 20 mM NaCl (final volume, 1.6 ml) were incubated in 8-ml bottles under an atmosphere of H_2-CO_2 (80:20, vol/vol; 127 kPa) or N_2-CO_2 (80:20, vol/vol; 127 kPa) on a rotary shaker for 10 min at 30°C. At time zero, 2 μl of carrier-free $^{22}NaCl$ (0.7 μCi/μl) was added. Samples (100 μl) were withdrawn by syringe as indicated in the legend to Fig. 4, transferred to membrane filters (25 mm in diameter; pore size, 0.45 μm; Sartorius, Göttingen, Federal Republic of Germany) and washed four times with 1 ml of buffer. The filters were counted in a liquid scintillation counter with 10 ml of Rialuma (J. T. Baker B. V., Deventer, Holland). Unspecific binding of $^{22}Na^+$ on the filter was reduced by overnight preincubation of the filters in 20 mM NaCl. The intracellular Na^+ concentration was calculated after correction for unspecific binding of $^{22}Na^+$ on the filters and with an intracellular volume of 3.2 μl/mg of protein (R. Boenigk, Diploma thesis, Universität Göttingen, 1988).

Chemicals and gases. Imidazole was purchased from Serva, Heidelberg, Federal Republic of Germany. Enzymes were from Boehringer, Mannheim, Federal Republic of Germany. Monensin was purchased from Sigma,

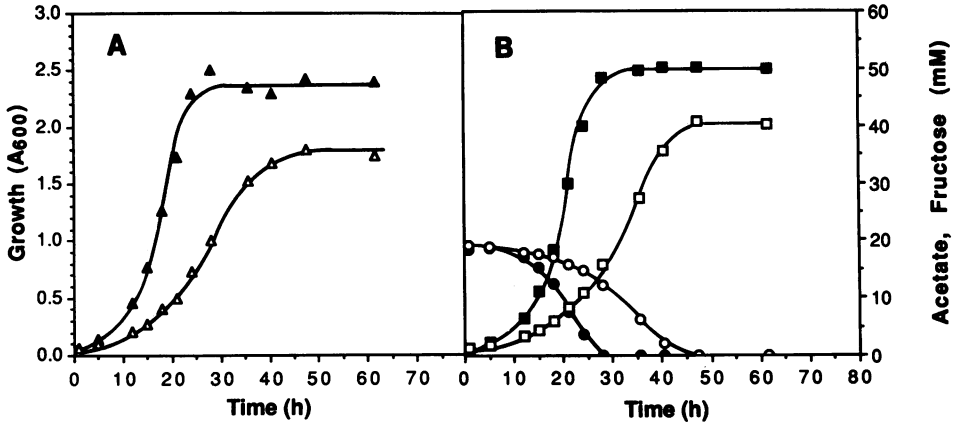

FIG. 2. (A) Growth (▲ and △) of *A. woodii* and (B) effect of NaCl concentration on fructose (○, ●) and acetate (□, ■) formation. A 50-ml amount of medium with 20 mM fructose was inoculated with 2 ml of a log-phase culture of *A. woodii* and incubated without shaking at 30°C. Solid symbols, 20 mM NaCl; open symbols, 0.2 mM NaCl.

Taufkirchen, Federal Republic of Germany. Propyliodide was from Merck, Darmstadt, Federal Republic of Germany. ^{22}NaCl was from New England Nuclear Corp., Dreieich, Federal Republic of Germany. Gases were from Messer-Griesheim, Kassel, Federal Republic of Germany.

RESULTS

Sodium dependence of growth on fructose, H_2-CO_2, or methanol. Cells of *A. woodii* were transferred to medium supplemented with various amounts of NaCl. Growth on fructose under an atmosphere of N_2-CO_2 was stimulated by the presence of NaCl in the medium; the organisms reached a maximal A_{600} of 2.5 at 20 mM NaCl and grew with a doubling time of 4.2 h, whereas an A_{600} of 1.8 and a doubling time of 7.1 h were reached at 0.2 mM NaCl (Fig. 2). The ratio of acetate formed to fructose consumed decreased from 2.7 to 2.1 when the NaCl concentration was lowered from 20 to 0.2 mM. H_2 production was stimulated in the absence of NaCl; 5 and 12 μmol of H_2 were produced from 1 mmol of fructose at 20 and 0.2 mM NaCl, respectively. Small amounts of ethanol were produced (approx. 25 μmol of ethanol at 20 mM and 100 μmol at 0.2 mM NaCl per mmol of fructose). Formate and succinate were not found in detectable amounts. Contrary to growth on fructose, growth on H_2-CO_2 was strictly dependent on the presence of NaCl. At 0.7 mM NaCl, no growth occurred; higher NaCl concentrations resulted in increased growth rates, and the maximum rate was observed at 20 to 30 mM NaCl (Fig. 3). The concentration of Na^+ required for one-half of the maximum growth rate was 6 mM. The minimum doubling time at NaCl concentrations above 20 mM was 5.5 h. A dependence on NaCl similar to that for growth on H_2-CO_2 was observed with methanol as the substrate (data not shown).

Localization of the sodium-dependent step in acetogenesis. To characterize the sodium dependence of growth and acetate formation more precisely, experiments with resting cells were performed. Cell suspensions of *A. woodii* were

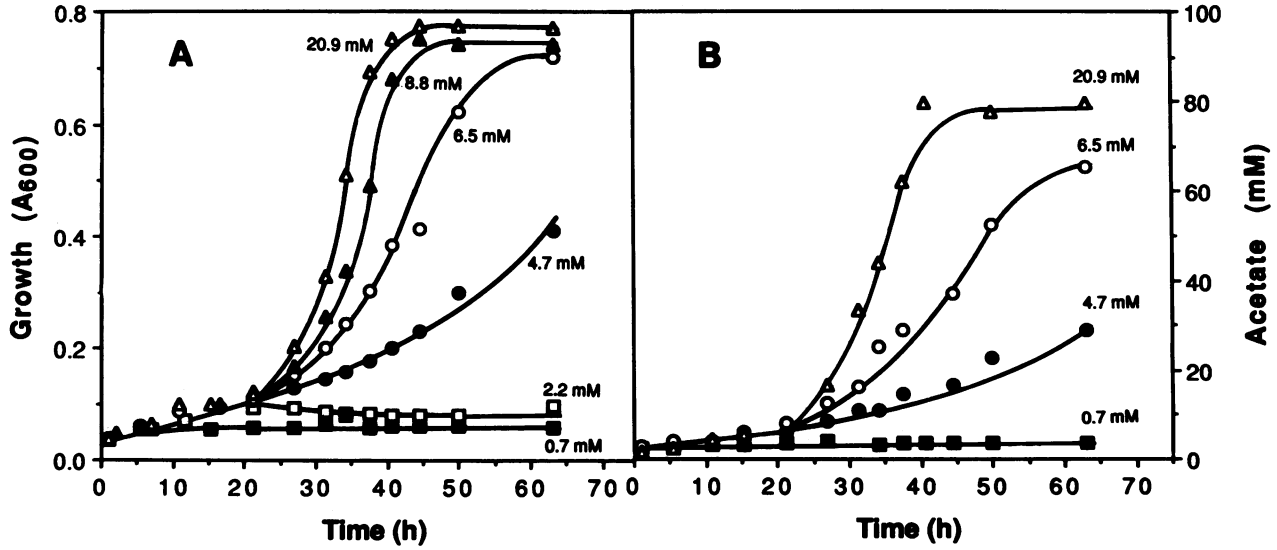

FIG. 3. (A) Growth of *A. woodii* on H_2-CO_2 and (B) acetate formation. Medium (50 ml) containing various concentrations of NaCl under an atmosphere of H_2-CO_2 (101 kPa) was inoculated with 2 ml of a culture of *A. woodii*. The cultures were put on a rotary shaker after 22 h. Consumed gas was replaced at an A_{600} of between 0.3 and 0.4. The concentrations of NaCl are indicated.

TABLE 1. Effect of Na^+ on acetate formation from various substrate combinations

Substrate[a]	Sodium[b]	Acetate formation (nmol/min per mg)
Fructose, N_2-CO_2	+	72.7
	−	51.2
H_2-CO_2	+	181.4
	−	21.6
Methanol, N_2-CO_2	+	37.8
	−	<1
Methanol, H_2-CO	+	54.1
	−	60.9
Formaldehyde, N_2-CO_2	+	10.9
	−	<1
Formaldehyde, H_2-CO	+	29.9
	−	8.5

[a] Concentrations of substrates were: fructose, 10 mM; methanol, 5 mM; formaldehyde, 2.5 mM; H_2-CO_2 (80:20, vol/vol; 111 kPa); N_2-CO_2 (80:20, vol/vol; 111 kPa); H_2-CO (92:8, vol/vol; 121 kPa). The protein content of the final cell suspensions was 0.7 to 1.5 mg/ml.

[b] +, 20 mM NaCl added; −, no sodium salts added and the sodium concentrations determined were lower than 0.2 mM.

incubated in buffer under an atmosphere of H_2-CO_2. Acetate formation started immediately after injection of cells and proceeded with a linear rate for at least 3 h. As was observed with growing cultures, acetate formation was dependent on the presence of NaCl. At 20 mM NaCl, an 8- to 10-fold stimulation of acetate formation was observed compared with the rate without the addition of sodium salts (Table 1). Na_2SO_4 but neither KCl nor choline chloride could substitute for NaCl, indicating that sodium ions are essential for growth as well as for acetate formation. Lithium ions could substitute for sodium ions. The concentration of Na^+ required for one-half of the maximum rate of acetogenesis was 4 mM; the corresponding concentration of Li^+ was approximately 10-fold higher. In contrast to the observed strong Na^+ dependence for H_2-CO_2, acetate formation from fructose was only slightly dependent on sodium ions. The rate of acetate formation in the absence of Na^+ was approximately two-thirds (70.4%) of the maximal rate at 20 mM (Table 1). In view of this difference, Na^+ must be primarily required for autotrophic synthesis of acetate. In order to localize the Na^+-dependent step during autotrophic acetate formation, experiments with various substrate combinations were conducted (Table 1).

A. woodii is able to convert formaldehyde or methanol to acetate in the presence of molecular hydrogen and carbon monoxide or in the presence of carbon dioxide (5). The sodium dependence of these fermentations was studied. Resting cells of *A. woodii* incubated with formaldehyde in the presence of 20 mM NaCl under an atmosphere of H_2-CO produced acetate with a linear rate for 2 h. Without Na^+, very little acetate was formed. Acetate formation from methanol under H_2-CO, however, was sodium independent. When the gas atmosphere was changed to N_2-CO_2, a strict Na^+ requirement was seen with both substrates. An effect of pH caused by CO_2 could be excluded, because the initial pH in the experiments was always 7. With formaldehyde under H_2-CO, *A. woodii* produced more acetate than would be expected from the stoichiometric conversion of formaldehyde plus CO, indicating that carbon monoxide must be converted to additional acetate by disproportionation to H_2 plus CO_2. This coconsumption was not observed with methanol under H_2-CO. From H_2-CO (92:8, vol/vol) alone, no acetate was formed.

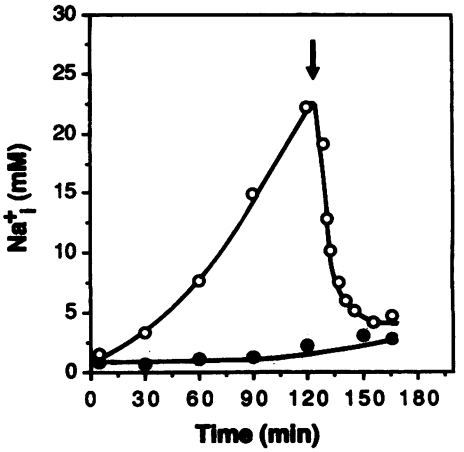

FIG. 4. Sodium extrusion as a result of acetogenesis from H_2-CO_2. One cell suspension (○) was incubated under an atmosphere of N_2-CO_2. At the time indicated by the arrow, 5 ml of H_2 was added. The other cell suspension (●) was incubated under H_2-CO_2. For experimental details, see Materials and Methods. Protein content of the cell suspension: 3.2 mg/ml. Na^+_i, Intracellular sodium concentration.

It has been shown that the reduction of CO_2 to CO as catalyzed by carbon monoxide dehydrogenase is an energy-dependent reaction (5). Therefore, it was of interest to study the effect of Na^+ on this reaction. Resting cells of *A. woodii* were incubated under an atmosphere of N_2 in the presence of 10 mM propyliodide, which is known to inhibit acetate formation from CO (9). After addition of CO (final concentration, 98.8% N_2, 1.2% CO), the cells formed H_2 from CO irrespective of the presence or absence of NaCl at rates between 50 and 60 nmol/min per mg of protein. Acetate formation did not occur. These experiments are not in favor of a possible role of Na^+ as the driving force for CO_2 reduction. This interpretation is supported by the observation that the sodium dependence of acetate formation from methanol under N_2-CO_2 could be overcome by the addition of H_2 (data not shown). Therefore, the sodium dependence must be connected to methanol oxidation which has to occur if no external H donor is available for acetate synthesis from methanol plus CO_2. Acetate formation in the absence of NaCl from methanol plus CO_2 plus H_2 shows unequivocally that the reduction of CO_2 to CO with H_2 does not require Na^+.

Generation of a transmembrane sodium gradient during acetogenesis from H_2-CO_2 and effect of monensin. Cells of *A. woodii* were allowed to produce acetate from H_2-CO_2 in the presence of 20 mM $^{22}NaCl$. Under these conditions, the intracellular Na^+ concentration was lower than 1 mM, so that a transmembrane sodium gradient existed. It varied between 22 and 44, with an average value of 32 (Na^+_{out}/Na^+_{in}; $\Delta pNa = -91$ mV). On the other hand, upon incubation under an atmosphere of N_2-CO_2, the Na^+ gradient slowly disappeared; in the experiment depicted in Fig. 4, the intracellular concentration of Na^+ reached 23 mM after 120 min. Addition of H_2 led to a rapid efflux of Na^+, and after 30 min the original gradient was almost established again (Fig. 4). Analogous results were obtained with fructose as the substrate (data not shown). Addition of 18 μM monensin to a cell suspension in buffer at 20 mM NaCl under H_2-CO_2 immediately dissipated the transmembrane Na^+ gradient (Fig. 5A). At the same time, acetogenesis from H_2-CO_2 at 20

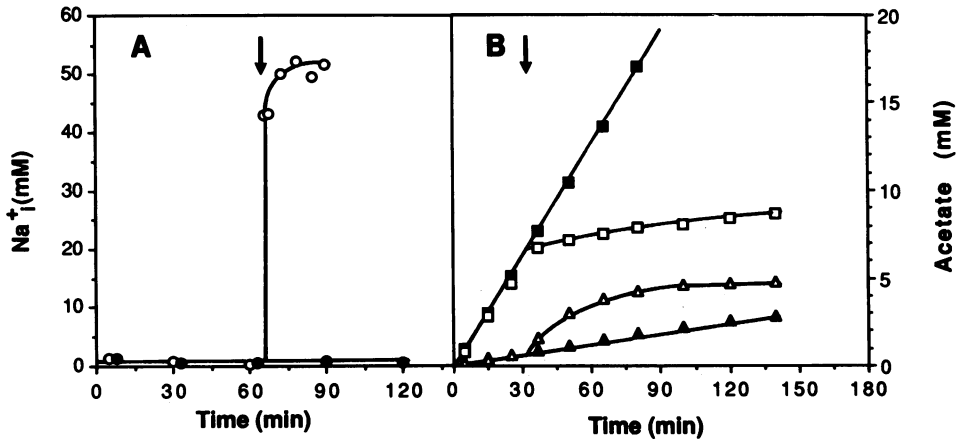

FIG. 5. Effect of monensin on the sodium gradient (A) and on acetate formation (B) of cell suspensions metabolizing H_2-CO_2. In both experiments, addition of monensin (indicated by the arrow; final concentration, 18 μM) is shown by open symbols. No addition is indicated by solid symbols. (A) Cells were incubated at 20 mM NaCl under an atmosphere of H_2-CO_2. (B) Cells were incubated under an atmosphere of H_2-CO_2 at 20 mM NaCl (squares) or 0.2 mM NaCl (triangles). The protein content of the cell suspensions was (A) 2.0 mg/ml or (B) 1.1 mg/ml. Symbols: ●,○, intracellular sodium concentration (Na^+_i); ■,□ and ▲,△, acetate from H_2-CO_2.

mM NaCl was inhibited. At 0.2 mM NaCl, however, acetogenesis was transiently stimulated (Fig. 5B).

DISCUSSION

The catabolic flexibility of *Acetobacterium woodii* allows the reaction site at which sodium ions are required to be narrowed down. When these organisms ferment fructose, Na^+ is only stimulatory. However, growth in low sodium is accompanied by a shift of the acetate-fructose ratio from 2.7 to 2.1. This shift is indicative of a possible role of Na^+ not in glycolysis but in reduction of CO_2 to acetate, a conclusion which is supported by the observed strict dependence on Na^+ of acetate formation from CO_2 plus H_2. A further narrowing of the Na^+-requiring reaction was made possible by the use of formaldehyde and methanol as substrates. Acetate formation from methanol plus H_2-CO was not Na^+ dependent. The fermentation of methanol involves the methylation of a corrinoid protein by a transferase reaction (29), subsequent carbonylation, and formation of acetate via acetyl-coenzyme A and acetyl phosphate (Fig. 1). Apparently none of these reactions require Na^+. However, when methanol is replaced by formaldehyde, an Na^+ requirement was detectable. This indicates that Na^+ plays a role in the conversion of methylenetetrahydrofolate, which is formed from formaldehyde and tetrahydrofolate (14), to methyltetrahydrofolate. The corresponding reductase has recently been shown to be membrane bound (11), which is in accordance with a possible function of this enzyme as a primary sodium pump. Acetogenesis from methanol under N_2-CO_2 was strictly Na^+ dependent. Under these conditions, methyl groups are expected to be oxidized through the same enzymes which are involved in the reductive pathway (16). However, it should be mentioned in this connection that evidence has been obtained for a novel methanol dehydrogenase with *Clostridium thermoautotrophicum* and *Eubacterium limosum* (7), which contains the coenzyme pyrroloquinoline quinone.

A. woodii is able to create a rather steep Na^+ gradient across the cytoplasmic membrane. Intracellular Na^+ has been found to be 20 to 40 times lower than extracellular Na^+. During incubation under N_2-CO_2, this gradient is slowly dissipated, but it is immediately reestablished when the gas atmosphere is replaced by H_2-CO_2. Monensin, which functions as a very effective Na^+/H^+ antiporter, dissipates this gradient and simultaneously inhibits acetogenesis. If the NaCl concentration is high, this inhibition is instantaneous. At low sodium, stimulation of the low rate of acetogenesis is observed first, followed by inhibition. Such a stimulation by monensin at low NaCl concentrations was also seen in methanogenesis from H_2-CO_2 by *Methanobacterium thermoautotrophicum* (25) and *Methanosarcina barkeri* (V. Müller, unpublished results). A possible explanation is that in low Na^+, the processes of methanogenesis and acetogenesis are limited by the availability of intracellular Na^+ for the sodium pump. Na^+ is then brought into the cells by the antiporter activity of monensin. After some time, however, the cytoplasmic membrane is deenergized, probably resulting in a drop of the energy charge so that ATP is not available any more for the ATP-requiring steps of acetogenesis. This conclusion is supported by the fact that inhibition by monensin of acetate formation from formaldehyde under H_2-CO in high sodium is much weaker and the transient stimulation at low sodium is stronger than that observed from H_2-CO_2 (data not shown).

The importance of sodium bioenergetics in methanogenic bacteria was discovered when methanogenesis from methanol by *Methanosarcina barkeri* was studied (18, 21). It was then shown that oxidation of methyl groups to methylene groups is driven by Na^+ influx and that the sodium gradient required for this reaction is generated by an active Na^+/H^+ antiporter (19). When methanogenesis from H_2 plus CO_2 and from H_2 plus formaldehyde was studied, it could be unequivocally shown that the reverse reaction—the reduction of methylene groups to methyl groups—is coupled to sodium extrusion, and it is this reaction which apparently has its parallel in acetogenesis as carried out by *A. woodii*.

Further characterization of the mechanism for sodium extrusion and utilization of the sodium gradients is now in progress in our laboratory.

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LITERATURE CITED

1. **Balch, W. E., S. Schoberth, R. S. Tanner, and R. S. Wolfe.** 1977. *Acetobacterium*, a new genus of hydrogen-oxidizing, carbon dioxide-reducing, anaerobic bacteria. Int. J. Syst. Bacteriol. **27**:355–361.
2. **Bergmeyer, H. U. (ed.).** 1984. Methods of enzymatic analysis, vol. 6, metabolites 1: carbohydrates. Verlag Chemie, Weinheim.
3. **Blaut, M., V. Müller, K. Fiebig, and G. Gottschalk.** 1985. Sodium ions and an energized membrane required by *Methanosarcina barkeri* for the oxidation of methanol to the level of formaldehyde. J. Bacteriol. **164**:95–101.
4. **Bryant, M. P.** 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. **25**:1324–1328.
5. **Diekert, G., E. Schrader, and W. Harder.** 1986. Energetics of CO formation and CO oxidation in cell suspensions of *Acetobacterium woodii*. Arch. Mikrobiol. **144**:386–392.
6. **Dorn, M., J. R. Andreesen, and G. Gottschalk.** 1978. Fermentation of fumarate and L-malate by *Clostridium formicoaceticum*. J. Bacteriol. **133**:26–32.
7. **Duine, J. A., J. J. Frank, J. A. Jongejan, and M. Dijkstra.** 1984. Enzymology of the bacterial methanol oxidation step, p. 91–96. *In* R. L. Crawford and R. S. Hanson (ed.), Microbial growth on C_1 compounds. American Society for Microbiology, Washington, D.C.
8. **Fuchs, G.** 1986. CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol. Rev. **39**:181–213.
9. **Ghambeer, R. K., H. G. Wood, M. Schulman, and L. G. Ljungdahl.** 1971. Total synthesis of acetate from CO_2. III. Inhibition by alkylhalides of the synthesis from CO_2, methyltetrahydrofolate, and methyl-B_{12} by *Clostridium thermoaceticum*. Arch. Biochem. Biophys. **143**:471–484.
10. **Gottschalk, G.** 1989. Bioenergetics of methanogenic and acetogenic bacteria, p. 383–396. *In* H. Schlegel and B. Bowien (ed.), Autotrophic bacteria. Springer-Verlag, Berlin.
11. **Hugenholtz, J., D. M. Ivey, and L. G. Ljungdahl.** 1987. Carbon monoxide-driven electron transport in *Clostridium thermoautotrophicum* membranes. J. Bacteriol. **169**:5845–5847.
12. **Hungate, R. E.** 1969. A roll tube method for cultivation of strict anaerobes, p. 117–132. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 3b. Academic Press, Inc., New York.
13. **Jarrell, K. F., S. E. Bird, and G. D. Sprott.** 1984. Sodium-dependent isoleucine transport in the methanogenic archaebacterium *Methanococcus voltae*. FEBS Lett. **166**:357–361.
14. **Kallen, R. G., and W. P. Jencks.** 1966. The mechanism of the condensation of formaldehyde with tetrahydrofolic acid. J. Biol. Chem. **241**:5851–5863.
15. **Keltjens, J. T., and C. van der Drift.** 1986. Electron transfer reactions in methanogens. FEMS Microbiol. Rev. **39**:259–303.
16. **Ljungdahl, L. G.** 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. Annu. Rev. Microbiol. **40**: 415–450.
17. **Müller, V., M. Blaut, and G. Gottschalk.** 1986. Utilization of methanol plus hydrogen by *Methanosarcina barkeri* for methanogenesis and growth. Appl. Environ. Microbiol. **52**:269–274.
18. **Müller, V., M. Blaut, and G. Gottschalk.** 1987. Generation of a transmembrane gradient of Na^+ in *Methanosarcina barkeri*. Eur. J. Biochem. **162**:461–466.
19. **Müller, V., M. Blaut, and G. Gottschalk.** 1988. The transmembrane electrochemical gradient of Na^+ as driving force for methanol oxidation in *Methanosarcina barkeri*. Eur. J. Biochem. **172**:601–606.
20. **Müller, V., C. Winner, and G. Gottschalk.** 1988. Electron-transport-driven sodium extrusion during methanogenesis from formaldehyde and molecular hydrogen by *Methanosarcina barkeri*. Eur. J. Biochem. **178**:519–525.
21. **Perski, H. J., J. Moll, and R. K. Thauer.** 1981. Sodium dependence of growth and methane formation in *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **130**:319–321.
22. **Perski, H. J., P. Schönheit, and R. K. Thauer.** 1982. Sodium dependence of methane formation in methanogenic bacteria. FEBS Lett. **143**:323–326.
23. **Schmidt, K., S. Liaanen-Jensen, and H. G. Schlegel.** 1963. Die Carotinoide der Thiorhodaceae. Arch. Microbiol. **46**:117–126.
24. **Schönheit, P., and D. B. Beimborn.** 1985. Presence of Na^+/H^+ antiporter in *Methanobacterium thermoautotrophicum* and its role in Na^+ dependent methanogenesis. Arch. Microbiol. **142**: 354–361.
25. **Schönheit, P., and D. B. Beimborn.** 1986. Monensin and gramicidin stimulate CH_4 formation in *Methanobacterium thermoautotrophicum* at low external Na^+ concentration. Arch. Microbiol. **146**:181–185.
26. **Terraciano, J. S., W. J. A. Schreurs, and E. R. Kashket.** 1987. Membrane H^+ conductance of *Clostridium thermoaceticum* and *Clostridium acetobutylicum*: evidence for electrogenic Na^+/H^+ antiport in *Clostridium thermoaceticum*. Appl. Environ. Microbiol. **53**:782–786.
27. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
28. **Tschech, A., and N. Pfennig.** 1984. Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. Arch. Microbiol. **137**:163–167.
29. **Van der Meijden, P., C. van der Drift, and G. D. Vogels.** 1984. Methanol conversion in *Eubacterium limosum*. Arch. Microbiol. **138**:360–364.