JOURNAL OF BACTERIOLOGY, Aug. 1981, p. 305-312
0021-9193/81/080305-08$02.00/0

Vol. 147, No. 2

Reduced Cadmium Transport Determined by a Resistance Plasmid in *Staphylococcus aureus*

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Received 18 November 1980/Accepted 4 May 1981

The presence of a plasmid harboring a gene for Cd^{2+} resistance led to markedly reduced Cd^{2+} uptake via the energy-dependent Mn^{2+} transport system in *Staphylococcus aureus* strain 17810R. Cd^{2+} uptake by the resistant strain via this high-affinity system was seen only at very low Cd^{2+} concentrations. At high concentrations, Cd^{2+} was taken up by the resistant strain via a different low-affinity uptake system. Cd^{2+} uptake via this system was energy dependent but was not blocked by Mn^{2+}. Loss of the plasmid from the resistant strain resulted in Cd^{2+} sensitivity and unblocking of Cd^{2+} transport via the Mn^{2+} carrier in the plasmidless derivative strain 17810S. The energy-dependent Cd^{2+} uptake by the sensitive strain was inhibited by Mn^{2+} with kinetics indicating competitive inhibition. It is suggested that the second, low-affinity uptake system for Cd^{2+} in the resistant strain is the energy-dependent cadmium/proton antiporter, which at low Cd^{2+} concentrations functions in net Cd^{2+} efflux.

Penicillinase plasmids in *Staphylococcus aureus* carry additional genetic determinants which confer resistance to heavy metal ions: mercury, cadmium, lead, arsenic, and antimony (11, 13, 15, 18). Mechanisms of mercury, cadmium, and recently, arsenic have been studied (2, 19, 20, 25-27). Resistance to mercury and organic mercurials in *S. aureus* (19, 26) is due to enzymatic detoxification and volatilization.

Plasmid-determined resistance to cadmium has been found only in *S. aureus* (4, 13, 19). It is the most common resistance marker on the penicillinase plasmid in this organism (4, 11-14, 16, 22). Several laboratories have undertaken studies on the mechanism of plasmid-mediated Cd^{2+} resistance in *S. aureus* (1, 2, 8, 19, 25, 27). Resistant *S. aureus* strains take up less Cd^{2+} than do the sensitive organisms (1, 2, 8, 24, 25, 27). The molecular mechanism of the reduced uptake of Cd^{2+} in the resistant *S. aureus* has not been resolved. Chopra suggested that Cd^{2+} resistance is due to a change in orientation of membrane proteins or phospholipids leading to shielding of the —SH groups (2), the possible binding sites for Cd^{2+}. Weiss et al. (27) suggested a mechanism dependent upon a blockage of energy-dependent Cd^{2+} transport. Data presented in this paper confirm that the reduced uptake of Cd^{2+} lies at the transport level. The presence of the cadmium resistance plasmid in the resistant *S. aureus* strain 17810R resulted in highly reduced Cd^{2+} uptake; loss of the plasmid allowed high-affinity Cd^{2+} uptake via the energy-dependent Mn^{2+} transport system. The resistant cells were protected against Cd^{2+} accumulation at lower concentrations (up to 100 μM Cd^{2+}). At higher concentrations, Cd^{2+} was taken up by the resistant cells via a low-affinity system which was not blocked either by Mn^{2+} or valinomycin (24), but was inhibited by dinitrophenol and by low temperature (4°C).

MATERIALS AND METHODS

S. aureus strain 17810R was a 1964 hospital isolate (14, 16) and was provided by Keith Dyke (Oxford, United Kingdom) along with its cured variant strain, 17810S. The Cd^{2+} resistance plasmid from strain 17810R is now called pII17810 and falls into the δ incompatibility group, for which the prototype is plasmid pII147 (16).

Growth conditions. Early-exponential-phase cells of both strains were obtained for transport studies by the following method. A small inoculum of cells from an agar slant was cultured in nutrient broth (Serum Institute, Warsaw, Poland) overnight at 37°C. A 5-ml amount of this culture was transferred into 50 ml of broth and shaken for about 3 h, until the turbidity (Spekol photocolorimeter; 550 nm) corresponded to 1 mg of dry weight per ml. The culture was rapidly cooled and kept overnight at 4°C. It was then warmed to 37°C and mixed with 50 ml of fresh, prewarmed broth; incubation was continued with shaking until the optical density again indicated 1 mg of dry weight per ml. The cells were centrifuged at 6,000 rpm for 10 min and resuspended in broth at about 160 turbidity units, which corresponded to about 0.5 mg of dry weight. At the end of each uptake experiment, 10 ml of cell suspensions was filtered, cells were thoroughly

washed with deionized water, and the actual dry weight was determined. In experiments carried out at Washington University, St. Louis, cells were grown in brain heart infusion (Difco Laboratories, Detroit, Mich.) and uptake experiments were performed on the same day. Turbidity of the cell suspensions for uptake experiments was 90 Klett units (0.2 mg of dry cells per ml).

Assay of $^{115m}Cd^{2+}$ or $^{109}Cd^{2+}$ uptake. Cell suspensions were incubated on a shaker in the presence of various concentrations of $^{115m}Cd^{2+}$ or $^{109}Cd^{2+}$. Samples (0.5 ml each) were withdrawn at the indicated time intervals, rapidly filtered through the prewet membrane cellulose filters (0.45- or 0.6-μm pore size), and washed twice with 5 ml of room-temperature broth. Filters were dried and immersed in toluene-based scintillation fluid and counted in a scintillation counter SL, Intertechnique, France, or in a Packard Tri-Carb liquid scintillation spectrometer, model 3375. Cell-free filters took up less than 1% of the amount of Cd^{2+} taken up by sensitive cells at 10 μM or 100 μM Cd^{2+}, and even at 1 mM Cd^{2+}, only 0.4% bound to cell-free filters. This corresponds to 2 nmol per filter, or less than 5% of the uptake by resistant cells under these conditions.

Chemicals. All chemicals of analytical grade were the product of either POCH, Gliwice, Poland, or Fisher Scientific Co., Fair Lawn, N.J. Membrane filters (0.6 μm) were produced by Chemapol, Czechoslovakia and filters with a pore size of 0.45 μm by Sartorius, West Germany. Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) was purchased from Sigma Chemical Co., St. Louis, Mo. Valinomycin was the product of Calbiochem, La Jolla, Calif. $^{115m}Cd^{2+}$ (specific activity, 50 Ci/mol) was obtained from the Institute of Nuclear Research, Poland. Carrier-free $^{109}Cd^{2+}$ and carrier-free $^{54}Mn^{2+}$ were obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

Uptake experiments with radioactive $^{115m}Cd^{2+}$ in broth (Fig. 1) showed that the differential sensitivities of *S. aureus* strains to cadmium during growth (25) are due to their differential abilities to accumulate this cation. With 10 μM Cd^{2+}, less than 1 nmol of Cd^{2+} per mg of dry weight was taken up by the resistant strain, as compared with 17 nmol of Cd^{2+} per mg of dry weight taken up by strain 17810S. With 100 μM Cd^{2+}, uptake of this cation by the sensitive strain increased about fourfold, whereas the resistant strain still did not accumulate toxic amounts of Cd^{2+} (Fig. 1; 24, 25). At 1 mM Cd^{2+}, the extent of Cd^{2+} uptake by strain 17810S doubled, whereas the plasmid-mediated protective mechanism in strain 17810R seemed partially overcome; approximately 40 nmol of Cd^{2+} per mg of dry weight was taken up, which was still only half of that taken up by strain 17810S (Fig. 1).

Figure 2 shows a 1-min lag period of the initial uptake of Cd^{2+} by the resistant strain in Serum Institute nutrient broth, which was not observed

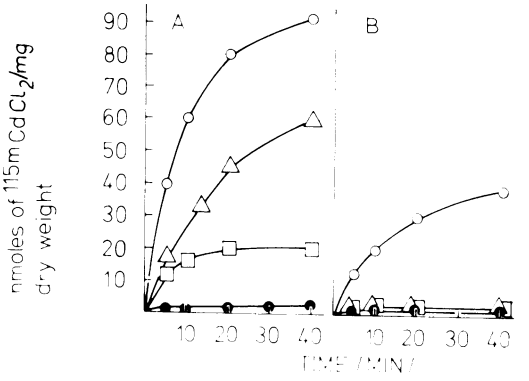

FIG. 1. *Time course of $^{115m}Cd^{2+}$ uptake by S. aureus strains 19810S (A) and 17810R (B). The uptake assay was performed at 37°C as described in the text. Symbols: ●, 1 μM Cd^{2+}; □, 10 μM Cd^{2+}; △, 100 μM Cd^{2+}; and ○, 1 mM Cd^{2+}.*

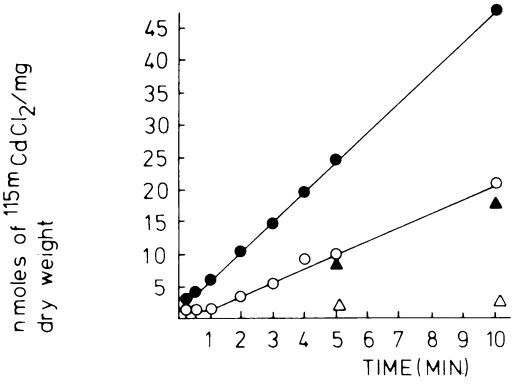

FIG. 2. *Initial rate of $^{115m}Cd^{2+}$ uptake at 1 mM. The uptake assay was performed at 37°C as described in the text. S. aureus strain 17810R: control cells (○); cells with 10 mM dinitrophenol added at −5 min (△). S. aureus strain 17810S: control cells (●); cells with 10 mM dinitrophenol added at −5 min (▲).*

in the plasmidless organism. Cd^{2+} uptake by both strains at 1 mM Cd^{2+} was inhibited by dinitrophenol (Fig. 2).

Effect of temperature on $^{115m}Cd^{2+}$ uptake by *S. aureus* strains. Uptake of Cd^{2+} by strain 17810S was highly reduced at 4°C (Table 1). The amount of $^{115m}Cd^{2+}$ taken up by the resistant strain at 4°C was also lower than that at 27 or 37°C (Table 1). Inhibition of Cd^{2+} uptake by both strains at a low temperature (4°C) and with dinitrophenol suggests that metabolic energy is required for this uptake. The strains did not differ in the extent of Cd^{2+} uptake at 4°C, but they did differ in the rate of this uptake (Fig. 3). In the sensitive organism, Cd^{2+} uptake at 1 mM (at 4°C) was completed within 5 min, whereas

TABLE 1. *Temperature dependence of $^{115m}Cd^{2+}$ uptake by S. aureus*[a]

Strain	Temp (°C)	Uptake (nmol of $^{115m}Cd^{2+}$ per mg of dry wt)		
		1 mM Cd^{2+}	100 μM Cd^{2+}	10 μM Cd^{2+}
17810R	4	6.9	0.11	0.07
	27	18	0.39	0.28
	37	44	0.83	0.63
17810S	4	7.4	0.12	0.09
	27	43.5	29.3	13.9
	37	99	65	17.1

[a] Cell suspensions in broth were incubated with various cadmium concentrations at various temperatures for 40 min and then filtered and washed as described in the text.

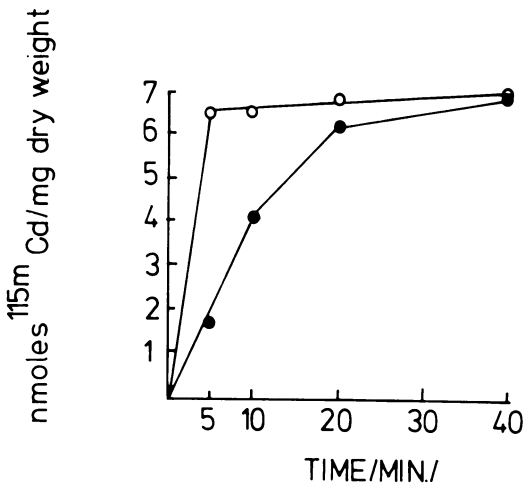

FIG. 3. *Initial rate of Cd^{2+} uptake at 1 mM Cd^{2+} by S. aureus strains 17810R (●) and 17810S (○) at a low temperature (4°C). The uptake assay was performed as indicated in the text.*

with resistant cells, 20 min were required to reach a similar steady-state level.

Concentrative uptake of $^{115m}Cd^{2+}$ by *S. aureus* strains. For calculation of the ratio between cadmium taken up by the cells and that left in the supernatant fluid (Table 2), the value obtained by Collins and Hamilton (3) for the internal water volume in *S. aureus* (1.55 μl of water per mg of dry weight) was used. Both strains took up $^{115m}Cd^{2+}$ at 37°C against a concentration gradient. The sensitive strain accumulated Cd^{2+} against a 7,300:1 concentration gradient at 10 μM added Cd^{2+}; for the resistant organism, the corresponding value was only 40:1.

None of the cations tested (K^+, Na^+, Mg^{2+}, Ca^{2+}, and Zn^{2+}) at 100 μM had any effect on uptake of 10 μM $^{115m}Cd^{2+}$ by the sensitive strain except Mn^{2+} (Fig. 4). $^{115m}Cd^{2+}$ accumulated by the sensitive cells was also exchangeable with external Mn^{2+} or Cd^{2+} and was released by toluene (Fig. 5), which breaks the permeability barrier of the cells.

Kinetics of $^{115m}Cd^{2+}$ uptake by *S. aureus* strains. The initial influx rate of $^{115m}Cd^{2+}$ into cells of the sensitive strain followed Michaelis-Menten saturation kinetics in Serum Institute nutrient broth, with an apparent K_m of 9.6 μM Cd^{2+} and a V_{max} of 2.73 nmol min^{-1} mg of dry $weight^{-1}$ (Fig. 6). In contrast, the initial influx rate of Cd^{2+} into the parent strain 17810R

TABLE 2. *Distribution ratio of $^{115m}Cd^{2+}$ in S. aureus cells and the medium*[a]

Strain	$^{115m}Cd^{2+}$ (mM) in:			Ratio: in cells/outside cells
	Initial sample	Supernatant	Cells	
17810R	0.01	0.0097	0.39	40
	0.1	0.099	0.85	9
	1.0	0.98	25.9	26
17810S	0.01	0.0015	11.0	7,300
	0.1	0.070	38.8	550
	1.0	0.95	63.8	67

[a] Uptake assay was performed in broth as described in the text. Cells were incubated with $^{115m}Cd^{2+}$ at 37°C for 40 min. Radioactivity retained by the cells and that in the supernatant fluid was counted.

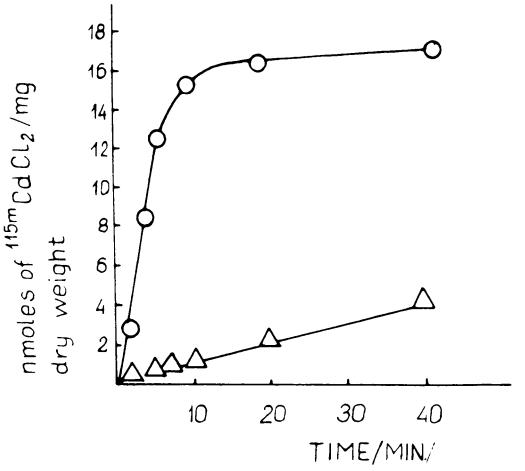

FIG. 4. *Effect of various cations on $^{115m}Cd^{2+}$ uptake by S. aureus strain 17810S. The uptake assay was performed at 37°C as described in the text. Cells were incubated with 100 μM of various salts at −2 min before addition of 10 μM $^{115m}Cd^{2+}$. Symbols: ○, no additions or with KCl, NaCl, $MgCl_2$, $ZnCl_2$, or $CaCl_2$; and △, with $MnCl_2$.*

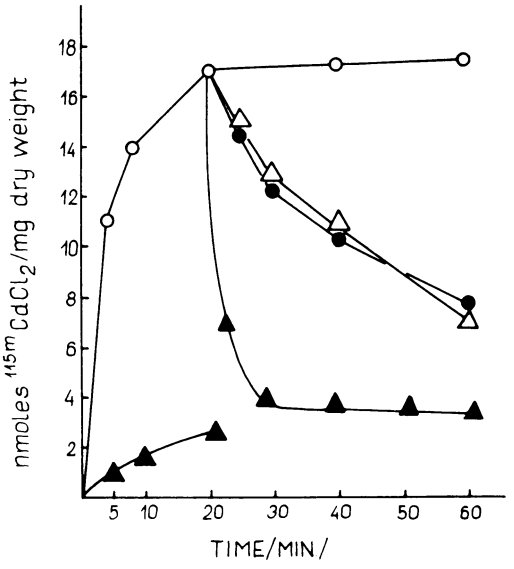

FIG. 5. *$^{115m}Cd^{2+}/Cd^{2+}$ or $^{115m}Cd^{2+}/Mn^{2+}$ exchange in S. aureus strain 17810S. Cells were incubated at 37°C with 10 μM $^{115m}Cd^{2+}$ for 20 min (○). Then 100 μM $CdCl_2$ (△) or 100 μM $MnCl_2$ (●) was added, and efflux of $^{115m}Cd^{2+}$ was assayed at various time intervals at 37°C; 0.6% toluene (vol/vol) was added at either 2 min before or 20 min after addition of $^{115m}Cd^{2+}$ (▲). Samples (0.5 ml each) were withdrawn, filtered, and washed.*

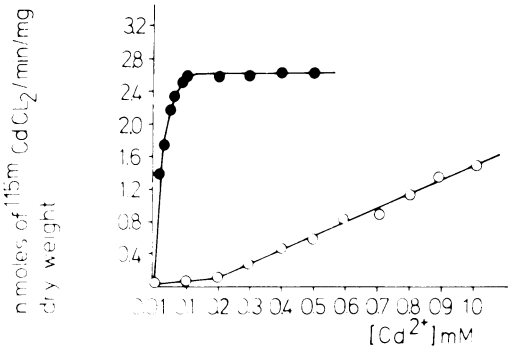

FIG. 6. *Initial rate of $^{115m}Cd^{2+}$ uptake as a function of Cd^{2+} concentration by S. aureus strain 17810R (○) and 17810S (●). Cell suspensions were incubated at 37°C with indicated concentrations of $^{115m}Cd^{2+}$ for 10 min and then filtered and washed.*

showed a biphasic character (Fig. 6). At lower concentrations (10 to 100 μM Cd^{2+}), which were not toxic for the resistant cells (24, 25), Cd^{2+} uptake in Serum Institute nutrient broth was negligible; less than 1 nmol of Cd^{2+} per mg of dry weight was taken up. At concentrations above 100 μM Cd^{2+}, uptake of this cation by resistant cells did not show saturation kinetics (Fig. 6). This uptake resulted in a progressive inhibition of respiration of the resistant cells (24).

Additional kinetic experiments were performed in St. Louis in brain heart infusion to clarify the specificity of the Cd^{2+} and Mn^{2+} uptake in both strains. An extensive series of such experiments with strain 17810R and 17810S, as well as with those strains studied by Weiss et al. (27), showed that both the strains and the medium (Serum Institute nutrient broth, Difco nutrient broth [24], and Difco brain heart infusion) affected cation uptake rates. Although Cd^{2+} transport by the resistant strain 17810R was negligible at low Cd^{2+} levels in Serum Institute nutrient broth (Fig. 6), it was readily measured in Difco nutrient broth and brain heart infusion, even though it was still substantially reduced when compared with uptake by the sensitive cells (24). This enabled direct kinetic comparisons between the two strains (Fig. 7).

The properties of the Mn^{2+} transport system

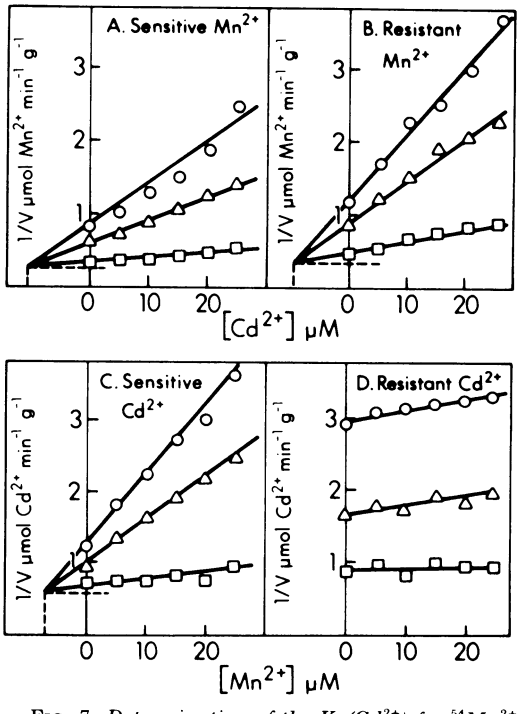

FIG. 7. *Determination of the K_i (Cd^{2+}) for $^{54}Mn^{2+}$ transport and the K_i (Mn^{2+}) for $^{109}Cd^{2+}$ transport in S. aureus strains 17810S (A and C) and 17810R (B and D), respectively, in brain heart infusion (Difco) at 37°C. Cells were prewarmed for 3 min, both cations were added simultaneously, and uptake was terminated after 1 min by filtration. Symbols: ○, 5 μM Mn^{2+} or Cd^{2+}; △, 10 μM Mn^{2+} or Cd^{2+}; and □, 30 μM Mn^{2+} or Cd^{2+}. K_m and V_{max} values were obtained from Lineweaver-Burk plots.*

were similar in both strains (Fig. 7A and B) with K_m's of 16.0 and 15.4 μM and V_{max}'s of 5.0 and 4.3 μmol min^{-1} g^{-1} (dry weight) for the sensitive and resistant strain, respectively. Cd^{2+} was a competitive inhibitor of Mn^{2+} transport, with a K_i of 12.5 μM Cd^{2+} for the sensitive and 10 μM C^{2+} for the resistant strain (Fig. 7A and B). The K_m for Cd^{2+} transport (5.4 μM Cd^{2+}; Fig. 7C and data not shown) was lower than that for Mn^{2+} transport (16 μM Mn^{2+}) in the sensitive strain, showing a higher affinity for the toxic Cd^{2+} ions. Although there were no significant differences between the sensitive and resistant strain with regard to Mn^{2+} transport kinetic parameters, they did differ with regard to Cd^{2+} transport. At low Cd^{2+} concentrations, the resistant strain took up less Cd^{2+} than did the sensitive strain (compare 5 μM Cd^{2+} results in Fig. 7C and D). At higher Cd^{2+} concentrations, the sensitive strain showed saturation kinetics, with a K_m of 5.4 μM Cd^{2+}, but the resistant strain showed no sign of saturation kinetics up to 100 μM Cd^{2+} in brain heart infusion (data not shown), similar to the results in Serum Institute nutrient broth (Fig. 6). Mn^{2+} was a competitive inhibitor of Cd^{2+} uptake in the sensitive cells, with a K_i of 8 μM Mn^{2+} (Fig. 7C), but Mn^{2+} was essentially without effect on Cd^{2+} uptake by the resistant cells (Fig. 7D).

At Cd^{2+} concentrations (0.5 to 5.0 μM Cd^{2+}) lower than those given in the legend to Fig. 7, Mn^{2+} was a competitive inhibitor of Cd^{2+} transport in the resistant cells, with kinetic constants (except for V_{max}) similar to those of the sensitive strain (Fig. 7C and 8). A suggestion of this inhibition by Mn^{2+} at low Cd^{2+} concentrations is also seen in the 5 μM Cd^{2+} data in Fig. 7D. These kinetic results will be interpreted below in terms of a single Cd^{2+} uptake system in the sensitive cells, but two functioning Cd^{2+} uptake systems in the resistant cells.

Another difference between uptake by the sensitive and by the resistant cells was the response to pH. Sensitive cells accumulated Cd^{2+} in buffers (sodium acetate or Tris-hydrochloride) at pH's above 4 and maintained approximately constant amounts of Cd^{2+} in the range of pH 5 to 9.5 (Fig. 9). The resistant cells did not accumulate significant Cd^{2+} in buffers below pH 7, but accumulated almost as much Cd^{2+} as did the sensitive cells at alkaline pH.

The higher affinity of the Mn^{2+}/Cd^{2+} transport system for Cd^{2+} than for Mn^{2+} was also demonstrated in exchange experiments. When sensitive cells accumulating from 1 μM $^{54}Mn^{2+}$ or $^{109}Cd^{2+}$ in brain heart infusion were exposed to 1 μM nonradioactive Cd^{2+} or Mn^{2+}, respectively, $^{54}Mn^{2+}$ was released from the cells but $^{109}Cd^{2+}$ was retained (data not shown). Similar

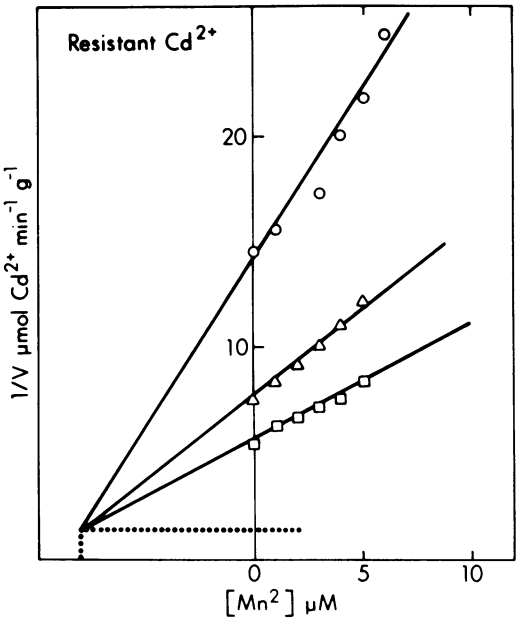

FIG. 8. *Determination of the K_i (Mn^{2+}) for $^{109}Cd^{2+}$ transport in S. aureus strain 17810R. The experimental procedure was as described in the legend to Fig. 7. Symbols: ○, 0.5 μM Cd^{2+}; △, 1 μM Cd^{2+}; and □, 1.5 μM Cd^{2+}.*

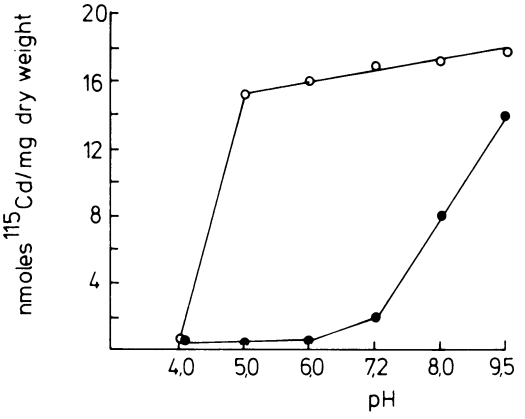

FIG. 9. *pH effect on $^{115m}Cd^{2+}$ uptake by S. aureus strains 17810R (●) and 17810S (○). Log cells were prepared as described in the text and then resuspended at 0.5 mg of dry weight per ml in 0.1 M buffers (sodium acetate [pH 4.0 to 6.0] or Tris-hydrochloride [pH 7.2 to 9.5]) with no exogenous energy source. Cells were incubated with 10 μM $^{115m}Cd^{2+}$ for 20 min at 37°C.*

results were obtained with the resistant strain at 5 μM Mn^{2+} or Cd^{2+}. Tenfold excess of nonradioactive Mn^{2+} was required for release (exchange) of accumulated $^{115m}Cd^{2+}$ (Fig. 5).

The fact that K_m and V_{max} values for Cd^{2+}

transport were lower in the sensitive strain obtained in Serum Institute nutrient broth (Fig. 6) than those obtained in brain heart infusion (Fig. 7C) may be due to the different media used. Differences in minimal inhibitory concentrations were medium dependent (24, 25; Z. Tynecka and R. D. Perry, unpublished data).

$^{109}Cd^{2+}$ uptake by the resistant strain, although markedly reduced at 1 μM (Fig. 10A), was further reduced by CCCP, by valinomycin plus K^+, and by $MnCl_2$ to about the same degree as it was in the sensitive strain (Fig. 10B). $^{54}Mn^{2+}$ uptake by both strains was equally inhibited by CCCP, by valinomycin plus K^+, and by $CdCl_2$ (Fig. 10).

DISCUSSION

Microorganisms take up cations via specific, energy-dependent transport systems (5–7, 9, 17, 21, 23). Toxic metals have been shown to enter microbial cells via transport systems which normally take up physiological cations (10, 19, 25, 27). Accumulation of a given cation may be impaired either by mutation or by possession of additional genetic material, for example, the Cd^{2+} resistance plasmid in *S. aureus* (1, 2, 8, 19, 24–27).

Cadmium-sensitive plasmidless *S. aureus* strain 17810S takes up Cd^{2+} in response to membrane potential via a specific, energy-dependent Mn^{2+} transport system. Uptake of Cd^{2+} by this system results in a severe poisoning of the cells, as judged by a marked inhibition of respiration and of growth even at very low Cd^{2+} concentrations (24, 25).

The presence of a Cd^{2+} resistance determinant in the resistant *S. aureus* 17810R resulted in markedly reduced Cd^{2+} uptake. The resistant strain appeared, however, to take up low levels of Cd^{2+} by two different uptake systems: (i) high-affinity Cd^{2+} uptake via the Mn^{2+} carrier in response to membrane potential and (ii) low-affinity Cd^{2+} uptake without involvement of the Mn^{2+} carrier or of the membrane potential. The presence of two uptake systems for Cd^{2+} in the resistant strain is reflected in the biphasic character of the Cd^{2+} uptake curve (Fig. 6). The high-affinity Cd^{2+} uptake by the resistant strain was seen only at low Cd^{2+} concentrations (Fig. 8 and 10); the kinetic parameters and sensitivity to CCCP and valinomycin were similar to those in the sensitive strain. Mn^{2+} was a competitive inhibitor of Cd^{2+} transport in both strains (Fig. 7C and 8).

The resistant strain seems to have a block on the high-affinity Cd^{2+} transport which permits the cells to take up only negligible amounts of Cd^{2+} in Serum Institute nutrient broth (Fig. 1).

The reduced Cd^{2+} accumulation by the resistant strain seems to be due to the operation of energy-dependent Cd^{2+} efflux system catalyzing exchange of the cellular Cd^{2+} for protons (24). This Cd^{2+}/proton antiporter seems to protect the resistant cells against Cd^{2+} toxicity (24, 25). The activity of the Cd^{2+} efflux system was reflected by a 1-min lag at 37°C in the initial Cd^{2+}

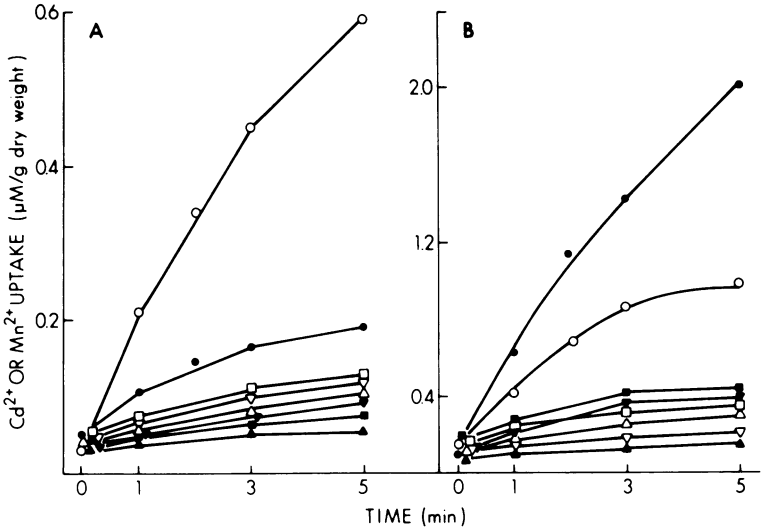

FIG. 10. *Uptake of $^{109}Cd^{2+}$ and $^{54}Mn^{2+}$ (at 1 μM) by S. aureus strains 17810R (A) and 17810S (B). Assay of $^{54}Mn^{2+}$ (open symbols) or $^{109}Cd^{2+}$ uptake (closed symbols) was performed at 37°C as described in the text. Symbols: ○, ●, control cells; △, ▲, cells with 40 μM CCCP; □, ■, cells with 40 μM valinomycin plus 5 mM KCl; ▽, ▼, cells with 20 μM $CdCl_2$ or $MnCl_2$.*

uptake at 1 mM by the resistant cells (Fig. 2). During that short time, the efflux system seemed adequate to exclude net Cd^{2+} accumulation by the resistant cells. Under these conditions, less than 1 nmol of Cd^{2+} per mg of dry weight was taken up, which is about the same as that taken up at 10 or 100 μM Cd^{2+} during 40 min (Fig. 1). After the 1-min lag, the efflux system was gradually impaired, leading to a net, but still reduced, Cd^{2+} uptake by the resistant cells. This uptake, however, occurred via the low-affinity uptake system and was not inhibited by Mn^{2+} or by valinomycin (24). Cd^{2+} uptake by this system resulted in inhibition of respiration of the resistant cells (24).

We suggest that at higher concentrations, Cd^{2+} competes with protons and is taken up by the plasmid-determined antiporter, converting a Cd^{2+}/proton exchange system into a Cd^{2+}/Cd^{2+} exchange system. The pH dependence of Cd^{2+} uptake by the resistant strain (Fig. 9) supports the idea that Cd^{2+} accumulation by the resistant cells occurs mainly via a plasmid-determined cadmium/proton antiport system, so that H^+ effectively competes with Cd^{2+} for this system. The normal function of this antiporter is to exclude Cd^{2+}. The antiporter, operating in response to pH gradient (24), was sensitive to *p*-chloromercuribenzoate (24) and dinitrophenol (Fig. 2) but not to Mn^{2+} (Fig. 7D) or valinomycin (24). When the external Cd^{2+} was removed, the antiporter seemed to resume its normal function, performing exchange of the cellular Cd^{2+} for protons. This resulted in Cd^{2+} efflux, accompanied by reversal of inhibition of respiration in the resistant cells (24). Cd^{2+} uptake by the sensitive strain is not affected by *p*-chloromercuribenzoate (24), but is inhibited by dinitrophenol, by Mn^{2+}, and by valinomycin (24). Inhibition of respiration by Cd^{2+} is irreversible in this strain (24), due to the lack of the Cd^{2+} efflux system.

ACKNOWLEDGMENTS

Our thanks are due to T. Klopotowski for constructive discussions throughout the work, to W. A. Hamilton and F. M. Harold for reading the manuscript and for critical, valuable comments on it, and to K. G. H. Dyke for strains 17810R and 17810S. One of us (Z.T.) wishes to thank S. Silver for his exceptional hospitality during the stay in St. Louis and for many valuable suggestions.

This work was supported by the Polish Academy of Science within the Project 09.7. The kinetic experiments and preparation of the manuscript were done at Washington University, St. Louis, with support from the National Science Foundation (grant PCM79-03986).

LITERATURE CITED

1. **Chopra, I.** 1970. Decreased uptake of cadmium by a resistant strain of *Staphylococcus aureus*. J. Gen. Microbiol. **63**:265–267.
2. **Chopra, I.** 1975. Mechanism of plasmid-mediated resistance to cadmium in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **7**:8–14.
3. **Collins, S. H., and W. A. Hamilton.** 1976. Magnitude of protonmotive force in respiring *Staphylococcus aureus* and *Escherichia coli*. J. Bacteriol. **126**:1224–1231.
4. **Dyke, K. G. H., M. T. Parker, and M. H. Richmond.** 1970. Penicillinase production and metal ion resistance in *Staphylococcus aureus* cultures isolated from hospital patients. J. Med. Microbiol. **3**:125–136.
5. **Hamilton, W. A.** 1975. Energy coupling in microbial transport. Adv. Microb. Physiol. **12**:1–53.
6. **Harold, F. M.** 1972. Conservation and transformation of energy by bacterial membranes. Bacteriol. Rev. **36**:172–230.
7. **Harold, F. M.** 1977. Ion currents and physiological functions in microorganisms. Annu. Rev. Microbiol. **31**:181–203.
8. **Kondo, I., T. Ishikawa, and H. Nakahara.** 1974. Mercury and cadmium resistance mediated by the penicillinase plasmid in *Staphylococcus aureus*. J. Bacteriol. **117**:1–7.
9. **Mitchell, P.** 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol. Rev. **41**:445–502.
10. **Nelson, D. L., and E. P. Kennedy.** 1971. Magnesium transport in *Escherichia coli*. Inhibition by cobaltous ion. J. Biol. Chem. **246**:3042–3049.
11. **Novick, R. P.** 1967. Penicillinase plasmids of *Staphylococcus aureus*. Fed. Proc. **26**:29–38.
12. **Novick, R. P., and D. Bouanchaud.** 1971. Extrachromosomal nature of drug resistance in *Staphylococcus aureus*. Ann. N.Y. Acad. Sci. **182**:279–294.
13. **Novick, R. P., and C. Roth.** 1968. Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J. Bacteriol. **95**:1335–1342.
14. **Peyru, G., L. F. Wexler, and R. P. Novick.** 1969. Naturally occurring penicillinase plasmids in *Staphylococcus aureus*. J. Bacteriol. **98**:215–221.
15. **Richmond, M. H., and M. John.** 1964. Co-transduction by a staphylococcal phage of the genes responsible for penicillinase synthesis and resistance to mercury salts. Nature **202**:1360–1361.
16. **Shalita, Z., E. Murphy, and R. P. Novick.** 1980. Penicillinase plasmids of *Staphylococcus aureus*: structural and evolutionary relationships. Plasmid **3**:291–311.
17. **Silver, S.** 1977. Manganese transport in microorganisms, p. 105–149. *In* E. D. Weinberg (ed.), Microorganisms and metals. Marcel Dekker, New York.
18. **Silver, S.** 1978. Transport of cations and anions, p. 221–234. *In* B. P. Rosen (ed.), Bacterial transport. Marcel Dekker, New York.
19. **Silver, S.** 1980. Mechanism of plasmid-determined resistance to mercury, cadmium and arsenic in *Staphylococcus aureus*. *In* J. Jeljaszewicz (ed.), IVth International Symposium on staphylococci and staphylococcal diseases, in press. Gustav Fischer Verlag, Stuttgart.
20. **Silver, S., K. Budd, K. M. Leahy, W. V. Shaw, D. Hammond, R. P. Novick, G. R. Willsky, M. H. Malamy, and H. Rosenberg.** 1981. Inducible plasmid-determined resistance to arsenate, arsenite, and antimony(III) in *Escherichia coli* and *Staphylococcus aureus*. J. Bacteriol. **146**:983–996.
21. **Silver, S., and M. Kralovic.** 1969. Manganese accumulation by *Escherichia coli*: evidence for a specific transport system. Biochem. Biophys. Res. Commun. **34**:640–645.
22. **Smith, K. and R. P. Novick.** 1972. Genetic studies on plasmid-linked cadmium resistance in *Staphylococcus aureus*. J. Bacteriol. **112**:761–772.
23. **Summers, A. O., and S. Silver.** 1978. Microbial transformation of metals. Annu. Rev. Microbiol. **32**:637–672.
24. **Tynecka, Z., Z. Gos, and J. Zajac.** 1981. Energy-de-

pendent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. J. Bacteriol. **147**:313-319.

25. **Tynecka, Z., J. Zajac, and Z. Gos.** 1975. Plasmid-dependent impermeability barrier to cadmium ions in *Staphylococcus aureus*. Acta Microbiol. Pol. **7**:11-20.
26. **Weiss, A. A., S. D. Murphy, and S. Silver.** 1977. Mercury and organomercurial resistances determinant by plasmids in *Staphylococcus aureus*. J. Bacteriol. **132:** 197-208.
27. **Weiss, A. A., S. Silver, and T. G. Kinscherf.** 1978. Cation transport alteration associated with plasmid-determined resistance to cadmium in *Staphylococcus aureus*. Antimicrob. Agents Chemother. **14**:856-865.