Nickel Transport in *Methanobacterium bryantii*†

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Methanobacterium bryantii, grown autotrophically on H_2-CO_2, transported nickel against a concentration gradient by a high-affinity system ($K_m = 3.1$ μM). The system had a pH optimum of 4.9 and a temperature optimum of 49°C with an energy of activation of 7.8 kcal/mol (ca. 32.6 kJ/mol). A headspace of H_2-CO_2 (4:1, vol/vol) was required for maximum rate of transport. The system was highly specific for nickel and was unaffected by high levels of all monovalent and divalent ions tested (including Mg^{2+}) with the sole exception of Co^{2+}. Kinetic experiments indicated that accumulated nickel became increasingly incorporated into cofactor F_{430} and protein. Nickel transport was inhibited by nigericin, monensin, and gramicidin but not by carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone, carbonyl cyanide-*m*-chlorophenyl hydrazone, *N,N'*-dicyclohexylcarbodiimide, valinomycin plus potassium, or acetylene. The ineffectiveness of carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone, carbonyl cyanide-*m*-chlorophenyl hydrazone, and *N,N'*-dicyclohexylcarbodiimide may be related to difficulties in the penetration of these compounds through the outer cell barriers. Nickel uptake was greatly stimulated by an artificially imposed pH gradient (inside alkaline). The data suggest that nickel transport is not dependent on the membrane potential or on intracellular ATP, but is coupled to proton movement.

A nickel requirement for growth of bacteria has been reported in only a few cases (37), most notably for chemolithotrophic growth of many hydrogen-oxidizing bacteria (4, 14, 36). Recent studies conducted on methanogens, members of the so-called *Archaebacteriae* (13), have indicated that nickel is required for growth of all methanogens tested (8, 31, 32), including *Methanobacterium bryantii* (19). It has also been recently shown that the conversion of acetate to CH_4 and CO_2 by a mixed methanogenic population from an anaerobic fixed-film digestor is stimulated by the addition of nickel (26).

The precise role of nickel in those circumstances where it is an essential growth requirement is obscure. Nickel is required for active hydrogenase formation in *Alcaligenes eutrophus*, and it may be required for CO_2 fixation in that organism as well (14). In methanogens, nickel has been reported to stimulate hydrogenase activity in *Methanobacterium thermoautotrophicum* (10), and it has been recently shown that this enzyme contains nickel (16). Nickel has also been shown to be a component of a unique cofactor, coenzyme F_{430}, in all methanogens tested (7, 8, 40). Very recently, it has been demonstrated that nickel is required to prevent

rapid lysis of *M. bryantii* in a synthetic medium (19). It has not been shown that any of these requirements is the sole and essential reason for the strict nickel requirement for growth.

In *Escherichia coli*, Nelson (personal communication to Jasper and Silver [21]) has demonstrated that nickel appears to be transported by a high-affinity magnesium system. Both magnesium and cobalt competitively inhibited nickel uptake in these experiments, and a reduction in nickel uptake was observed at 0°C or in the presence of azide or cyanide. Based on the few nickel transport studies performed on bacteria, it is generally believed that nickel is transported by an energy-dependent system naturally responsible for magnesium uptake (for a review, see 22).

It was of interest to study nickel transport in *M. bryantii* because of the nickel requirement for growth and the apparent nickel involvement in maintaining cell wall integrity (19). In addition, since at least some methanogens lack a proton gradient (inside alkaline) but instead possess a small pH gradient (inside acid) at neutral external pH (20, 30), it was also of interest to examine some aspects of the mechanism of nickel uptake by this organism.

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MATERIALS AND METHODS

Organism, media, and growth conditions. *M. bryantii* (6) was obtained from M. P. Bryant. Cultures were maintained at 35°C by weekly transfers in a carbonate-buffered synthetic broth containing vitamins and minerals (S medium) (5) under an atmosphere of H_2-CO_2 (4:1, vol/vol). The medium was prereduced (18) with cysteine-hydrochloride (1.27 mM) and sodium sulfide (0.83 mM). Most of the sulfide is lost during medium preparation, resulting in a final concentration in the 10 to 100 μM range. JM medium (19), a recently described modification of S medium which contains increased amounts of phosphate, NH_4Cl, Na_2 $MoO_4 \cdot 2H_2O$, and $NiCl_2 \cdot 6H_2O$, was used in some experiments.

Cells were routinely grown from a 10% (vol/vol) inoculum in 100-ml volumes in modified 1-liter Wheaton bottles at 35°C under an atmosphere of H_2-CO_2 (4:1, vol/vol). Cultures were shaken at 150 rpm, and the bottles were pressurized daily with H_2-CO_2 (4:1, vol/vol) to 170 kPa. Growth of the organisms was followed turbidimetrically at 600 nm with cuvettes of 1-cm light path.

Chemicals. The following radioactive compounds were obtained from New England Nuclear Corp. of Canada, Lachine, Quebec, Canada: [³H]triphenyl-methylphosphonium bromide, $^{63}NiCl_2$, and D-[U-¹⁴C]glucose. [¹⁴C]methylamine was obtained from Amersham Corp., Oakville, Ontario, Canada. Valinomycin, tetraphenylboron, gramicidin, and carbonyl cyanide-*m*-chlorophenyl hydrazone (CCCP) were purchased from Sigma Chemical Co., St. Louis, Mo.; 2,4-dinitrophenol (DNP) was purchased from Matheson, Coleman and Bell, Norwood, Ohio; and N,N'-dicyclohexylcarbodiimide (DCCD) was purchased from Schwarz/Mann, Orangeburg, N.Y. Nigericin and monensin were kind gifts of J. Kwong (Eli Lilly Canada, Inc., Scarborough, Ontario, Canada), and carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone (FCCP) was the kind gift of P. G. Heytler (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.).

General anaerobic techniques. Methods for monitoring and maintaining anaerobiosis, anaerobic centrifugation to concentrate cells, and preparation of anaerobic buffers have been described previously (20).

Intracellular space. The intracellular space of *M. bryantii* was determined to be 2.08 μl/mg (dry weight) (average of eight determinations) based on the penetration of [¹⁴C]glucose (35).

General procedure for nickel uptake. Cells (generally 5-ml final volumes) concentrated by anaerobic centrifugation were transferred to 60-ml serum bottles filled with H_2-CO_2 (4:1, vol/vol). After the addition of $^{63}NiCl_2$ (final concentration, 15 μM unless otherwise stated), 0.5-ml samples were removed at timed intervals over a 10-min period, filtered (0.45-μm Millipore EH or HA filters), and washed with 5 ml of 0.1 M LiCl. The filters were placed in 5 ml of Aquasol (New England Nuclear) and counted on the tritium channel of a liquid scintillation counter.

pH optimum for nickel uptake. *M. bryantii* cells were centrifuged, and the pellets were suspended in an anaerobic buffer at 35°C for 15 min before the addition of $^{63}Ni^{2+}$. The buffers used were 100 mM sodium acetate (pH 4 to 5), 100 mM MES (morpholineethane-sulfonic acid) (pH 4.7 to 6.5), 100 mM sodium phos-

phate (pH 6 to 7), and 100 mM HEPES (N-2-hydroxy-ethylpiperazine-N'-2-ethanesulfonic acid) (pH 6.5 to 7.5).

Temperature optimum for nickel uptake. The anaerobic pellet from 15 ml of S medium-grown cells (mid-log phase) was suspended in 5 ml of anaerobic 50 mM HEPES buffer (pH 7.0) and transferred to a 60-ml serum bottle under H_2-CO_2 (4:1, vol/vol). After temperature equilibration for approximately 10 min, $^{63}Ni^{2+}$ was added, and uptake was followed as described above. The temperature range studied was 10 to 60°C.

Nickel uptake under various atmospheres. The anaerobic pellet from 20 ml of mid-log-phase *M. bryantii* cells was suspended in 5 ml of anaerobic 100 mM sodium phosphate buffer (pH 6.5). The concentrated cells were transferred to 60-ml serum bottles containing the desired gas atmosphere and flushed thoroughly with the desired gas. The bottles were sealed and incubated at 35°C for 90 min before nickel uptake was determined.

Effect of other ions on nickel uptake. Mid-log-phase cells grown in S medium were centrifuged anaerobically, and the pellets were suspended in anaerobic 100 mM HEPES buffer (pH 7.0), resulting in a threefold concentration of cells. HEPES buffer was used since it does not form complexes with metal ions (15). The cells were transferred to 60-ml serum bottles under H_2-CO_2 (4:1, vol/vol) and temperature equilibrated at 35°C. To determine the effect of various ions on nickel transport, the ion being tested was added to the cells to a final concentration of 1 mM immediately before the addition of 15 μM $^{63}Ni^{2+}$. The ions used were ammonium, calcium, cobaltous, ferrous, magnesium, manganese, nickelous, potassium (all as the chloride salts), and zinc (as the sulfate salt). The anions carbonate, phosphate, and sulfate (as the potassium salts) were also tested.

Effect of rapid pH shift on nickel uptake. *M. bryantii* cells were concentrated by anaerobic centrifugation, transferred in S medium to 120-ml serum bottles, flushed thoroughly with N_2, and capped under N_2. After 2 h at 35°C, the cells were transferred to an anaerobic apparatus similar to that described by Patel and Agnew (27) such that a pH probe was immersed in the sample for constant pH monitoring. $^{63}Ni^{2+}$ (10.7 μM) was added by syringe to begin the experiment. After 6 min, 8 μl of concentrated HCl was added to cause a pH drop from approximately 7.5 to 2.5. Nickel uptake was followed by the filtration assay with a 0.5-ml sample per filter. For controls, nickel uptake was determined with cells suspended under N_2 in S medium to which no acid was added and also with cells suspended under N_2 in S medium adjusted with HCl for 3 min before nickel addition. All experiments were performed at ambient temperature.

Effect of inhibitors on nickel uptake. All inhibitors except DCCD and acetylene were added for 15 min before the addition of $^{63}Ni^{2+}$, and uptake was followed as described above. DCCD was added for 90 min before the addition of nickel. Acetylene was present for 2 h before nickel addition, an incubation period more than sufficient to deplete the intracellular ATP concentration to less than the detection limit of 0.2 nmol/mg of protein (G. D. Sprott, K. F. Jarrell, K. M. Shaw, and R. Knowles, J. Gen. Microbiol., in press). Separate controls were run for the DCCD- and acety-
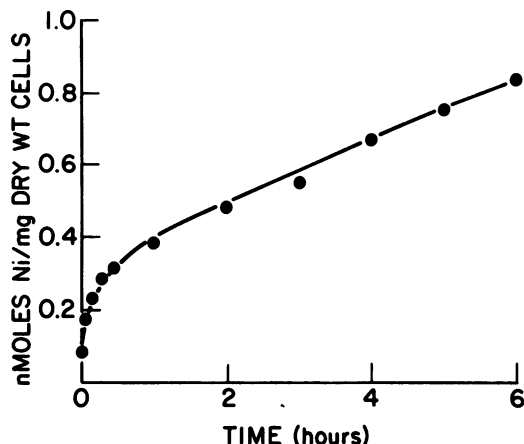
FIG. 1. Time course of nickel uptake by *M. bryantii* in S medium (pH 6.5). Uptake was followed by the filter assay after the addition of 15 μM $^{63}NiCl_2$. By 6 h, approximately 5% of the extracellular nickel was cell associated.

lene-treated cells. The inhibitors used were FCCP (10 μM), valinomycin (20 μM) plus KCl (0.5 M), nigericin (20 μM), monensin (20 μM), DNP (1 mM), DCCD (0.1 mM), CCCP (20 μM), gramicidin (10 μg/ml), and dissolved acetylene (290 μM).

Determination of the electrical potential. The electrical potential was estimated in cells suspended in S medium from the distribution of the lipophilic cation triphenylmethylphosphonium (10 μM, 10 μCi/μmol) in the presence of the counterion, tetraphenylboron (2 μM), by using a filter system (20). In some experiments, the results were compared to those obtained by centrifuging cells anaerobically through oil (1) by using polypropylene tubes pretreated with 0.1 M sodium sulfide solution.

Kinetics of nickel incorporation into factor F_{430}. *M. bryantii* was grown by using a shaking water bath at 35°C in three modified 1-liter Wheaton bottles, each containing 100 ml of JM medium without added nickel. After 2 days of growth (absorbance at 600 nm, ~0.6), 5 μM $^{63}NiCl_2$ was added, and incubation was continued. After 10 min, 1 h, and 4 h, a bottle of cells was harvested (at 10,000 × *g* for 10 min), the pellet was washed once with distilled water, and the cofactors were isolated from the pellet essentially as described by Diekert et al. (9).

RESULTS

Nickel uptake was examined in *M. bryantii* cells grown in S medium (containing no added Ni^{2+}) or in JM medium (containing 5 μM added Ni^{2+}). The rate of nickel uptake was somewhat greater (<twofold) with S medium-grown cells than with JM medium-grown cells and was most rapid in the early log phase. The rate of uptake per milligram (dry weight) of cells decreased as the culture aged. Nickel uptake into cells grown

to the stationary phase in JM medium was very poor (data not shown). Nickel uptake into stationary-phase S medium-grown cells could not be performed because rapid lysis occurs before the stationary phase (19). For all further work, mid-log-phase cells grown in S medium were used.

Nickel uptake was studied by using a filtration assay and also by anaerobic centrifugation with prereduced polypropylene tubes. A similar total nickel accumulation was observed with either system. It was therefore unlikely that the washing procedure used in the filtration method was removing accumulated nickel. Because of the comparative ease of the filtration method, it was used for the remainder of the study.

Time course of nickel uptake. A time course of uptake of $^{63}Ni^{2+}$ by *M. bryantii* cells in S medium (pH 6.5) indicated that within 1 h the rate of nickel uptake had decreased. However, nickel continued to be accumulated throughout the entire course of the experiment (6 h) at a reduced rate (Fig. 1), suggesting incorporation into cellular material.

Kinetics of nickel uptake. Nickel uptake by *M. bryantii* followed Michaelis-Menten kinetics over the concentration range from 0.5 to 15 μM, whereas at higher levels of nickel (50 to 100 μM), there was some (~15 to 20%) inhibition of uptake. At 35°C and pH 6.5, the K_m obtained from a double-reciprocal plot of the data was 3.1
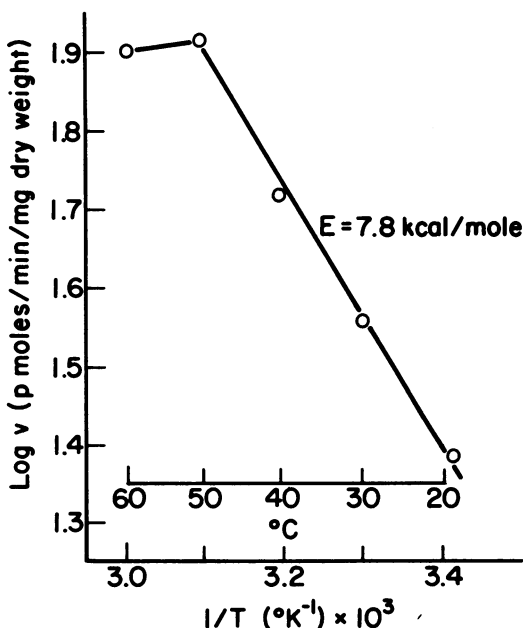

FIG. 2. Effect of temperature on nickel uptake. Cells were preincubated for 10 min at the indicated temperature before the addition of 15 μM $^{63}NiCl_2$.
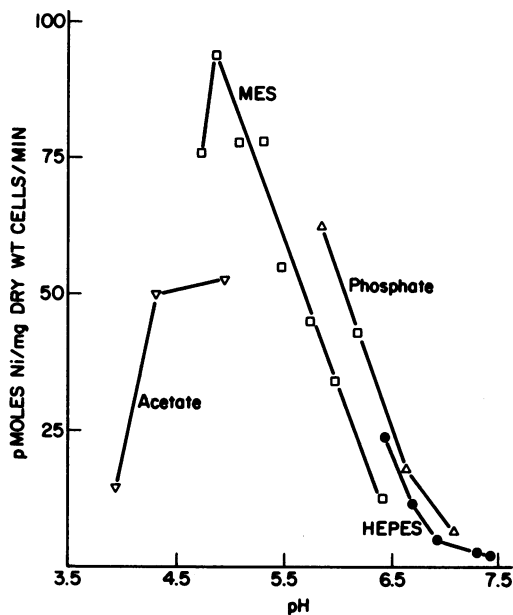
FIG. 3. Effect of pH on nickel uptake. S medium-grown cells were centrifuged anaerobically and suspended in the indicated buffer at 35°C for 15 min before the addition of 15 μM nickel. The rate of nickel uptake was followed over a 10-min period by using a filter assay.

± 1.8 μM. The V_{max} was 24 ± 7.5 pmol/min per mg (dry weight) of cells under these conditions.

Temperature and pH optima for nickel uptake. Nickel uptake by *M. bryantii* was temperature dependent, with an optimum of about 49°C (Fig. 2). Uptake was not observed at temperatures below 10°C. The energy of activation was calculated to be 7.8 kcal/mol (ca. 32.6 kJ/mol). Transport assays were routinely performed at 35°C, the temperature used for growth. The uptake of nickel at 35°C was strongly dependent on pH, with a sharp maximum at approximately pH 4.9 (Fig. 3). The initial rate of nickel uptake at pH 5 was usually four to fivefold higher than that observed at pH 6.5 (the approximate growth pH). Uptake was very poor at even slightly alkaline pH. Similar results were obtained when the pH was adjusted by the addition of small amounts of concentrated HCl or NaOH to cells suspended in growth medium:

Effect of headspace gas on nickel uptake. Maximum uptake of nickel occurred only under a headspace containing both CO_2 and H_2. Under an atmosphere lacking either or both of these gases, the rate of uptake was reduced by 50 to 70% (Fig. 4). These experiments were conducted on cells suspended in 100 mM sodium phosphate buffer (pH 6.5), since the pH of this buffer was relatively constant (within 0.11 pH units)

under any of the tested headspaces. When similar experiments were conducted on cells in S medium, N_2 and H_2 headspaces caused an apparent total inhibition of nickel uptake. This, however, could be explained by the alkalinization of the S medium from pH 6.5 to 7.5 caused by the absence of CO_2. Nickel uptake was not observed when the cells were exposed to O_2 (20% [vol/vol] for 90 min) or when the cells were heat treated (at 90°C for 10 min) before nickel addition.

Specificity of nickel uptake. The rate of nickel accumulation by *M. bryantii* cells suspended in anaerobic 100 mM HEPES buffer (pH 7) was unaffected by a variety of unlabeled cations added at levels (1 mM) 67-fold higher than that of $^{63}Ni^{2+}$ (15 μM). The ions having no effect were NH_4^+, K^+, Ca^{2+}, Fe^{2+}, Mn^{2+}, Mg^{2+}, and Zn^{2+} (Fig. 5). Co^{2+} had a strong inhibitory effect at a concentration of 1 mM and progressively less effect at 100 μM and 10 μM. The rate of nickel uptake was not stimulated by several anions tested, including phosphate, carbonate, and sulfate tried as possible counterions.

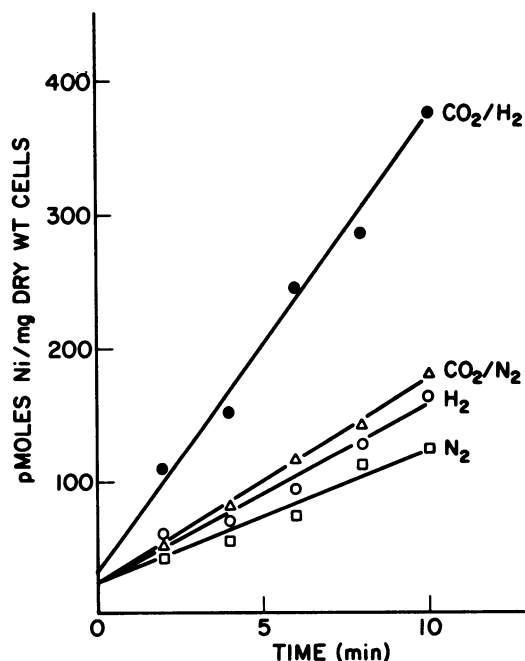**Nickel uptake in response to an artificial pH gradient.** When *M. bryantii* cells were incubated

FIG. 4. Effect of gas phase on nickel uptake. Cells in 100 mM sodium phosphate buffer (pH 6.5) were transferred to 60-ml serum bottles under the indicated gas phase and preincubated for 90 min before the addition of 15 μM $^{63}Ni^{2+}$. The pHs of the cell suspensions at the end of the experiment were 6.41 under CO_2-H_2 or CO_2-N_2 and 6.52 under N_2 or H_2.
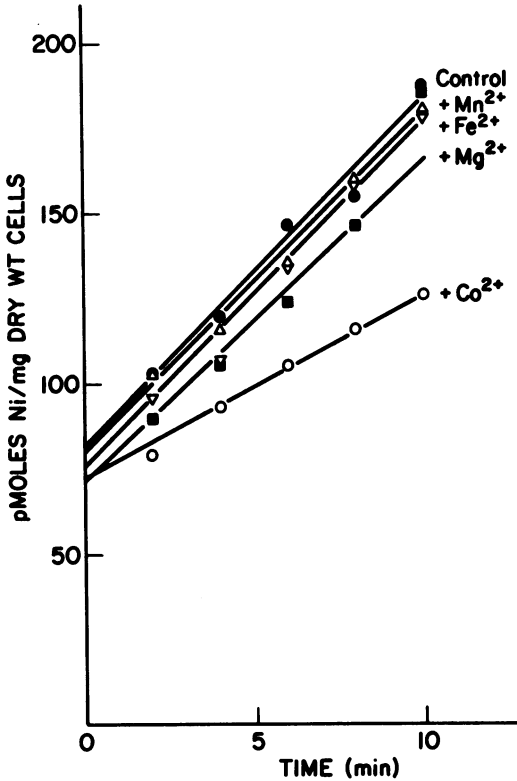
FIG. 5. Specificity of the nickel transport system. Cells were centrifuged, and the pellets were suspended in anaerobic 100 mM HEPES buffer (pH 7.0) at 35°C. The test ions were added at a final concentration of 1 mM immediately before the addition of 15 μM $^{63}Ni^{2+}$. ●, Control; ■, with Mg^{2+}; △, with Mn^{2+}; ▽, with Fe^{2+}; ○, with Co^{2+}. Other ions tested and found to be ineffective in altering the rate of nickel uptake were NH_4^+, Ca^{2+}, Zn^{2+}, K^+, phosphate, sulfate, and carbonate. The K_m for nickel was 3.1 μM.

under N_2 for 2 h and then tested for $^{63}Ni^{2+}$ transport, no uptake was observed (Fig. 6). However, if such cells were subjected to a rapid pH drop (from approximately 7.5 to 2.5) caused by injection of concentrated HCl, there was a dramatic uptake of $^{63}Ni^{2+}$, reaching a peak concentration gradient of 63 (in/out). Constant monitoring of the pH showed that the lower limit was achieved within 1 to 2 s of HCl injection, which excluded the possibility that the peak represented an increase in $^{63}Ni^{2+}$ uptake as the cells were transiently exposed to the optimal pH for uptake during mixing after HCl addition. The effect of acid injection was transient, since efflux of the label was evident. Further, when the cells were subjected to this pH drop for 3 min before nickel addition, no uptake was observed.

Effects of metabolic inhibitors and ionophores. The effects of various metabolic inhibitors and

ionophores on nickel uptake by *M. bryantii* are listed in Table 1. The effects were tested in growth medium of approximately pH 6.5 (or, on occasion, in 100 mM HEPES buffer [pH 7.0]) and in 100 mM MES buffer of pH 5.1 to 5.3, which is near the pH optimum for uptake. Under both conditions, the most effective inhibitors were nigericin, usually causing an electroneutral K^+-H^+ exchange (17), and monensin, usually causing an electroneutral Na^+-H^+ exchange. Gramicidin, a rather nonspecific cation conductor (17), was only effective at neutral pH. DNP, a proton ionophore (29), was partially effective,
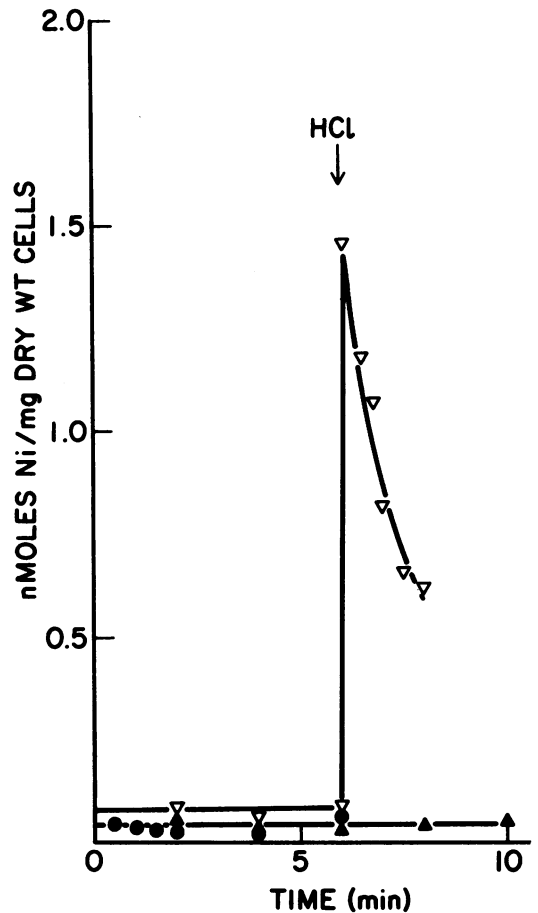

FIG. 6. Effect of an artificial pH gradient on nickel uptake. Cells were preincubated under a 100% N_2 atmosphere for 2 h. Uptake was studied on cells under N_2 (▲); on cells under N_2 acidified for 3 min before nickel addition (●); and on cells under N_2 to which acid was added at 6 min (▽). HCl injection caused the following typical pH response recorded for the reaction mixture at the indicated times after injection (▽) (pH was 7.5 before HCl injection): 1 to 2 s, pH 2.5; 15 s, pH 2.5; 30 s, pH 2.5; 1 min, pH 2.7; and 2 min, pH 2.8.

TABLE 1. Effect of metabolic inhibitors and ionophores on nickel uptake

Inhibitor	Concn		Inhibition (%)[a] in:	
			Growth medium (pH 6.5)	MES buffer (pH 5.2)
CCCP	20	μM	2	2
FCCP	10	μM	1	ND[b]
Gramicidin D	10	μg/ml	51	15
Nigericin	20	μM	72	62
Monensin	20	μM	56	63
Valinomycin + 0.5 M KCl	20	μM	11	24
Acetylene	290	μM	10	9
DCCD	0.1	mM	0	ND
DNP	1	mM	37	ND

[a] The uninhibited rate of nickel uptake in growth medium and MES buffer was 27 and 92 pmol/min per mg of cells (dry weight), respectively.
[b] ND, Not determined.

but other supposed proton ionophores, such as FCCP and CCCP, never affected nickel transport. CCCP at 50 μM still had no effect on nickel uptake, even though *M. bryantii* is reported to be sensitive to the ionophore at this level (28). Similarly, CCCP did not affect significantly either CH_4 synthesis or intracellular ATP concentrations (data not shown). Acetylene, recently shown to cause a dramatic decline in the intracellular ATP concentration of *M. bryantii* (G. D. Sprott, K. F. Jarrell, and R. Knowles, Abstr. Annu. Meet. Can. Soc. Microbiol. 1981, H3p, p. 121), had little effect on nickel uptake, as did DCCD, which is usually, but not always (33), an inhibitor of ATPase. Valinomycin in the presence of 0.5 M KCl (a level which approximates the free potassium level inside *M. bryantii* cells) (unpublished data) eliminated the electrical potential, which was measured to be 102 or 113 mV (by the filtration and centrifugation assays, respectively; negative inside) from the distribution of triphenylmethylphosphonium. However, the effect of valinomycin plus potassium on nickel uptake was small.

Distribution of cell-associated $^{63}Ni^{2+}$. A large percentage of the accumulated $^{63}Ni^{2+}$ was precipitable with 70% (vol/vol) acetone (Fig. 7). This percentage decreased with time, but even when cells were grown in $^{63}Ni^{2+}$-supplemented medium for 4 days, more than 20% of the label was precipitable with 70% (vol/vol) acetone. The acetone supernatants were loaded onto a quaternary aminoethyl Sephadex A-25 column and washed. Of the total counts, only 9 to 16% either were not absorbed onto the column or were lost during washing with 50 mM glycine-KOH buffer (pH 9.5). The column-associated radioactivity

was eluted in two distinct peaks with 50 mM glycine-KOH buffer (pH 9.5) containing increasing concentrations of NaCl. Peak I corresponded to nickel ions and nickel hydroxides (7), whereas peak II coeluted with factor F_{430} (Fig. 8). Over the course of the experiment, the amount of label eluting with F_{430} increased dramatically. This appears to be due partly to a burst of F_{430} synthesis (Fig. 8), which is probably due to the addition of nickel to the medium (9). After 4 h, about 40% of the total cell-associated counts coeluted with F_{430}. This is almost as high as the 43% found when the cells were grown in the presence of $^{63}Ni^{2+}$ for 4 days (Fig. 7) and compares with only 3% found associated with F_{430} after 10 min with the label. The level of free nickel in the cells, if represented by the first peak eluted from the quaternary aminoethyl Sephadex column, stayed fairly constant at about 10% of the total cell-associated counts.

Concentration gradients of Ni^{2+}. The distribution of $^{63}Ni^{2+}$ across the cell membrane was determined from measurements of the amount of free nickel present in washed cells (Fig. 8, peak
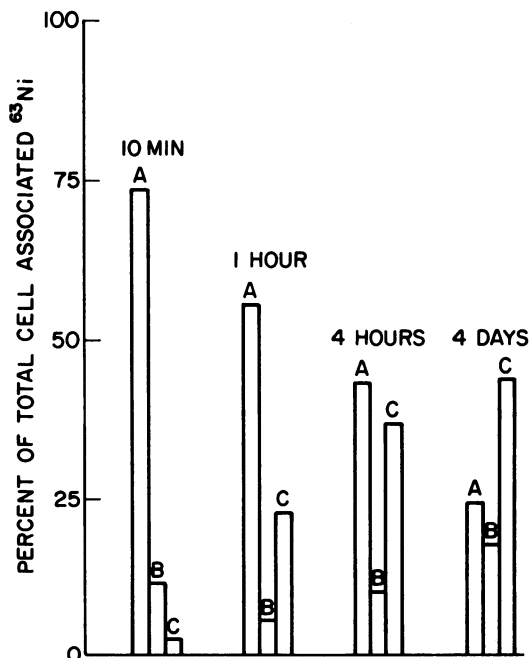

FIG. 7. Distribution of cell-associated $^{63}Ni^{2+}$. Cells were incubated with 5 μM $^{63}Ni^{2+}$ for the indicated time and centrifuged, and the pellets were washed twice. Fractions were then isolated (see text). (A) Acetone-precipitable $^{63}Ni^{2+}$; (B) free $^{63}Ni^{2+}$; (C) F_{430}-associated $^{63}Ni^{2+}$. The total cell-associated counts present at each of the times were: 10 min, 251,000 cpm; 1 h, 573,000 cpm; 4 h, 1.9×10^6 cpm; 4 days, 8.8×10^6 cpm.
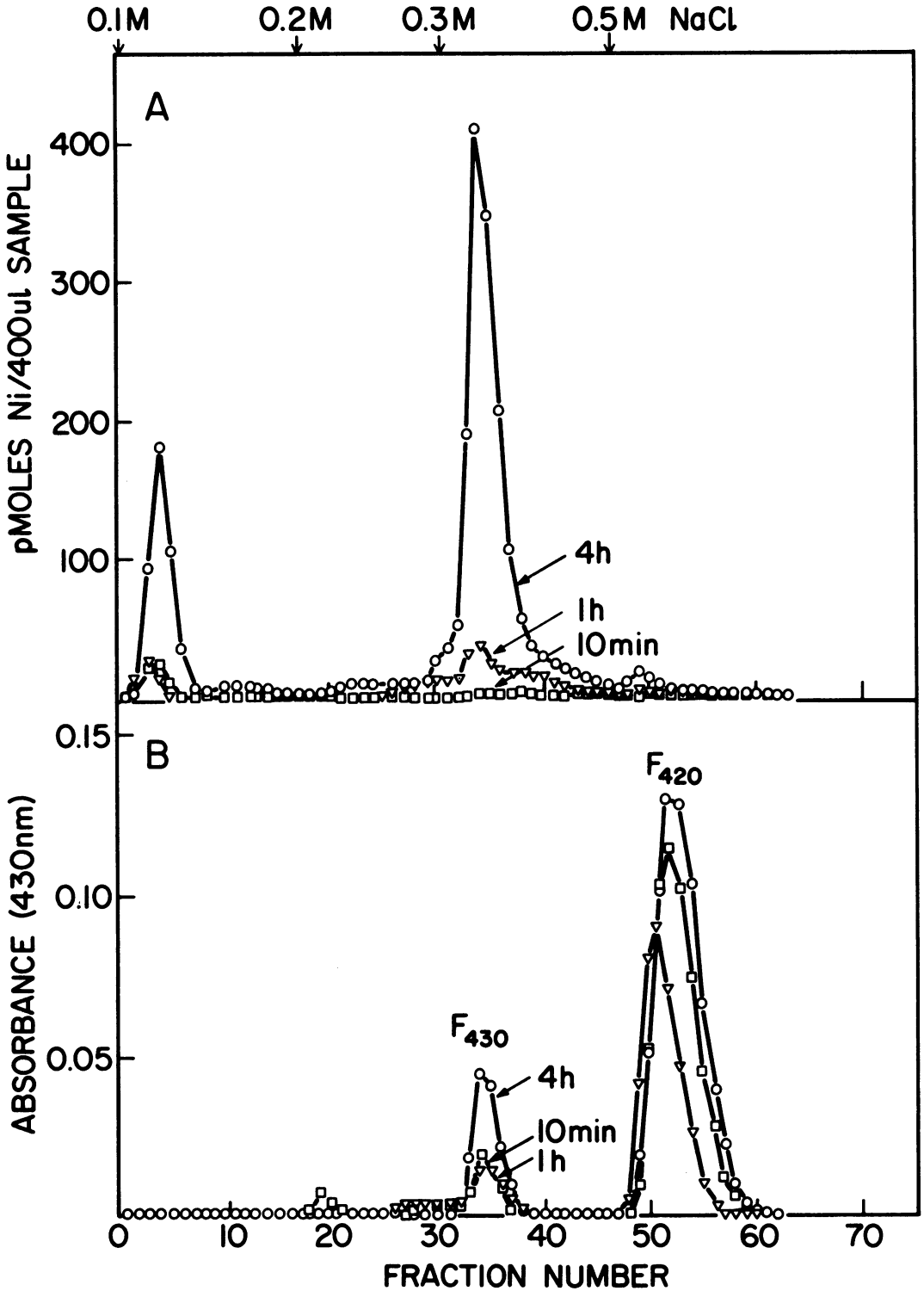
FIG. 8. $^{63}Ni^{2+}$ incorporation into F_{430}. Cells were incubated for 10 min (□), 1 h (∇), or 4 h (○) in the presence of 5 μM $^{63}Ni^{2+}$, and then cofactors were extracted (see text). The 70% acetone supernatant was applied to a quaternary aminoethyl Sephadex A-25 column, and the radioactivity was eluted with 50 mM glycine-KOH buffer (pH 9.5) containing increasing amounts of NaCl.

I). Whereas the total nickel taken up by the cells indicated an in/out concentration of 70- to 90-fold after uptake had proceeded for 10 min in S medium (pH 6.5), only about 10% of the total counts were free (Fig. 7). Thus, a gradient of free nickel of seven to nine existed. Cells grown for 4 days in the presence of 5 or 0.3 μM nickel (initial concentrations) established gradients (free intracellular $^{63}Ni^{2+}/^{63}Ni^{2+}$ remaining in the medium) of about 30- and 30,000-fold, respectively.

DISCUSSION

The results of the present study document the presence of an active transport system for nickel in *M. bryantii*. Other than a study by Balch and Wolfe (3) on coenzyme M transport by *Methanobacterium ruminantium* (*Methanobrevibacter ruminantium*) (2) and a brief report on adenine nucleotide translocase in *M. thermoautotrophicum* (11), this is the only study of a transport system in methanogens. The nickel transport system of *M. bryantii* was pH and temperature dependent, required the presence of H_2 and CO_2 (conditions resulting in methanogenesis) for maximum uptake, established appreciable concentration gradients of free Ni^{2+}, and could be inhibited by nigericin, monensin, and gramicidin. The system was specific for nickel and was unaffected by all cations tested except Co^{2+}. Interestingly, Mg^{2+} did not inhibit nickel uptake. In most, if not all, other reported cases of nickel transport in bacteria, Ni^{2+} is transported as an alternate, low-affinity substrate by the Mg^{2+} transport system (21, 22, 39).

The nickel transport system of *M. bryantii* had a high affinity ($K_m = 3.1$ μM) and a low rate of accumulation ($V_{max} = 24$ pmol/min per mg of cells [dry weight]). These characteristics are quite similar to those of specific transport systems in other bacteria for essential micronutrient cations, such as Mn^{2+} in *E. coli*, *Bacillus subtilis*, and *Rhodopseudomonas capsulata* (for a review, see 34). This high-affinity system presumably accounts for the ability of *M. bryantii* to grow in media containing only contaminating levels of nickel.

Nickel is an essential growth factor in *M. bryantii* (19) as well as in other methanogens (8, 31, 32). The majority of nickel (~50 to 70%) taken up by the cells is associated with coenzyme F_{430} (8, 9; this study), which contains Ni^{2+} (7, 40). Ellefson and Wolfe (12) have recently suggested that F_{430} may be the prosthetic group of methyl coenzyme M reductase. A large percentage of the total nickel, however, is also associated with the protein fraction (8), and it has very recently been shown that at least one protein, the hydrogenase from *M. thermoautotrophicum*, contains nickel (16). Since nickel is required to prevent rapid lysis of *M. bryantii* in a

synthetic medium (19), nickel protein(s) may have a role in cell wall maintenance or synthesis.

Kinetic studies demonstrated the increasing incorporation of $^{63}Ni^{2+}$ into F_{430}. After 10 min of incubation with $^{63}Ni^{2+}$, only 3% of the cell-associated $^{63}Ni^{2+}$ was F_{430} associated, whereas after 4 h, over 40% of the label was found in F_{430}, a value similar to that found for *M. bryantii* after growth of the cells in $^{63}Ni^{2+}$-supplemented medium for 4 days. The addition of nickel also seemed to stimulate F_{430} synthesis over a 4-h period. A similar burst of F_{430} synthesis was observed by Diekert et al. (9) when nickel was added to a culture of *M. thermoautotrophicum* growing in nickel-deficient medium.

Attempts to determine the mechanism of nickel transport were presented. First, a direct requirement for ATP in nickel transport, such as that reported for calcium transport in *Streptococcus faecalis* (23), seems to be precluded by the general ineffectiveness of acetylene in inhibiting uptake. Dissolved acetylene at 65 μM caused a decrease in the intracellular ATP concentration in *M. bryantii* to below the detection limit (Sprott et al., in press). The effect on nickel transport was minimal at concentrations as high as 290 μM (Table 1). Second, a major involvement of membrane potential in nickel transport seems unlikely since, in the presence of valinomycin and potassium, which eliminated any detectable membrane potential determined by triphenylmethylphosphonium distribution, nickel transport was not greatly affected. This would seem to eliminate the possibility of nickel uniport with accumulation due to an electrical potential (inside negative). Third, nigericin, monensin, and gramicidin (at pH 6.5) were all effective in inhibiting nickel uptake. Nigericin and monensin usually facilitate electroneutral exchange of H^+ for K^+ and Na^+, respectively, whereas gramicidin is a rather nonspecific cation ionophore (17). All three ionophores induced a large uptake of H^+, resulting in acidification of the cytoplasm of *M. bryantii* (Jarrell and Sprott, unpublished data). Furthermore, the proton conductor DNP inhibited nickel transport to a limited extent (Table 1), whereas the ATPase inhibitor DCCD had little effect on nickel transport or ATP concentration in cell suspensions of this methanogen (100 μM DCCD for 1 h) (G. D. Sprott and K. F. Jarrell, Can. J. Microbiol., in press). The protonophores FCCP and CCCP had no effect on nickel uptake. It is presently uncertain whether the latter inhibitors penetrate the unusual walls (24) and membranes (25, 38) of these bacteria.

Further work is in progress on the effects of the ionophores used here on the protonmotive force in *M. bryantii*. Although a model for Ni^{2+} transport is premature pending these results, it

seems probable that Ni^{2+} transport in *M. bryantii* is coupled to H^+ movement.

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