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Nickel Transport by the Thermophilic Acetogen *Acetogenium kivui*

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Exogenous ^{63}Ni was incorporated into carbon monoxide dehydrogenase when *Acetogenium kivui* ATCC 33488 was cultivated in the presence of $^{63}NiCl_2$. The capacity for nickel ($^{63}NiCl_2$) transport was greatest with cells harvested from the mid- to late exponential phases of growth. Nickel transport was linear during the transport assay period and displayed saturation kinetics. The apparent K_m and V_{max} for nickel transport by H_2-cultivated cells approximated 2.3 μM Ni and 670 pmol of Ni transported per min per mg (dry weight) of cells, respectively. The nickel transport system was not appreciably affected by the other divalent cations that were tested, and transported nickel was not readily exchangeable with exogenous nickel. Nickel transport was stimulated by glucose or H_2 and was decreased by various metabolic inhibitors; however, nickel uptake by glucose- and H_2-cultivated cells displayed differential sensitivities to ATPase inhibitors.

Acetogenic bacteria are important in the flow of carbon and energy in anaerobic habitats (8, 15). Since nickel plays an essential role in the Wood pathway of acetogenesis at the level of carbon monoxide dehydrogenase (acetyl coenzyme A synthetase) (24), the transport and incorporation of nickel are fundamental processes in acetogenic bacteria. Nickel transport by the acetogen *Clostridium thermoaceticum* is energy dependent and specific for nickel; furthermore, cellular energy reserves appear to be used under some conditions to power nickel uptake (18). *Acetogenium kivui* is a nonclostridial thermophilic acetogen that has been isolated from the sediments of Lake Kivu, Zaire (14). In the present study, *A. kivui* was used to further resolve the nature of acetogenic metabolism and transport of nickel.

MATERIALS AND METHODS

Organism and cultivation. Unless otherwise indicated, *A. kivui* ATCC 33488 was cultivated at 55°C in a defined medium with a 100% CO_2 initial gas phase at atmospheric pressure (14). Undefined medium was defined medium supplemented with 1 g of yeast extract per liter. Medium contained either 11 mM glucose or H_2 (100% CO_2 at atmospheric pressure with an additional 300 kPa of H_2 overpressure) as an energy substrate.

Nickel transport assays. Nickel transport assays were conducted anaerobically by a modification of previously described methods (3, 18). In brief, cells were harvested, washed once, and suspended in transport buffer to a final optical density of 1.0 at 660 nm (equivalent to 0.66 mg of cell dry weight per ml). The transport assay buffer was 100 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 6.5) containing 11 mM glucose, 0.5 mM sodium dithionite, and 0.01% resazurin. The transport assay gas phase was 100% N_2 at atmospheric pressure. When nickel uptake was assayed in the absence of exogenous energy sources, glucose was deleted from the assay buffer. When H_2 was used as an energy source, glucose was deleted, and the assay gas phase was H_2-CO_2 (50:50) at 144 kPa of overpressure. After approximately 25 min of preincubation at 55°C, $^{63}NiCl_2$ was injected to a final concentration of 10 μM (approximately 14 μCi/μmol). Three minutes (the standard assay time) after the injection of $^{63}NiCl_2$, samples (1 ml) of the cell suspension were removed with a syringe and filtered through membrane filters (pore size, 0.45 μm; GN-6 Metricel; Gelman Sciences, Inc., Ann Arbor, Mich.) (3). The filters were rinsed four times (1 ml per rinse) with 100 mM Tris hydrochloride (pH 7.2) containing 10 mM EDTA, dried for 15 min at 60°C, and counted in 4 ml of Bio-Safe II scintillation cocktail (Research Products International Corp., Mount Prospect, Ill.) with a liquid scintillation counter (LS6800; Beckman Instruments, Inc., Fullerton, Calif.). The transport rates reported here are based on the amount of nickel taken up between time zero and 3 min. Assays were performed in triplicate, and the values reported are the means; the standard error of the means did not exceed 10% of the control.

Analytical methods. Metabolic inhibitors were from Sigma Chemical Co. (St. Louis, Mo.), and $^{63}NiCl_2$ was from Dupont, NEN Research Products (Boston, Mass.). Growth was measured as the A_{660} with a spectrophotometer (Spectronic 501; Bausch & Lomb, Inc., Rochester, N.Y.), and cell dry weights were determined as described previously (19).

Cell extracts for polyacrylamide gel electrophoretic analysis were prepared by lysozyme digestion (17) of cells cultivated in defined medium with either glucose or H_2; medium was supplemented with $^{63}NiCl_2$ (6×10^5 cpm/ml of medium). Gels were loaded with 50 μl of cell extract (containing 0.13 mg of protein and 2×10^4 cpm of ^{63}Ni), electrophoresed at pH 9, stained for carbon monoxide dehydrogenase and hydrogenase, and sliced for analysis of ^{63}Ni as described previously (5, 6).

RESULTS

Incorporation of nickel by *A. kivui*. In cell extracts of glucose-cultivated cells, carbon monoxide dehydrogenase was the predominant nickel-containing protein (Fig. 1). Carbon monoxide dehydrogenase was also the predominant nickel-containing protein observed in cell extracts of H_2-cultivated cells (data not shown). Although nickel was incorporated primarily into carbon monoxide dehydrogenase, additional nickel-containing protein bands were indicated (Fig. 1). When gels were stained for hydrogenase, three bands with hydrogenase activity were observed, one of which coincided with the unidentified nickel peak (slice 17) preceding the band with carbon monoxide dehydrogenase activity.

Effects of culture age, pH, and temperature on nickel

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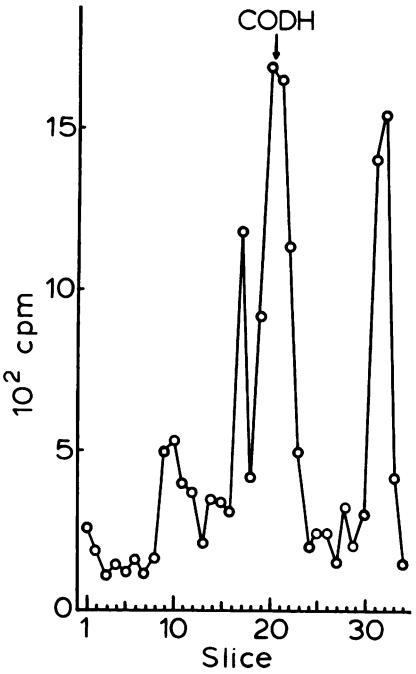

FIG. 1. Analysis of ^{63}Ni in polyacrylamide gels of cell extracts from glucose-cultivated cells. The carbon monoxide dehydrogenase (CODH) in situ activity band was centered at slices 20 and 21, and the ^{63}Ni peak at slice 32 coincided with the bromophenol blue dye front (also coincident with the location of ferredoxin). Recovery of radioactivity approximated 85%.

transport. Cells harvested from the mid- to late exponential phases of growth displayed the highest capacity for nickel transport (Fig. 2). The following nickel transport rates (in picomoles of Ni transported per minute per milligram [dry weight] of cells) were obtained with glucose-cultivated cells at the assay pHs indicated (at 55°C): pH 5.4, 376; pH 6.5, 538; pH 7.5, 403; pH 8.3, 347; pH 9.2, 180. Temperature influenced the transport rates as follows (at pH 6.5): 40°C, 82; 55°C, 318; 65°C, 352; 75°C, 204. Based on these results,

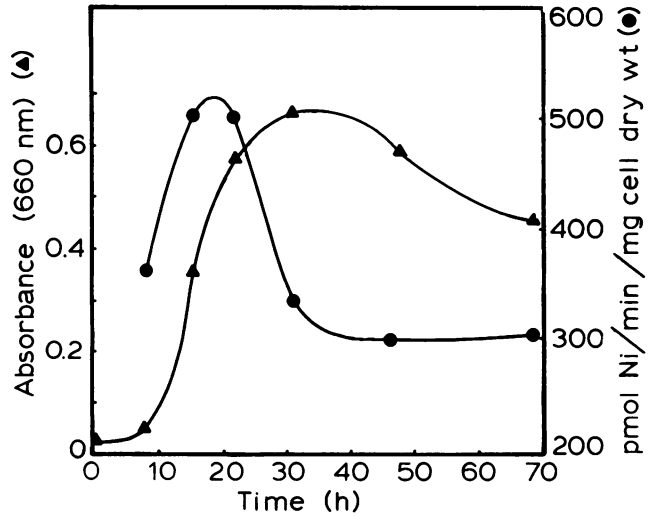

FIG. 2. Effect of culture age on the capacity for nickel transport by *A. kivui*. During growth (▲), cells were harvested and assayed for their ability to transport $^{63}NiCl_2$ (●).

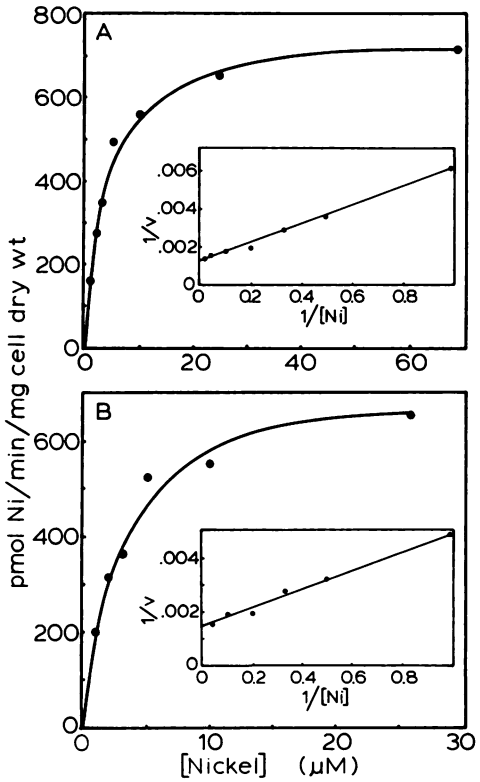

FIG. 3. Kinetics of nickel transport. (A) Glucose-cultivated cells with glucose as the energy source. (B) H_2-cultivated cells with H_2 as the energy source. v, Velocity.

subsequent experiments were conducted with mid- to late-exponential-phase cells, and the standard assay pH and temperature used were 6.5 and 55°C, respectively.

Kinetics of nickel transport. Nickel transport was linear (to at least 18 min; data not shown), and both glucose- and H_2-cultivated cells displayed similar saturation kinetics over the ranges of 0 to 60 μM and 0 to 30 μM of Ni, respectively (Fig. 3). The apparent K_m and V_{max} for nickel transport by glucose-cultivated cells approximated 4.4 μM Ni and 830 pmol of Ni transported per min per mg (dry weight) of cells, respectively; the apparent K_m and V_{max} for H_2-cultivated cells approximated 2.3 μM Ni and 670 pmol of Ni transported per min per mg (dry weight) of cells, respectively.

Effects of other divalent cations. The divalent cations cobalt, iron, magnesium, or manganese (at 75 μM) did not affect nickel transport rates (with 3 μM Ni). In contrast, calcium at 3, 15, and 75 μM decreased nickel uptake by 20, 40, and 30%, respectively; this pattern was not indicative of competitive inhibition.

Exchangeability of transported nickel. Following a 3-min nickel transport assay with 3 μM $^{63}NiCl_2$, unlabeled $NiCl_2$ was added to the assay vial to a final concentration of 50 μM. At 1 and 20 min postinjection of the unlabeled nickel, greater than 90% of the ^{63}Ni taken up during the initial 3-min transport period remained associated with the cell, i.e., did not readily exchange with exogenous nickel. Extracellular and transported (intracellular) forms of nickel, therefore, appeared to be dissimilar. In this regard, most of the ^{63}Ni of cell extracts prepared from cells following a 3-min nickel transport assay electrophoresed as low-M_r anionic species coincident with the dye front (data not shown); the nature of the low-M_r species remains unknown. Assuming that the

TABLE 1. Effect of metabolic inhibitors on growth and nickel transport[a]

Inhibitor (concn [μM])	Glucose cells		H_2 cells	
	Growth[b]	Transport[c]	Growth[b]	Transport[c]
Control (none added)	0.68	100	0.51	100
DCCD (700)	0.00	60	0.00	110
TBT (200)	0.00	65	0.00	200
CCCP (1,000)	0.00	42	0.00	37
Dinitrophenol (800)	0.00	61	0.03	78
Nigericin (14)	0.00	116	0.00	77
Monensin (14)	0.00	138	0.00	101
KCN (200)	0.42	0	0.00	13
O_2[d]	—[e]	14	—[e]	ND[f]
None		6[g], 5[h]		6[h]

[a] Transport assay was with 10 μM Ni. Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; TBT, tributyltin chloride; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone. Cells were cultivated and assayed with the indicated energy source. H_2 cells were cultivated in undefined medium. Inhibitors were present during the preincubation period.
[b] Maximum A_{660}.
[c] Percentage of control.
[d] Nickel transport assay gas phase was 100% O_2.
[e] —, Not applicable; *A. kivui* is an obligate anaerobe (14).
[f] ND, Not determined.
[g] Assay temperature was 4°C.
[h] Cells were autoclaved anaerobically for 10 min prior to assay.

intracellular volume of *A. kivui* is similar to that of *C. thermoaceticum* (approximately 1.5 μl per mg [dry weight] of cells) (1), nickel was concentrated approximately 350-fold against the apparent nickel concentration gradient during a standard nickel transport assay.

Effect of metabolic inhibitors on nickel transport. Concentrations of metabolic inhibitors which were inhibitory to growth were used to determine whether nickel transport was metabolically coupled (Table 1). In general, putative ATPase inhibitors (*N,N'*-dicyclohexylcarbodiimide and tributyltin chloride), protonophores (carbonyl cyanide *m*-chlorophenylhydrazone and dinitrophenol), KCN, O_2, and low temperature were inhibitory to nickel transport. However, the ionophores nigericin and monensin did not inhibit nickel transport under the assay conditions that were used. Nickel uptake was essentially abolished when cells were autoclaved prior to the assay.

Effect of exogenous energy source on nickel transport. Glucose and H_2 were stimulatory to nickel transport by *A. kivui* (Table 2), thus corroborating the energy-dependent nature of the nickel transport system. However, transport by glucose-cultivated cells was not stimulated by glucose when the standard 25-min preincubation period was decreased to 10 min or less; preincubation periods longer than 25 min with glucose did not increase transport rates further. In contrast, transport rates without glucose were essentially identical after 1, 10, 25, and 90 min of preincubation.

When the capacity of glucose-cultivated cells to transport nickel was assayed in the absence of exogenous glucose, transport was linear (to at least 18 min; data not shown). Nickel transport under these conditions was reduced significantly by low temperature, O_2, and metabolic inhibitors (Table 3).

TABLE 2. Effect of exogenous energy sources on nickel transport

Cultivation medium and transport assay energy source	Nickel transport rate[a]
Undefined glucose	
None	162
Glucose	527
Defined glucose	
None	214
Glucose	557
Undefined hydrogen	
None[b]	281
Hydrogen	635

[a] Picomoles of Ni transported per minute per milligram (dry weight) of cells.
[b] Assay gas phase was 100% CO_2.

TABLE 3. Effect of physiological parameters and metabolic inhibitors on nickel transport in the absence of exogenous energy source[a]

Assay condition	Nickel transport (% of control)
Control[b]	100
At 4°C	8
With O_2 (100% gas phase)	27
With TBT (200 μM)	29
With DCCD (700 μM)	46
With CCCP (1,000 μM)	66
With KCN (200 μM)	2

[a] Cells were cultivated in defined glucose medium. Abbreviations of compounds are as described in footnote *a* of Table 1.
[b] Standard assay condition without exogenous energy source.

DISCUSSION

In general, chemoorganotrophic bacteria transport nickel with decreased specificity via a magnesium transport system (3, 12, 13, 22, 23), while bacteria with autotrophic potentials translocate nickel via transporters which have high affinities for the metal (7, 9, 11, 16, 18, 20). In the present study, neither magnesium nor other divalent metals inhibited nickel transport by *A. kivui*. This finding, together with the low K_m values for nickel, suggest that the nickel transport system of *A. kivui* translocates nickel with a high specificity. The K_m values obtained for *A. kivui* were similar to those obtained for the acetogen *C. thermoaceticum* (18). The K_m values obtained for other organisms with autotrophic potentials are 3.1 μM Ni for *Methanobacterium bryantii* (11), 17 and 0.34 μM Ni for *Alcaligenes eutrophus* (16), 26 and 50 μM Ni for *Bradyrhizobium japonicum* (20), 17 nM Ni for *Anabaena cylindrica* (4), 5.5 μM Ni for *Rhodopseudomonas capsulta* (21), and 7 μM Ni for *Rhodobacter capsultatus* (R. Smith, A. Colbeau, M. Leclerc, and P. M. Vagnais, Abstr. Lithoautotrophy, A Centenary Meeting in Memory of S. N. Winogradsky, abstr. no. P30, p. 70, 1987).

Results obtained with both *A. kivui* and *C. thermoaceticum* (10, 18) indicate that these acetogens have nickel transport systems that are metabolically coupled and that may, under some conditions, be powered by energy reserves in the absence of an exogenous energy source. In this regard, *C. thermoaceticum* has been shown to contain high levels of PP_i which are hydrolyzed concomitantly with nickel transport (10, 18). In contrast, the nonacetogen *Clostridium pasteurianum* is more dependent on an exogenous energy source for nickel transport (3).

The capacity to transport nickel in the absence of extracellular energy sources has also been observed with *B. japonicum* (20), *Alcaligenes eutrophus* (16), and *Rhodopseu-*

domonas capsulata (21). Glycogen was postulated to be the energy reserve utilized by *Rhodopseudomonas capsulata* (21). Nickel uptake (adsorption) in short-term assays by the sheathed methanogen *Methanothrix concilii* was postulated to be energy independent since transport was not abolished when methanogenesis was inhibited and purified sheath preparations yielded nickel-binding profiles similar to those of intact (sheathed) cells (2). Studies to date do not exclude the possibility that acetogens may also be capable of energy-independent nickel uptake, nor do they exclude the possibility that energy-independent cell binding may be prerequisite to transport.

Putative inhibitors of ATPase (*N*,*N*′-dicyclohexylcarbodiimide and tributyltin chloride) which inhibited nickel transport by glucose-cultivated cells were somewhat stimulatory with H_2-cultivated cells (Table 2). In the nickel transport assay, ATPase in glucose (fermentative?) cells may be operating in the direction of proton extrusion and maintenance of the proton motive force, while ATPase in H_2 (respiring?) cells may operate primarily in the direction of ATP synthesis, in which case the proton motive force (and, hence, the capacity to catalyze the energy-dependent transport of nickel) would not be directly dependent on ATPase. An understanding of how tightly coupled nickel transport is to the metabolic state of the cell (regardless of whether external or internal energy sources are used) will require further resolution of the mechanism(s) of acetogenic energy conservation.

Electrophoretic analysis of the intracellular nature of nickel indicated that nickel was incorporated into carbon monoxide dehydrogenase and other proteins, one of which may be hydrogenase. To date, carbon monoxide dehydrogenase is the only nickel-containing protein identified from acetogenic bacteria.

ACKNOWLEDGMENTS

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