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Cadmium-Resistant Mutant of *Bacillus subtilis* 168 with Reduced Cadmium Transport

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Cd^{2+} and Mn^{2+} accumulation was studied with wild-type *Bacillus subtilis* 168 and a Cd^{2+}-resistant mutant. After 5 min of incubation in the presence of 0.1 μM $^{109}Cd^{2+}$ or $^{54}Mn^{2+}$, both strains accumulated comparable amounts of $^{54}Mn^{2+}$, while the sensitive cells accumulated three times more $^{109}Cd^{2+}$ than the Cd^{2+}-resistant cells did. Both $^{54}Mn^{2+}$ and $^{109}Cd^{2+}$ uptake, which apparently occur by the same transport system, demonstrated cation specificity; 20 μM Mn^{2+} or Cd^{2+} (but not Zn^{2+}) inhibited the uptake of 0.1 μM $^{109}Cd^{2+}$ or $^{54}Mn^{2+}$. $^{54}Mn^{2+}$ and $^{109}Cd^{2+}$ uptake was energy dependent and temperature sensitive, but $^{109}Cd^{2+}$ uptake in the Cd^{2+}-resistant strain was only partially inhibited by an uncoupler or by a decrease in temperature. $^{109}Cd^{2+}$ uptake in the sensitive strain followed Michaelis-Menten kinetics with a K_m of 1.8 μM Cd^{2+} and a V_{max} of 1.5 μmol/min · g (dry weight); $^{109}Cd^{2+}$ uptake in the Cd^{2+}-resistant strain was not saturable. The apparent K_m value for the saturable component of $^{109}Cd^{2+}$ uptake by the Cd^{2+}-resistant strain was very similar to that of the sensitive strain, but the V_{max} was 25 times lower than the V_{max} for the sensitive strain. The K_m and V_{max} for $^{54}Mn^{2+}$ uptake by both strains were very similar. Cd^{2+} inhibition of $^{54}Mn^{2+}$ uptake had an apparent K_i of 3.4 and 21.5 μM Cd^{2+} for the sensitive and Cd^{2+}-resistant strains, respectively. Mn^{2+} had an apparent K_i of 1.2 μM Mn^{2+} for inhibition of $^{109}Cd^{2+}$ uptake by the sensitive strain, but the Cd^{2+}-resistant strain had no defined K_i value for inhibition of Cd^{2+} uptake by Mn^{2+}.

Cd^{2+} is toxic to bacteria (13). A mechanism of resistance to Cd^{2+} has been studied in *Staphylococcus aureus*. *S. aureus* is normally sensitive to concentrations of Cd^{2+} above about 2 μM, but if a plasmid which confers resistance to Cd^{2+} is introduced into the organism, it is resistant to up to 200 μM Cd^{2+} (14). The plasmid codes for a Cd^{2+} efflux (antiporter) transport system which rapidly transports Cd^{2+} out of the cells (9, 11).

Recently, a Cd^{2+}-resistant mutant of *Bacillus subtilis* 168 was isolated (10). Both the parental and mutant strains are plasmidless. The mutant strain was obtained by successive transfers of the parental strain into medium containing up to 90 μM Cd^{2+}. Under steady-state conditions, the Cd^{2+}-sensitive parental strain accumulated 10 times the level of Cd^{2+} that the Cd^{2+}-resistant mutant strain accumulated. This Cd^{2+}-resistant strain appears to be the first chromosomally determined Cd^{2+}-resistant mutant isolated. Cd^{2+} resistance in this strain is expressed constitutively (10).

The existence of chromosomally governed Cd^{2+} resistance is quite reasonable. Cd^{2+} must enter the cell to be toxic (7, 13). Cd^{2+} can be accumulated in cells by transport system(s) responsible for the uptake of essential cation(s) like Mn^{2+} (8, 12, 14). Therefore, a mutation affecting the active transport system responsible for Cd^{2+} accumulation which resulted in altered cation specificity or loss of the uptake system altogether could provide Cd^{2+} resistance.

An analogous situation exists in *Escherichia coli*, in which chromosomal mutations affecting the constitutive phosphate transport system resulted in arsenate resistance (6). *E. coli* has two phosphate transport systems. The constitutive system has a wide substrate specificity (it will also transport the toxic anions arsenate and vanadate). A second inducible phosphate transport system has a 100-fold higher affinity for phosphate than the constitutive system. Cells which lose the constitutive phosphate transport system by mutation therefore become relatively resistant to arsenate (6).

Cd^{2+} transport has been studied in only two procaryotes to date, *S. aureus* (5, 12, 14) and *Lactobacillus plantarum* (1). For both species, Cd^{2+} is accumulated by the Mn^{2+} active transport system, and Cd^{2+} and Mn^{2+} are competitive inhibitors of $^{54}Mn^{2+}$ and $^{109}Cd^{2+}$ uptake, respectively. The cation specificity of the Mn^{2+} transport system of the two organisms differed somewhat. However, Zn^{2+} (a cation closely related to Cd^{2+}) did not inhibit $^{109}Cd^{2+}$ or $^{54}Mn^{2+}$ uptake in *S. aureus* or in *L. plantarum*.

The purpose of this study was to determine the mechanism of resistance to Cd^{2+} in the mutant strain of *B. subtilis* 168. Surowitz et al. (10), who isolated the mutant strain, had already concluded that the resistance was due to lesser accumulation of Cd^{2+}. However, their studies involved atomic absorption measurements of Cd^{2+} accumulated over periods of hours and did not determine the basic properties of the transport system(s) involved. This information is important for an understanding of the relationship between toxic elements and microorganisms.

MATERIALS AND METHODS

Bacterial strains. *B. subtilis* 168 1A1 is a plasmidless tryptophan auxotroph, and strain 1A1R is a constitutive Cd^{2+} resistant mutant of 1A1 (10). Both the parental and the mutant strains were obtained from Robert M. Pfister, Department of Microbiology, Ohio State University, Columbus, Ohio.

Media and growth experiments. Cells were grown in tryptone broth (8 g of tryptone per liter and 5 g of NaCl per liter) for all experiments except sporulation experiments, in which tryptone broth was supplemented with 25 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 1 to 50 μM Mn^{2+}. Germination of spores was done on a medium consisting of 8 g of nutrient broth (Difco Laboratories, Detroit, Mich.) per liter and 2.5 g of yeast extract (Difco) per liter. Starter cultures were grown

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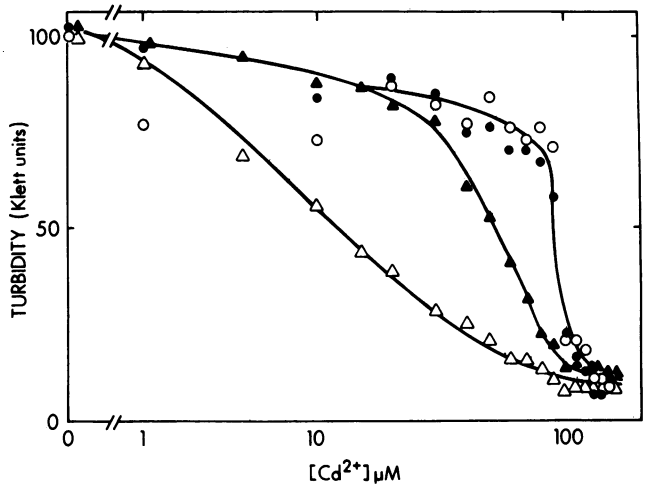

FIG. 1. Growth of sensitive and Cd^{2+}-resistant cells in the presence of cadmium and the capacity of Mn^{2+} ions to spare both strains from the toxic effects of Cd^{2+}. Overnight cultures were diluted 1:100 into broth and incubated at 37°C until the late-log phase of growth. Culture turbidity was measured after 5 h in the presence of increasing concentrations of Cd^{2+}. Symbols: ○, Cd^{2+}-resistant cells; ●, Cd^{2+}-resistant cells plus 50 μM Mn^{2+}; △, sensitive cells; ▲, sensitive cells plus 50 μM Mn^{2+}.

overnight with aeration, a 1% (vol/vol) inoculum was transferred to fresh medium, and the cell suspensions were grown until the mid-log phase of growth in the absence of Cd^{2+} or in the presence of Cd^{2+} and in the presence or absence of 50 μM Mn^{2+}. Turbidity measurements were done with a Klett-Summerson photoelectric colorimeter (no. 54 Kodak Wratten filter).

Transport assays. The cells were harvested by centrifugation at 16,000 × *g* for 10 min at 4°C and resuspended in a small volume of fresh medium at 4°C at 25 mg (dry weight) per ml. The final cell density during the transport assays was 0.5 mg (dry weight) per ml. Transport assays were conducted in broth at 37°C with aeration by shaking. Samples were filtered through 0.45-μm filters and then rinsed twice with 5-ml volumes of broth.

Reagents and chemicals. Reagent grade chemicals and deionized water were used in all experiments. [^{14}C]uracil and $^{54}Mn^{2+}$ were obtained from New England Nuclear Corp., Boston, Mass. $^{109}Cd^{2+}$ and scintillation counting fluid were obtained from Amersham Corp., Arlington Heights, Ill. Carbonyl cyanide *m*-chlorophenyl-hydrazone (CCCP) was obtained from Sigma Chemical Co., St. Louis, Mo. Membrane filters were obtained from Nuclepore Corp., Pleasanton, Calif. All samples were counted in a liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

RESULTS

Effect of Cd^{2+} on growth and the sparing effect of Mn^{2+}. The effect of Cd^{2+} on the growth of sensitive and Cd^{2+}-resistant *B. subtilis* cells and the ability of Mn^{2+} to protect the sensitive cells from Cd^{2+} were tested (Fig. 1). The Cd^{2+}-resistant strain grew in the presence of 40 to 80 times the concentrations of Cd^{2+} that the sensitive cells would tolerate. Weiss et al. (14) and Archibald and Duong (1) showed that the Mn^{2+} active transport system is responsible for Cd^{2+} accumulation by *S. aureus* and *L. plantarum*, respectively. If Cd^{2+} is also accumulated by the Mn^{2+} transport system in *B. subtilis*, then at some concentration(s) of Cd^{2+} used in the growth inhibition experiments added Mn^{2+} may spare the cells from the toxic effect of Cd^{2+} by competitively blocking Cd^{2+} uptake by the cells. The extent of the sparing effect would depend on the K_m, K_i, and V_{max} values of both Mn^{2+} and Cd^{2+} uptake by each strain and also to some extent on the mechanism(s) of Cd^{2+} toxicity. The sparing effect of Mn^{2+} with the Cd^{2+}-sensitive cells was greatest from 5 to about 20 μM Cd^{2+}. The resistant cells grew to the same extent in the presence of Cd^{2+} and in the presence and absence of $MnCl_2$.

Effect of Mn^{2+} on sporulation. The formation of spores by *B. subtilis* cells is dependent on several factors. One is the concentrations of minerals such as Mn^{2+}, Ca^{2+}, K^+, and Mg^{2+} in the medium in which the cells are grown (2). Both strains formed spores at approximately the same frequency in low and in high Mn^{2+} (data not shown).

Cation specificity of transport. Cells of both the sensitive and the Cd^{2+}-resistant strains took up about the same amount of $^{54}Mn^{2+}$ over the 5-min time course of an uptake experiment (Fig. 2A and C). However, sensitive cells took up about 3.4 times more $^{109}Cd^{2+}$ than Cd^{2+}-resistant cells did. These results are consistent with those of steady state experiments in which Cd^{2+}-resistant cells accumulated much less Cd^{2+} than Cd^{2+}-sensitive cells did (10). For both sensitive and resistant cells, a 200-fold molar excess of nonradioactive Mn^{2+} or Cd^{2+} affected the accumulation of both 0.1 μM $^{54}Mn^{2+}$ and 0.1 μM $^{109}Cd^{2+}$ (Fig. 2). The addition of 20 μM Mn^{2+} or Cd^{2+} inhibited $^{54}Mn^{2+}$ or $^{109}Cd^{2+}$ uptake by sensitive cells while 20 μM Zn^{2+} caused a small reduction in the uptake of both substrates. For the Cd^{2+}-resistant cells, the results were somewhat different: 20 μM Mn^{2+} inhibited $^{54}Mn^{2+}$ uptake, but Cd^{2+} had a somewhat lesser effect in

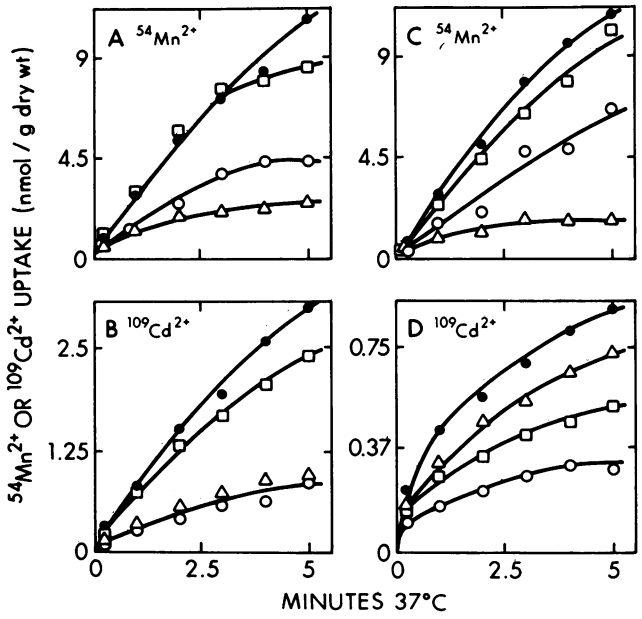

FIG. 2. Effect of divalent cations on $^{54}Mn^{2+}$ (A and C) or $^{109}Cd^{2+}$ (B and D) uptake by sensitive cells (A and B) or Cd^{2+}-resistant cells (C and D). The cells were prepared as described in the text and then preincubated for 2 min at 37°C. At −30 s, 20 μM nonradioactive Cd^{2+}, Mn^{2+}, or Zn^{2+} was added. At time 0, 0.1 μM $^{54}Mn^{2+}$ or $^{109}Cd^{2+}$ was added. Samples (0.25 mg [dry weight]) were filtered and washed as indicated. Symbols: ●, 0.1 μM $^{54}Mn^{2+}$ or $^{109}Cd^{2+}$; ○, +20 μM $CdCl_2$; □, +20 μM $ZnCl_2$; △, +20 μM $MnCl_2$. Note the change in the ordinate scales for panels B and D.

reducing $^{54}Mn^{2+}$ accumulation in Cd^{2+}-resistant cells as compared with that in sensitive cells (cf. Fig. 2A and C). Unlike the results with sensitive cells (Fig. 2B), the uptake of $^{109}Cd^{2+}$ by resistant cells was only slightly reduced by 20 μM Mn^{2+}. The small effect of Zn^{2+} on $^{54}Mn^{2+}$ uptake by Cd^{2+}-resistant cells was comparable to that observed with sensitive cells, but Zn^{2+} significantly reduced the low level of $^{109}Cd^{2+}$ uptake by the Cd^{2+}-resistant cells. Cd^{2+} was clearly the most effective of the three cations in reducing the uptake of $^{109}Cd^{2+}$ in the resistant strain.

The $^{54}Mn^{2+}$ uptake system for both strains and $^{109}Cd^{2+}$ accumulation by the sensitive strain were all sensitive to a decrease in the temperature to 4°C or to the addition of the uncoupler CCCP (Fig. 3). Cd^{2+} accumulation by the Cd^{2+}-resistant strain was almost completely resistant to the effect of the uncoupler, while a decrease in the temperature to 4°C caused a 75% reduction in $^{109}Cd^{2+}$ accumulation but not the 95% reduction obtained with the sensitive strain (cf. Fig. 3B and D). Increasing the concentration of CCCP (to 100 μM) or increasing the preincubation period in the presence of CCCP from 2 to 5 min or both did not reduce the amounts of $^{109}Cd^{2+}$ or $^{54}Mn^{2+}$ accumulation further. In control experiments, cells of both strains preincubated for 2 min at 4°C or with CCCP at 37°C did not take up and incorporate significant amounts of [^{14}C]uracil.

Kinetics of uptake. Kinetic parameters of $^{109}Cd^{2+}$ accumulation by both strains of *B. subtilis* were determined. A defined maximal initial velocity of $^{109}Cd^{2+}$ uptake was obtained with the sensitive strain (Fig. 4). Lineweaver-Burk plots were drawn (not shown), and the K_m and V_{max} values were determined (Table 1). The rate of $^{109}Cd^{2+}$ uptake by the Cd^{2+}-resistant strain continued to increase as the concentration of $^{109}Cd^{2+}$ increased and did not follow Michaelis-Menten kinetics. However, a careful examination of the data from Fig. 4 and similar experiments showed that there was a saturable component at concentrations ranging from 0.25 to 2.5 μM $^{109}Cd^{2+}$. A Lineweaver-Burk plot (not shown) was drawn based only on the initial velocities of uptake at concentrations of $^{109}Cd^{2+}$ from 0.25 to 1.0 μM (the saturable component of the curve in Fig. 4), and an apparent K_m and V_{max} for the Cd^{2+}-resistant strain were determined (Table 1). The K_ms for $^{109}Cd^{2+}$ uptake were similar for both strains, but the V_{max} for the saturable component of Cd^{2+} uptake with the resistant strain was 30 times less than the V_{max} for the sensitive strain. When the initial rate for $^{109}Cd^{2+}$ uptake by the Cd^{2+}-resistant strain was determined at concentrations of $^{109}Cd^{2+}$ greater than those shown in Fig. 4, the rate did not saturate. However, even at 50 μM $^{109}Cd^{2+}$ the initial rate of $^{109}Cd^{2+}$ uptake by the Cd^{2+}-resistant strain was only 0.48 μmol min · g (dry weight). The kinetic parameters of the

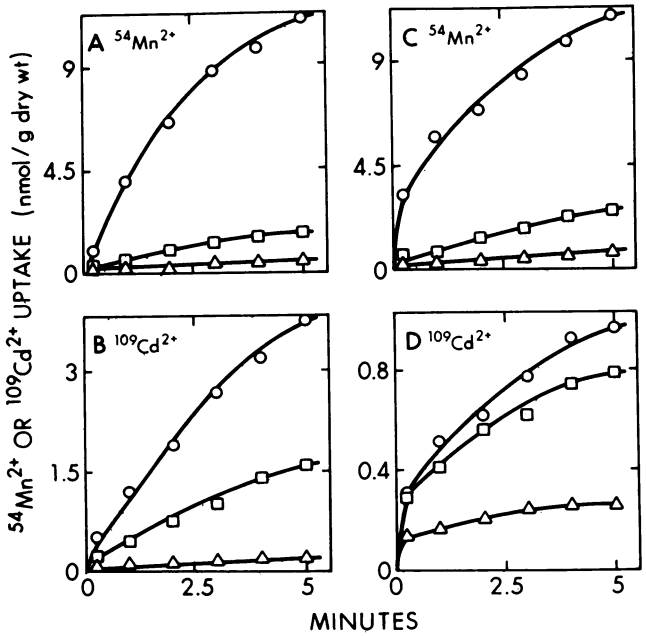

FIG. 3. Inhibition of $^{54}Mn^{2+}$ (A and C) or $^{109}Cd^{2+}$ (B and D) uptake by sensitive (A and B) or resistant (C and D) cells by the addition of 20 μM CCCP (at 37°C) (□) or reducing the temperature of the uptake suspension to 4°C (△) as compared with control cells at 37°C (○). Experimental procedures were as described in the legend of Fig. 2 except that cells were preincubated for 2 min at 4°C or in the presence or absence of CCCP at 37°C.

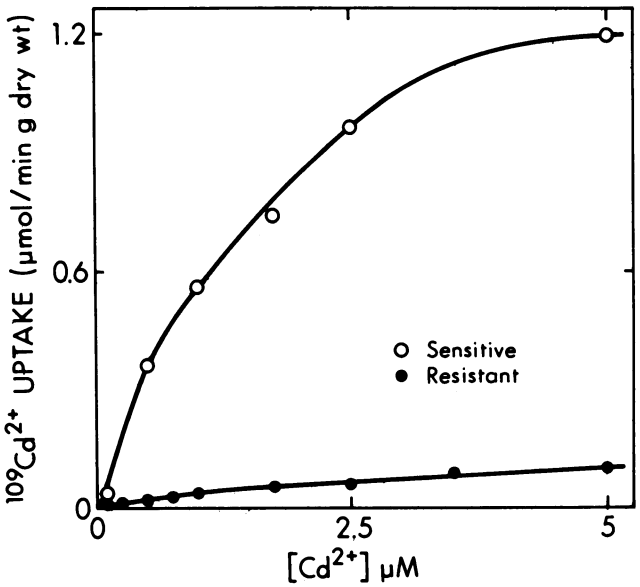

FIG. 4. Effect of increasing substrate concentration on $^{109}Cd^{2+}$ uptake by sensitive cells and Cd^{2+}-resistant cells. The experimental procedures were as described in the legend of Fig. 2 except that the concentrations of $^{109}Cd^{2+}$ added ranged from 0.1 to 5 μM and both the sampling intervals and the mass of cells filtered were changed depending upon the total concentration of Cd^{2+} used.

TABLE 1. Kinetic parameters of cadmium and manganese transport in resistant and sensitive cells

Parameters	Cells[a]	
	Wild-type sensitive	Resistant mutant
Mn^{2+}		
K_m (μM Mn^{2+})	1.12 ± 0.31 (4)	1.31 ± 0.26 (3)
V_{max} (μmol/min · g [dry wt])	0.097 ± 0.001 (4)	0.11 ± 0.011 (3)
K_i (μM Cd^{2+})	3.4 ± 1.4 (2)	21.5 ± 1.4 (2)
Cd^{2+}		
K_m (μM Cd^{2+})	1.77 ± 0.40 (3)	≈1.4[b]
V_{max} (μmol/min · g [dry wt])	1.53 ± 0.11 (3)	≈0.06[b]
K_i (μM Mn^{2+})	1.2 ± 0.28 (2)	None defined

[a] Mean value ± standard deviation (number of determinations).
[b] From analysis of data in Fig. 4 after subtraction of the nonsaturable component.

$^{54}Mn^{2+}$ active transport system were also determined (Table 1). Both the sensitive and the Cd^{2+}-resistant strain followed Michaelis-Menten kinetics for $^{54}Mn^{2+}$ uptake. Linear Lineweaver-Burk plots were obtained (Fig. 5), and similar K_m and V_{max} values were determined for both strains (Table 1).

The results presented so far indicate that $^{54}Mn^{2+}$ and $^{109}Cd^{2+}$ were accumulated by the same system. To establish that Mn^{2+} and Cd^{2+} are competitive inhibitors of $^{109}Cd^{2+}$ and $^{54}Mn^{2+}$ uptake, respectively, Dixon plots were obtained (Fig. 6). Cd^{2+} was a competitive inhibitor of $^{54}Mn^{2+}$ uptake by cells of both strains (Fig. 6C and D). However, the K_i (21.5 μM Cd^{2+}) for $^{54}Mn^{2+}$ uptake by the Cd^{2+}-resistant strain was larger than the K_i (3.4 μM Cd^{2+}) for $^{54}Mn^{2+}$ uptake by the sensitive strain (Table 1). Mn^{2+} was a competitive inhibitor of $^{109}Cd^{2+}$ uptake by sensitive cells, with a K_i of 1.2 μM Mn^{2+}. For the Cd^{2+}-resistant strain, the initial velocities of $^{109}Cd^{2+}$ uptake in the presence of Mn^{2+} did not follow simple kinetics, and no K_i values were defined. The unusual Dixon plot in Fig. 6B is somewhat similar to the Dixon plots of $^{54}Mn^{2+}$ uptake by *E. coli* cells in the presence of either Cd^{2+} or Zn^{2+} (3).

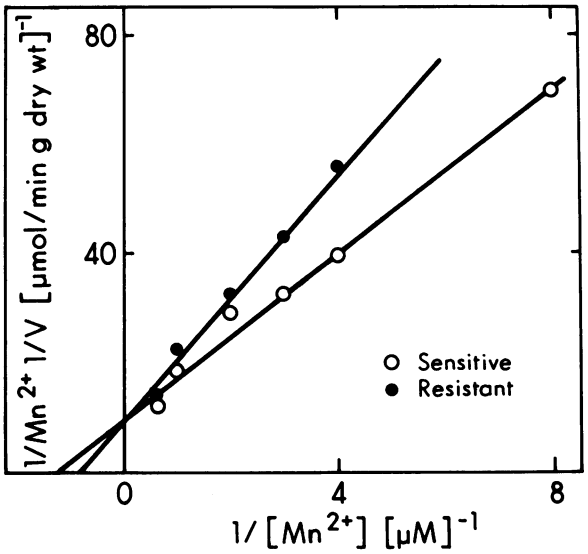

FIG. 5. Kinetics of $^{54}Mn^{2+}$ uptake by sensitive and Cd^{2+}-resistant cells. The experimental procedures were as described in the legend of Fig. 2 except that the concentrations of $^{54}Mn^{2+}$ added ranged from 0.125 to 1.5 μM and both the sampling intervals and the mass of cells filtered were changed depending upon the total concentration of Mn^{2+} used.

DISCUSSION

$^{109}Cd^{2+}$ was accumulated by the Mn^{2+} active transport system in sensitive *B. subtilis* cells. The evidence to support this claim is as follows. The capacity of Mn^{2+} to protect growing cells from the inhibitory effects of Cd^{2+} (Fig. 1) suggests a direct competition between Cd^{2+} and Mn^{2+} for transport carriers in the sensitive cells. The cation specific-

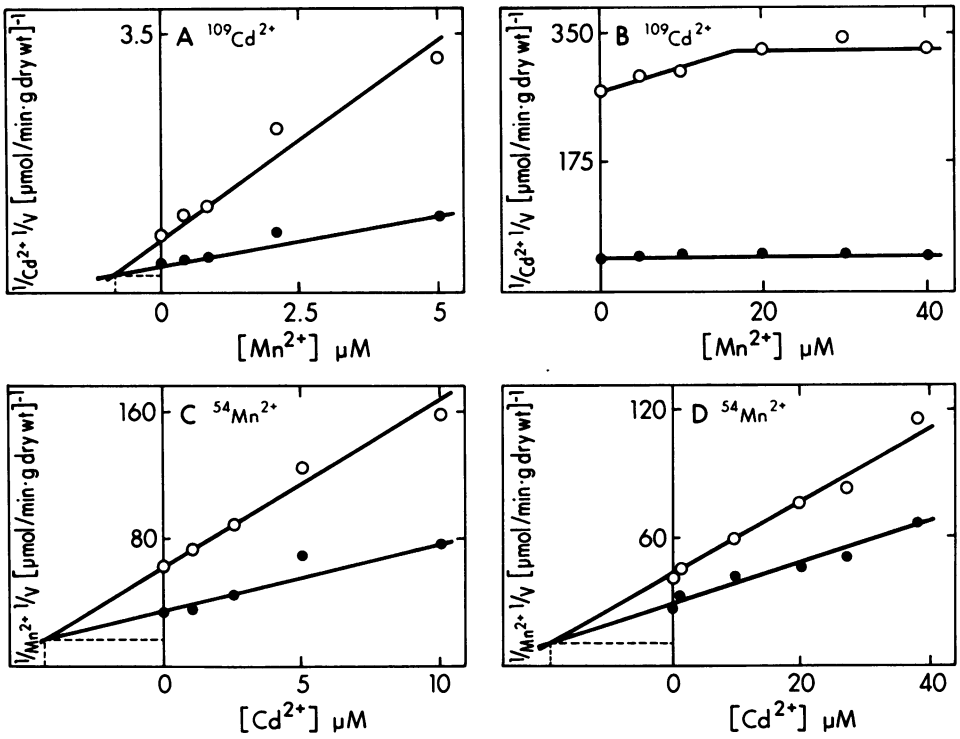

FIG. 6. Dixon plot determinations of the K_i (Mn^{2+}) for inhibition of $^{109}Cd^{2+}$ uptake (A and B) by sensitive (A) and Cd^{2+}-resistant (B) cells and the K_i (Cd^{2+}) for inhibition of $^{54}Mn^{2+}$ uptake (C and D) by sensitive (C) or resistant (D) cells. The experimental procedures were as described in the legend of Fig. 2 except that the concentrations of $^{109}Cd^{2+}$ and $^{54}Mn^{2+}$ varied and both the sampling intervals and the mass of cells filtered were changed depending upon the cation concentration used. Concentrations of $^{109}Cd^{2+}$ used in panel: A, 0.75 (○) and 2.5 (●) μM; B, 0.1 (○) and 1 (●) μM. Concentrations of $^{54}Mn^{2+}$ used in panel: C, 0.33 (○) and 0.75 (●) μM; D, 0.5 (○) and 1 (●) μM. V_{max} values (dashed lines) were determined from Lineweaver-Burk plots.

ities of the $^{54}Mn^{2+}$ and $^{109}Cd^{2+}$ uptake systems were the same (Fig. 2). Cd^{2+} and Mn^{2+} uptake followed Michaelis-Menten kinetics (Fig. 4 and 5), and Dixon plots showed that Cd^{2+} and Mn^{2+} were competitive inhibitors of $^{54}Mn^{2+}$ and $^{109}Cd^{2+}$ uptake, respectively. The K_m for $^{54}Mn^{2+}$ transport by sensitive cells was essentially the same as the K_i for Mn^{2+} as an inhibitor of $^{109}Cd^{2+}$ uptake by sensitive cells (Table 1). Similar results were obtained with a comparison of the K_m for $^{109}Cd^{2+}$ uptake and the K_i for Cd^{2+} as an inhibitor of Mn^{2+} uptake (Table 1). *B. subtilis* and the other two gram-positive organisms examined so far (1, 14) each accumulated Cd^{2+} by the Mn^{2+} active transport system. Since each bacterium is from a different genus, the data suggest a basic similarity in the micronutrient (Mn^{2+}) transport system of gram-positive bacteria.

The mechanism for Cd^{2+} resistance in the mutant *B. subtilis* strain appears to be reduced Cd^{2+} uptake (with no change in $^{54}Mn^{2+}$ uptake). Figure 5 and Table 1 show that the kinetics of $^{54}Mn^{2+}$ uptake by Cd^{2+}-resistant cells were comparable to those of sensitive cells, except that the K_i for Cd^{2+} as an inhibitor of $^{54}Mn^{2+}$ uptake by Cd^{2+}-resistant cells was about six times larger than the K_i for Cd^{2+} as a competitive inhibitor of $^{54}Mn^{2+}$ uptake by sensitive cells. Cd^{2+}-resistant and -sensitive cells differed significantly with respect to the kinetic parameters of $^{109}Cd^{2+}$ uptake. In particular, the V_{max} for the saturable component of $^{109}Cd^{2+}$ uptake by the Cd^{2+}-resistant strain was 30 times smaller than the V_{max} for the sensitive strain (Table 1). In addition, the Cd^{2+}-resistant strain had no defined K_i for Mn^{2+} as an inhibitor of $^{109}Cd^{2+}$ uptake, while the sensitive strain had a well-defined K_i of 1.2 μM Mn^{2+} (Fig. 6). The cation specificity, temperature dependence, and susceptibility to CCCP of $^{109}Cd^{2+}$ uptake by Cd^{2+}-resistant cells differed from those for sensitive cells, yet these same parameters of uptake were equivalent for $^{54}Mn^{2+}$ accumulation by sensitive and Cd^{2+}-resistant cells (Fig. 2 and 3). In *S. aureus* and *E. coli*, it seems likely that Cd^{2+} must be accumulated into the cytoplasm to be toxic (4, 5, 7, 13, 14). Therefore, reduced Cd^{2+} uptake by Cd^{2+}-resistant cells of *B. subtilis* is a plausible mechanism of resistance.

A more specific hypothesis for the basis of Cd^{2+}-resistance would be that a component(s) of the Mn^{2+} active transport system in the Cd^{2+}-resistant strain has been altered (by chromosomal mutation) so that it no longer recognizes Cd^{2+} as a substrate or no longer transports Cd^{2+} across the membrane or both. Evidence in favor of this model includes: (i) the change in specificity of the Mn^{2+} active transport system to cations in the Cd^{2+}-resistant strain (Fig. 2), (ii) the lack of sensitivity to the uncoupler CCCP and to 4°C when accumulating $^{109}Cd^{2+}$ (Fig. 3), (iii) the reduced initial velocity and final amounts of $^{109}Cd^{2+}$ accumulated by the Cd^{2+}-resistant strain (Fig. 2 through 5, Table 1), and (iv) that Mn^{2+} inhibition of Cd^{2+} uptake in the resistant strain did not follow simple kinetics.

The K_m value is a measure of the affinity of a substrate for an enzyme or transport protein. The apparent K_m for $^{109}Cd^{2+}$ uptake by the small saturable component in the Cd^{2+}-resistant cells was similar to the K_m for $^{109}Cd^{2+}$ uptake in sensitive cells, suggesting that perhaps the affinity of Cd^{2+} for the recognition component of the Mn^{2+} active transport system was not altered. However, there is reason to question the significance of this apparent K_m value. In particular, if the K_m for $^{109}Cd^{2+}$ accumulation was unchanged in both sensitive and resistant cells, why was the K_i for Cd^{2+} as an inhibitor of $^{54}Mn^{2+}$ uptake in the resistant cells about 10 times larger than the apparent K_m for Cd^{2+} uptake in resistant cells?

In conclusion, evidence has been presented which shows that $^{109}Cd^{2+}$ was accumulated by means of the Mn^{2+} active transport system in sensitive cells of *B. subtilis*. The mechanism of resistance of the Cd^{2+}-resistant strain of *B. subtilis* was reduced $^{109}Cd^{2+}$ accumulation while $^{54}Mn^{2+}$ transport remained unchanged. During the revision of this manuscript, an abstract appeared (B. E. Burke and R. M. Pfister, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, K178, p. 201) that also describes Mn^{2+} and Cd^{2+} uptake by the Cd^{2+}-resistant *B. subtilis* mutant.

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