Competitive Inhibition of an Energy-Dependent Nickel Transport System by Divalent Cations in Bradyrhizobium japonicum JH†

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Both nickel-specific transport and nickel transport by a magnesium transporter have been described previously for a variety of nickel-utilizing bacteria. The derepression of hydrogenase activity in Bradyrhizobium japonicum JH and in a gene-directed mutant of strain JH (in an intracellular Ni metabolism locus), strain JHK7, was inhibited by MgSO₄. For both strains, Ni²⁺ uptake was also markedly inhibited by Mg²⁺, and the Mg²⁺-mediated inhibition could be overcome by high levels of Ni²⁺ provided in the assay buffer. The results indicate that both B. japonicum strains transport Ni²⁺ via a high-affinity magnesium transport system. Dixon plots (1/V versus inhibitor) showed that the divalent cations Co²⁺, Mn²⁺, and Zn²⁺, like Mg²⁺, were competitive inhibitors of Ni²⁺ uptake. The Kᵢ₅ for nickel uptake inhibition by Mg²⁺, Co²⁺, Mn²⁺, and Zn²⁺ were 48, 22, 12, and 8 µM, respectively. Cu²⁺ strongly inhibited Ni²⁺ uptake, and molybdate inhibited it slightly. Respiratory inhibitors cyanide and azide, the uncoupler carbonyl cyanide m-chlorophenylhydrazone, the ATPase inhibitor N₅,N₅′-dicyclohexylcarbodiimide, and ionophores nigericin and valinomycin significantly inhibited short-term (5 min) Ni²⁺ uptake, showing that Ni²⁺ uptake in strain JH is energy dependent. Most of these conclusions are quite different from those reported previously for a different B. japonicum strain belonging to a different serogroup.

Nickel is an element essential for the growth of many microorganisms, because this element is a component of enzymes involved in ureolysis, H₂ metabolism, methane production, and carbon monoxide utilization (12). Therefore, Ni-requiring microorganisms must somehow internalize nickel from their environment. Ni transport has been studied in many bacteria (5), such as Escherichia coli (16), Methanobacterium bryantii (15), Alcaligenes eutrophus (21), Clostridium pasteurianum (3), Clostridium thermoacetatum (22), and Acetogenium kivui (33), and in the cyanobacterium Anabaena cylindrica (4). In E. coli, which has three Ni-containing hydrogenases (26), Ni seems to be transported primarily via the magnesium uptake system (14, 16). In the best-studied system with regard to Ni transport, that of A. eutrophus, two Ni transport systems have been described (21)—a nickel-specific system and an uptake process which also transports magnesium. Subsequently, a nickel-specific transport gene (hoxN) from A. eutrophus was isolated and sequenced, and it encodes a predicted 33.1-kDa membrane-associated polypeptide (6, 7).

The N₂-fixing symbiont of soybeans, Bradyrhizobium japonicum, possesses a Ni-containing hydrogenase (1, 11, 30) that is expressed predominantly in the symbiotic bacteroids but also in free-living cultures under defined derepression conditions (23). Nickel not only serves as a required component of hydrogenase but also regulates hydrogenase synthesis in this bacterium (19, 20). Ni²⁺ transport and accumulation have been studied in B. japonicum SR and in the hydrogenase-constitutive strain derived from SR, strain SR470 (24, 29). Little of the accumulated Ni was exchangeable with exogenously added NiCl₂, and the bulk of the Ni uptake seemed to be energy independent (29). Also, Mg²⁺ had little inhibitory effect on Ni²⁺ uptake (29). Hydrogenase is the major sink for nickel when cells are derepressed, but nickel incorporates into soluble proteins under non-hydrogenase-derepression conditions. This soluble pool can serve as a storage source for nickel until hydrogenase is later synthesized by incubation of cells under derepression conditions (24).

A nickel metabolism locus that encodes a factor responsible for insertion of Ni into hydrogenase has recently been identified within the 23-kb hydrogenase cluster of B. japonicum (9). A mutation at this locus (strain JHK7) causes the strain to require high Ni levels for hydrogenase activity as well as for hydrogenase synthesis at the transcriptional level (9). To understand Ni metabolism further, we have studied the transport characteristics of strains JH and JHK7. Specifically, we report here that (i) Mg²⁺ significantly inhibits Ni transport in both strains; (ii) this inhibition can be relieved by increasing the level of Ni²⁺ in the assay buffer; (iii) Ni²⁺ transport is competitively inhibited by the divalent cations Mg²⁺, Co²⁺, Mn²⁺, and Zn²⁺; and (iv) cyanide, azide, N₅,N₅′-dicyclohexylcarbodiimide (DCCD), carbonyl cyanide M-chlorophenylhydrazone (CCCP), nigericin, and valinomycin all significantly inhibit Ni²⁺ transport, showing that Ni²⁺ transport in B. japonicum JH is energy dependent.

MATERIALS AND METHODS

Chemicals. High-purity chemicals (Puratronic grade 1, certified to 99.99% purity) used for making nickel-free trace element stock and all other reagent-grade chemicals for media were described previously (9). 2-(N-Morpholino)-ethanesulfonic acid (MES), DCCP, CCCP, valinomycin, and all other chemicals used were of analytical grade.
nigericin, NaCN, and Na₂S were purchased from Sigma Chemical Co., St. Louis, Mo. Radioactive ⁶³NiCl₂ (specific activity, 10.58 mCi/mg; supplied as 50 µCi/µl) was purchased from New England Nuclear, Boston, Mass. ⁶³NiCl₂ was diluted to 5 µCi/µl with Ni-free deionized water before use as described previously (9). Controlled pore glass-8-hydroxyquinoline, used to remove contaminating trace divalent cations (8), was from Pierce Chemical Co. Gases were purchased from Linde Gases or Potomac Airgas, Baltimore, Md.

All glassware used for this study was soaked in 2 M nitric acid overnight and subsequently rinsed four times with deionized water as described previously (9).

Bacteria and growth. _B. japonicum_ strain USDA 110 (13), was used as the wild-type strain. Strain JHK7 is a gene-directed mutant (Ni metabolism locus was deleted) of strain JH (9). Both strains were grown in modified Bergersen (MB) medium as described before (2). Derepression medium containing no carbon (No-C) was described previously (9, 31). Cells were grown heterotrophically or derepressed at 30°C with shaking at 150 rpm. Cell numbers were routinely estimated by measuring the optical density at 540 nm and comparing it to standard curves of optical density versus viable cell number (9).

Derepression of hydrogenase. Ni-free mid-log-phase _B. japonicum_ strain JHK7 cells were harvested by centrifugation, washed twice in derepression medium containing no carbon, nickel, or magnesium (No-C, No-Ni²⁺, No-Mg²⁺ medium) and resuspended in fresh No-C, No-Ni²⁺, No-Mg²⁺ medium to 3 × 10⁶ to 5 × 10⁶ cells/ml. Cells were then rendered (10 ml) into 150-ml serum bottles, and NiCl₂ (0.5 µM) and different amounts of MgSO₄ (as indicated in the figures) were added to the bottles by using a pipette. The bottles were tightly closed with butyl rubber stoppers and aluminum crimps and then flushed with an anaerobic gas mixture (10% H₂, 5% CO₂, 85% N₂) for 30 min by use of inflow and outflow needles through the rubber stoppers as previously described (9). O₂ at a 1% partial pressure (final concentration) was added by using a syringe, and the bottles were then incubated at 30°C at 150 rpm for 20 h for hydrogenase derepression (9). Hydrogenase activity (H₂ uptake activity) was measured amperometrically as described previously (32) with O₂ as the electron acceptor (25, 27).

**Ni accumulation and Ni uptake assay.** For nickel accumulation assays, Ni-free mid-log-phase JH and JHK7 cells were harvested, washed twice in 50 mM MES buffer (pH 6.0) (29), and resuspended in fresh MES buffer, and then 50-ml portions of the cell suspension (3.5 × 10⁸ cells/ml) were dispensed into 125-ml flasks. This buffer system is designed for optimized Ni uptake rates by _B. japonicum_ (29). ⁶³NiCl₂ at 1 µM (final concentration), which equals 0.67 µCi/ml of cell suspension, was added to each flask. The flasks were then incubated at 30°C with shaking at 150 rpm. Samples (3 ml) were removed by using a pipette at the indicated time (see the figures) for filter assays as described previously (9). The filters were placed in 5-ml scintillation vials to which 4 ml of Optifluor (DuPont, NEN Products) was then added for counting (9, 29). For Ni²⁺ uptake in the presence or absence of MgSO₄ (see Fig. 3), strain JH cells were harvested, washed, and resuspended in 50 mM MES buffer (pH 6.0) as mentioned above and 5 ml of the cell suspension was dispensed into each 50-ml flask. A mixture of ⁶³NiCl₂ (1 µM), along with the required level of cold NiCl₂ and other metal ions as indicated, was added to each flask. The flasks were immediately incubated for 5 min at 150°C with shaking at 150 rpm, and samples were rapidly filtered through 25-mm-diameter type HA filters (1 ml for each filter) (pore size, 0.45 µm; Millipore Corp., Bedford, Mass.) as previously described (9).

**Effect of metal ions on Ni uptake.** Mid-log-phase cells were harvested, washed, and resuspended in 50 mM MES buffer (pH 6.0) as described above. Cell suspensions (5 ml at 4.0 × 10⁸ cells/ml) were dispensed into 50-ml flasks. A mixture of ⁶³NiCl₂ (1 µM, or 0.67 µCi/ml of cell suspension) and the indicated divalent metal ion (see Table 1) was added to the flask. For the competition studies (see Fig. 4), a mixture of ⁶³NiCl₂ (1 µM) plus nonradioactive NiCl₂ (to make concentrations of 10, 25, and 40 µM nickel) and different amounts of the cation was added to the flask containing 5 ml of cell suspension. Flasks were immediately incubated for 5 min at 30°C with shaking at 150 rpm (see above), and filter assays were performed (9).

**Effect of inhibitors on Ni uptake.** Ni-free mid-log-phase cells were harvested, washed, and resuspended in 50 mM MES buffer as described above. The inhibitor was added to the 50-ml flask containing 5 ml of cell suspension (4 × 10⁸ cells/ml), and flasks were incubated at 30°C for 30 min. Then 1.0 µM ⁶³Ni (0.67 µCi/ml of cell suspension) was added, and the amount of ⁶³Ni incorporated in the cells was determined after 5 min (30°C, with shaking at 150 rpm). The ⁶³Ni assayed was the result of internalized Ni because the cells were washed extensively on the filters (9). Background counts of radioactive nickel binding to the filter (<1,000 cpm) were subtracted from the average of the samples with cells. The 1,000 cpm represents about 1.5 pmol of Ni²⁺ uptake. Data are means of duplicates for H₂ uptake and triplicates for nickel uptake unless otherwise indicated.

**RESULTS**

The previous studies on Ni transport utilized wild-type strain SR and a mutant derived from SR, strain SR470 (29). However, it has not been possible to obtain gene-directed mutations of strain SR (since it is Kan') or of its derivative strain, LO (9), to permit definitive molecular genetics studies. Therefore, we have used strain JH, a strain in which we can obtain gene-directed mutations at loci involved in Ni metabolism (9). We have found the characteristics of Ni²⁺ uptake in strain JH to be different in many respects from those of strain SR and its derivative, SR470 (29).

**Effect of magnesium on hydrogenase activity.** A specific nickel transport gene is not present in _B. japonicum_ on the basis of lack of homology to the _hostN_ gene from _A. eutrophus_ (9). This result then raises the question of how nickel is transported into _B. japonicum_ JH cells. One possibility was that the strain lacks an Ni-specific transporter and that nickel is taken up via a magnesium transport system, as has been seen in some other bacteria (14, 17). Initially, the effect of magnesium on derepression of hydrogenase activity with low (0.5 µM) Ni²⁺ concentrations was studied (Fig. 1).

_B. japonicum_ JH (wild type) showed maximum hydrogenase activity (256 nmol of H₂ oxidized per h per 10⁸ cells) at 100 µM MgSO₄, and the activity decreased with increasing Mg²⁺ concentrations to 35 nmol of H₂ per h per 10⁸ cells at 10 mM MgSO₄. Hydrogenase activity in the Ni-metabolism-locus-deleted mutant strain (JHK7), which requires higher levels of nickel for hydrogenase expression than the wild strain JH (9), also showed inhibition by MgSO₄ (data not shown), although all specific hydrogenase activities were lower in the mutant strain than in the wild type, as is expected for a mutant that is deficient in intracellular Ni metabolism into hydrogenase.
Effect of MgSO₄ on Ni accumulation. To determine whether the decreased hydrogenase activity was directly due to inhibition of Ni²⁺ uptake by magnesium, a 1-h time course of nickel uptake by strains JH and JHK7 in the presence and absence of MgSO₄ (400 μM) was conducted (Fig. 2). For both wild-type JH and mutant JHK7, Ni²⁺ accumulation was strongly inhibited by 400 μM MgSO₄ in the assay buffer (Fig. 2), which was the concentration normally present in MB medium. The final internal Ni level accumulated in the presence of MgSO₄ at 1 h was 18 and 15% of that in the absence of MgSO₄ for strains JH and JHK7, respectively (Fig. 2). There was no significant difference in the Ni²⁺ uptake rate between strain JH and JHK7 (Ni-metabolism-locus-deleted mutant) in either the presence or absence of MgSO₄ (Fig. 2); this finding is in agreement with our previous findings (9) that the Ni²⁺ metabolism gene product is involved not in Ni transport but in an intracellular Ni metabolism step into hydrogenase.

The strong inhibition of Ni²⁺ accumulation by magnesium in B. japonicum suggests that Ni²⁺ is mainly taken up via a magnesium transport system, and the two cations may compete for the same transport site. This competition obviously affects hydrogenase activity expression as well (Fig. 1).

Ni²⁺ uptake (in short-term 5-min assays) was measured with increasing amounts of substrate (nickel) in the presence and absence of MgSO₄ (Fig. 3). The inhibition of Ni²⁺ uptake by a constant magnesium concentration (40 μM) was partially relieved by increasing nickel levels in the assay (Fig. 3). Similar results were obtained by using a higher MgSO₄ concentration (3 mM) (data not shown). These results support the idea that Ni²⁺ and Mg²⁺ compete with each other at the (short-term) transport level rather than only at the (long-term [Fig. 2]) accumulation level.

Effect of other metal ions on Ni²⁺ uptake. The effect of other (potentially competing) metals on Ni²⁺ uptake was measured in strains JH and JHK7 in the presence of 1 μM Ni²⁺ (Table 1). In addition to Mg²⁺, the divalent cations Co²⁺, Cu²⁺, Mn²⁺, and Zn²⁺ at 40 μM inhibited Ni²⁺ uptake by 74, 91, 62 and 85%, respectively; a similar inhibition pattern was observed for strain JHK7 (Table 1). These inhibitors were not toxic to the cells, because growth (on the basis of optical density) was not affected by these metals (Co²⁺, Cu²⁺, Mn²⁺, and Zn²⁺ at 40 μM [data not shown]). Molybdate (40 μM) inhibited strain JH Ni²⁺ uptake by 37%; Fe³⁺ and Ca²⁺ inhibited Ni²⁺ uptake slightly. Since no significant difference was found for Ni²⁺ uptake metal ion inhibition patterns between strains JH and JHK7, further studies focused on strain JH only. Nevertheless, the results presented for strain JHK7 reinforce our conclusion (9) that this mutant has Ni transport abilities similar to those of the wild type.

Competition studies of Ni²⁺ uptake by Mg²⁺, Co²⁺, Mn²⁺, and Zn²⁺. To determine whether inhibition of Ni²⁺ uptake...
TABLE 1. Effect of metal ions on Ni uptake in JH and JHK7 in the presence of 1 μM 63NiCl2.

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Conc (μM)</th>
<th>Ni uptake(^a) (% of control)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>JH</td>
</tr>
<tr>
<td>Control</td>
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<tr>
<td>CaCl(_2)</td>
<td>40</td>
<td>95</td>
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<tr>
<td></td>
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<tr>
<td>CoSO(_4)</td>
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</tr>
<tr>
<td></td>
<td>400</td>
<td>7</td>
</tr>
<tr>
<td>CuSO(_4)</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>3</td>
</tr>
<tr>
<td>Fe(_2)(SO(_4))(_3)</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>75</td>
</tr>
<tr>
<td>MnCl(_2)</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>12</td>
</tr>
<tr>
<td>ZnSO(_4)</td>
<td>40</td>
<td>15</td>
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<td></td>
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<td>7</td>
</tr>
<tr>
<td>Na(_2)MoO(_4)</td>
<td>40</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>73</td>
</tr>
<tr>
<td>MgSO(_4)</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>43</td>
</tr>
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</table>

\(^a\) The Ni uptake assay is described in Materials and Methods.

Ni uptake was significantly inhibited by these carbon sources (data not shown). The reason for this inhibition is unclear; perhaps succinate and/or gluconate can chelate the divalent nickel, rendering it unable to enter the cell. The inhibitors shown in Table 2 also inhibited the expression of hydrogenase activity when cells were subjected to derepression conditions (data not shown). Although other potential inhibitors and ionophores are available that mediate their effects in a manner similar to those listed in Table 2, the results obtained were quite clear, and the use of additional inhibitors was deemed to be unnecessary.

DISCUSSION

Nickel is required by many bacteria, and its role has been studied most for the H\(_2\)-oxidizing bacteria. Both Ni-specific transport and Ni transport by a magnesium transporter have been reported. Our recent study (9) showed that a Ni\(^{2+}\) transport gene was not found in B. japonicum by using boxN (specific Ni\(^{2+}\) transport gene) from A. eutrophus as a probe (6, 7). In support of the lack of such a transporter, our current results showed that hydrogenase activity decreases with the addition of MgSO\(_4\) during derepression and that Ni\(^{2+}\) transport is strongly inhibited by magnesium. The inhibition of Ni\(^{2+}\) uptake by Mg\(^{2+}\) was relieved by increasing the amount of Ni\(^{2+}\) provided in the assay buffer. However, hydrogenase activity and Ni\(^{2+}\) transport could not be inhibited completely by Mg\(^{2+}\), and so a low level of Ni-specific transport cannot be ruled out entirely. Nevertheless, all of the results reported here suggest that Ni\(^{2+}\) is mainly transported in B. japonicum JH by a system that also transports Mg\(^{2+}\). In addition to Mg\(^{2+}\), the divalent cations Co\(^{2+}\), Mn\(^{2+}\), and Zn\(^{2+}\) were highly inhibitory to Ni\(^{2+}\) uptake in B. japonicum JH, and this inhibition is probably the result of binding of the cations to the Mg\(^{2+}\) carrier that transports Mg\(^{2+}/Ni\(^{2+}\). These results resemble those reported for the "major" Ni\(^{2+}\) transport system of A. eutrophus (21) and for Ni\(^{2+}\) transport in E. coli (16). Some inhibition of Ni\(^{2+}\) uptake by some of these metal ions was reported for B. japonicum SR470 (29), although the extent of inhibition was not nearly that which we report here for strain JH. The competitive inhibition of Ni\(^{2+}\) uptake by Co\(^{2+}\), Mg\(^{2+}\), and Mn\(^{2+}\) in B. japonicum JH is similar to the metal inhibition effects reported for E. coli (16, 17) and (with the exception of Mn\(^{2+}\)) C. pasteurianum (3) but unlike the inhibition described for C. thermoacetica (22), A. kivui (33), and (with the exception of Co\(^{2+}\)) M. bryantii (15). The competitive inhibition that we report for Zn\(^{2+}\)-versus-Ni\(^{2+}\) transport has been reported in only one other organism, A. eutrophus (21). Energy-dependent Zn\(^{2+}\) transport systems have been reported for eucaryotic microbes; Ca\(^{2+}\) and Mn\(^{2+}\) are often competitive inhibitors of Zn\(^{2+}\) uptake (14). It appears that different bacteria use quite different divalent metal-sequencing components and mechanisms, and our results suggest that highly significant differences can occur even within two B. japonicum strains. Strains JH and SR are derivatives of strains USDA 110 and USDA 122, respectively—strains that fall into different strain serogroups.

Cytochrome c oxidase inhibitors NaCN and NaN\(_3\) strongly inhibited short-term Ni uptake. Therefore, the bulk of nickel taken up by B. japonicum JH is probably energy dependent. The ATPase inhibitor DCCD markedly inhibited

Effect of respiratory inhibitors on Ni uptake. To determine whether Ni uptake in B. japonicum JH is an energy-dependent transport process, the effect of respiratory inhibitors was studied (Table 2). The cytochrome c oxidase inhibitors NaCN and NaN\(_3\) significantly inhibited Ni uptake (68 and 75%, respectively). The ATPase inhibitor DCCD and the protonophore CCCP inhibited Ni\(^{2+}\) uptake by 67 and 91%, respectively. The K\(^{+}\)/H\(^+\) antiporter nigericin and the electrogenic ionophore valinomycin, each with 50 mM KCl to dissipate the membrane potential, also inhibited Ni\(^{2+}\) uptake (51 and 32%, respectively) compared with the control without inhibitor (Table 2). On the basis of these inhibitor results it seems that Ni\(^{2+}\) uptake in B. japonicum JH is energy dependent. However, the addition of exogenous energy sources that stimulate respiratory activity, such as succinate or gluconate (20 mM), to the cell suspensions did not stimulate Ni\(^{2+}\) uptake. In fact, nickel uptake was significantly inhibited by these carbon sources (data not shown). The reason for this inhibition is unclear; perhaps succinate and/or gluconate can chelate the divalent nickel, rendering it unable to enter the cell. The inhibitors shown in Table 2 also inhibited the expression of hydrogenase activity when cells were subjected to derepression conditions (data not shown). Although other potential inhibitors and ionophores are available that mediate their effects in a manner similar to those listed in Table 2, the results obtained were quite clear, and the use of additional inhibitors was deemed to be unnecessary.

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Ni uptake, suggesting that the proton motive force for ATP production via ATPase is required for Ni²⁺ transport. The protonophore CCCP and ionophores valinomycin and nigericin also significantly inhibited Ni uptake, also suggesting that some of the nickel taken up is coupled to proton movement. These results are different from those in the report in which these inhibitors were used on B. japonicum SR470; it was concluded that most of the Ni²⁺ transported was not energy generation dependent in that strain. Also, nickel uptake in Azotobacter chroococcum was apparently energy independent (28). On the basis of inhibitor studies, Jasper and Silver (16) demonstrated that Ni²⁺ uptake via a high-affinity magnesium system was energy dependent. Bryson and Drake (3) also showed energy-dependent Ni²⁺ transport (via a magnesium transporter system) in C. pasteurianum; DCCD, CCCP, valinomycin, and nigericin all inhibited transport of Ni²⁺ in C. pasteurianum. In conclusion, Ni²⁺ transport in B. japonicum JH is mainly via a magnesium transport system that also, evidently at the initial binding step(s), transports some other divalent cations. These ions are competitive inhibitors of Ni transport, and results of inhibitor studies strongly suggest that the uptake system is energy dependent.

FIG. 4. Dixon plots of inhibition of Ni²⁺ uptake by MgSO₄ (A), CoSO₄ (B), MnCl₂ (C), and ZnSO₄ (D) by strain JH with three nickel concentrations. Symbols: O, 10 μM NiCl₂; △, 25 μM NiCl₂; □, 40 μM NiCl₂; 1 μM ⁶⁷NiCl₂ (0.67 μCi/ml of cell suspension) was added to initiate each assay. The data were analyzed by linear regression and plotted by using Sigma-Plot (Sigma-Plot Scientific Graph System).

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>% Inhibition of Ni uptake*</th>
</tr>
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<tbody>
<tr>
<td>None (control)</td>
<td>0</td>
</tr>
<tr>
<td>Sodium cyanide (10 mM)</td>
<td>68</td>
</tr>
<tr>
<td>Sodium azide (10 mM)</td>
<td>75</td>
</tr>
<tr>
<td>DCCD (200 μM)</td>
<td>67</td>
</tr>
<tr>
<td>CCCP (200 μM)</td>
<td>91</td>
</tr>
<tr>
<td>Nigericin (20 μM) + KCl (50 mM)</td>
<td>51%</td>
</tr>
<tr>
<td>Valinomycin (20 μM) + KCl (50 mM)</td>
<td>32%</td>
</tr>
</tbody>
</table>

* Ni-free-grown cells (JH) were harvested, washed twice, and resuspended in 50 mM MES buffer (pH 6.0). The inhibitor was added to the flask containing 5 ml of cells, and the mixture was incubated for 30 min at 30°C, with shaking at 150 rpm; 1 μM ⁶⁷Ni (0.67 μCi/ml of cell suspension) was added, and the suspension was incubated again for 5 min. Samples were then taken out and filtered.

* The data represent the percent inhibition of nickel uptake on the basis of three or four replicates relative to the control cells without inhibitor. The nickel uptake rate of the control cells was 1.2 pmol of ⁶⁷Ni per min per 10⁸ cells.

* The slight effect of KCl (50 mM) alone on Ni²⁺ uptake was subtracted.
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REFERENCES


