SHORT COMMUNICATIONS

BBA 43189

Oligomycin-like inhibition of yeast respiration by *N,N'*-dicyclohexylcarbodiimide and the nature of energy coupling in intact yeast cells

Although a strict balance between energy-producing and energy-consuming reactions seems to exist in growing microbial cells, the high rate of their catabolic processes under resting, non-growing conditions appears to be puzzling[1]. Either a loose coupling between the respiratory chain and the ATP-forming reactions of the oxidative phosphorylation system or the hydrolysis of the ATP formed can be envisaged. On the basis of the inhibition by oligomycin, which inhibits tightly coupled respiration only[2,3], different animal cells have been thought to be loosely coupled under resting conditions (for literature, see ref. 4). Since, however, the bacterial oxidative phosphorylation system appears to be insensitive to oligomycin[5] and yeast cells seem to be permeable to oligomycin under particular conditions only[6], oligomycin cannot be used in a similar way as a tool in the study of resting energy metabolism of microbial cells. For this purpose it was tempting to use *N,N'*-dicyclohexylcarbodiimide (DCCD) which was found to inhibit energy-transfer processes in animal mitochondria[7] similarly to the action of oligomycin[2,3], and to interfere in energy metabolism in chloroplasts[8].

500 μM DCCD totally prevented aerobic growth of yeast *Saccharomyces cerevisiae* Yeast Foam in liquid synthetic medium (Yeast Nitrogen Base, Difco) with 2 % glucose or lactate as carbon and energy source. With lower concentrations of DCCD, growth resumed after a lag of 35 h and 10 h with 200 μM and 50 μM DCCD, re-

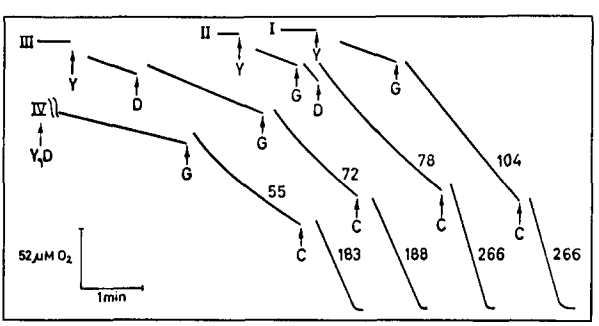

Fig. 1. Effect of DCCD on glucose oxidation by yeast cells in a polarographic experiment. The incubation mixture (1.5 ml) contained 50 mM potassium phosphate buffer (pH 4.3) and 10 mM KCl. At points indicated by arrows, aerobically grown yeast *S. cerevisiae* Yeast Foam (Y; 2.1 mg dry wt.), glucose (G; 10 mM), DCCD (D; 400 μM in 1% methanol) and CCCP (C; 10 μM) were added. The numbers at curves indicate oxygen consumption in nmoles/min. In Expt. IV, the cells were preincubated for 5 min with DCCD prior to glucose addition.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

spectively. On solid media, no induction of "petites colonies" mutants by DCCD was noted.

Respiration on glucose of aerobically grown yeast, which was stimulated by uncouplers, was inhibited by DCCD and the inhibition was released by uncouplers as shown by the polarographic recordings presented in Fig. 1. This indicates that DCCD acted on the yeast cells in a manner similar to oligomycin on animal cells. When DCCD was added after glucose to the yeast suspension and then, after 2–3 min, 10 μM carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was added, a complete release of inhibition was observed even in the presence of 5 mM DCCD. When, however, the cells were preincubated with DCCD for 2–5 min and glucose was then added, after a while a more pronounced inhibition occurred which was only partly relieved by the uncoupler.

The extent of inhibition of resting glucose respiration by different concentrations of DCCD as measured polarographically is shown in Fig. 2. The inhibition was exerted by DCCD in concentrations of 20–5000 μM. It can be seen that about 30% of the resting respiration can be inhibited by DCCD, which is similar to the inhibition level of resting respiration of animal cells treated with oligomycin[4].

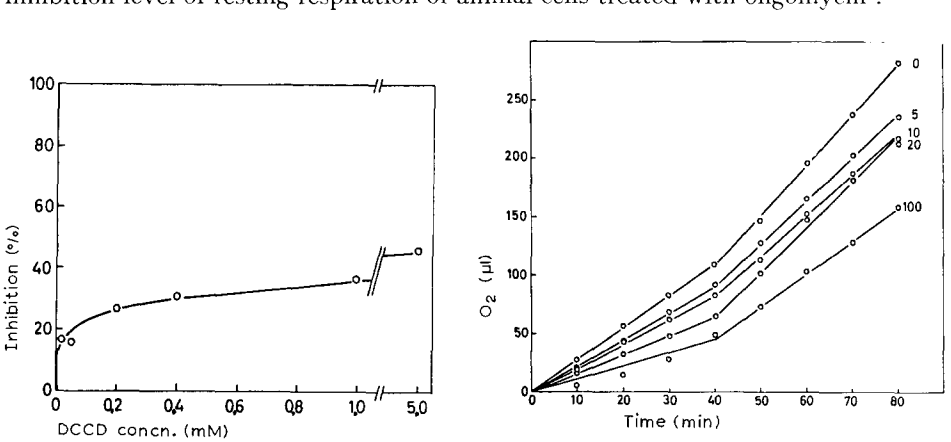

Fig. 2. Dependence of the inhibition of glucose oxidation by yeast cells on DCCD concentration. Conditions as in Fig. 1; DCCD (in 1% methanol) was added 30 sec after glucose and the respiratory rate was determined 90 sec after the addition of DCCD.

Fig. 3. Effect of DCCD on glucose oxidation by yeast cells in a manometric experiment. The main compartment of the Warburg flasks contained in 1.0 ml: 40 mM potassium phosphate (pH 4.3), 8 mM KCl, 20 mM glucose, 1% methanol, DCCD in concentrations indicated in the figure and 1.1 mg (dry wt.) of aerobically grown yeast. The central well contained 0.2 ml 2 M NaOH. 30°; gas phase: air. The measurements commenced after 10 min of thermal equilibration At 40 min, CCCP was tipped from the side arms to a final concentration of 10 μM. Numbers at curves indicate μM concentrations of DCCD.

The additional inhibition of respiration by DCCD which could not be relieved by uncouplers was more apparent when glucose oxidation was measured manometrically (Fig. 3). If allowance is made for this additional inhibition, it can be calculated from Fig. 3 that in this case too about 30% of the respiratory activity was inhibited by DCCD by its oligomycin-like action.

This additional inhibitory effect of DCCD was also apparent in an inhibition of yeast fermentation. Under the conditions of manometric experiments the anaerobic

glucose fermentation was inhibited 8 and 15 % by 20 and 100 μM DCCD, respectively.

In order to test whether bacterial cells react with DCCD, the respiration on glucose by cells of *Escherichia coli*, grown aerobically in glucose medium, suspended in 0.1 M phosphate buffer (pH 7.2), was tested. The respiration was found to be more than doubled in the presence of 10 μM CCCP (see also ref. 9). DCCD in concentrations as high as 2 mM did not inhibit the glucose respiration as measured polarographically either when added after glucose addition or if the cells had been preincubated with DCCD for 5 min.

From these results it can be concluded that (1) under strictly controlled conditions, DCCD exerts oligomycin-like action on intact yeast cells; as expected from its high chemical reactivity[10], it can also apparently affect other metabolic processes in the yeast cells not related to energy metabolism; (2) from their sensitivity to inhibition by DCCD, resting yeast cells can be considered to be energetically loosely coupled, similarly to animal cells[4]; (3) DCCD, like oligomycin[5], does not seem to inhibit catabolic processes in bacteria, which may stress the differences in the energy-transfer mechanism of the oxidative phosphorylation apparatus in bacteria and in animal and yeast mitochondria.

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