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EFFECT OF Cd^{2+} ON ATP SYNTHESIS COUPLED TO ELECTRON TRANSFER IN CADMIUM-RESISTANT AND -SENSITIVE *STAPHYLOCOCCUS AUREUS* * *

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In the Cd^{2+}-resistant *Staphylococcus aureus* 17810R which contains the plasmid-coded Cd^{2+} efflux system, accumulation of Cd^{2+} was highly reduced. Consequently, neither respiration nor ATP synthesis coupled to electron transfer were inhibited. The plasmidless *S. aureus* strain 17810S accumulated Cd^{2+} *via* the Mn^{2+} porter down the membrane potential ($\Delta\psi$) which resulted in inhibition of respiration and of ATP synthesis.

According to the chemiosmotic model of Mitchell [1], maintenance of biosynthetic direction of the reversible, membrane-bound ATP synthetase of prokaryotes and eukaryotes requires generation by the respiratory chain of an electrochemical proton gradient ($\Delta\tilde{\mu}_H{}^+$) of about 210 mV. Decrease of the magnitude of $\Delta\tilde{\mu}_H{}^+$ results in reversal of the ATP synthetase action to the hydrolytic direction. Either synthesis or hydrolysis of ATP is coupled to the obligatory proton flow *via* the proton channel F_o of the $F_o{\cdot}F_1$ complex. ATP synthesis is blocked by inhibitors of either electron transfer or of proton flow through F_o and is uncoupled by protonophores or, in mammalian mitochondria, also by Cd^{2+} [2, 3].

In this work we present the effect of 10 μM Cd^{2+} on ATP synthesis coupled to electron transfer in the cadmium-resistant and cadmium-sensitive strains of *Staphylococcus aureus*. So far, this problem has not been investigated in bacteria.

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MATERIALS AND METHODS

S. aureus 17810R carrying a Cd^{2+} resistance plasmid pII17810 and its plasmidless variant strain *S. aureus* 17810S, sensitive to Cd^{2+}, were grown aerobically as described previously [4]. Cells were deprived of endogenous energy reserves in 0.1 M Na^+/K^+-phosphate buffer, pH 7, containing 5 mM $MgCl_2$ at 37°C for 3 h on a shaker.

Assay of ^{109}Cd uptake was performed as described previously [4]. Oxygen uptake was measured manometrically according to [5]. Membrane potential ($\Delta\psi$) was estimated from distribution of 100 μM ^{86}Rb (1075 MBq/mmol) plus 10 μM valinomycin, while ΔpH from distribution of 100 μM [^{14}C]benzoate (407 MBq/mmol). ATP content was measured using firefly lantern extract (Sigma) according to [6].

RESULTS AND DISCUSSION

ATP synthesis in both *S. aureus* strains studied was equally inhibited by *N,N*-dicyclohexylcarbodiimide (DCCD), 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Table 1). This indicates that ATP synthesis in both organisms occurred *via* oxidative phosphorylation coupled to $\Delta\mu_{H+}$ of about 191-200 mV generated by substrate oxidation.

Table 1

Effect of inhibitors and ionophores on ATP synthesis in S. aureus strains

Starved cells suspended in 0.1 M Na^+/K^+-phosphate buffer, pH 7, containing 5 mM $MgCl_2$ were pretreated for 10 min with the inhibitors before addition of the respiratory substrates. Intracellular level of ATP was estimated according to [6], after 20 min preloading with 10 mM glutamate or pyruvate. The background values of ATP remaining in starved cells were subtracted. Results are mean of at least 3 determinations.

Strains	Additions	Net ATP synthesis (mM)			
		glutamate	inhibition (%)	pyruvate	inhibition (%)
S. aureus 17810R	None	10.52	—	11.02	—
	100 μM DCCD	1.24	88	0.71	94
	100 μM HQNO	1.17	89	1.18	89
	50 μM CCCP	0.0	100	0.0	100
	10 μM Cd^{2+}	10.76	0	10.99	0
S. aureus 17810S	None	10.96	—	11.28	—
	100 μM DCCD	1.33	88	0.99	91
	100 μM HQNO	0.45	96	0.39	96
	50 μM CCCP	0.0	100	0.0	100
	10 μM Cd^{2+}	0.0	100	0.0	100

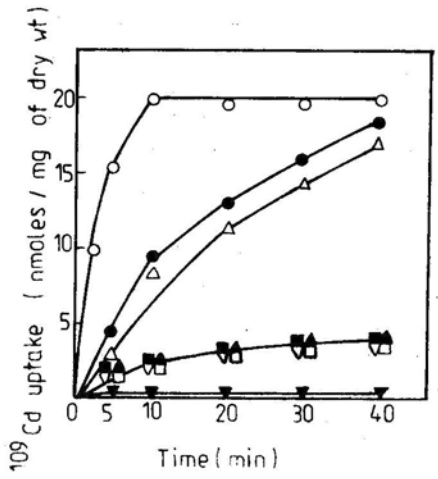

Fig. 1

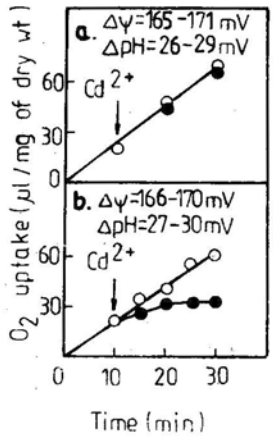

Fig. 2

Fig. 1. Uptake of ^{109}Cd by *S. aureus* 17810R and 17810S. Starved cells suspended in 0.1 M Na^+/K^+-phosphate buffer, pH 7, containing 5 mM $MgCl_2$ were preloaded with 10 mM glutamate at 37°C for 20 min before addition of 10 μM ^{109}Cd. Strain 17810S: ○, control cells; cells pretreated for 10 min with: ●, 100 μM DCCD; Δ, 100 μM HQNO; ▲, 100 μM DCCD + 100 μM HQNO; □, 100 μM $MnCl_2$; ■, 40 μM valinomycin + 50 mM KCl; ▽, 50 μM CCCP; ▼, control of strain 17810R. Results are mean of 3 experiments

Fig. 2. Effect of Cd^{2+} on glutamate oxidation in *S. aureus* 17810R (a) and 17810S (b): ○, control cells; ●, with 10 μM Cd^{2+}. The values are the average of 3 determinations. Estimation of $\Delta\psi$ and ΔpH were as described in Methods

Starved cells of strain 17810S preloaded with glutamate, accumulated 20 nmol Cd^{2+}/mg of dry weight *via* the Mn^{2+} porter down $\Delta\psi$, since Mn^{2+}, valinomycin + K^+ or CCCP markedly reduced this uptake (Fig. 1). Neither HQNO nor DCCD inhibited Cd^{2+} uptake to the level seen with CCCP. Complete inhibition was achieved by combined action of HQNO + DCCD. This means that two proton pumps, the redox chain or ATPase, energized Cd^{2+} transport. However, one proton pump could not drive the rapid initial Cd^{2+} influx (Fig. 1).

We suggest that in control cells of strain 17810S, Cd^{2+} accumulation down $\Delta\psi$ generated by the redox chain, resulting in inhibition of glutamate oxidation (Fig. 2b), could result in a decrease of $\Delta\tilde{\mu}_{H+}$ followed by reversal of ATP synthetase action to the hydrolytic direction and inhibition of ATP synthesis (Table 1). H^+ extrusion *via* F_o could generate $\Delta\psi$, thereby accelerating the initial Cd^{2+} influx (Fig. 1). It seems that two proton pumps cooperate in energizing the rapid Cd^{2+} accumulation, instead of producing ATP. Similar results were obtained when pyruvate was the energy donor (Table 1). We suggest that, by analogy to mammalian mitochondria [2], the toxic effect of

Cd^{2+} may be due to blocking the vicinal dithiols present in the cytoplasmic system of oxidative decarboxylation of pyruvate or glutamate (as α-ketoglutarate). Thus, 10 μM Cd^{2+} may inhibit ATP synthesis in strain 17810S at the level of $\Delta\tilde{\mu}_{H+}$ generation. Yagi & Hatefi [3] claim that Cd^{2+}-mediated uncoupling may be unique to mitochondria due to the presence of vicinal dithiol in subunit F_B of the F_0 fragment, which is absent in bacteria.

In strain 17810R, due to the activity of the Cd^{2+} efflux system operating as a $2H^+/Cd^{2+}$ antiporter [7], accumulation of Cd^{2+} was highly reduced (Fig. 1) and the internal dithiols were not blocked, as neither glutamate oxidation (Fig. 2a) nor ATP synthesis (Table 1) were inhibited by Cd^{2+}. Similar results were obtained with pyruvate (Table 1). Our data suggest that the Cd^{2+} efflux system protects efficiently oxidative phosphorylation against Cd^{2+} toxicity.

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