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Kierownik: doc. dr farm. Zofia Tynecka

Zofia TYNECKA, Anna MALM

The Effect of DCCD, HQNO or Cd^{2+} on Glutamate Oxidation in *Staphylococcus aureus*

Wpływ DCCD, HQNO i Cd^{2+} na utlenianie glutaminianu u *Staphylococcus aureus*

Влияние DCCD, HQNO и Cd^{2+} на окисляние глутамата *Staphylococcus aureus*

Facultative anaerobes, like *Staphylococcus aureus* or *Escherichia coli*, synthesize adenosinetriphosphate (ATP) via oxidative phosphorylation when oxygen is available (3, 5). Energy for this process is derived from a substrate oxidation via electron transport chain (3, 5). According to the chemiosmotic model by Mitchell (7, 9), protons extruded via the respiratory proton pump, create a protonmotive force (pmf) consisting of membrane potential and *pH* gradient. This is the driving force for return of protons via the proton channel F_0 of the adenosinetriphosphatase (ATP-ase) complex F_0F_1 located in the membrane (3, 7, 8, 10). This enzyme catalyzes adenosinediphosphate (ADP) phosphorylation when protons translocated through F_0 achieve the active center at F_1. N,N'-dicyclohexylcarbodiimide (DCCD) or oligomycin block proton flow through the proton channel in mitochondria and stop ATP synthesis via oxidative phosphorylation (4, 7). In bacteria, only DCCD blocks specifically this process (10). Neither oligomycin nor DCCD have any inhibitory effect on the activity of the respiratory system in uncoupled mitochondria (4, 7). However, in tightly coupled mitochondria, DCCD or oligomycin suppress respiration which supports ATP synthesis (4, 7). According to Mitchell (7), this is due to a back-pressure exerted by protons on redox-chain when they cannot reenter the cell via F_0F_1 blocked by DCCD. This phenomenon is called a respiratory control (4, 6, 7). Little is known about the respiratory control in bacteria (6). These free-living organisms are highly adaptive to changes in environment and can ad-

just their metabolism to a new surrounding. When oxidative phosphorylation is blocked, the facultative anaerobe can synthesize ATP via substrate-level phosphorylation (3, 5).

The aim of this paper was to look into the effect of DCCD and also of HQNO or Cd^{2+} on glutamate oxidation in two *S. aureus* strains. HQNO blocks specifically electron flow between cytochrome "b_1" and cytochrome oxidase "o" in this organism (14). Cd^{2+} is an inhibitor of respiratory systems (2, 16, 17), but not of the ATP-ase (2, 16). However, by blocking respiration, Cd^{2+} stops also indirectly ATP synthesis via oxidative phosphorylation. The effect of the three inhibitors on glutamate oxidation was studied in an attempt to get some information about the respiratory control in two *S. aureus* strains. One of the organisms studied is resistant to Cd^{2+} due to a possession of a resistance plasmid (16); the other strain is a plasmidless, Cd^{2+}-sensitive derivative of the parent, resistant organism (15, 16).

MATERIALS AND METHODS

Strains. *S. aureus* strain 17810R was a 1964 hospital isolate and was provided by Keith Dyke (Oxford, United Kingdom) along with its cured variant strain 17810S. The Cd^{2+} resistance plasmid from strain 17810R is now called pII17810 and falls into the beta incompatibility group, for which the prototype is plasmid pII147 (15).

Growth conditions. Early exponential-phase cells of both strains were obtained as follows: a small inoculum of cells from an agar slant was cultured in 3% nutrient broth overnight at 37°C. A 5 ml amount of this culture was transferred into 50 ml of broth and shaken for about 3 hrs until the turbidity (Specol photocolorimeter, 550 nm) corresponded to 1 mg of dry weight per 1 ml. The culture was rapidly cooled and kept overnight at 4°. Then it was warmed to 37° and mixed with 50 ml of fresh, prewarmed broth. Incubation was continued until the culture reached the same turbidity.

Manometric experiments. The effects of DCCD, HQNO or $CdCl_2$ on L-glutamate oxidation in both strains were studied in Warburg respirometer. Cells were collected by centrifugation, washed twice in 0.1 M phosphate buffer, *pH* 7 and suspended in the same buffer to give approximately 0.5 mg of dry weight per 1 ml. Cell suspensions were pretreated for 10 min. with 100 μM DCCD, 100 μM HQNO, or various concentrations of $CdCl_2$, at 37°. The main Warburg vessel contained 2.5 ml of bacterial suspensions, while the side-arm bulb — 0.5 ml of 0.06 M L-glutamate. 0.2 ml of 20% KOH was added to the center well. The suspensions were equilibrated for 10 min. at 37°C. L-glutamate was added after 10 min. incubation and the readings were taken every 10 min. during 1 hr.

Chemicals. All chemicals of analytical grade were the products of POCH, Gliwice, Poland. N, N'-dicyclohexylcarbodiimide (DCCD) was purchased from Koch-Light Ltd., England, whereas 2-heptyl-4-hydroxyquinoline N-oxide (HQNO) was obtained from Sigma, USA.

RESULTS

As shown in Figs. 1 and 2, both the Cd^{2+}-resistans and -sensitive *S. aureus* oxidize glutamate at a similar rate (about 100 µl O_2 per 1 mg dry weight/hr. HQNO inhibits markedly oxidation of this amino acid in both strains by about 70%. This suggests that glutamate oxidation may proceed in the strains via an identical, HQNO-sensitive electron transport chain.

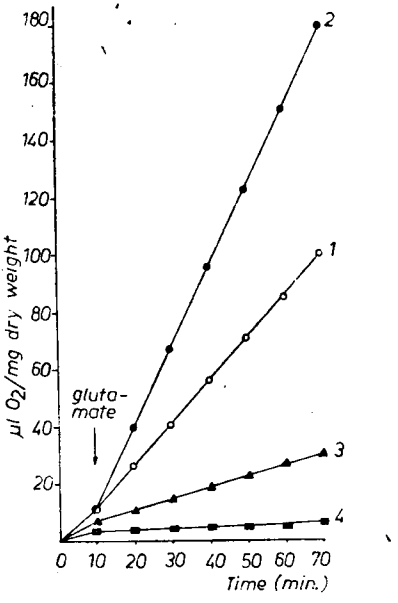

Fig. 1. The effect of DCCD or HQNO on glutamate oxidation by *S. aureus* 17810R; 1 — control, 2 — 100 µM DCCD, 3 — 100 µM HQNO, 4 — 100 µM DCCD + 100 µM HQNO

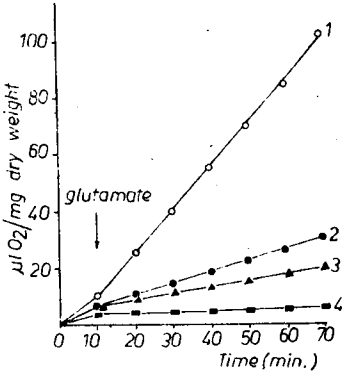

Fig. 2. The effect of DCCD or HQNO on glutamate oxidation by *S. aureus* 17810S; 1 — control, 2 — 100 µM DCCD, 3 — 100 µM HQNO, 4 — 100 µM DCCD + 100 µM HQNO

According to Figs. 1 and 2, the effect of DCCD on glutamate oxidation differ greatly, depending on the strain. In the Cd^{2+}-sensitive organism (Fig. 2), DCCD reduces markedly (by about 70%) oxygen uptake. In sharp contrast to this, glutamate oxidation in the Cd^{2+}-resistant strain is highly stimulated by DCCD (Fig. 1). Increased oxidation of this amino acid by the DCCD-treated resistant cells is abolished by HQNO. This suggests that the DCCD-stimulated oxidation of glutamate in *S. aureus* 17810R proceeds via the same electron transport chain, as in the control cells.

Stimulation of glutamate oxidation by DCCD in Cd^{2+}-resistant orga-

nism indicates that this ATP-ase inhibitor does not suppress the respiratory system oxidizing glutamate. This suggests that also in the sensitive strain, inhibition of glutamate oxidation by DCCD is not due to a specific lesion in the electron transport chain. By analogy to the tightly-coupled mitochondria (4, 7), this inhibitory effect of DCCD may result from a secondary effect caused by inhibition of the ATP-ase. We suggest that oxidation of glutamate in the Cd^{2+}-sensitive strain may be tightly coupled to ATP synthesis via oxidative phosphorylation, and inhibition of ATP-ase suppresses also respiration. In contrast, stimulation of glutamate oxidation by DCCD in the resistant strain suggests, that in this organism, glutamate oxidation may be rather loosely coupled to ATP synthesis. In this case, inhibition of ATP-ase by DCCD does not stop respiration, but even stimulate it (Fig. 2).

Figures 3 and 4 illustrate the effect of various concentrations of $CdCl_2$ on glutamate oxidation in both strains. The difference between strains is seen in the presence of 10 μM Cd^{2+}. Under these conditions, oxygen uptake is blocked in the sensitive organism, but not in the resistant one. This may be due to the presence of the Cd^{2+}-resistance system, which extrudes Cd^{2+} as soon as it is taken up via the Mn^{2+} carrier (16). In this way, no net accumulation of Cd^{2+} takes place (15) and no inhibition of glutamate oxidation is seen (Fig. 3). The sensitive organism which lacks the Cd^{2+}-resistance mechanism, accumulates Cd^{2+} via the Mn^{2+} carrier (11, 15, 16) and this results in inhibition of glutamate oxidation (Fig. 4). Higher Cd^{2+} concentration (100 μM Cd^{2+}) blocked glutamate oxidation in both strains (Figs. 3 and 4).

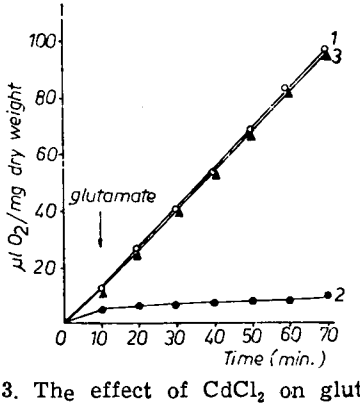

Fig. 3. The effect of $CdCl_2$ on glutamate oxidation by *S. aureus* 17810R. 1 — control, 2 — 100 μM $CdCl_2$, 3 — 10 μM $CdCl_2$

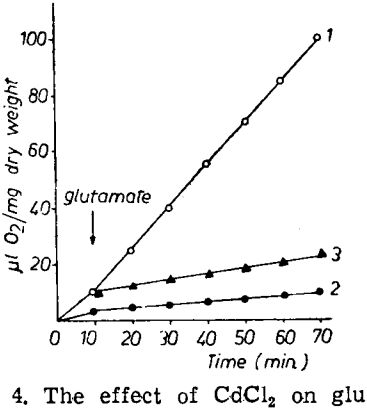

Fig. 4. The effect of $CdCl_2$ on glutamate oxidation by *S. aureus* 17810S; 1 — control, 2 — 100 μM $CdCl_2$, 3 — 10 μM $CdCl_2$

DISCUSSION

Glutamate appears to be an important source for growth of *S. aureus* in nutrient broth (12, 13). The equal rate of glutamate oxidation in both strains allows us to suggest that oxidation of this amino acid may be coupled to ATP synthesis via oxidative phosphorylation. However, the differential effect of DCCD on glutamate oxidation suggests, that this coupling differ greatly depending on the strain. In the sensitive organism, coupling seems to be tight since inhibition of ATP-ase by DCCD causes also cessation of glutamate oxidation. This would be a pure example of respiratory control in bacteria, a phenomenon unique in these free-living organisms.

In tightly coupled mitochondria, respiratory control may be released by 2,4-dinitrophenol (DNP) — an uncoupler which translocates protons inwards and uncouples oxidation from phosphorylation (4, 7). In this case, oxidative phosphorylation is blocked due to dissipation of the pmf, while respiration proceeds at an increased rate.

An example of bacterium lacking the respiratory control, is the parent organism, resistant to Cd^{2+}. Inhibition of ATP-ase in this strain, reveals the loose coupling between oxidation and phosphorylation, which is seen as a high stimulation of glutamate oxidation by DCCD. We suggest that protons extruded by the DCCD-treated cells during glutamate oxidation do not seem to exert a back pressure on the glutamate oxidizing system. Our hypothesis is that these protons can return to the cytoplasm via the antiporter and release the respiratory control. Besides, the antiporter seems to protect the resistant cells against Cd^{2+} poisoning by preventing the net accumulation of Cd^{2+} via the Mn^{2+} carrier.

Removal of the plasmid *cad* marker from the resistant organism resulted in a variant strain, sensitive to Cd^{2+} (15, 16). In consequence, cadmium accumulated by these cells via the Mn^{2+} carrier is not extruded due to lack of the antiporter (16). This results in a severe poisoning of the cells (16). Another consequence for the sensitive cells is, that they have no pathway for proton reentry and release of respiratory control with glutamate as a substrate.

We conclude, that the loosely coupled parent organism was rendered tightly coupled simply by genetic loss of genes coding antiport synthesis. Under normal conditions, the antiporter may be a regulatory mechanism controlling the cytoplasmic level of Mn^{2+}. There were reports describing antiporters for several cations (1, 18). Only by chance, due to the chemical similarity between Cd^{2+} and Mn^{2+}, the antiporter may play a role of the cadmium resistance system (15, 16).

REFERENCES

1. Brey R. N. et al.: Cation/Proton Antiport Systems in *Escherichia coli*. Biochem. a. Biophys. Res. Commun. **83**, 1588, 1978.
2. Brierley G. P.: The Uptake and Extrusion of Monovalent Cations by Isolated Heart Mitochondria. Molecular a. Cellular Biochem. **10**, 41, 1976.
3. Haddock B. A., Jones C. W.: Bacterial Respiration. Bacteriol. Rev. **41**, 47, 1977.
4. Hanstein W. G.: Uncoupling of Oxidative Phosphorylation. Biochim. et Biophys. Acta **456**, 129, 1976.
5. Harold F. M.: Ion Currents and Physiological Functions in Microorganisms. Ann. Rev. Microbiol. **31**, 181, 1977.
6. John F., Hamilton W. A.: Release of Respiratory Control in Particles from *Micrococcus denitrificans* by Ion-translocating Antibiotics. Eur. J. Biochem. **23**, 528, 1971.
7. Mitchell P.: Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation. Biol. Rev. **41**, 445, 1966.
8. Mitchell P.: A Chemiosmotic Molecular Mechanism for Proton Translocating Adenosine Triphosphatases, FEBS Letters **43**, 189, 1974.
9. Mitchell P.: Compartmentation and Communication in Living Systems. Eur. J. Biochem. **95**, 1, 1979.
10. Okamoto H. et al.: Purified Proton Conductor in Proton Translocating Adenosine Triphosphatase of a Thermophilic Bacterium. J. Biol. Chem. **252**, 6125, 1977.
11. Perry R. D., Silver S.: Cadmium and Manganese Transport in *Stahpylococcus aureus* Membrane Vesicles. J. Bacteriol. **150**, 973, 1982.
12. Ramsey H. H.: Endogenous Respiration of *Staphylococcus aureus*. J. Bacteriol. **83**, 507, 1962.
13. Strasters K. C., Winkler K. C.: Carbohydrate Metabolism in *Staphylococcus aureus*. J. Gen. Microbiol. **33**, 213, 1963.
14. Taber H. W., Morrison M.: Electron Transport in Staphylococci. Arch. Biochem. Biophys. **105**, 367, 1964.
15. Tynecka Z. et al.: Reduced Cadmium Transport Determined by a Resistance Plasmid in *Staphylococcus aureus*. J. Bacteriol. **147**, 305, 1981.
16. Tynecka Z. et al.: Energy-dependent Efflux of Cadmium Coded by a Plasmid Resistance Determinant in *Staphylococcus aureus*. J. Bacteriol. **147**, 313, 1981.
17. Vallee B. L., Ulmer D. D.: Biochemical Effects of Mercury, Cadmium, and Lead. Ann. Rev. Biochem. **41**, 91, 1972.
18. West J. C., Mitchell P.: Proton/Sodium Ion Antiport in *Escherichia coli*. Biochem. J. **144**, 87, 1974.

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STRESZCZENIE

Zbadano wpływ DCCD — inhibitora ATP-azy — oraz inhibitorów oddechowych — HQNO i Cd^{2+} — na utlenianie glutaminianu u dwóch szczepów *S. aureus*. Szczep 17810R jest oporny na kadm, gdyż posiada energiozależny proton-kadm, an-

typort kodowany przez geny plazmidowe. *S. aureus* 17810S jest wrażliwym na Cd^{2+} bezplazmidowym wariantem, nie posiadającym antyportu. Obydwa szczepy utleniały glutaminian w jednakowym stopniu poprzez wrażliwy na HQNO system transportujący elektrony. Stymulacja utleniania glutaminianu przez DCCD u szczepu plazmidowego oraz hamowanie u bezplazmidowego warianta sugerują, że utlenianie tego aminokwasu może być sprzężone z oksydacyjną fosforylacją, ale w różny sposób. U szczepu bezplazmidowego sprzężenie to jest ścisłe, natomiast u opornego — luźne. Konsekwencje posiadania lub braku antyportu są dwojakie. U szczepu plazmidowego kadm jest natychmiast usuwany z komórki, co uwidacznia się brakiem zahamowania utleniania glutaminianu w obecności 10 μM Cd^{2+}. Ponadto w warunkach zahamowania ATP-azy przez DCCD protony wnikają poprzez antyport, powodując zwolnienie kontroli oddechowej. U bezplazmidowego warianta brak antyportu powoduje zablokowanie utleniania glutaminianu w obecności 10 μM Cd^{2+}. Ponadto utlenianie glutaminianu podlega kontroli oddechowej w obecności DCCD; wskutek braku szlaku dla powrotu protonów.

РЕЗЮМЕ

Исследовалось влияние DCCD — ингибитора АТР-азы, ингибиторов дыхания — HQNA и Cd^{2+} на окисление глутамата двумя штаммами *S. aureus*. У штамма 17810R, который устойчивый к ионам кадмия, выступает энергио-зависимый антипорт протон-кадмий, обусловленный плазмидовыми генами. *S. aureus* 17810S — это бесплазмидовый вариант, чувствительный к Cd^{2+}, не имеющий антипорта. Оба штамма окисляли глутамат в одинаковой степени системой дыхательной цепи, которая чувствительная к HQNO. Стимулирование окисления глутамата DCCD, выступающее у плазмидового штамма, а также заторможение у бесплазмидового варианта, указывает, что окисление этой аминокислоты и окислительное фосфорилирование спряжены между собой по-разному. У бесплазмидового варианта это сопряжение точное, у устойчивого штамма — свободное. Последствия выступления антипорта или его недостаток разные. Плазмидовый штамм удаляет кадмий из клетки немедленно, у него не выступает заторможение окисления глутамата в присутствии 10 μ M Cd^{2+}. У бесплазмидового варианта недостаток антипорта, потому у него заторможение окисления глутамата в присутствии 10 μM Cd^{2+}. Кроме того, окисление глутамата зависимое от дыхательного контроля в присутствии DCCD вследствие того протоны не могут вернуться.