BioMetals 1995, **8**, 197–204

Energetic basis of cadmium toxicity in *Staphylococcus aureus*

Zofia Tynecka & Anna Malm

Department of Pharmaceutical Microbiology, Medical Academy, Lublin, Poland

Received 4 April 1994; received in revised form 22 July 1994; accepted for publication 5 September 1994

In washed cells of cadmium-sensitive *Staphylococcus aureus* 17810S oxidizing glutamate, initial Cd^{2+} influx via the Mn^{2+} porter down membrane potential ($\Delta\psi$) was fast due to involvement of energy generated by two proton pumps—the respiratory chain and the ATP synthetase complex working in the hydrolytic direction. Such an unusual energy drain for rapid initial Cd^{2+} influx is suggested to be due to a series of toxic events elicited by Cd^{2+} accumulation down $\Delta\psi$ generated via the redox proton pump: (i) strong inhibition of glutamate oxidation accompanied by a decrease of electrochemical proton gradient ($\Delta\mu_{H^+}$) formation via the respiratory chain, (ii) automatic reversal of ATP synthetase from biosynthetic to hydrolytic mode, which was monitored by a decrease of $\Delta\mu_{H^+}$-dependent ATP synthesis, (iii) acceleration of the initial Cd^{2+} influx down $\Delta\psi$ generated by the reversed ATP synthetase, the alternative proton pump hydrolyzing endogenous ATP. The primary, cadmium-sensitive targets in strain 17810S seem to be dithiols located in the cytoplasmic glutamate oxidizing system, prior to the membrane-embedded NADH oxidation system. Inhibition by Cd^{2+} of $\Delta\mu_{H^+}$-dependent ATP synthesis and of pH gradient (ΔpH)-linked [^{14}C]glutamate transport is a secondary effect due to cadmium-mediated inhibition of $\Delta\mu_{H^+}$ generation at the cytoplasmic level. In washed cells of cadmium-resistant *S. aureus* 17810R oxidizing glutamate, Cd^{2+} accumulation was prevented due to activity of the plasmid-coded Cd^{2+} efflux system. Consequently, $\Delta\mu_{H^+}$-producing and -requiring processes were not affected by Cd^{2+}.

Keywords: cadmium resistance, cadmium toxicity, energetic processes, primary targets, secondary effects, *Staphylococcus aureus*

Introduction

Cadmium, a toxic element of unknown physiological function, is potentially hazardous for both prokaryotic and eukaryotic cells (Vallee & Ulmer 1972, Sanadi 1982, Trevors *et al.* 1986, Hughes & Poole 1989). Among bacteria, *Staphylococcus aureus* appeared to be particularly sensitive to Cd^{2+} (Babich & Stotzky 1977, Morozzi *et al.* 1986). In this organism, Cd^{2+} was accumulated in the cytoplasm via the Mn^{2+} porter down membrane potential—$\Delta\psi$ (Chopra 1971, 1975, Weiss *et al.* 1978, Tynecka *et al.* 1975, 1981a,b). This resulted in inhibition of respiration (Tynecka *et al.* 1975, 1981b, Tynecka & Szcześniak 1991), respiration-dependent inorganic phosphate transport (Tynecka and Szcześniak 1991), aerobic growth (Babich & Stotzky 1977, Weiss *et al.* 1978, Morozzi *et al.* 1986, Tynecka *et al.* 1989a) and protein synthesis (Chopra 1975, Morozzi *et al.* 1986). However, the precise mechanism of Cd^{2+} toxicity to bacterial cells is so far poorly understood.

The present investigation was undertaken to study the mechanism of Cd^{2+} toxicity to the following energetic processes in washed cells of cadmium-sensitive *S. aureus* 17810S: (i) generation of an electrochemical proton gradient ($\Delta\mu_{H^+}$) via the respiratory chain and (ii) utilization of $\Delta\mu_{H^+}$ energy for $\Delta\mu_{H^+}$-requiring processes, such as ATP synthesis via oxidative phosphorylation or solute transport through the cytoplasmic membrane. Attempts have been made to define the primary Cd^{2+} sensitive targets and also the secondary effect of Cd^{2+} in energy producing and energy conserving systems of this organism. To verify our results, a control organism, the parent strain *S. aureus* 17810R, resistant to Cd^{2+} due to possession of the energy-dependent *cadA*-coded efflux system (Tynecka *et al.* 1981b) was included in the present studies. The efflux system extruded Cd^{2+} from the cytoplasm into the environment immediately after its entry via the Mn^{2+} porter, which prevented Cd^{2+} accumulation and its toxicity (Tynecka *et al.* 1975, 1981a,b, 1989a, Tynecka & Szcześniak 1991).

Address for correspondence: Z. Tynecka, Department of Pharmaceutical Microbiology, Medical Academy, Lubartowska 85, 20-123 Lublin, Poland. Fax: (+48) 81 28903.

Materials and methods

Strains and culture conditions

The cadmium-sensitive, plasmid-less variant strain *S. aureus* 17810S and its parent cadmium-resistant strain *S. aureus* 17810R, carrying a penicillinase plasmid pI17810 (Shalita *et al.* 1980), were described previously (Tynecka *et al.* 1975, 1981a). All experiments were performed on early-exponential phase cells of both strains grown aerobically in 3% nutrient broth at 37 °C, which were obtained according to Tynecka *et al.* (1981a). Cells were harvested by centrifugation (2000 *g* for 10 min), washed twice with 0.1 M potassium/ sodium or sodium phosphate buffer (pH 7) and resuspended in one of the buffers at a density of 0.2 mg dry weight ml^{-1}. In each experiment, 5 mM $MgCl_2$ was included into the buffers.

Starvation procedure

To obtain cells with depleted pools of the endogenous respiratory substrates and of ATP, washed cells of both strains were aerated in potassium/sodium phosphate buffer at 37 °C for 3 h. After starvation, cells were centrifuged and resuspended in the same buffer or in sodium phosphate buffer, and preincubated for 20 min at 37 °C with 10 mM glutamate or in some experiments with 10 mM NADH as exogenous energy sources.

Preparation of cell lysates

In order to get lysates from strain 17810S, starved cells of this organism suspended in potassium/sodium phosphate buffer were treated with lysostaphin (5 units ml^{-1}) for 5 min at 37 °C.

Measurement of respiration

Oxygen consumption by cells of both strains or by cell lysates was determined at 37 °C in potassium/sodium phosphate buffer in a Gilson 5/6H Oxygraph (Gilson Medical Electronics, Middleton, WI). Cell suspensions or lysates were placed in a 1.7 ml temperature-controlled chamber fitted with a Clark Oxygen Electrode (Yellow Springs, Yellow Springs, OH). The oxygen electrode was calibrated by the method of Robinson & Cooper (1970).

ATP assay

Determination of the cellular ATP content in potassium/ sodium phosphate buffer was performed at 37 °C in freshly harvested cells, starved cells or in the glutamate-preincubated cells of both strains. Intracellular ATP was extracted by iced 3N perchloric acid and the extract was neutralized with 3N KOH. ATP was assayed with firefly lantern extract (FLE-50) as described by Maloney & Wilson (1975). The intra-cellular concentration of ATP was calculated using a value of 1.55 μl cell water mg^{-1} dry weight, according to Jeacocke *et al.* (1972).

Measurement of membrane potential ($\Delta\psi$)

Membrane potential was estimated in freshly harvested cells, in starved cells or in the glutamate-preincubated cells, suspended in sodium phosphate buffer. Cell suspensions were incubated on a shaker at 37 °C with 100 μM ^{86}Rb plus 10 μM valinomycin. Samples (0.5 ml) were withdrawn at 0, 1, 2, 3, 4 and 5 min, rapidly filtered through the membrane filters (0.45 μm pore size; Sartorius, Goettingen, Germany) and washed twice with 5 ml of the same buffer. Then, filters were dried and counted in a toluene-based scintillation fluid in a scintillation counter (LS 6000TA; Beckman, Fullerton, CA). The magnitude of $\Delta\psi$ was calculated from the Nernst equation: $\Delta\psi = 61.7\ Rb_{in}/Rb_{out}$, using the steady-state concentration values (Kashket 1985). Concentration gradients were estimated on the basis of the intracellular water content according to Jeacocke *et al.* (1972).

Assay of ^{109}Cd uptake

^{109}Cd uptake at 10 μM in potassium/sodium phosphate buffer was performed at 37 °C in freshly harvested cells, starved cells or in the glutamate- or NADH-preincubated cells of both strains, according to Tynecka *et al.* (1981a).

Assay of [^{14}C]glutamate uptake

[^{14}C]glutamate uptake at 10 mM was studied in cells of both strains oxidizing endogenous respiratory substrates in potassium/sodium phosphate buffer, containing chloramphenicol (Tynecka *et al.* 1989b).

Chemicals

Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), *N,N'*-dicycloheksylocarbodiimide (DCCD), FLE-50, 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO), lyso-staphin, NADH, nigericin and valinomycin were purchased from Sigma (St Louis, MO). $^{86}RbCl$ (1.075 GBq mmol $^{-1}$) was obtained from the Institute of Nuclear Research, Swierk, Poland. [U-^{14}C]glutamate (7.4 GBq $mmol^{-1}$) and carrier-free $^{109}CdCl_2$ were obtained from Amersham (Amersham, UK).

CCCP, DCCD, HQNO, nigericin or valinomycin were used as methanolic solutions.

Results

In the absence of exogenous energy donors, *S. aureus* utilizes an endogenous amino acid pool (Ramsey 1962, Tynecka 1968, Krzemiński *et al.* 1972) or ATP for membrane energization (Tynecka *et al.* 1989b). Endogenous amino acids, of which glutamate is the quantitatively predominant compound, are the source of endogenous respiration in this organism (Ramsey 1962, Tynecka 1968, Krzemiński *et al.* 1972).

In the cadmium-sensitive *S. aureus* 17810S oxidizing endogenous amino acids (Figure 1A), initial influx of Cd^{2+} was fairly rapid and the steady-state equilibrium was attained in 20 min (Figure 1B). The majority of Cd^{2+} taken up (about 80%) was accumulated in the cytoplasm by its normal route, that is via the energy-dependent Mn^{2+} transport system down $\Delta\psi$ of 165 mV (Table 1), since Mn^{2+}, valinomycin + K^+ or CCCP inhibited Cd^{2+} influx (Figure 1B). Some energy-independent Cd^{2+} uptake insensitive to Mn^{2+} and ionophores but

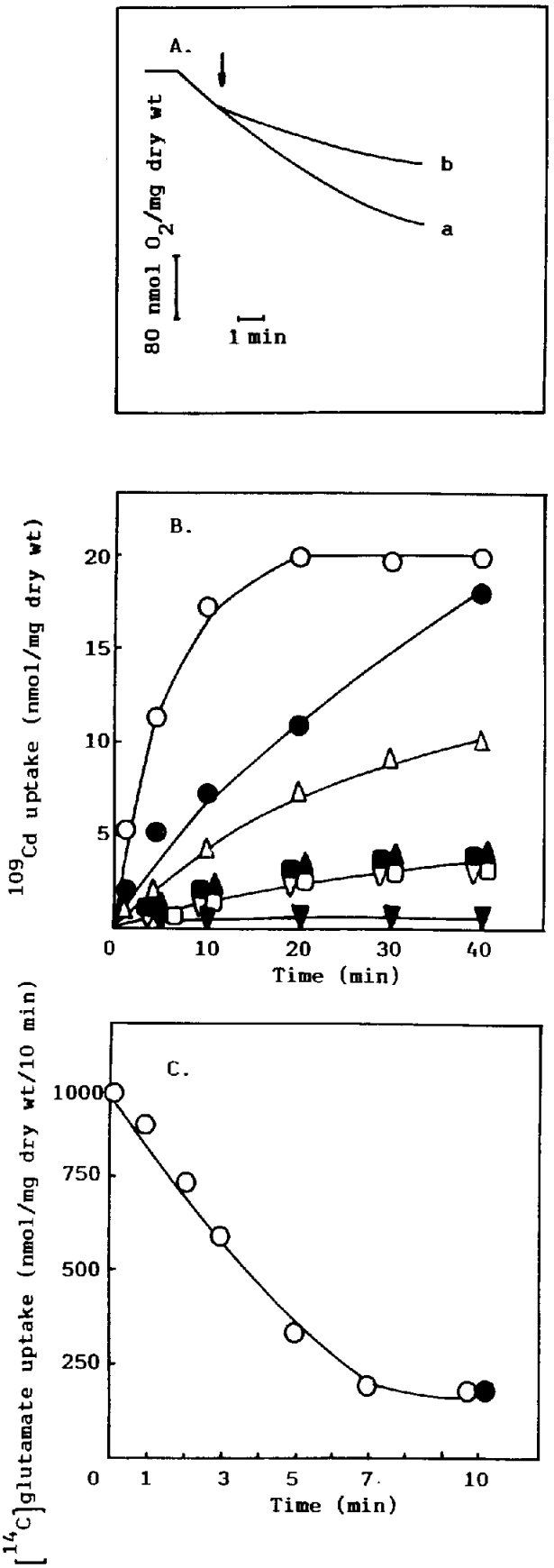

Table 1. Membrane potential ($\Delta\psi$) generated by *S. aureus* strains

State of cells	$\Delta\psi$ (mV)	
	S. aureus 17810S	*S. aureus* 17810R
Unstarved cells	165	162
Starved cells	122	120
Starved cells oxidizing glutamate	160	158

The values are the average of more than three experiments.

sensitive to 4 °C was observed in strain 17810S (Figure 1B). This uptake may represent Cd^{2+} binding to the temperature-dependent, negatively-charged groups located within the cell surface.

HQNO, a known inhibitor of the bacterial electron transfer chain, decreased markedly the initial rate of Cd^{2+} influx and also reduced the magnitude of Cd^{2+} accumulation in strain 17810S by about 50% (Figure 1B). When activity of ATP synthetase in this organism was abolished with DCCD, a known inhibitor of the proton flow through the proton channel F_0, the initial rate of Cd^{2+} influx was also markedly decreased and the steady-state equilibrium was not attained until about 40 min (Figure 1B). Simultaneous addition of HQNO and DCCD inhibited totally the energy-dependent Cd^{2+} accumulation (Figure 1B). The effects of these two inhibitors on Cd^{2+} accumulation in strain 17810S suggest that neither the respiratory chain nor the reversed ATP synthetase, when acting alone, were able to energize the rapid initial Cd^{2+} influx. Only in control cells in which both endogenous respiration and ATP synthetase were functioning, was the initial rate of Cd^{2+} influx very fast (Figure 1B).

Progressive Cd^{2+} accumulation by cells of strain 17810S (Figure 1B) resulted in progressive inhibition of endogenous respiration (Figure 1A). Due to this, a progressive inhibition of [^{14}C]glutamate transport was observed (Figure 1C). Dependence of [^{14}C]glutamate transport on pH gradient (ΔpH) was evidenced by inhibition with nigericin (Figure 1C).

Figure 1. (**A**) Effect of Cd^{2+} on endogenous respiration in unstarved cells of *S. aureus* 17810S. Control cells (curve a); cells with 10 μM $CdCl_2$ (curve b); Cd^{2+} was added to cell suspension at a time indicated by the arrow. (**B**) ^{109}Cd uptake by unstarved cells of *S. aureus* 17810S. Cells were pretreated with inhibitors and ionophores for 10 min before addition of 10 μM ^{109}Cd. Symbols: (○), control cells at 37 °C; cells with: (●), 100 μM DCCD; (△), 100 μM HQNO; (▲), 100 μM DCCD + 100 μM HQNO; (□), 1100 μM $MnCl_2$; (■), 10 μM valinomycin + 50 mM KCl; (▽), 10 μM CCCP; (▼), cells at 4 °C. (**C**) Time-dependent effect of Cd^{2+} on [^{14}C]glutamate uptake by unstarved cells of *S. aureus* 17810S. Cells were preincubated with 10 μM Cd^{2+} for different periods of time, and then [^{14}C]glutamate uptake was assayed for 10 min (○). (●) Cells pretreated for 10 min with 0.6 μM nigericin before addition of [^{14}C]glutamate. Results are representative of three experiments.

an ionophore which collapses selectively ΔpH. This is in agreement with data by Niven & Hamilton (1974).

Starvation of both *S. aureus* strains of endogenous energy reserves resulted in almost complete exhaustion of endogenous respiration [Figures 2 (inset) and 5A] and in a marked drop of endogenous ATP content from about 11 to about 2 mM. Also, a significant reduction of $\Delta\psi$ from about 160 mV in freshly harvested cells to about 120 mV after starvation was noted in both organisms (Table 1).

In starved cells of strain 17810S, the initial rate of Cd^{2+} influx was highly decreased and the steady-state Cd^{2+} level was about 50% that of unstarved cells (Figure 2). Energy-dependent Cd^{2+} accumulation in starved cells was abolished with DCCD (Figure 2), which indicates that under these conditions Cd^{2+} accumulation was energized solely by $\Delta\psi$ generated via hydrolysis of endogenous ATP.

Preincubation of starved cells of both strains with a single amino acid (10 mM glutamate) resulted in restoration of the respiratory activity (Figures 3A and 5A) and resynthesis of ATP (Figures 3C and 5C) coupled to an electrochemical proton gradient ($\Delta\mu_H$-) generated by the electron transfer chain. This was evidenced by inhibition of ATP synthesis with DCCD, HQNO or CCCP (Figures 3C and 5C). In the glutamate-preincubated cells of both organisms the magnitude of $\Delta\psi$ increased from about 120 to about 160 mV, reaching values similar to those in cells oxidizing endogenous amino acids (Table 1).

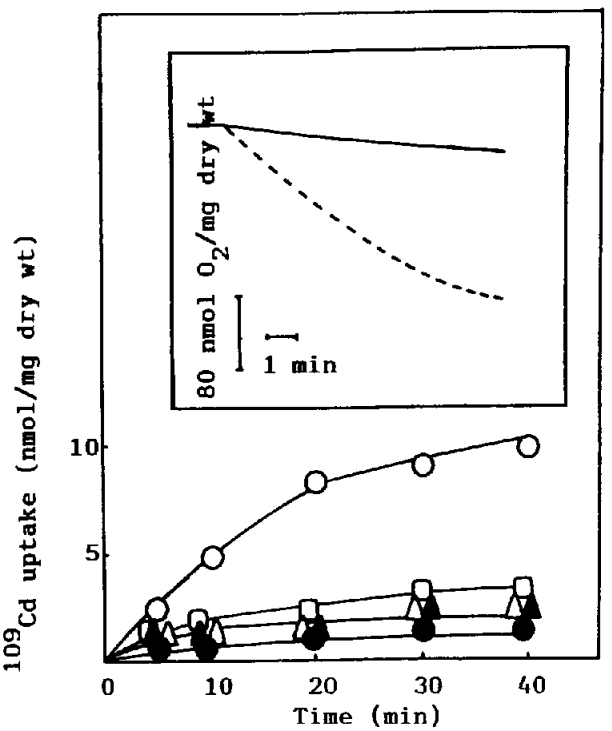

Figure 2. ^{109}Cd uptake by starved cells of *S. aureus* 17810S. Cells were pretreated for 10 min with: (●), 100 μM DCCD; (△), 100 μM $MnCl_2$; (▲), 10 μM valinomycin + 50 mM KCl; (□), 10 μM CCCP; before addition of 10 μM ^{109}Cd. (○) Control cells. Inset: effect of starvation on endogenous respiration in *S. aureus* 17810S (—); for comparison endogenous respiration of unstarved cells was presented (– – –). Results are means of three experiments.

Cells of strain 17810S oxidizing glutamate regained the ability to accumulate Cd^{2+} rapidly via its normal route (Figure 3B). HQNO or DCCD reduced markedly the fast initial Cd^{2+} influx and delayed the attainment of steady-state equilibrium (Figure 3B). Energy-independent Cd^{2+} binding was also observed (Figure 3B). Progressive Cd^{2+} accumulation by strain 17810S (Figure 3B) resulted in progressive inhibition of glutamate oxidation (Figure 3A); also ATP synthesis via oxidative phosphorylation was blocked by Cd^{2+} (Figure 3C).

As indicated in Figure 4A, whole cells of strain 17810S oxidized NADH to a small extent. This suggests that NADH crossed the cytoplasmic membrane of whole cells and was oxidized by the membrane-bound NADH oxidizing system, sensitive to HQNO (Figure 4A). Cd^{2+} (10 μM) did not affect NADH oxidation (Figure 4A), despite accumulation of about 18 nmol Cd^{2+} mg^{-1} dry weight (Figure 4C), down $\Delta\psi$ generated via NADH oxidation.

As evidenced in Figure 4(B), NADH oxidation in cell lysates of strain 17810S was about six times higher than that in whole cells (Figure 4A). This seems to be due to the better accessibility of NADH to the NADH dehydrogenase located on the inner surface of the cytoplasmic membrane. It should be noted that the rate of NADH oxidation by whole cells or by cell lysates was equally sensitive to HQNO; both processes were also equally insensitive to Cd^{2+} (Figure 4A and B).

In cadmium-resistant *S. aureus* 17810R, Cd^{2+} accumulation was prevented due to activity of the plasmid-coded cadmium efflux system under all conditions studied: in freshly harvested cells, in starved cells or in the glutamate-preincubated cells (Figure 5A, inset), although in each case the magnitude of $\Delta\psi$ was comparable with that in strain 17810S (Table 1). Due to inability to accumulate Cd^{2+} by the resistant organism, neither oxidation of endogenous amino acids and of exogenous glutamate (Figure 5A) nor ΔpH-dependent [^{14}C]glutamate transport (Figure 5B) and $\Delta\mu_H$-dependent ATP synthesis (Figure 5C) were affected by Cd^{2+}.

Discussion

We have shown in this paper that in aerobically grown, washed cells of cadmium-sensitive *S. aureus* 17810S oxidizing endo- or exogenous glutamate, the fast initial Cd^{2+} accumulation via the Mn^{2+} porter down $\Delta\psi$ was energized by two proton pumps, the HQNO-sensitive respiratory chain and the DCCD-sensitive ATP synthetase complex, hydrolyzing endogenous ATP. A question arises as to what is the mechanism of utilization of ATP energy for rapid Cd^{2+} accumulation in staphylococcal cells, which in the absence of cadmium synthesize ATP via oxidative phosphorylation.

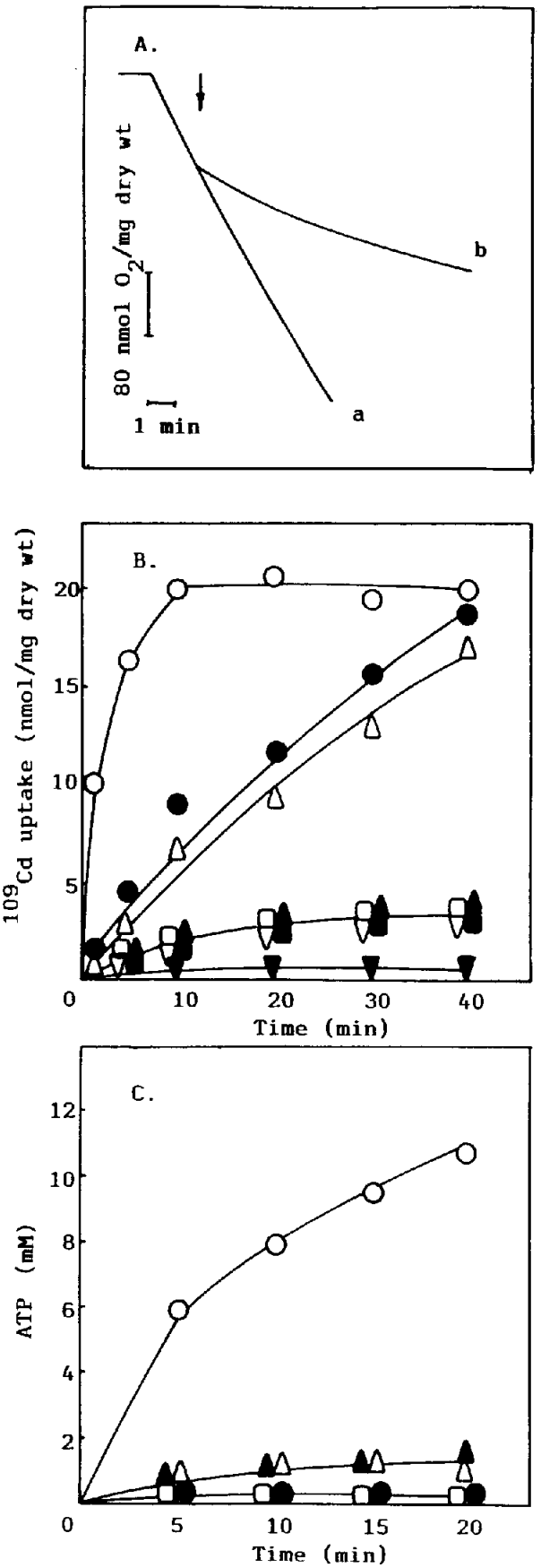

Figure 3. (**A**) Effect of Cd^{2+} on glutamate oxidation in starved cells of *S. aureus* 17810S. Starved cells were preincubated for 20 min with 10 mM glutamate before the oxygen uptake was followed. Control cells (curve a); cells with 10 μM Cd^{2+} (curve b). Cd^{2+} was added to cell suspension at a time indicated by the arrow. (**B**) ^{109}Cd uptake by starved cells of *S. aureus* 17810S oxidizing glutamate. Starved cells were preincubated for 20 min with 10 mM glutamate and then 10 μM ^{109}Cd uptake was assayed. For details, see legend to Figure 1(B). Symbols: (○), control cells at 37 C; cells with: (●), 100 μM DCCD; (△), 100 μM HQNO; (▲), 100 μM DCCD + 100 μM HQNO; (□), 100 μM $MnCl_2$; (■), 10 μM valinomycin + 50 mM KCl; (▽), 10 μM CCCP; (▼) cells at 4 C. (**C**) Effects of inhibitors and ionophores on ATP synthesis in starved cells of *S. aureus* 17810S oxidizing glutamate. Starved cells were pretreated for 10 min with the inhibitors before addition of 10 mM glutamate. The intracellular level of ATP was estimated after 5, 10, 15 or 20 min incubation of cell suspensions with 10 mM glutamate. The background values of ATP remaining in starved cells were subtracted. (○) Control cells; cells pretreated with: (●), 10 μM $CdCl_2$; (△), 100 μM DCCD; (▲), 100 μM HQNO; (□), 5 μM CCCP. Results are means of three determinations.

To answer that question, we should remember that in energy-transducing membranes of mitochondria or of respiring bacteria, the reversible ATP synthetase complex (F_0F_1) is maintained in the biosynthetic direction due to a thermodynamic back-pressure exerted by a high $\Delta\mu_{H^+}$ of about 210 mV generated via the respiratory chain (Mitchell 1966, Collins & Hamilton 1976, Nicholls 1984). When $\Delta\mu_{H^+}$ derived from electron transfer falls below this required value, the thermodynamic back-pressure is released and the ATP synthetase automatically reverses its direction into a hydrolytic mode and by pumping protons generates $\Delta\mu_{H^+}$ at the expense of ATP (Mitchell 1966, Rosen & Kashket 1978, Nicholls 1984).

It is well known that, in the absence of respiration, facultative anaerobes like *S. aureus* utilize ATP hydrolysis for membrane energization (Rosen & Kashket 1978). However, our data provide a novel observation that Cd^{2+}, being a substrate of the Mn^{2+} porter and at the same time a toxic compound, utilizes first the preformed respiratory $\Delta\psi$, then inhibits respiration and decreases $\Delta\mu_{H^+}$ production via the respiratory chain. In this way, Cd^{2+} arranges by itself automatic reversal of ATP synthetase into a hydrolytic mode which accelerates its initial influx down $\Delta\psi$ generated via ATP hydrolysis.

To consider the primary cadmium-sensitive targets in strain 17810S, we took into account the high sensitivity to Cd^{2+} of glutamate oxidation and insensitivity to Cd^{2+} of NADH oxidation which suggest that these targets can be located prior to the membrane-bound NADH dehydrogenase. This is in agreement with the suggestion by Surowitz *et al.* (1984) that the toxic effect of Cd^{2+} on respiration of *Bacillus subtilis* involved a metabolic mechanism preceding NADH oxidation in the electron transfer chain. It is

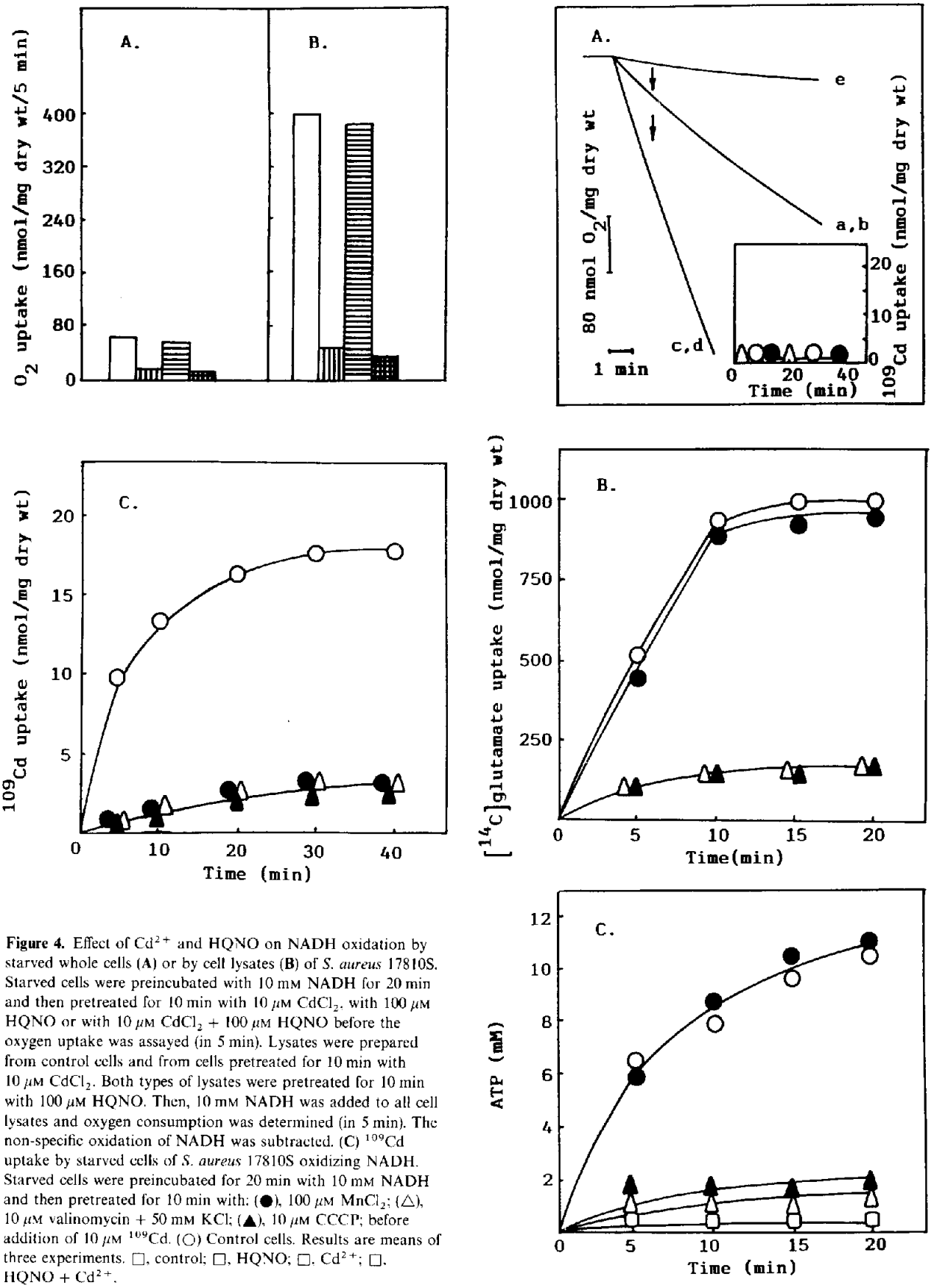

Figure 4. Effect of Cd^{2+} and HQNO on NADH oxidation by starved whole cells (**A**) or by cell lysates (**B**) of *S. aureus* 17810S. Starved cells were preincubated with 10 mM NADH for 20 min and then pretreated for 10 min with 10 μM $CdCl_2$, with 100 μM HQNO or with 10 μM $CdCl_2$ + 100 μM HQNO before the oxygen uptake was assayed (in 5 min). Lysates were prepared from control cells and from cells pretreated for 10 min with 10 μM $CdCl_2$. Both types of lysates were pretreated for 10 min with 100 μM HQNO. Then, 10 mM NADH was added to all cell lysates and oxygen consumption was determined (in 5 min). The non-specific oxidation of NADH was subtracted. (**C**) ^{109}Cd uptake by starved cells of *S. aureus* 17810S oxidizing NADH. Starved cells were preincubated for 20 min with 10 mM NADH and then pretreated for 10 min with: (●), 100 μM $MnCl_2$; (△), 10 μM valinomycin + 50 mM KCl; (▲), 10 μM CCCP; before addition of 10 μM ^{109}Cd. (○) Control cells. Results are means of three experiments. □, control; □, HQNO; □, Cd^{2+}; □, HQNO + Cd^{2+}.

known that *S. aureus* possesses cytoplasmic enzyme systems oxidizing glutamate and 2-oxoglutarate (Elek 1959, Strasters & Winkler 1963). We suggest that these enzymes may be the candidates for potential, primary cadmium-sensitive targets in strain 17810S. The first one may be the metal-binding site in the glutamate dehydrogenase, in which cadmium can substitute for zinc or calcium followed by enzyme inactivation (Vallee & Ulmer 1972, Hughes & Poole, 1989, Hudson & Daniel 1993). The second target may be the two vicinal dithiols in the oxidative decarboxylation system of 2-oxoglutarate in the Krebs cycle, one in the lipoyl dehydrogenase and the other in the lipoamide. This is in accord with data by Sanadi (1982) that in mammalian mitochondria, Cd^{2+} blocked these dithiols which resulted in inhibition of enzyme activity. We suggest that blockage of these primary targets in the glutamate oxidizing system of *S. aureus* 17810S by the accumulated Cd^{2+} could stop NADH production in the Krebs cycle and its subsequent oxidation in the respiratory chain. Consequently, $\Delta\mu_{H^+}$ generation via the respiratory chain would be inhibited, without inactivation of the membrane-bound NADH de-hydrogenase and electron transfer chain.

In view of the above considerations, we postulate that the cadmium-mediated inhibition of $\Delta\mu_{H^+}$ generation could affect secondarily $\Delta\mu_{H^+}$-dependent processes, such as ATP synthesis via oxidative phosphorylation and [^{14}C]glutamate transport, without direct blockage of the membrane-embedded ATP synthetase complex and of the H^+/glutamate symporter.

Some evidence supporting our suggestion comes from data obtained with *S. aureus* 17810R, used in these studies as a control organism, resistant to cadmium. In this strain, Cd^{2+} is extruded by the plasmid-coded, energy-dependent Cd^{2+} efflux system (Tynecka *et al.* 1981b) and cannot reach the primary sensitive targets within the glutamate oxidizing system. Due to this, neither NADH production in the Krebs cycle nor proton circulation via the respiratory chain and $\Delta\mu_{H^+}$ generation were affected, despite the presence of Cd^{2+} in the environment. Consequently, the ATP synthetase complex worked in the biosynthetic direction and produced ATP coupled to $\Delta\mu_{H^+}$ generated by the electron transfer chain. In addition, ΔpH-dependent [^{14}C]glutamate transport was not affected by Cd^{2+}. This means that when the primary targets are not blocked by Cd^{2+}, no secondary effects of Cd^{2+} could be seen. This also suggests that Cd^{2+} cannot poison the *S. aureus* cells 'from outside', but must first accumulate in the cytoplasm in order to be toxic.

Figure 5. (**A**) Effect of Cd^{2+} on endogenous respiration (curves a and b) or glutamate oxidation (curves c and d) in *S. aureus* 17810R. Cd^{2+} (10 μM) was added to cell suspensions at a time indicated by the arrow. Control cells, curves a and c; cells with Cd^{2+}, curves b and d. Oxygen uptake by starved cells (curve e). Inset: ^{109}Cd uptake at 10 μM by unstarved cells (○), by starved cells (●) or by starved cells oxidizing glutamate (△) in *S. aureus* 17810R. (**B**) Effects of Cd^{2+} and ionophores on [^{14}C]glutamate uptake by unstarved cells of *S. aureus* 17810R. Symbols: (○), control cells; cells pretreated for 10 min with: (●), 10 μM $CdCl_2$; (△), 0.6 μM nigericin; (▲), 10 μM CCCP; before addition of 10 mM [^{14}C]glutamate. (**C**) Effects of inhibitors and ionophores on ATP synthesis in starved cells of *S. aureus* 17810R oxidizing glutamate. For details, see legend to Figure 3(C). Symbols: (○), control cells; cells pretreated with: (●), 10 μM $CdCl_2$; (△), 100 μM DCCD; (▲), 100 μM HQNO; (□), 5 μM CCCP. Results are representative of three experiments.

Acknowledgments

This work was supported by a grant PB 0447/P2/93/04 from the State Committee for Scientific Research. Our thanks are due to Professor A. Summers for helpful discussions.

References

Babich A, Stotzky C. 1977 Sensitivity of various bacteria, including actinomycetes, and fungi to cadmium and the influence of pH on sensitivity. *Appl Environ Microbiol* **33**, 681–695.

Collins SH, Hamilton WA. 1976 Magnitude of the protonmotive force in respiring *Staphylococcus aureus* and *Escherichia coli*. *J Bacteriol* **126**, 1224–1231.

Chopra I. 1971 Decreased uptake of cadmium by resistant strain of *Staphylococcus aureus*. *J Gen Microbiol* **63**, 265–267.

Chopra I. 1975 Mechanism of plasmid-mediated resistance to cadmium in *Staphylococcus aureus*. *Antimicrob Ag Chemother* **7**, 8–14.

Elek SD. 1959 *Staphylococcus pyogenes and Its Relation to Disease*. Edinburgh: Livingstone.

Hughes MN, Poole RK. 1989 *Metal and Micro-organisms*. London: Chapman and Hall.

Hudson RC, Daniel RM. 1993 L-glutamate dehydrogenases: distribution, properties and mechanism. *Comp Biochem Physiol* **106B**, 767–792.

Jeacocke RE, Niven DF, Hamilton WA. 1972 The proton- motive force in *Staphylococcus aureus*. *Biochem J* **127**, 57P.

Kashket ER. 1985 The proton motive force in bacteria: a critical assessment of methods. *Annu Rev Microbiol* **39**, 219–242.

Krzemiński Z, Mikucki J, Szarapińska-Kwaszewska J. 1972 Endogenous metabolism of *Staphylococcus aureus*. *Folia Microbiol* **17**, 46–54.

Maloney PC, Wilson TH. 1975 ATP synthesis driven by a protonmotive force in *Streptococcus lactis*. *J Membr Biol* **25**, 285–310.

Mitchell P. 1966 Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol Rev* **41**, 444–502.

Morozzi G, Genci G, Scardazza F, Pitzurra M. 1986 Cadmium uptake by growing cells of Gram-positive and Gram-negative bacteria. *Microbios* **46**, 27–35.

Nicholls D. 1984 Mechanism of energy transduction. In: Ernster L, ed. *Bioenergetics*, Amsterdam: Elsevier; 29–47.

Niven DF, Hamilton WA. 1974 Mechanism of energy coupling to the transport of amino acids by *Staphylococcus aureus*. *Eur J Biochem* **44**, 517–522.

Ramsey HH. 1962 Endogenous respiration of *Staphylococcus aureus*. *J Bacteriol* **83**, 507–514.

Robinson J, Cooper JM. 1970 Method determining oxygen concentrations in biological media, suitable for calibration of the oxygen electrode. *Anal Biochem* **33**, 390–399.

Rosen BP, Kashket ER. 1978 Energetics of active transport. In: Rosen BP, ed. *Bacterial Transport*. New York: Marcel Dekker; 559–620.

Sanadi DR. 1982 Mitochondrial coupling factor B. Properties and role in ATP synthesis. *Biochim Biophys Acta* **683**, 39–56.

Shalita Z, Murphy E, Novick P. 1980 Penicillinase plasmids of *Staphylococcus aureus*: structural and evolutionary relation ships. *Plasmid* **3**, 291 311.

Strasters KC, Winkler KC. 1963 Carbohydrate metabolism of *Staphylococcus aureus*. *J Gen Microbiol* **33**, 213–229.

Surowitz KG, Titus JA, Pfister RM. 1984 Effects of cadmium accumulation on growth and respiration of cadmium-sensitive strain of *Bacillus subtilis* and a selected cadmium-resistant mutant. *Arch Microbiol* **140**, 107–112.

Trevors JT, Stratton GW, Gadd GM. 1986 Cadmium transport, resistance, and toxicity in bacteria, algae, and fungi. *Can J Microbiol* **32**, 447–464.

Tynecka Z. 1968 Free aminoacids of staphylococci. *Exp Med Microbiol* **20**, 228–232.

Tynecka Z, Szcześniak Z. 1991 Effect of Cd^{2+} on phosphate uptake by cadmium-resistant and cadmium-sensitive *Staphylococcus aureus*. *Microbios* **68**, 53 63.

Tynecka Z, Zajaç J, Goś Z. 1975 Plasmid-dependent impermeability barrier to cadmium ions in *Staphylococcus aureus*. *Acta Microbiol Polon* **7**, 11–20.

Tynecka Z, Goś Z, Zajaç J. 1981a Reduced cadmium transport determined by a resistance plasmid in *Staphylococcus aureus*. *J Bacteriol* **147**, 305–312.

Tynecka Z, Goś Z, Zajaç J. 1981b Energy-dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. *J Bacteriol* **147**, 313 319.

Tynecka Z, Malm A, Skwarek T. 1989a Effect of Cd^{2+} on growth of cadmium-resistant and -sensitive *Staphylococcus aureus*. *Acta Microbiol Polon* **38**, 117–129.

Tynecka Z, Malm A, Skwarek T, Szcześniak Z. 1989b Plasmid-linked protection of [^{14}C]glutamate transport and its oxidation against Cd^{2+} in *Staphylococcus aureus*. *Acta Microbiol Polon* **38**, 131–141.

Vallee BL, Ulmer DD. 1972 Biochemical effects of mercury, cadmium and lead. *Annu Rev Biochem* **41**, 91–128.

Weiss AA, Silver S, Kinscherf TG. 1978 Cation transport alteration associated with plasmid-determined resistance to cadmium in *Staphylococcus aureus*. *Antimicrob Ag Chemother* **14**, 856–865.