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Metal Ion Uptake by a Plasmid-Free Metal-Sensitive *Alcaligenes eutrophus* Strain

DIETRICH H. NIES†* AND SIMON SILVER

Department of Microbiology and Immunology, University of Illinois College of Medicine, Box 6998, Chicago, Illinois 60680

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The divalent metal cations of zinc, cadmium, cobalt, nickel, and manganese are transported into cells of *Alcaligenes eutrophus* strain AE104 by the energy-dependent magnesium transport system. Chromate is transported by the sulfate uptake system.

In contrast to heavy-metal-resistant *Alcaligenes eutrophus* CH34, sensitive derivative strain AE104 does not contain the plasmids pMOL28 and pMOL30 (8), which encode resistance to several metal ions (3, 8). Plasmid pMOL30 encodes an inducible efflux pump responsible for resistance to Co^{2+}, Zn^{2+}, and Cd^{2+} (8, 10). Plasmid pMOL28 encodes an inducible efflux pump responsible for resistance to Co^{2+} and Ni^{2+} (10, 13, 14) and chromate resistance via inducible reduced accumulation (10). The export of the metal ions by the resistance systems has been previously described (10). This paper describes the uptake of Co^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, CrO_4^{2-}, and SO_4^{2-} by sensitive strain AE104.

A. eutrophus AE104 (metal sensitive, plasmid free) was previously described (8, 9). Cells were grown at 30°C in a Tris-buffered minimal salts medium (8) with 2 g of sodium gluconate per liter as carbon source. Turbidity measurements were done with a Klett-Summerson photoelectric colorimeter (no. 54 Kodak Wratten filter). Transport assays were performed as previously described (10). After 10-fold dilution into fresh medium, cells were incubated with shaking at 30°C for 15 to 18 h. Cells were harvested by centrifugation at 12,000 × *g* for 5 min at 4°C and suspended in the same volume of 10 mM Tris hydrochloride buffer (pH 7.0). Transport assays were conducted in 10 mM Tris hydrochloride buffer (pH 7.0) at 30°C with aeration by shaking. Samples (500 μl) were filtered through 0.45-μm-pore-diameter filters (Nuclepore Corp., Pleasanton, Calif.) and rinsed twice with 5-ml volumes of buffer. Reagent-grade chemicals and deionized water were used. $^{54}Mn^{2+}$, $^{60}Co^{2+}$, $^{65}Zn^{2+}$, $^{109}Cd^{2+}$, and scintillation counting fluid were obtained from Amersham Corp. (Arlington Heights, Ill.); $^{51}CrO_4^{2-}$ was obtained from ICN Pharmaceuticals Inc. (Irvine, Calif.); and $^{35}SO_4^{2-}$ and $^{63}Ni^{2+}$ were obtained from New England Nuclear Corp. (Boston, Mass.). All samples were counted in a liquid scintillation spectrometer (Tri-Carb 3375; Packard Instrument Corp., Downers Grove, Ill.). Carbonyl cyanide-*m*-chlorophenylhydrazone, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone, *N*,*N*′-dicyclohexylcarbodiimide, venturicidin, 2,4-dinitrophenol, and EDTA were obtained from Sigma Chemical Corp. (St. Louis, Mo.).

The uptake of chromate by strain AE104 was competitively inhibited by sulfate (K_i, 30 μM SO_4^{2-}), and uptake of sulfate was competitively inhibited by chromate (K_i, 26 μM CrO_4^{2-}; Fig. 1). However, the K_ms for sulfate and chromate uptake were about four times higher than the respective K_i values (Table 1). This result may be an artifact of the assay conditions or may reflect the existence of a secondary transport system.

Uptake of chromate and sulfate was derepressed in cells cultivated in 30 μM sulfate instead of 300 μM or 3 mM sulfate (Fig. 2). These kinetic experiments are consistent with a single SO_4^{2-}-repressible shared substrate (SO_4^{2-} or CrO_4^{2-}) transport system, comparable to the situation in *Pseudomonas fluorescens* (11).

The transport of Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} by strain AE104 followed Michaelis-Menten saturation kinetics, and V_{max} and K_m values were calculated (Table 1). Cation uptake in strain AE104 was energy dependent. While 500 μM venturicidin and 50 μM *N*,*N*′-dicyclohexylcarbodiimide showed no effect on cation uptake, 1 mM 2,4-dinitrophenol inhibited uptake betwen 23% (Ni^{2+} uptake) and 84% (Co^{2+} uptake; data not shown). Carbonyl cyanide-*m*-chlorophenylhydrazone and carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone did not inhibit at 10 μM but did inhibit cation transport at 100 μM or 1 mM (data not shown). Therefore, the membrane potential probably drives cation uptake by strain AE104.

Uptake of $^{54}Mn^{2+}$, $^{60}Co^{2+}$, $^{65}Zn^{2+}$, $^{109}Cd^{2+}$, and $^{63}Ni^{2+}$ (1 μM each) was inhibited by 1 mM Mg^{2+} (1,000:1 ratio) or by $MnCl_2$, $ZnCl_2$, $CoCl_2$, $NiCl_2$, or $CdCl_2$ (100 μM each; data not shown). In cells of strain AE104 cultivated under magnesium starvation, the uptake of Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} was enhanced compared with that of control cells (Fig. 2). When the magnesium concentration in the growth medium was reduced from 1 mM to 100 μM, uptake of divalent metal cations increased about sevenfold (Fig. 2).

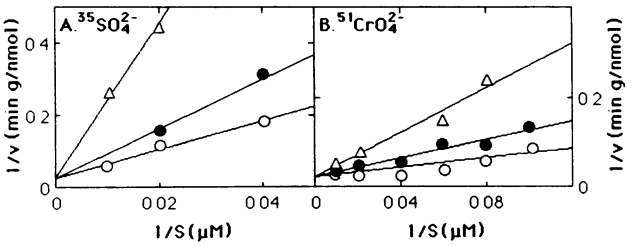

FIG. 1. Cross-inhibition of sulfate and chromate uptake. Uptake of sulfate (A) and chromate (B) was measured in the presence of 0 (○), 20 (●), and 50 (△) μM chromate and sulfate. Each line was calculated from six sulfate concentrations, but only the data points for the highest sulfate concentrations are displayed.

* Corresponding author.
† Present address: Freie Universitat Berlin, Konigin-Luise Str. 12-16A, 1000 Berlin 33, Federal Republic of Germany.

TABLE 1. Kinetic parameters of ion uptake by strain AE104

Ion	Mean ± SD (no. of expts)		
	K_m (μM)	V_{max} (μmol/min per g [dry wt])	K_i
Zn^{2+}	137 ± 87 (5)	3.7 ± 2.1 (5)	10 ± 1.5 μM Mg^{2+} (2)
Cd^{2+}	136 ± 22 (5)	3.4 ± 1.2 (5)	6.4 ± 0.4 μM Mg^{2+} (2)
Co^{2+}	40 ± 29 (5)	1.9 ± 0.3 (5)	4.2 ± 0.7 μM Mg^{2+} (2)
Ni^{2+}	40 ± 23 (5)	1.6 ± 0.4 (5)	20 ± 11 μM Mg^{2+} (2)
Mn^{2+}	58 ± 27 (4)	1.5 ± 0.9 (4)	24 ± 12 μM Mg^{2+} (2)
CrO_4^{2-}	151 ± 28 (2)	0.14 ± 0.06 (2)	26 ± 6 μM SO_4^{2-} (2)
SO_4^{2-}	112 ± 36 (2)	0.029 ± 0.008 (2)	30 ± 13 μM CrO_4^{2-} (2)

When the magnesium concentration was lowered to 10 μM, uptake of the divalent metal cations increased about 20-fold (Fig. 2).

The kinetics of uptake of Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} by strain AE104 are consistent with transport by a single system with a low substrate specificity. Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} compete with each other and with Mg^{2+} for transport by this system. Unfortunately, the radionuclide of magnesium (^{28}Mg; half life, 21 h) is not readily available, so probably the most natural substrate for this system ($^{28}Mg^{2+}$) was not tested. However, Mg^{2+} was a competitive inhibitor for Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} uptake with an average K_i of 13 ± 9 μM Mg^{2+} (Table 1).

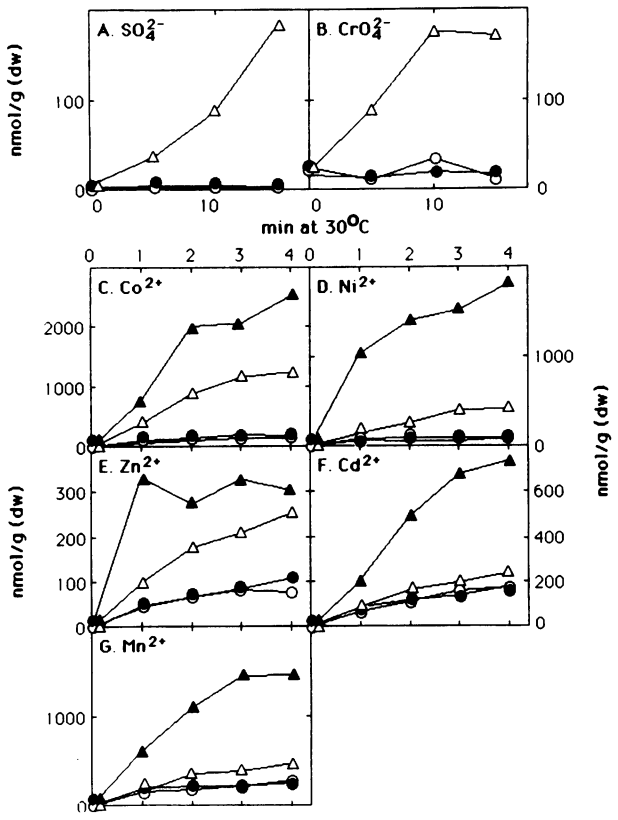

FIG. 2. Regulation of cation and anion uptake. Strain AE104 was cultivated overnight in modified Tris-buffered mineral salts medium. Cells were harvested in the late exponential growth phase and suspended in 10 mM Tris hydrochloride buffer (pH 7.0), and the uptake of SO_4^{2-} (A), CrO_4^{2-} (B), Co^{2+} (C), Ni^{2+} (D), Zn^{2+} (E), Cd^{2+} (F), and Mn^{2+} (G) was measured at a concentration of 1 μM each. For anion uptake experiments (A and B), the sulfate concentration in the growth medium was 3 mM (○), 300 μM (●), or 30 μM (△) SO_4^{2-}. For cation uptake experiments (C, D, E, F, and G), the magnesium concentration and the amount of trace element solution SL6 (12) in the growth medium were SL6 and 1 mM $MgCl_2$ (○), no SL6 and 1 mM $MgCl_2$ (●), no SL6 and 100 μM $MgCl_2$ (△), or no SL6 and 10 μM $MgCl_2$ (▲). All uptake experiments were performed in 10 mM Tris hydrochloride buffer (pH 7.0) with shaking at 30°C.

The activity of this magnesium transport system is strongly regulated by the environmental magnesium concentration. This broad cation transport system is responsible for the major supply of strain AE104 with divalent metal cations. When strain AE104 was repressed by growth on high Mg^{2+}, we could not detect high-affinity, high-specificity transport systems as found in other bacteria for Mn^{2+} transport (4, 15) or for Ni^{2+} transport (7).

Co^{2+} and Mn^{2+} compete with Mg^{2+} for uptake by *Escherichia coli* (4, 17). Ni^{2+} uptake by *A. eutrophus* H16 (7), *Clostridium pasteurianum* (1), *Rhodobacter capsulatus* (16), and other bacteria (17) is also mediated by the Mg^{2+} transport system. Cd^{2+} transport by *E. coli* is inhibited by Zn^{2+} but not by Mn^{2+}, Ni^{2+}, or Co^{2+} (6).

This situation of one broad-substrate-range divalent-cation transport system responsible for uptake of divalent metal cations may be generalized for procaryotes, although additional transport systems with higher ion selectivity exist. Zn^{2+} uptake, but not Cd^2 uptake, by the Mg^{2+} transport system has been suggested for other bacteria (17). Energy-dependent Zn^{2+} uptake has been reported for *E. coli* (2) but could not be reproduced (5). Therefore, this paper may present the first secure data about zinc transport in procaryotes.

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