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Energy-Dependent, High-Affinity Transport of Nickel by the Acetogen *Clostridium thermoaceticum*

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The nickel transport system of *Clostridium thermoaceticum* was investigated with $^{63}NiCl_2$ and an anaerobic microfiltration transport assay. Transport was optimal at pH 7 to pH 7.5 and 65°C and decreased in the presence of metabolic uncouplers and inhibitors. Exogenous nickel was concentrated 3,000-fold over the apparent nickel concentration gradient during typical transport assays. Stored cellular energy appeared to provide a short-term energy source to power nickel transport, and starvation experiments demonstrated external energy source stimulation of nickel translocation. The apparent K_m and V_{max} for nickel transport by carbon monoxide-dependent chemolithotrophic cells approximated 3.2 μM Ni and 400 pmol of Ni transported per min per mg of cells (dry weight), respectively. Magnesium, calcium, cobalt, iron, manganese, and zinc did not inhibit the transport of nickel.

Nickel is a biologically active trace metal, its biological roles ranging from the induction of carcinomas to the catalysis of essential metabolic processes. In general, the biological activities of nickel are dependent upon cellular internalization of the metal (8). Two principal types of energy-dependent nickel transport system have been described (8, 10, 16). One is characterized by a high-affinity magnesium transporter which translocates other divalent cations (e.g., nickel) with decreased affinity. The second type is a high-affinity nickel transport system which is less affected by other divalent cations, in particular, magnesium.

As an essential element of carbon monoxide (CO) dehydrogenase (acetyl coenzyme A synthetase), nickel plays a vital role in the Wood pathway of acetogenesis (6, 9, 20, 33). In this study, we report that nickel translocation by *Clostridium thermoaceticum* (i) is energy dependent, (ii) is not inhibited by other divalent cations, and (iii) may be powered by cellular energy reserves.

MATERIALS AND METHODS

Cultivation. *C. thermoaceticum* ATCC 39073 was cultivated at 55°C in a defined medium containing low phosphates (3.7 mM) and 1 μM $NiCl_2$ (as described previously, with the exclusion of yeast extract [5]). The following energy sources were used: glucose, 10 mM; methanol, 60 mM; syringic acid, 10 mM; and CO, 144 kPa (14 lb/in^2) over the atmospheric pressure at room temperature.

Clostridium thermoautotrophicum JW701/3 was cultivated at 58°C in a defined medium (25, 26), *Peptostreptococcus productus* U-1 was cultivated at 37°C in an undefined medium (22), and *Acetogenium kivui* was cultivated at 55°C in an undefined medium (19).

Nickel transport assay. Nickel transport was determined by a modification of the assay previously described for the characterization of nickel transport by *Clostridium pasteurianum* (3). All cell manipulations were performed anaerobically. Cells were harvested, washed once, suspended in transport buffer (100 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES], pH 7.0, containing 0.5 mM sodium dithionite and 0.01% resazurin), and preincubated at 55°C for 15 min in crimp-sealed vials in a shaking water bath (100 oscillations per min). Unless otherwise indicated, glucose (final concentration, 11 mM) was added as an energy source during this preincubation period. Final cell density was adjusted to an optical density of 1.0 at 660 nm (equivalent to 0.45 mg of cell dry weight per ml). After the preincubation period, $^{63}NiCl_2$ was injected to various final concentrations (approximately 30,000 dpm/nmol). At appropriate intervals (the standard assay time was 4 min), aliquots of the cell suspension were removed with a syringe and subjected to microfiltration analysis with GN-6 Metricel membrane filters (pore size, 0.45 μm; Gelman Sciences, Inc., Ann Arbor, Mich.) for the measurement of ^{63}Ni uptake (3). Assays were performed in triplicate, and the values reported are the means; the standard error of the means approximated 4% of the control.

Analytical methods. Growth was monitored at 660 nm with a Spectronic 88 or Spectronic 501 (Bausch & Lomb, Inc., Rochester, N.Y.). Cell dry weights were determined as previously described (25). Protein was estimated by the Bradford method (2). Acetate was quantitated by high-performance liquid chromatography as previously described (3). Cellular extracts for polyacrylamide gel electrophoretic analysis were prepared by lysozyme digestion (23), and electrophoresis and in situ gel staining for CO dehydrogenase were done as previously described (7). $^{63}NiCl_2$ was purchased from New England Nuclear Corp., Boston, Mass.

RESULTS AND DISCUSSION

Optimal conditions for and kinetics of nickel transport. Cells from the mid-log phase of growth displayed the highest rates of nickel transport (data not shown), and subsequent studies were performed with cells obtained from this period of growth. Nickel transport was optimal at pH 7 to pH 7.5 and 65°C (data not shown). However, to more closely approximate the standard growth conditions of the organism, we conducted the standard nickel transport assay at pH

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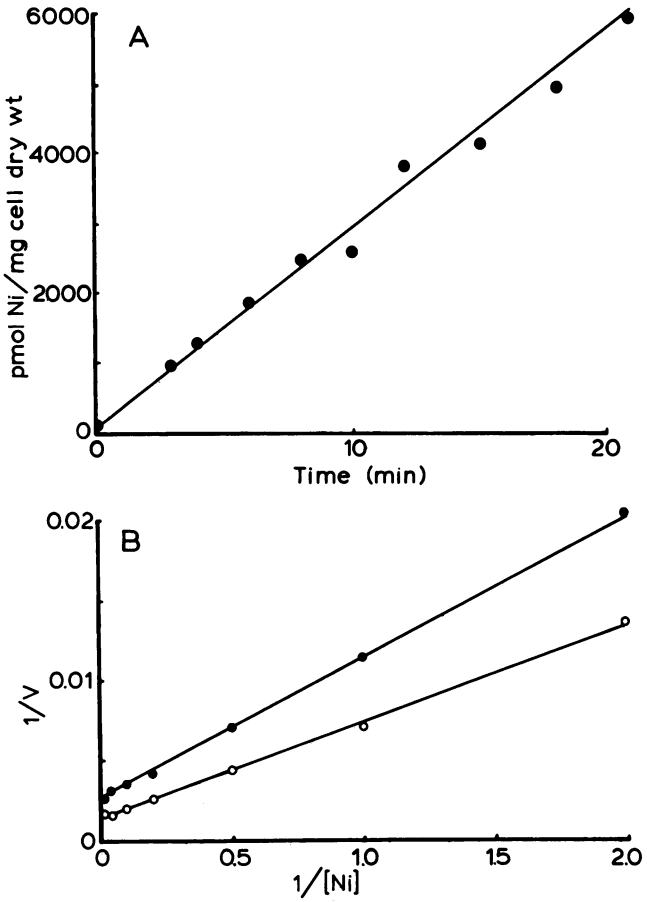

FIG. 1. Linearity (A) and Lineweaver-Burk plot (B) of nickel transport by *C. thermoaceticum*. (A) Glucose-cultivated cells with 25 μM $^{63}NiCl_2$. (B) Glucose-cultivated cells with 11 mM glucose (○) and CO-cultivated cells with an assay gas phase of CO-CO_2-N_2 (7:5:14) at 144 kPa (14 lb/in^2) over the atmospheric pressure (●).

7.0 and 55°C. Transport was not inhibited by nickel concentrations up to 100 μM, was linear, and followed Michaelis-Menten kinetics (Fig. 1). The apparent K_m values for CO- and glucose-cultivated strain ATCC 39073 were estimated to be 3.2 and 3.8 μM Ni, respectively, while the corresponding V_{max} values approximated 400 and 670 pmol of Ni transported per min per mg of cells (dry weight), respectively. The apparent K_m and V_{max} values for both methanol- and syringate-cultivated cells approximated 10 μM Ni and 1,200 pmol of Ni transported per min per mg of cells (dry weight), respectively (data not shown). In comparison, nickel transport by the methanogen *Methanobacterium bryantii* yields a K_m of 3.1 μM Ni and a V_{max} of 24 pmol of Ni transported per min per mg of cells (dry weight) (14). The cyanobacterium *Anabaena cylindrica* exhibits extremely low K_m and V_{max} values for nickel (17 nM Ni and 0.37 pmol of Ni transported per min per mg of cells [dry weight], respectively) (4).

Recovery of transported nickel in cell extracts. On the basis of an intracellular volume of 1.27 μl per mg of cells (dry weight) (1), exogenous nickel was concentrated by the cells approximately 3,000-fold over the apparent nickel concentration gradient during the transport assay. Polyacrylamide gel electrophoretic analysis of cellular extracts prepared from such cells revealed that less than 1% of the total transported nickel was present in CO dehydrogenase; most of the nickel was accounted for as low-molecular-weight species which electrophoresed coincidently with the dye front (data not shown).

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

Inhibitor (μM)[a]	Growth (maximum A_{660})	Nickel transport (% of control)
Control (none added)	0.50	100
DCCD (700)	0.15	25
CCCP (1,000)	0.02	68
Nigericin (14)	0.02	47
Harmaline (100)	0.34	43
KCN (200)	0.2	0

[a] Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

Effects of metabolic inhibitors and starvation on nickel transport. Various metabolic inhibitors decreased nickel transport (Table 1). However, nickel transport was not consistently affected when glucose was deleted from transport assays of glucose-cultivated cells. On the assumption that stored cellular energy was involved in powering nickel transport in the absence of an exogenous energy source, cells were subjected to glucose starvation prior to injection of $^{63}NiCl_2$ in the transport assay. After 90 min of starvation, external energy sources were found to significantly stimulate nickel transport by both glucose- and methanol-cultivated cells (Table 2). In addition, concentrations of CO as high as 30% stimulated nickel transport approximately threefold by CO-cultivated cells; higher concentrations of CO were inhibitory (data not shown).

Alcaligenes eutrophus (21), *Bradyrhizobium japonicum* (28), and *Rhodopseudomonas capsulata* (29) have been shown to transport nickel in the initial absence of exogenous energy sources. In the case of *R. capsulata*, glycogen was postulated as the internal energy reserve utilized (29). In the present study, acetate was not formed by *C. thermoaceticum* in the absence of glucose (data not shown), suggesting that cells were not using carbohydrate reserves for nickel transport in the absence of an exogenous energy source. High-energy phosphates, such as inorganic PP_i (11), may, in part, account for the ability of *C. thermoaceticum* to transport nickel in the absence of an exogenous energy source. *Methanobacterium thermoautotrophicum* produces both PP_i (18) and 2,3-cyclopyrophospho-glycerate as high-energy phosphates (17, 27). Further work will be required to resolve the potential energy reserves of acetogens.

TABLE 2. Effects of starvation and external energy sources on nickel transport[a]

Cultivation medium	Transport assay energy source (mM)	Starvation time (min)	Nickel transport	
			% of total[b]	Rate[c]
Glucose	None	0[d]	5.5	376
Glucose	None	90	4.7	319
Glucose	Glucose (10)	90	12.5	855
Methanol	None	0[d]	6.3	432
Methanol	None	90	0.4	24
Methanol	Methanol (60)	90	17.5	1,008

[a] Cells were incubated with or without the indicated energy source prior to the injection of $^{63}NiCl_2$ (final concentration, 15 μM).

[b] Percent of the total exogenous ^{63}Ni available in the assay.

[c] Picomoles of nickel transported per minute per milligram of cells (dry weight).

[d] At zero time, exogenous energy sources did not appreciably affect nickel transport.

Effects of metals on nickel transport. To assess the specificity of the nickel transport system, we evaluated various divalent cations for the ability to inhibit nickel transport. Calcium, cobalt, iron, magnesium, manganese, and zinc (added as dichloride salts) had no appreciable effects at concentrations 15-fold greater than that of nickel (data not shown). In contrast, nickel transport by *C. pasteurianum* is strongly inhibited by magnesium, cobalt, and zinc, an inhibition pattern indicative of a magnesium transporter which transports nickel with a low affinity (3). This observation, in addition to the relatively low K_m for nickel, indicates that nickel translocation by *C. thermoaceticum* is catalyzed by a transporter with a high affinity for nickel.

Nickel transport by other acetogens. As with *C. thermoaceticum*, the initial capacities of *C. thermoautotrophicum* and *P. productus* to transport nickel were not affected by exogenous energy sources but were strongly inhibited by metabolic uncouplers and inhibitors (data not shown). Unlike nickel transport by the other acetogens tested, nickel transport by *A. kivui* was stimulated by exogenous energy sources without starvation prior to the transport assay (data not shown).

In general, bacterial nickel transport is energy dependent; chemolithotrophic bacteria possess high-affinity nickel transport systems (13, 14, 21, 28; this study), while chemoorganotrophic bacteria appear to transport nickel with a lower affinity by a magnesium transport system (3, 15, 24, 31, 32). Proton motive force is likely to be involved in nickel transport, although which component of the proton motive force serves as the main driving force in *C. thermoaceticum* is unresolved. Under the assay conditions used in the present study, ATPase (12) appeared to be involved, since transport (by glucose-cultivated cells) decreased in response to the putative ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide. It has been postulated that an Na^+/H^+ antiporter plays a role in energy conservation by *C. thermoaceticum* (30); it may be of related importance that the putative Na^+/H^+ antiporter inhibitor harmaline inhibited nickel transport.

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