Biometals (2016) 29:651–663
DOI 10.1007/s10534-016-9941-5

CrossMark

Cd^{2+} extrusion by P-type Cd^{2+}-ATPase of *Staphylococcus aureus* 17810R via energy-dependent Cd^{2+}/H^+ exchange mechanism

Zofia Tynecka · Anna Malm · Zofia Gość-Szcześniak

Received: 25 February 2016 / Accepted: 12 June 2016 / Published online: 21 June 2016
© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract Cd^{2+} is highly toxic to *Staphylococcus aureus* since it blocks dithiols in cytoplasmic 2-oxoglutarate dehydrogenase complex (ODHC) participating in energy conservation process. However, *S. aureus* 17810R is Cd^{2+}-resistant due to possession of *cadA*-coded Cd^{2+} efflux system, recognized here as P-type Cd^{2+}-ATPase. This Cd^{2+} pump utilizing cellular energy—ATP, $\Delta\mu_H^+$ (electrochemical proton potential) and respiratory protons, extrudes Cd^{2+} from cytoplasm to protect dithiols in ODHC, but the mechanism of Cd^{2+} extrusion remains unknown. Here we propose that two Cd^{2+} taken up by strain 17810R via Mn^{2+} uniporter down membrane potential ($\Delta\psi$) generated during glutamate oxidation in 100 mM phosphate buffer (high P_iB) are trapped probably by high affinity sites in cytoplasmic domain of Cd^{2+}-ATPase, forming SCdS. This stops Cd^{2+} transport towards dithiols in ODHC, allowing undisturbed NADH production, its oxidation and energy conservation, while ATP could change orientation of SCdS towards facing transmembrane channel. Now, increased number of P_i-dependent protons pumped electrogenically via respiratory chain and countertransported through the channel down $\Delta\psi$, extrude two trapped cytoplasmic Cd^{2+}, which move to low affinity sites, being then extruded into extracellular space via $\Delta\psi$-dependent Cd^{2+}/H^+ exchange. In 1 mM phosphate buffer (low P_iB), external Cd^{2+} competing with decreased number of P_i-dependent protons, binds to ψ_s of Cd^{2+}-ATPase channel, enters cytoplasm through the channel down $\Delta\psi$ via Cd^{2+}/Cd^{2+} exchange and blocks dithiols in ODHC. However, Mg^{2+} pretreatment preventing external Cd^{2+} countertransport through the channel down $\Delta\psi$, allowed undisturbed NADH production, its oxidation and extrusion of two cytoplasmic Cd^{2+} via Cd^{2+}/H^+ exchange, despite low P_iB.

Keywords Cd^{2+} resistance · Cd^{2+}-ATPase · Energy dependent Cd^{2+}/H^+ exchange · *Staphylococcus aureus*

Introduction

Cadmium is highly toxic to living organisms, since it blocks sulphhydryl groups in essential proteins (Vallee and Ulmer 1972; Moulis and Thevenod 2010). Some bacteria carry plasmid-linked *cadA* gene (Novick and Roth 1968; Dyke et al. 1970) conferring Cd^{2+} resistance expressed as decreased ^{109}Cd uptake (Chopra 1975; Tynecka et al. 1975; Silver et al. 1982). Tynecka et al. (1981a, 1981b) were the first to report that the highly decreased ^{109}Cd uptake by growing cells of Cd^{2+}-resistant *Staphylococcus aureus* 17810R was due to pH gradient (ΔpH)-dependent, nigericin-sensitive *cadA*-coded Cd^{2+} efflux system.

Z. Tynecka (✉) · A. Malm · Z. Gość-Szcześniak
Department of Pharmaceutical Microbiology with Laboratory for Microbiological Diagnostics, Medical University, Chodźki 1, 20-093 Lublin, Poland
e-mail: mikrob.farm@umlub.pl

Subsequently, Silver and coworkers (Nucifora et al. 1989; Silver et al. 1989) showed that the *cadA* gene from staphylococcal plasmid pI258 coded the P-type Cd^{2+}-ATPase, belonging to family of membrane-bound, cation-translocating pumps found in eukaryotes and prokaryotes. These pumps located across the membrane maintain homeostasis of essential cations (e.g. Mg^{2+}, Ca^{2+}, K^{+}, Na^{+}) or protons (Apell 2003; Kühlbrandt 2004; Pedersen 2007), and confer resistance to heavy metals (e.g. Cd^{2+}, Zn^{2+}, Cu^{2+}) (Rosen 2002; Nies 2003; Kühlbrandt 2004; Silver and Phung 2005; Argüello et al. 2007, 2011). The best characterized is the P-type Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) for which detailed biochemical and biophysical data (Apell 2003; Toyoshima 2008) and about 50 crystal structures are available (Toyoshima et al. 2013). However, it is still controversial, how ATP energy is transduced to vectorial Ca^{2+} movement (Scarborough 2003; Toyoshima 2009).

According to sequencing data by Silver and coworkers (Nucifora et al. 1989; Silver et al. 1989), the four cysteine residues present in staphylococcal CadA protein are essential for Cd^{2+}-ATPase activity: the conserved Cys23X_2Cys26 in cytoplasmic domain—a possible high affinity Cd^{2+} binding site, and in conserved Cys371ProCys373 inside transmembrane channel, involved probably in Cd^{2+} extrusion. The CysX_2Cys motif is related to copper-binding region in Cu^{2+}-ATPases (Fan and Rosen 2002) and to mercury-binding region in proteins involved in Hg^{2+} resistance (Barkay et al. 2003). According to Tsai et al. (1992), staphylococcal P-type Cd^{2+}-ATPase requires only ATP. Here is shown, that the *cadA*-coded Cd^{2+} efflux system in Cd^{2+}-resistant *S. aureus* 17810R (Tynecka et al. Tynecka et al. 1981a, 1981b; Tynecka and Szcześniak 1991) is a P-type Cd^{2+}-ATPase requiring: ATP, electrochemical proton potential ($\Delta\mu_{H}^{+}$), high phosphate buffer (P_iB) and P_i-dependent protons or Mg^{2+}. The mechanism of Cd^{2+} extrusion by this staphylococcal Cd^{2+}-ATPase is proposed.

Materials and methods

Bacterial strains and culture conditions

Cd^{2+}-resistant *S. aureus* 17810R, carrying *cadA* gene on penicillinase plasmid pII17810 (Shalita et al. 1980), was described previously (Tynecka et al. 1981a, 1981b). Experiments were performed at 37 °C using early exponential phase cells grown aerobically in 3 % nutrient broth and suspended in 100 mM potassium/sodium phosphate buffer, pH 7 (P_iB). Cell suspensions were vigorously aerated for 3 h at 37 °C without exogenous electron donor to deprive cells of endogenous energy reserves (Tynecka and Malm 1995; Tynecka et al. 2001). Next, cells were suspended in P_iB of various concentrations, depending on the experiment, at a density of 0.2 mg dry weight/ml and preincubated with 10 mM glutamate for 10 min at 37 °C (glutamate oxidizing cells). In some experiments, cells were suspended in other buffers: 100 mM triethanolamine/phosphate, pH 7, 100 mM Tris/HCl, pH 7.2 or 100 mM MOPS/NaOH, pH 7. Cd^{2+}-sensitive variant strain *S. aureus* 17810S lacking *cadA* gene, also described previously (Tynecka et al. 1981a, 1981b), was used in some experiments as a control organism.

Reagents

Inhibitors: 2-heptyl-4-hydroxyquinoline N-oxide (HQNO) and dicyclohexylcarbodiimide (DCCD), and ionophores: valinomycin, nigericin or carbonyl cyanide m-chlorophenyl hydrazone (CCCP) were from Sigma (St. Louis, MO). The following radiolabeled compounds were used: ^{109}Cd (carrier-free) or sodium [U-^{14}C]glutamate (7.4 GBq/mmol)—from Amersham, UK, $^{86}RbCl$ (1.075 GBq/mmol), sodium [^{14}C]benzoate (407 MBq/mmol), [^{3}H]inulin (3.7 GBq/mmol) or [γ-^{32}P]ATP (111 TBq/mmol)—from NEN™ Life Science Products (Boston, MA), while $^{32}P_i$—inorganic orthophosphate (740 MBq/mmol)—from the Institute of Nuclear Research, Świerk, Poland.

Uptake experiments

Uptake of ^{109}Cd at 10 μM (as $CdCl_2$) by glutamate oxidizing cells of strain 17810R and strain 17810S was assayed by filtration procedure, as described previously (Tynecka et al. 1981a, 1981b). These cells suspended in 100 or 1 mM P_iB were preincubated at 37 °C for 10 min, with appropriate compounds: $MgCl_2$, $MnCl_2$ or ionophores—nigericin, valinomycin + KCl or CCCP, depending on the experiment, before addition of 10 μM $^{109}CdCl_2$. In order to determine K_m and V_{max} of ^{109}Cd uptake in strain

17810R, the initial influx rate of ^{109}Cd uptake in 1 mM P_iB within 1 min at various $CdCl_2$ concentrations was measured.

Uptake of $^{32}P_i$ (inorganic orthophosphate) by glutamate oxidizing cells of strain 17810R suspended in 100 or 1 mM P_iB was assayed by filtration procedure, as described previously (Tynecka and Szcześniak 1991).

Assay of ^{109} Cd efflux

^{109}Cd efflux was assayed by filtration procedure, as described previously (Tynecka et al. 1981b). ^{109}Cd efflux from washed, glutamate oxidizing cells of strain 17810R was performed after cell preincubation for 20 min with 10 μM $^{109}CdCl_2$ in 1 mM P_iB. After removal of external $^{109}CdCl_2$ by cell washing at 4 °C, cells were resuspended in 100 mM P_iB or in other buffers: 100 mM triethanolamine/phosphate, pH 7, 100 mM Tris/HCl, pH 7.2 or 100 mM MOPS/NaOH, pH 7. DCCD and ionophores: CCCP, valinomycin + KCl or nigericin were added, depending on the experiment. In each experiment 10 mM glutamate was added and all suspensions were prewarmed to 37 °C, before ^{109}Cd efflux was measured. ^{109}Cd efflux from unwashed, glutamate oxidizing cells was also performed after cell preincubation for 20 min with 10 μM $^{109}CdCl_2$ in 1 mM P_iB. Then, P_iB concentration was increased at steady-state from 1 mM to 100 mM without cell washing, before ^{109}Cd efflux was measured. Appropriate compounds: $MgCl_2$ or ionophores: CCCP, valinomycin + KCl or nigericin were added at steady-state, depending on the experiment.

Assay of ^{109}Cd content in subcellular fractions

^{109}Cd distribution among subcellular fractions obtained from glutamate oxidizing cells of strain 17810R and strain 17810S preloaded with 10 μM $^{109}CdCl_2$ in 100 or 1 mM P_iB and preincubated at 37 °C with appropriate compounds: $MgCl_2$ or ionophores—CCCP, valinomycin + KCl or nigericin, depending on the experiment, was determined according to Tynecka et al. (2001).

Assay of enzyme activity

Activity of 2-oxoglutarate dehydrogenase complex (ODHC) was measured in the cytoplasmic fraction obtained from glutamate oxidizing cells of strain 17810R suspended in 100 or 1 mM P_iB and preincubated at 37 °C with $CdCl_2$, according to the method described previously (Tynecka and Malm 1996).

Assay of membrane potential (Δψ) and pH gradient (ΔpH)

The values of Δψ and ΔpH in glutamate oxidizing cells of strain 17810R suspended in 100 or 1 mM P_iB were determined by a filtration procedure from the steady-state distribution of 100 μM ^{86}Rb in the presence of 10 μM valinomycin or of 20 μM sodium [^{14}C]benzoate, respectively, as described previously (Tynecka et al. 1999). [^{3}H]inulin served as a marker for extracellular water.

Phosphorylation assay

Membrane fraction of strain 17810R was obtained according to the procedure described previously (Tynecka and Malm 1996; Tynecka et al. 2001). Phosphorylation assay was performed as described elsewhere (Tsai and Lynn Linet 1993) with some modifications. To 200 μl of membrane fraction (2.4 mg protein/ml), 2 μl of 1.2 mM EDTA were added, followed by incubation for 10 min, and then 2 μl of 50 μM $CdCl_2$ or equivalent volume of deionized water, followed by incubation for 5 min. The reaction was started by addition of 10 μCi of [γ-^{32}P]ATP and 2 μl of 0.8 M $MgCl_2$. The reaction mixture was incubated at room temperature, then the reaction was stopped after 60 s by addition of equivalent volume of ice-cold 20 % TCA. After 10 min incubation on ice, the membranes were collected by centrifugation (14,000 rpm, 5 min). In order to assay the effect of alkali or hydroxylamine, the pellets were incubated with 100 μl of 0.5 M KOH for 5 min on ice or with 200 μl of 0.1 M sodium acetate containing 260 mM hydroxylamine for 10 min at room temperature. After incubation, equivalent volume of ice-cold 10 % TCA was added. In each case, the collected pellets were washed with water and then twice with 50 mM H_3PO_4/NaOH, pH 2.4. Then, the pellets were dissolved in 10 % SDS at 100 °C and suspended in a standard sample buffer used for acidic SDS-PAGE, as described elsewhere (Fairbanks and Avruch 1972). Gels were run at 40 mA for 4–5 h at

room temperature. After electrophoresis, autoradiography of the dried gels was performed at 4 °C for 48 h.

Reproducibility of results

The experimental data shown in each figure are the mean ± SD from at least three independent experiments.

Results

Highly decreased ^{109}Cd accumulation in Cd^{2+}-resistant *S. aureus* 17810R oxidizing glutamate in 100 mM phosphate buffer, pH 7 (high P_iB).

First, membrane proteins of *S. aureus* 17810R harbouring *cadA* gene were phosphorylated by [γ-^{32}P]ATP (Fig. 1). The protein band of about 100 kDa was strongly phosphorylated, when Cd^{2+} was present. Intensity of this band was decreased by alkali or hydroxylamine, which is typical for phosphoenzyme intermediate of P-type ATPases (Tsai and Lynn Linet 1993). This suggests that the band strongly phosphorylated in strain 17810R in the presence of Cd^{2+} (Fig. 1) may correspond to CadA protein, having also molecular weight of about 80 kDa (Nucifora et al. 1989; Tsai and Lynn Linet 1993).

Cd^{2+}-resistant *S. aureus* 17810R took up only 0.5 ± 0.15 nmol ^{109}Cd/mg dry wt (Fig. 2a) and accumulated in cytoplasm merely 0.37 ± 0.1 nmol ^{109}Cd/mg protein (Fig. 2b). Under similar conditions, the Cd^{2+}-sensitive variant strain *S. aureus* 17810S lacking *cadA* gene, took up 20 ± 1.2 nmol ^{109}Cd/mg dry wt (Fig. 2a) and accumulated in cytoplasm 21 ± 1.5 nmol ^{109}Cd/mg protein (Fig. 2b) down $\Delta\psi$ (membrane potential) via high affinity Mn^{2+} uniporter sensitive to Mn^{2+} or valinomycin + K^+ (Fig. 2a). As was already reported (Tynecka et al. 1981a, 1981b; Tynecka and Malm 1995, 1996: Tynecka et al. 1989), two Cd^{2+} accumulated by strain 17810S in a transport cycle, blocked vicinal dithiols in dihydrolipoate and dihydrolipoate dehydrogenase in the cytoplasmic 2-oxoglutarate dehydrogenase complex (ODHC) in Krebs cycle located in the first energy coupling site of respiratory chain (Tynecka et al. 1999). These dithiols are the only Cd^{2+}-sensitive targets in glutamate-linked energy conservation system in strain 17810S; their blocking stopped endogenous NADH production, its oxidation via respiratory chain, generation of electrochemical proton potential ($\Delta\mu_H^+$) and consequently $\Delta\mu_H^+$-dependent processes without direct blocking of solute transporters and ATP synthase (Tynecka and Malm 1995, 1996; Tynecka et al. 1989, 2001).

The Cd^{2+}-resistant strain 17810R did not accumulate Cd^{2+} (Fig. 2a, b), although cells of strain 17810R and 17810S oxidizing glutamate generated $\Delta\mu_H^+$ of similar value expressed as protonmotive force (Δp) of about −191 ± 5 mV. Data in Fig. 2a, b suggest that two Cd^{2+} transported by strain 17810R via Mn^{2+} uniporter down $\Delta\psi$ of −161 ± 5 mV were extruded by Cd^{2+} efflux system described by Tynecka et al. 1981a, 1981b, which was recognized here as a P-type Cd^{2+}-ATPase. Cd^{2+} extrusion by this Cd^{2+} pump via Cd^{2+}/H^+ exchange before reaching Cd^{2+}-sensitive targets—dithiols in ODHC, allowed undisturbed NADH production (5.4 ± 0.6 nmol NADH/min/mg protein), and consequently its oxidation via respiratory chain, $\Delta\mu_H^+$ generation and energy conservation (data not shown), rendering host cells Cd^{2+}-resistant.

Since nigericin, collapsing ΔpH but stimulating $\Delta\psi$, did not increase Cd^{2+} uptake by strain 17810R (Fig. 2a), this suggested that Cd^{2+} extrusion by Cd^{2+}-ATPase from glutamate oxidizing cells was not energized by ΔpH. According to chemiosmotic principles (Mitchell 1966), the enhanced transport of inorganic phosphate (P_i) by strain 17810R via $H^+/^{32}P_i$ symport consuming ΔpH (Tynecka and Szcześniak 1991), could stimulate generation of membrane potential ($\Delta\psi$). It is probable that according to Rosenberg and Friedberg (1984) the H^+/P_i cotransport by strain 17810R in high P_iB could result in phosphate

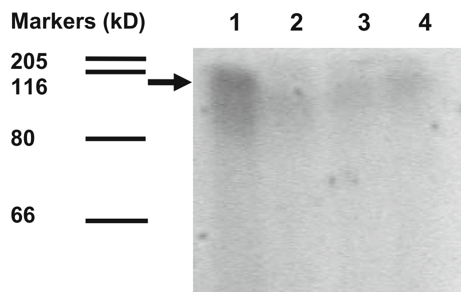

Fig. 1 Phosphorylation of membrane proteins in *S. aureus* 17810R by [^{32}P]ATP. Lane 1—membrane proteins + 50 μM Cd^{2+}, lane 2—membrane proteins + 50 μM Cd^{2+} + 260 mM hydroxylamine, lane 3—membrane proteins + 50 μM Cd^{2+} + 0.5 M KOH, lane 4—membrane proteins without Cd^{2+}, the molecular mass markers are also presented. A position of CadA protein is indicated by an arrow

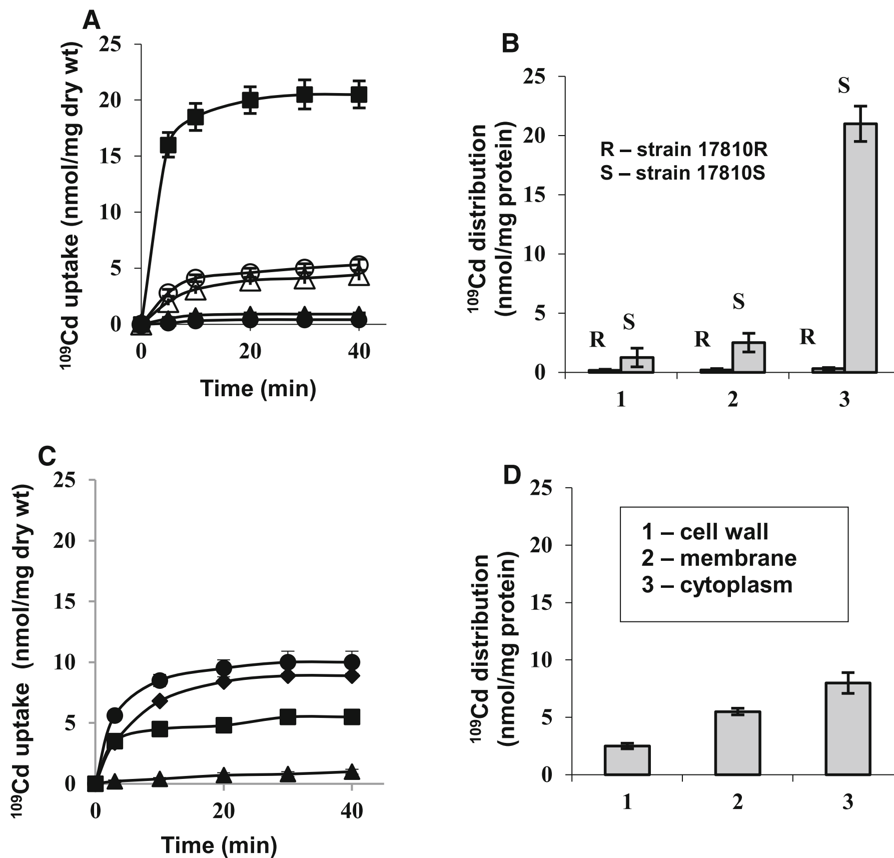

Fig. 2 ^{109}Cd uptake and its distribution in subcellular fractions in *S. aureus* 17810R oxidizing glutamate in 100 mM phosphate buffer, pH 7 (high P_iB) or in 1 mM phosphate buffer, pH 7 (low P_iB). In some experiments Cd^{2+}-sensitive variant strain *S. aureus* 17810S was used. (**a**) Uptake of ^{109}Cd in high P_iB: control cells of strain 17810R (*filled circles*), cells of strain 17810R preincubated with 0.5 μM nigericin (*filled triangles*), control cells of strain 17810S (*filled squares*), cells of strain 17810S preincubated with 100 μM Mn^{2+} (*empty circles*) or 5 μM valinomycin + 50 mM K^+ (*empty triangles*). (**b**) Distribution of ^{109}Cd in subcellular fractions of strain 17810R and strain 17810S in high P_iB. (**c**) Uptake of ^{109}Cd in low P_iB: control cells of strain 17810R (*filled circles*), cells of strain 17810R preincubated with 100 μM Mn^{2+} (*filled diamonds*), 1 mM Mg^{2+} (*filled squares*) or 5 mM Mg^{2+} (*filled triangles*). (**d**) Distribution of ^{109}Cd in subcellular fractions of strain 17810R in low P_iB

polymerization and accumulation of additional protons in cytoplasm. We suggest that these P_i-dependent protons pumped electrogenically via respiratory chain could return through the transmembrane channel of Cd^{2+}-ATPase down $\Delta\psi$ and extruded two cytoplasmic Cd^{2+} into extracellular space via $\Delta\psi$-dependent Cd^{2+}/H^+ exchange. This was confirmed by dependence of Cd^{2+} extrusion on high P_iB, since below 25 mM P_iB, linear Cd^{2+} uptake by strain 17810R insensitive to Mn^{2+} was observed (data not shown). To explain the mechanism of Cd^{2+} extrusion by P-type Cd^{2+}-ATPase in high P_i, ^{109}Cd uptake by strain 17810R was first characterized in 1 mM P_iB and then requirements for its net extrusion were studied.

Uptake of ^{109}Cd by Cd^{2+}-resistant *S. aureus* 17810R oxidizing glutamate in 1 mM phosphate buffer, pH 7 (low P_iB).

The markedly decreased $^{32}P_i$ uptake by strain 17810R from 350 nmol $^{32}P_i$/mg dry wt/20 min in high P_iB to 150 nmol $^{32}P_i$/mg dry wt/20 min in low P_iB could result in decreased number of P_i-dependent protons pumped electrogenically via respiratory chain. Under these conditions, strain 17810R took up 10 ± 1.3 nmol ^{109}Cd/mg dry wt, insensitive to Mn^{2+} (Fig. 2c); about 8 ± 0.9 nmol Cd^{2+}/mg protein were found in cell wall and membrane and similar amount of ^{109}Cd—in cytoplasm (Fig. 2d), which was only about half of that accumulated by strain 17810S

in high P_iB (Fig. 2b). We suggest that in low P_iB the external Cd^{2+} could compete with decreased number of P_i-dependent protons for entry into cytoplasm down $\Delta\psi$ through transmembrane channel of Cd^{2+}-ATPase. Therefore, the first cytoplasmic Cd^{2+} could be extruded from strain 17810R via exchange with external Cd^{2+} via Cd^{2+}/Cd^{2+} exchange, while the second cytoplasmic Cd^{2+} was absent, suggesting its net extrusion. External ^{109}Cd uptake in low P_iB showed linear dependence on Cd^{2+} concentration (data not shown) and high $K_m = 112 \pm 2.3$ μM and $V_{max} = 9.1 \pm 1.2$ nmol Cd^{2+}/mg dry wt/min, suggesting that Cd^{2+}-ATPase channel may function now as low affinity second pathway transporting external Cd^{2+} down $\Delta\psi$ instead of protons towards Cd^{2+}–sensitive targets—dithiols in ODHC.

External Cd^{2+} accumulated by strain 17810R in low P_iB blocked dithiols in ODHC, which stopped NADH production (from 5.4 ± 0.6 to 0.2 ± 0.1 nmol NADH/min/mg protein) and consequently its oxidation via respiratory chain, but $\Delta\mu_H^+$ generation was unaffected ($\Delta p = -210 \pm 4$ mV). This suggests that according to Mitchell (1966), Cd^{2+} toxicity to cell respiration could result in conversion of the reversible F_oF_1-ATP synthase into hydrolytic direction, which working now as Cd^{2+}-insensitive, anaerobic proton pump—F_oF_1-ATPase (Tynecka et al. 1990), could continue $\Delta\mu_H^+$ generation. We suggest that $\Delta\psi$ of -195 ± 4 mV could energize transport of the second cytoplasmic Cd^{2+} via Mn^{2+} uniporter, while ΔpH of 15 ± 2 mV could support its extrusion via Cd^{2+}/H^+ exchange, as confirmed by absence of the second Cd^{2+} in cytoplasm (Fig. 2c, d). Thus, Cd^{2+}-ATPase extruded in low P_iB also two cytoplasmic Cd^{2+}, but only external Cd^{2+} reached dithiols in ODHC through the channel via Cd^{2+}/Cd^{2+} exchange, disturbing energy conservation and Cd^{2+} resistance of strain 17810R.

We also considered in strain 17810R a controversial problem– existence of low affinity sites on external surface of P-type ATPases (McIntosh 2000; Apell 2003; Scarborough 2003; Toyoshima 2009). First, Silver and coworkers (Nucifora et al. 1989, Silver 1996) recognized during sequencing studies some negatively charged amino acid residues (Glu, Asp) on extracellular surface of CadA protein. It is known (Williams 1978; Barber 1980) that such residues create at physiological pH the surface potentials (ψ_s) on biological membranes, protected by cations of various protective abilities ($Mg^{2+} > Ca^{2+} > K^+ > Na^+$), depending on their concentration and/or affinity. We suggest that Cd^{2+}-ATPase channel in strain 17810R may also possess two negatively charged residues forming surface potential (ψ_s) functioning as low affinity sites to which protons or external Cd^{2+} may bind before entering the channel, but this depends on P_iB concentration.

According to Fig. 2c, 1 mM Mg^{2+} prevented external Cd^{2+} uptake by strain 17810R in 50 %, while 5 mM Mg^{2+} (further called Mg^{2+}) stopped it. These data confirm existence of low affinity ψ_s sites on extracellular surface of Cd^{2+}-ATPase channel in strain 17810R. Protection of ψ_s by Mg^{2+} against external Cd^{2+} binding and its countertransport through the channel towards dithiols in ODHC allowed undisturbed energy conservation and Cd^{2+} resistance. In contrast, Cd^{2+} uptake by strain 17810S was Mg^{2+}-insensitive (data not shown), suggesting ψ_s absence in Mn^{2+} uniporter.

The ionophore studies in low P_iB showed that nigericin, collapsing ΔpH, doubled Cd^{2+} uptake by strain 17810R (Fig. 3a). Probably, by stopping ΔpH-dependent efflux of the second cytoplasmic Cd^{2+} energized by the reversed Cd^{2+}-insensitive F_oF_1-ATPase, nigericin could unmask $\Delta\psi$-dependent Cd^{2+} transport via Mn^{2+} uniporter, sensitive to Mn^{2+} (Fig. 3a). Now, strain 17810R accumulating in cytoplasm two Cd^{2+} down $\Delta\psi$—via transmembrane channel (Cd^{2+}/Cd^{2+} exchange) and via Mn^{2+} uniporter (Fig. 3a, b), became Cd^{2+}-sensitive, like strain 17810S (Fig. 2a, b). However, Mg^{2+} pretreatment of strain 17810R before nigericin addition, prevented external Cd^{2+} binding to ψ_s of Cd^{2+}-ATPase and also stopped Cd^{2+} countertransport through the channel, rendering host cells Cd^{2+}-resistant, despite low P_iB (Fig. 3a).

Valinomycin + K^+ collapsing $\Delta\psi$, did not affect external Cd^{2+} uptake by strain 17810R (Fig. 3a), although $\Delta\psi$-dependent Cd^{2+} transport via Mn^{2+} uniporter into cytoplasm of strain 17810S was stopped by this ionophore (Fig. 2a). Therefore, valinomycin-insensitive Cd^{2+} uptake by strain 17810R may represent only $\Delta\psi$-independent external Cd^{2+} binding to cell wall and only to one ψ_s site of Cd^{2+}-ATPase, prevented by Mg^{2+} (Fig. 3a, c), while external Cd^{2+} binding to the second ψ_s site was probably prevented by protons countertransported down unaffected ΔpH.

CCCP also doubled ^{109}Cd uptake by strain 17810R (Fig. 3a), although Cd^{2+} accumulation in cytoplasm

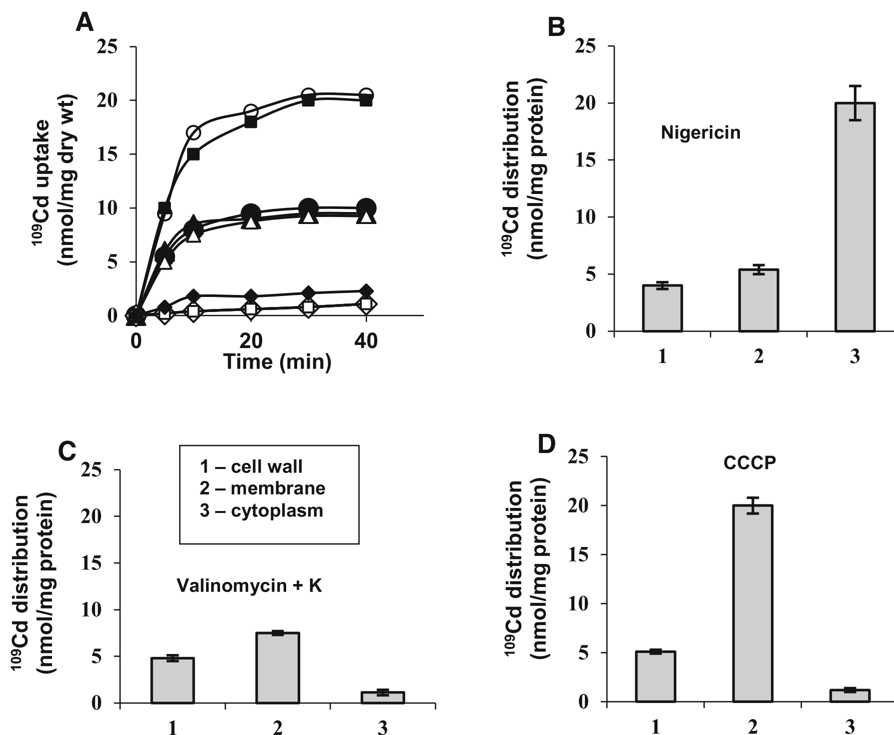

Fig. 3 Effects of ionophores on ^{109}Cd uptake and its distribution in subcellular fractions in *S. aureus* 17810R oxidizing glutamate in 1 mM phosphate buffer, pH 7 (low P_iB) with or without 5 mM Mg^{2+}. (**a**) ^{109}Cd uptake by control cells (*filled circles*) or cells preincubated with 0.5 μM nigericin (*filled squares*), 0.5 μM nigericin + 100 μM Mn^{2+} (*empty triangles*) or 5 mM Mg^{2+} + 0.5 μM nigericin (*filled diamonds*), cells preincubated with 5 μM valinomycin + 50 mM K^+ (*empty triangles*) or 5 mM Mg^{2+} + 5 μM valinomycin + 50 mM K^+ (*empty diamonds*), cells preincubated with 10 μM CCCP (*empty circles*) or 5 mM Mg^{2+} + 10 μM CCCP (*empty squares*). Distribution of ^{109}Cd in subcellular fractions after cell preincubation with 0.5 μM nigericin (**b**), 5 μM valinomycin + 50 mM K^+ (**c**) or 10 μM CCCP (**d**)

was stopped (Fig. 3d), since Δψ for Cd^{2+} transport through the channel was blocked by CCCP. This means that two external Cd^{2+} could bind without energy to two ψ_s sites in Cd^{2+}-ATPase channel (Fig. 3d), prevented by Mg^{2+} (Fig. 3a). These CCCP data strongly confirm existence of two low affinity ψ_s sites in Cd^{2+}-ATPase channel of strain 17810R.

Restoration of Cd^{2+} resistance in *S. aureus* 17810R by extrusion of Cd^{2+} preaccumulated in 1 mM phosphate buffer, pH 7 (low P_iB).

Cd^{2+}-preloaded cells of strain 17810R in low P_i were washed and resuspended in high P_iB. Since in these Cd^{2+}-poisoned cells of strain 17810R the NADH production was blocked and consequently its oxidation, therefore $\Delta\mu_H^+$ generation and Cd^{2+} extrusion via Cd^{2+}/H^+ exchange were also stopped. Therefore, under such conditions only the Cd^{2+}-insensitive proton pump—the reversed F_oF_1-ATPase could provide protons for Cd^{2+}-ATPase to start Cd^{2+} extrusion from dithiols in ODHC. We suggest that these protons could bind easily to ψ_s of Cd^{2+}-ATPase channel in washed cells, since there was no extracellular Cd^{2+} to compete. Finally, protons countertransported through the channel down Δψ displaced Cd^{2+} from dithiols in ODHC, which was evidenced by undisturbed ODHC activity (5.6 ± 0.8 nmol NADH/min/mg protein).

Cd^{2+} extrusion was inhibited in 50 % by DCCD, blocking H^+ channel of F_oF_1-ATPase and also by CCCP or valinomycin + K^+ collapsing Δψ (Fig. 4b). This suggests that Cd^{2+} could be removed from cell wall and ψ_s of strain 17810R without energy, but Cd^{2+} extrusion from dithiols in ODHC requiring H^+ and Δψ, was stopped as evidenced by inhibited ODHC activity with all three compounds (from 1.4 ± 0.2 to 1.5 ± 0.3 nmol NADH/min/mg protein). Only nigericin collapsing ΔpH, allowed Cd^{2+} extrusion down undisturbed Δψ (Fig. 4b). Since CCCP or valinomycin + K^+ prevented Δψ-dependent proton

countertransport through the channel and also stopped Cd^{2+} extrusion, this suggests that dithiols in ODHC ($S^{-}S^{-}$) may function in glutamate-linked energy conservation process probably as $\Delta\psi$ generation site.

According to Fig. 4c, other 100 mM buffers containing glutamate-Tris/HCl, pH 7.2 or MOPS/NaOH, pH 7.0, did not initiate Cd^{2+} extrusion from washed cells of strain 17810R. Only 100 mM triethanolamine/phosphate buffer pH 7, triggered total Cd^{2+} efflux (Fig. 4c) sensitive to CCCP or valinomycin + K^{+} in 50 %, but insensitive to nigericin (Fig. 4d). These data strongly confirm requirement of high P_i and of P_i-dependent protons for net Cd^{2+} extrusion by Cd^{2+}-ATPase.

Cd^{2+} efflux triggered by 100 mM P_iB from unwashed cells of strain 17810R was incomplete (Fig. 5a), since only Cd^{2+} from cell wall and ψ_s could be released without energy, but not Cd^{2+} from cytoplasm requiring protons and $\Delta\psi$, as evidenced by blocked ODHC activity (1.4 ± 0.3 nmol NADH/min/mg protein). Only high P_iB plus Mg^{2+}—preventing external Cd^{2+} countertransport down $\Delta\psi$ through the channel, allowed Cd^{2+} extrusion from dithiols in ODHC via Cd^{2+}/H^{+} exchange (Fig. 5a), as evidenced

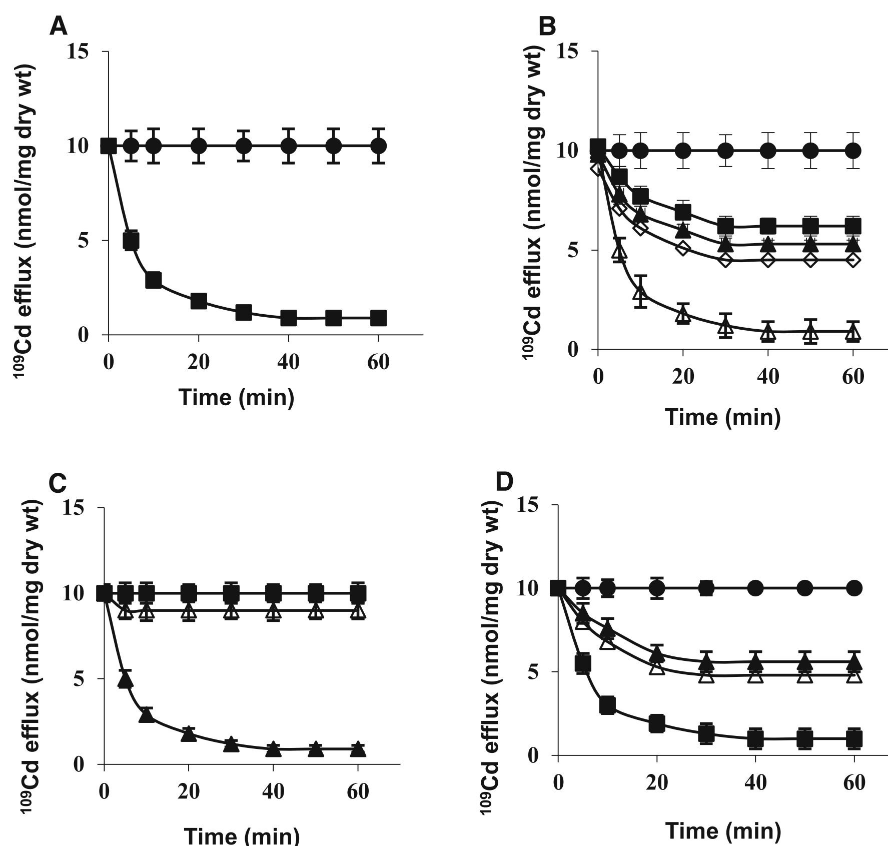

Fig. 4 ^{109}Cd efflux from washed cells of *S. aureus* 17810R preloaded with ^{109}Cd in 1 mM phosphate buffer, pH 7 (low P_iB + glutamate. (**a**) Cd^{2+}-preloaded, washed cells were resuspended in 1 mM P_iB + glutamate (*filled circles*) or in 100 mM P_iB + glutamate (*filled squares*). (**b**) Cd^{2+}-preloaded, washed cells were resuspended in 1 mM P_iB (*filled circles*) or in 100 mM P_iB + 10 μM CCCP (*filled squares*), 100 mM P_iB + 5 μM valinomycin + 50 mM K^{+} (*filled triangles*), 100 mM P_iB + 100 μM DCCD (*empty diamonds*) or 100 mM 100 mM P_iB + 0.5 μM nigericin (*empty triangles*), to each suspension glutamate was added. (**c**) Cd^{2+}-preloaded, washed cells were resuspended in 100 mM Tris/HCl, pH 7.2 (*filled squares*), 100 mM MOPS/NaOH, pH 7 (*empty triangles*) or in 100 mM triethanolamine/phosphate, pH 7 (*filled triangles*), to each buffer glutamate was added. (**d**) Cd^{2+}-preloaded, washed cells were suspended in 1 mM P_iB (*filled circles*) or in 100 mM triethanolamine/phosphate buffer + 10 μM CCCP (*filled triangles*), 100 mM triethanolamine/phosphate buffer + 5 μM valinomycin + 50 mM K^{+} (*empty triangles*) or 100 mM triethanolamine/phosphate buffer + 0.5 μM nigericin (*filled squares*), to each suspension glutamate was added

by unblocked ODHC activity (5.5 ± 0.8 nmol NADH/min/mg protein). This Cd^{2+} efflux was equally affected by ionophores (Fig. 5b), as that from washed cells (Fig. 4b).

Discussion

Bacterial Cd^{2+}-ATPases belong to the superfamily of P-type ATPases and to the P1 subfamily of soft metal ions pumps (Kühlbrandt 2004). Studies on Cd^{2+}-ATPase in *S. aureus* (Nucifora et al. 1989; Silver et al. 1989; Tsai et al. 2002) and in *Listeria monocytogenes* (Bal et al. 2003; Wu et al. 2006a) established amino acid sequence of CadA protein, its membrane topology and suggested involvement in Cd^{2+} extrusion of four cysteine residues present in this protein, but the mechanism of how Cd^{2+} is extruded from Cd^{2+}-resistant *S. aureus* remains so far unknown. Also studies on Cd^{2+}-ATPase in other microorganisms did not explain this mechanism (Schwager et al. 2012; Schurig-Briccio and Gennis 2012; Chien et al. 2013; Maynaud et al. 2014). As found here, the *cadA*-coded Cd^{2+} efflux system in *S. aureus* 17810R described by Tynecka et al. (1981a, 1981b), appeared to be the P-type Cd^{2+}-ATPase. Figure 6 presents a proposed scheme for Cd^{2+} extrusion via Cd^{2+}/H^{+} exchange mechanism by the native Cd^{2+}-ATPase in *S. aureus* 17810R oxidizing glutamate in high P_iB.

We propose that two Cd^{2+}transported by strain 17810R down $\Delta\psi$ via Mn^{2+} uniporter in high P_iB are trapped by high affinity sites—dithiols in cytoplasmic domain of staphylococcal Cd^{2+}-ATPase, which were recognized by Silver and coworkers (Nucifora et al. 1989; Silver et al. 1989). We suggest that Cd^{2+} trapping (SCdS) stops its transport towards dithiols in cytoplasmic ODHC, allowing undisturbed NADH production, its oxidation via respiratory chain and $\Delta\mu_{H}^{+}$ generation, while ATP could change SCdS orientation from facing cytoplasm towards facing transmembrane channel, probably by a tilting mechanism suggested for Ca^{2+}-ATPase (Albers 1967; Post et al. 1972; Higgins and Linton 2001). Finally, increased number of P_i-dependent protons pumped electrogenically via respiratory chain, could compete with external Cd^{2+} for binding to ψ_s of Cd^{2+}-ATPase. Then, entering the channel, protons displaced from high affinity sites the two trapped cytoplasmic Cd^{2+}, which were transferred through the channel towards low affinity sites ψ_s, being then extruded into extracellular space via Cd^{2+}/H^{+} exchange against electrochemical and concentration gradients (Tynecka et al. 1981a, 1981b), rendering host cells Cd^{2+}-resistant. In *Listeria monocytogenes* (Wu et al. 2006b) the *cadA*-coded Cd^{2+}-ATPase also extruded two Cd^{2+}.

According to the proposed concept (Fig. 6), the P_i-dependent protons and Cd^{2+} binding ligands in Cd^{2+}-ATPase channel of strain 17810R seem to play vital role in Cd^{2+} extrusion. We suggest that the negative charges exposed in the channel by successive proton movement to ψ_s, CysProCys and CysCys (recognized by Nucifora et al. 1989; Silver et al. 1989; Tsai et al.

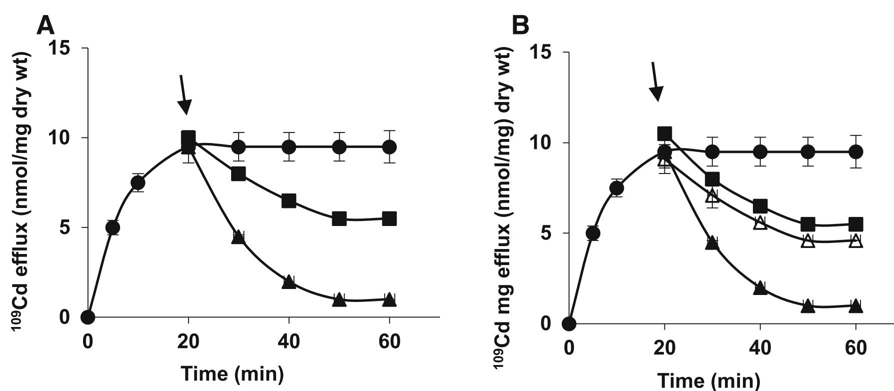

Fig. 5 ^{109}Cd efflux from unwashed cells of *S. aureus* 17810R preloaded with ^{109}Cd in 1 mM phosphate buffer, pH 7 (low P_iB) + glutamate. (**a**) At the time indicated by an arrow, P_iB concentration was increased from 1 mM to 100 mM (*filled squares*), to one portion of suspension 5 mM Mg^{2+} was added (*filled triangles*), cells suspended in 1 mM P_iB (*filled circles*). (**b**) At the time indicated by an arrow, P_iB concentration was increased from 1 mM to 100 mM with the following additions: 5 μM valinomycin + 50 mM K^{+} (*empty triangles*), 10 μM CCCP (*filled squares*) or 0.5 μM nigericin (*filled triangles*), to each suspension 5 mM Mg^{2+} was added; cells suspended in 1 mM P_iB (*filled circles*)

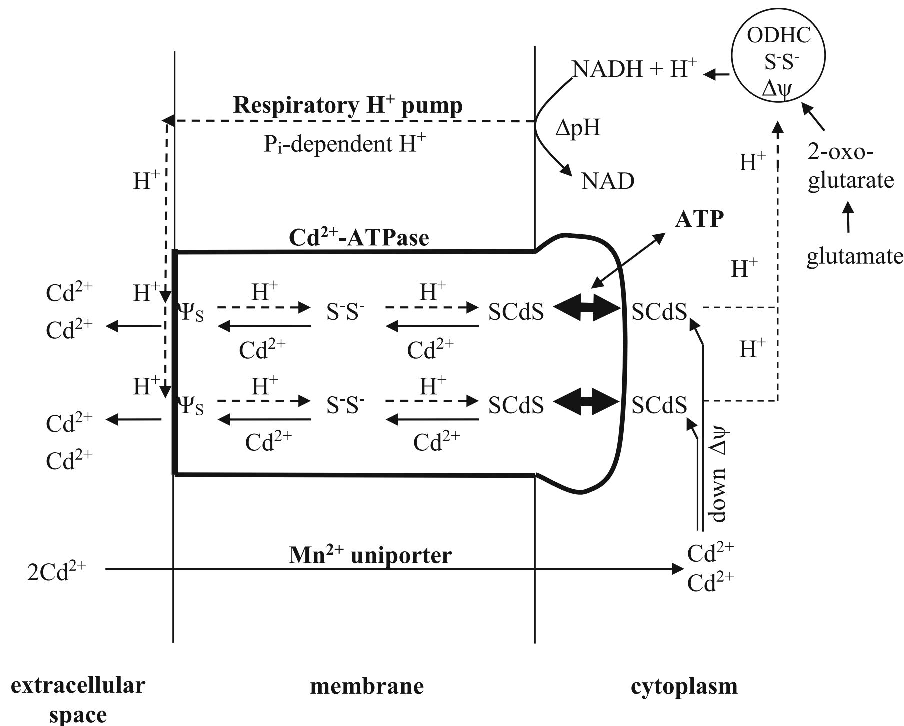

Fig. 6 The proposed mechanism of Cd^{2+} extrusion by P-type Cd^{2+}-ATPase from *S. aureus* 17810R oxidizing glutamate in 100 mM phosphate buffer, pH 7 (high P_iB). Two Cd^{2+} transported via Mn^{2+} uniporter down membrane potential ($\Delta\psi$) are trapped by high affinity sites—dithiols located in cytoplasmic domain of CadA protein, recognized by Silver and coworkers (Nucifora et al. 1989; Silver et al. 1989). SCdS formation may trigger phosphorylation of CadA protein by ATP, which changes SCdS orientation from facing cytoplasm into facing transmembrane channel. Cd^{2+} trapping stops Cd^{2+} transport towards dithiols in 2-oxoglutarate dehydrogenase complex (ODHC)—the only Cd^{2+}-sensitive targets in glutamate-linked energy conservation system, functioning most likely as $\Delta\psi$ generation site S^-S^-. This allows undisturbed NADH production, its oxidation and $\Delta\mu_H^+$ generation. Consequently, increased number of P_i-dependent protons pumped electrogenically via respiratory chain in high P_iB compete with external Cd^{2+} and bind to low affinity surface sites (ψ_s) of Cd^{2+}-ATPase channel. Finally, protons countertransported down $\Delta\psi$ through the channel, extrude two trapped cytoplasmic Cd^{2+} into extracellular space via $\Delta\psi$-dependent Cd^{2+}/H^+ exchange, rendering host cells Cd^{2+}-resistant, since the toxic Cd^{2+} could not reach the primary targets—dithiols in ODHC either via Mn^{2+} uniporter or via transmembrane channel

2002) can be the driving force for uphill Cd^{2+} pumping through the channel into opposite direction–the extracellular environment. Also Ca^{2+} efflux by Ca^{2+}-ATPase from mammalian mitochondria was P_i-dependent (Roos et al. 1980; Nicholls and Akerman 1982; Ligeti and Lukács 1984). Proton requirement for Cd^{2+} extrusion in strain 17810R, is in accord with Scarbourough‘s considerations (Scarborough 2003)—that after phosphorylation reaction, something must weaken ion binding site, allowing Ca^{2+} release into extracellular space. However, the role of protons in SR Ca^{2+}-ATPase has been controversial for many years (Ueno and Sekine 1981; Levy et al. 1990; Andersen and Vilsen 1995; Karjalainen et al. 2007; Toyoshima 2009; Fibich and Apell 2011; Bublitz et al. 2013).

Our earlier observations (Tynecka et al. 1981a, 1981b) suggested that protons and external Cd^{2+} could compete for entry into cytoplasm through the channel of Cd^{2+}-ATPase down $\Delta\psi$. Our present data confirm that due to decreased number of P_i-dependent protons in low P_iB the external Cd^{2+} could bind to ψ_s of Cd^{2+}-ATPase channel and then driven down $\Delta\psi$ through the channel via Cd^{2+}/Cd^{2+} exchange, blocks dithiols in ODHC, rendering host cells Cd^{2+}-sensitive, like strain 17810S.

However, we found that Mg^{2+} can protect strain 17810R against Cd^{2+} poisoning in low P_iB. According to William's model (Williams 1978) and our CCCP data, Mg^{2+} can prevent external Cd^{2+} binding to ψ_s and stops its countertransport through the channel

towards dithiols in ODHC. In energized cells, Mg^{2+} can be displaced transiently by respiratory protons, but still prevents external Cd^{2+} countertransport. Therefore, even a decreased number of P_i-dependent protons pumped electrogenically during glutamate oxidation in low P_iB, but protected by Mg^{2+}, could enter the channel to extrude two trapped cytoplasmic Cd^{2+} via energy-dependent Cd^{2+}/H^+ exchange. Discharge of $\Delta\mu_H^+$ by protons allowed Mg^{2+} return to ψ_s. Such Mg^{2+} oscillation can maintain undisturbed NADH production, its oxidation, energy conservation and Cd^{2+} resistance, despite low P_iB. Also Ca^{2+} influx and $\Delta\psi$ disruption in mitochondria were prevented by Mg^{2+} (Sharikabad et al. 2001; Racay 2008).

Net Cd^{2+} extrusion requires also steady-state thermodynamic equilibrium between activities of two energy-dependent membrane systems—Mn^{2+} uniporter and Cd^{2+}-ATPase. Some changes, e.g. increased Cd^{2+} concentration (Tynecka et al. 1981b), alkaline pH (Tynecka et al. 1981a) or decreased P_iB concentration shown here, disturb equilibrium and consequently Cd^{2+} resistance. Therefore, the Cd^{2+}-ATPase cooperating with P_i-dependent protons and utilizing cellular energy (ATP and $\Delta\mu_H^+$) can protect against Cd^{2+} poisoning the vital dithiols in ODHC.

However, we found that Cd^{2+}, which blocked dithiols in ODHC in low P_iB could be also extruded. As was already mentioned, in these Cd^{2+}-poisoned cells of strain 17810R, only the Cd^{2+}-insensitive, reversed F_oF_1-ATPase could pump protons to start the Cd^{2+} efflux process. Besides, we increased the P_iB concentration to 100 mM (high P_iB) and also inhibited external Cd^{2+} countertransport down $\Delta\psi$ through the channel either by cell washing or by Mg^{2+} pretreatment. In both situations, the countertransport of P_i-dependent protons through the channel was restored, leading to Cd^{2+} displacement from dithiols in ODHC. We suggest that the displaced Cd^{2+} could be trapped by high affinity sites of Cd^{2+}-ATPase, forming SCdS, while ATP could change SCdS orientation towards facing transmembrane channel. Now, Cd^{2+} displaced by protons from high affinity sites via Cd^{2+}/H^+ exchange moves towards low affinity sites (ψ_s) of Cd^{2+}-ATPase. From here, Cd^{2+} is displaced into extracellular space also via Cd^{2+}/H^+ exchange. Gradual Cd^{2+} extrusion by Cd^{2+}-ATPase restored gradually: NADH production, its oxidation, $\Delta\mu_H^+$ generation via respiratory chain, reversal of F_oF_1-ATPase into biosynthetic direction and energy conservation, rendering host cells again Cd^{2+}-resistant. DCCD—blocking H^+ channel of F_oF_1-ATPase or CCCP and valinomycin + K^+ collapsing $\Delta\psi$, prevented Cd^{2+} extrusion, confirming the requirement of P_i-dependent protons and of $\Delta\psi$ for the Cd^{2+} efflux process.

To summarize, these studies provide for the first time the novel data on the so far unknown mechanism of Cd^{2+} extrusion by *cadA*-coded P-type Cd^{2+}-ATPase in *S. aureus* 17810R, oxidizing glutamate in high P_iB. Energy-dependent Cd^{2+} extrusion by this pump via Cd^{2+}/H^+ exchange mechanism renders host cells Cd^{2+}-resistant, since the toxic Cd^{2+} could not reach the primary Cd^{2+}-sensitive targets—dithiols in ODHC via two routes—Mn^{2+} uniporter or transmembrane channel, allowing undisturbed glutamate-linked energy conservation process. Moreover, the vital role of P_i-dependent protons or Mg^{2+} and of cellular energy (ATP and $\Delta\mu_H^+$) in Cd^{2+} extrusion by Cd^{2+}-ATPase is underlined.

Acknowledgments Zofia Tynecka would like to thank Prof. Keith Dyke, Wadham College, University of Oxford, for the generous gift of Cd^{2+}-resistant *S. aureus* 17810R and its Cd^{2+}-sensitive variant strain *S. aureus* 17810S. These studies were supported by a grant 6 P04C 020 13 from the State Committee for Scientific Research, Warsaw, Poland. We dedicate this work to the memory of our friend and collegue Zofia Goś-Szcześniak.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Albers RW (1967) Biochemical aspects of active transport. Annu Rev Biochem 36:727–756

Andersen JP, Vilsen B (1995) Structure-function relationships of cation translocation by Ca^{2+}- and Na^+, K^+-ATPases studied by site-directed mutagenesis. FEBS Lett 359:101–106

Apell HJ (2003) Structure-function relationship in P-type ATPases—a biophysical approach. Rev Physiol Biochem Pharmacol 150:1–35

Argüello JM, Eren E, González-Guerrero M (2007) The structure and function of heavy metal transport P1B-ATPases. Biometals 20:233–248

Argüello JM, González-Guerrero M, Raimunda D (2011) Bacterial transition metal P(1B)-ATPases: transport mechanism and roles in virulence. Biochem 50:9940–9949

Bal N, Wu CC, Catty P, Guillain F, Mintz E (2003) Cd^{2+} and the N-terminal metal-binding domain protect the putative membranous CPC motif of the Cd^{2+}-ATPase of *Listeria monocytogenes*. Biochem J 369:681–685

Barber J (1980) Membrane surface charges and potentials in relation to photosynthesis. Biochim Biophys Acta 594:253–308

Barkay T, Miller SM, Summers AO (2003) Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol Rev 27:355–384

Bublitz M, Musgaard M, Poulsen H, Thögersen L, Olesen C, Schiött B, Morth JP, Möller JV, Nissen P (2013) Ion pathways in the sarcoplasmic reticulum Ca^{2+}-ATPase. J Biol Chem 288:10759–10765

Chien CC, Huang CH, Lin YW (2013) Characterization of a heavy metal translocating P-type ATPase gene from an environmental heavy metal resistance *Enterobacter* sp. Isolate. Appl Biochem Biotechnol 169:1837–1846

Chopra I (1975) Mechanism of plasmid-mediated resistance to cadmium in *Staphylococcus aureus*. Antimicrob Agents Chemother 7:8–14

Dyke KGH, Parker MT, Richmond MH (1970) Penicillinase production and metal-ion resistance in *Staphylococcus aureus* cultures isolated from hospital patients. J Med Microbiol 3:125–136

Fairbanks G, Avruch J (1972) Four gel systems for electrophoretic fractionation of membrane proteins using ionic detergents. J Supramol Struct 1:66–75

Fan B, Rosen BP (2002) Biochemical characterization of CopA, the *Escherichia coli* Cu(I)-translocating P-type ATPase. J Biol Chem 277:46987–46992

Fibich A, Apell HJ (2011) Kinetics of luminal proton binding to the SR Ca-ATPase. Biophys J 19:1896–1904

Higgins CF, Linton KJ (2001) The xyz of ABC transporters. Science 293:1782–1784

Karjalainen EL, Hauser K, Barth A (2007) Proton paths in the sarcoplasmic reliculum Ca^{2+}-ATPase. Biochim Biophys Acta 1767:1310–1318

Kühlbrandt W (2004) Biology, structure and mechanism of P-type ATPases. Nat Rev Mol Cell Biol 5:282–295

Levy D, Seigneuret M, Bluzat A, Rigaud J-L (1990) Evidence for proton countertransport by the sarcoplasmic reticulum Ca^{2+}-ATPase during calcium transport in reconstituted proteoliposomes with low ionic permeability. J Biol Chem 265:19524–19534

Ligeti E, Lukács GL (1984) Phosphate transport, membrane potential and movements of calcium in rat liver mitochondria. J Bioenerg Biomembr 16:101–113

Maynaud G, Brunel B, Yashiro E, Mergeay M, Cleyet-Marel JC, Le Quere A (2014) CadA of Mesorhizobium metallidurans isolated from a zinc-rich mining soil is a P(IB-2)-type ATPase involved in cadmium and zinc resistance. Res Microbiol 165:175–189

McIntosh DB (2000) Portrait of a P-type pump. Nature Struct Biol 7:532–535

Mitchell P (1966) Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. Biol Rev Camb Philos Soc 41:445–502

Moulis JM, Thevenod F (2010) New perspectives in cadmium toxicity—an introduction. Biometals 23:763–768

Nicholls D, Akerman K (1982) Mitochondrial calcium transport. Biochim Biophys Acta 683:57–88

Nies DH (2003) Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol Rev 27:313–339

Novick RP, Roth C (1968) Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. J Bacterio. 95:1335–1342

Nucifora G, Chu L, Misra TK, Silver S (1989) Cadmium resistance from *Staphylococcus aureus* plasmid pI258 *cadA* gene results from a cadmium-efflux ATPase. Proc Natl Acad Sci USA 86:3544–3548

Pedersen PL (2007) Transport ATPases into the year 2008: a brief overview related to types, structures, functions and roles in health and disease. J Bioenerg Biomembr 39:349–355

Post RL, Hegyvary C, Kume S (1972) Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. J Biol Chem 247:6530–6540

Racay P (2008) Effect of magnesium on calcium-induced depolarization of mitochondrial transmembrane potential. Cell Biol Int 32:136–145

Roos I, Crompton M, Carafoli E (1980) The role of inorganic phosphate in the release of Ca^{2+} from rat-liver mitochondria. Eur J Biochem 110:319–325

Rosen BP (2002) Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes. Comp Biochem Physiol A: Mol Integr Physiol 133:689–693

Rosenberg M, Friedberg I (1984) Respiratory control in *Micrococcus lysodeicticus*. J Bioenerg Biomembr 16:61–68

Scarborough GA (2003) Rethinking the P-type ATPase problem. Trends Biochem Sci 28:581–584

Schurig-Briccio LA, Gennis R (2012) Characterization of the P_{IB}-type ATPases present in *Thermus thermophiles*. J Bacteriol 194:4107–4113

Schwager S, Lumjiaktase P, Stöckli M, Weisskopf L, Eberl L (2012) The genetic basis of cadmium resistance of *Bulkholderia cenocepacia*. Environ Microbiol Rep 4:562–568

Shalita Z, Murphy E, Novick RP (1980) Penicillinase plasmids of *Staphylococcus aureus*: structural and evolutionary relationships. Plasmid 3:291–311

Sharikabad MN, Ostbye KM, Brörs O (2001) Increased $[Mg^{2+}]_o$ reduces Ca influx and disruption of mitochondrial potential during reoxygenation. Am J Physiol Heart Circ Physiol 281:113–123

Silver S (1996) Bacterial resistances to toxic metal ions—a review. Gene 179:9–19

Silver S, Phung LT (2005) A bacterial view of the periodic table: genes and proteins for toxic inorganic ions. J Ind Microbiol Biotechnol 32:587–605

Silver S, Perry RD, Tynecka Z, Kinscherf TG (1982) Mechanism of bacterial resistance to the toxic heavy metals antimony, arsenic, cadmium, mercury and silver. In: Mitsuchashi S (ed.) Proceedings of Third Tokyo symposium on drug resistance in bacteria, Japan Scientific Societes Press Tokyo, Thieme-Stratton Inc, New York, pp 347–361

Silver S, Nucifora G, Chu L, Misra TK (1989) Bacterial resistance ATPases: primary pumps for exporting toxic cations and anions. Trends in Biochem Sci 14:76–80

Toyoshima C (2008) Structural aspects of ion pumping by Ca^{2+}-ATPase of sarcoplasmic reticulum. Arch Biochem Biophys 476:3–11

Toyoshima C (2009) How Ca^{2+}-ATPase pumps ions across the sarcoplasmic reticulum membrane. Biochim Biophys Acta 1793:941–946

Toyoshima C, Iwasawa S, Ogawa H, Hirata A, Tsueda J, Inesi G (2013) Crystal structures of the calcium pump and sarcolipin in the Mg-bound E1 state. Nature 495:260–264

Tsai KJ, Lynn Linet A (1993) Formation of a phosphorylated enzyme intermediate by the *cadA* Cd^{2+}-ATPase. Arch Biochem Biophys 305:267–270

Tsai KJ, Yoon KP, Lynn AR (1992) ATP-dependent cadmium transport by the *cadA* cadmium resistance determinant in everted membrane vesicles of *Bacillus subtilis*. J Bacteriol 174:116–121

Tsai KJ, Lin YF, Wong MD, Yang HHC, Fu HL, Rosen BP (2002) Membrane topology of the pI258 CadA Cd(II)/Pb(II)/Zn(II)- translocating P-type ATPase. J Bioenerg Biomembr 34:147–156

Tynecka Z, Malm A (1995) Energetic basis of cadmium toxicity in *Staphylococcus aureus*. Biometals 8:197–204

Tynecka Z, Malm A (1996) Cadmium-sensitive targets in the aerobic respiratory metabolism of *Staphylococcus aureus*. J Basic Microbiol 36:447–452

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

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

Tynecka Z, Goś Z, Zając 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, Szcześniak Z (1989) Plasmid-linked protection of [^{14}C]-glutamate transport and its oxidation against Cd^{2+} in *Staphylococcus aureus*. Acta Microbiol Polon 38:131–141

Tynecka Z, Skwarek T, Malm A (1990) Anaerobic ^{109}Cd accumulation by cadmium-resistant and -sensitive *Staphylococcus aureus*. FEMS Microbiol Lett 69:159–164

Tynecka Z, Szcześniak Z, Malm A, Łoś R (1999) Energy conservation in aerobically grown *Staphylococcus aureus*. Res Microbiol 150:555–566

Tynecka Z, Korona-Głowniak I, Łoś R (2001) 2-oxoglutarate transport system in *Staphylococus aureus*. Arch Microbiol 176:143–150

Ueno T, Sekine T (1981) A role of H^+ flux in active Ca^{2+} transport into sarcoplasmic reticulum vesicles. II. H^+ ejection during Ca^{2+} uptake. J Biochem 89:1247–1252

Vallee BL, Ulmer DD (1972) Biochemical effects of mercury, cadmium and lead. Ann Rev Biochem 41:91–128

Williams RJP (1978) The multifarious couplings of energy transduction. Biochim Biophy. Acta 505:1–44

Wu CC, Gardarin A, Catty P, Guillain F, Mintz E (2006a) CadA, the Cd^{2+}-ATPase from *Listeria monocytogenes*, can use Cd^{2+} as co-substrate. Biochimie 88:1687–1692

Wu CC, Gardarin A, Martel A, Mintz E, Guillain F, Catty P (2006b) The cadmium transport sites of CadA, the Cd^{2+}-ATPase from *Listeria monocytogenes*. J Biol Chem 281:29533–29541