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ENERGY DONOR-DEPENDENT EFFECT OF Cd^{2+} ON [^{14}C] GLUTAMATE TRANSPORT IN *STAPHYLOCOCCUS AUREUS* *

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In starved cells of Cd^{2+}-sensitive *Staphylococcus aureus* 17810S preloaded with either glutamate or pyruvate, [^{14}C]glutamate transport was blocked by 10 μM Cd^{2+}, whereas in cells preloaded with lactate, [^{14}C]glutamate transport was not affected. This differential effect of Cd^{2+} could be due to the presence or absence of dithiols in the substrate oxidizing systems. In starved cells of Cd^{2+}-resistant strain 17810R preloaded with either of the three substrates, [^{14}C]glutamate transport was insensitive to 10 μM Cd^{2+}.

As shown previously [1], glutamate transport in Cd^{2+}-resistant *Staphylococcus aureus* 17810R and its Cd^{2+}-sensitive variant strain 17810S occurred *via* an electroneutral H^{+}-co-transport down the pH gradient (ΔpH) generated by oxidation of endogenous amino acids, which is in accordance with the chemiosmotic model [2, 3].

In strain 17810S, 10 μM Cd^{2+} was a strong inhibitor of glutamate transport [1] due to its accumulation *via* the Mn^{2+} uniporter down the membrane potential [4, 5] and inhibition of respiration [5]. In strain 17810R, the transport of this amino acid was not affected [1] because of highly reduced Cd^{2+} accumulation in the cytoplasm [4] and lack of inhibition of respiration [5]. According to Tynecka *et al.* [5], this inability to accumulate Cd^{2+} results from a steady-state between Cd^{2+} entry *via* the Mn^{2+} uniporter and Cd^{2+} exit *via* the plasmid-coded $2H^{+}/Cd^{2+}$ antiporter.

Data presented in this paper indicate that the toxic effect of Cd^{2+} on [^{14}C]glutamate transport in starved cells of strain 17810S was dependent on the energy donor used.

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MATERIALS AND METHODS

Staphylococcus aureus 17810R, carrying a cadmium resistance plasmid pII17810 and its plasmidless variant strain 17810S grown aerobically [4] were suspended in 0.1 M Na^+/K^+-phosphate buffer, pH 7, containing 5 mM $MgCl_2$. Cells were deprived of endogenous amino acids in the same buffer for 3 h at 37°C on a shaker. Oxygen uptake was studied manometrically according to [6]. Assay of ^{109}Cd (carrier-free) or [^{14}C]glutamate (7400 MBq/mmol) uptake was performed as described previously [1, 4]. ΔpH was measured from distribution of 100 μM [^{14}C]benzoate (407 MBq/mmol).

RESULTS AND DISCUSSION

Accumulation of [^{14}C]glutamate by starved cells of *S. aureus* 17810S and 17810R preloaded with glutamate or lactate occurred down ΔpH of about 27-30 mV, since nigericin or carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) strongly inhibited this uptake (Fig. 1). Similar results were obtained with pyruvate as the energy donor (not shown).

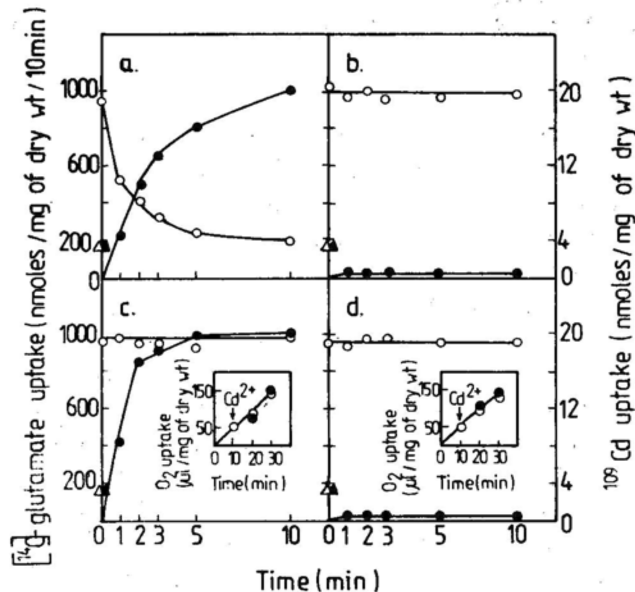

Fig. 1. ^{109}Cd and [^{14}C]glutamate uptake in *S. aureus* 17810S (a,c) and 17810R (b,d). Starved cells suspended in 0.1 M Na^+/K^+-phosphate buffer, pH 7, containing 5 mM $MgCl_2$ were preloaded with 10 mM glutamate (a,b) or lactate (c,d) for 20 min at 37°C. ○, [^{14}C]glutamate (10 mM) uptake (in 10 min) in cells preincubated with 10 μM Cd^{2+} for the time indicated; △, control + 0.5 μg/ml nigericin; ▲, control + 50 μM CCCP. ●, ^{109}Cd (10 μM) uptake. Insert: the effect of Cd^{2+} on lactate oxidation in *S. aureus* 17810S (c) and 17810R (d). ○, control; ●, with 10 μM Cd^{2+}. The results are representative of 3 experiments.

In strain 17810S preloaded with glutamate, progressive accumulation of Cd^{2+} (Fig. 1a) followed by progressive inhibition of glutamate oxidation [7] resulted in progressive inhibition of [^{14}C]glutamate transport to the level seen with nigericin or CCCP (Fig. 1a). Similar results were obtained with pyruvate (not shown). We suggest that the mechanism of Cd^{2+} toxicity for [^{14}C]glutamate transport in strain 17810S may be similar to that suggested for ATP synthesis coupled to electron transfer [7]. Cd^{2+} seems to stop generation of ΔpH required for [^{14}C]glutamate transport.

In strain 17810R preloaded with glutamate, Cd^{2+} accumulation was highly reduced (Fig. 1b) and, therefore, neither glutamate oxidation [7] nor [^{14}C]glutamate transport (Fig. 1b) were affected. Similar results were obtained with pyruvate (not shown). Removal of Cd^{2+} from cytoplasm by the $2H^+/Cd^{2+}$ antiporter [5] allowed the resistant cells to generate ΔpH, the driving force for [^{14}C]glutamate transport. Cd^{2+} extrusion is thus an efficient resistance system protecting the vital dithiols against Cd^{2+}.

Oxidation of lactate by strain 17810S was not blocked by 10 μM Cd^{2+} (Fig. 1c, insert) and, consequently, [^{14}C]glutamate transport was not inhibited despite Cd^{2+} accumulation (Fig. 1c). This means that ΔpH for [^{14}C]glutamate transport was generated. As reported by Short & Kaback [8], in membrane vesicles of *S. aureus* which are devoid of the cytoplasm, the transport of several amino acids is energized by L-lactate oxidation *via* the membrane-bound L-lactate dehydrogenase. This means that in lactate oxidation in *S. aureus* no additional cytoplasmic systems are involved. Therefore, the internal Cd^{2+} may have no cytoplasmic targets in strain 17810S.

In strain 17810R preloaded with lactate, neither lactate oxidation (Fig. 1d, insert) nor [^{14}C]glutamate transport (Fig. 1d) were affected by 10 μM Cd^{2+}. It appears that the parent strain 17810R may also lack the cytoplasmic targets for Cd^{2+} in the lactate oxidation system. However, with lactate, Cd^{2+} accumulation was also highly reduced (Fig. 1d). This suggests that the $2H^+/Cd^{2+}$ antiporter does not function as a Cd^{2+} resistance system during lactate oxidation, but merely may regulate the cytoplasmic level of Cd^{2+}, which behaves as a nontoxic cation. The presence of proton/cation antiporters regulating the cytoplasmic level of physiological cations in bacteria has been described [9].

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