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THE INHIBITORY EFFECT OF *N,N'*-DICYCLOHEXYLCARBODIIMIDE IN ACTIVE SUGAR UPTAKE BY *RHODOTORULA GLUTINIS*

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Cells of the yeast *Rhodotorula glutinis* on treatment with *N,N'*-dicyclohexylcarbodiimide (DCCD) at a concentration of about 0.5 mM fail to accumulate D-xylose, cause efflux of accumulated sugar and do not exhibit H^+/sugar symport. The results are interpreted as being due to depolarization of the membrane potential by DCCD.

The inhibitory role of DCCD in the synthesis and hydrolysis of ATP in biological systems is well known [1–5]. This has been explained as a consequence of its binding to membrane-bound ATPase [6–9]. Misra and Höfer [10] reported that DCCD suspends active sugar uptake by the yeast *Rhodotorula gracilis* and renders its plasma membrane impermeable to H^+. DCCD-inhibited amino acid transport in *Saccharomyces* has been reported [11]. This paper describes experiments on the uptake and efflux of sugar, and H^+/sugar symport in the yeast *R. glutinis* under normal and DCCD inhibitory conditions. The results are suggestive that the effects of DCCD on sugar uptake and efflux are due to depolarization of the membrane potential.

Cells of *R. glutinis* (ATCC 26 198 and CBS 6681) were grown and cultivated as described previously [10]. DCCD was dissolved in 90% (v/v) ethanol to give 100 mM as stock solution. Cells were treated for 60 min under aerobic conditions and compared with cells treated identically with equivalent amounts of ethanol, used as a solvent for DCCD. The measurement of pH changes and sugar uptake was followed as described earlier [12]. An aerobic yeast suspension (5.0 g wet wt./100 ml) in water was used and D-xylose served as substrate.

Energized sugar uptake by *Rhodotorula* has been reported to be an H^+/sugar symport [13]. The plasma membrane of this organism is capable of creating the necessary H^+ gradient [10]. The H^+ gradient together with the ensuing electrical potential constitutes the necessary source of energy, termed the proton-motive force, for the active transport of substrate (cf. Ref. 9). The effect of DCCD on sugar uptake by *R. glutinis* is illustrated in Fig. 1.

DCCD inhibits sugar transport. Tested at varying sugar concentrations, the amount of total sugar accumulated by cells never exceeds the diffusion equilibrium. At 100 mM, the amount of sugar accumulated in cells at steady state is about 5-times higher in control cells. Inhibition of leucine transport by DCCD in the yeast *Saccharomyces* has been reported [11]. The inhibition has been shown to be competitive, because at higher amino acid concentration there is no inhibition. No such effect was seen in the present experiments. It is

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

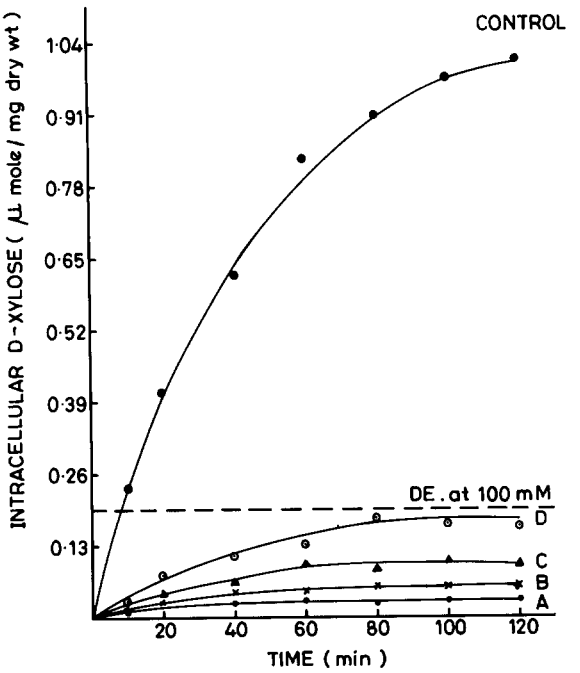

Fig. 1. D-Xylose uptake by *R. glutinis* cells. Yeast suspensions in water (about 8 mg dry wt./ml) were incubated with 0.5 mM DCCD for 1 h. Equivalent amounts of alcohol (as present in DCCD solution) were incorporated in normal cell suspensions and aerated for 60 min which served as control. The cell suspensions (about 4 mg dry wt./ml) were incubated with 75 mM phosphate buffer, pH 4.5, at 30°C with different concentrations of D-xylose (A, 12.5 mM; B, 25.0 mM; C, 50.0 mM; D, 100.0 mM). DE, diffusion equilibrium.

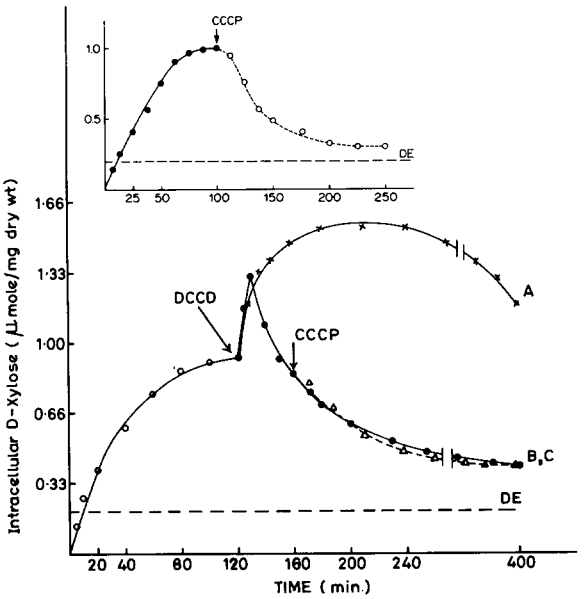

Fig. 2. Effect of DCCD on D-xylose transport by *R. glutinis* cells at steady state. DCCD (0.5 mM) was incorporated in systems B and C at the times indicated. An equivalent amount of alcohol, as present in DCCD solution, was incorporated simultaneously in normal cells (A) which served as control. Addition of $2 \cdot 10^{-5}$ M CCCP was made to system C after 40 min of DCCD action. Inset: control run with CCCP incorporation at steady state. DE, diffusion equilibrium.

worthwhile to note that in leucine-transport studies, the yeast cells were preincubated with DCCD for about 5 min. As with *Rhodotorula*, it is reported [10] that the effect of DCCD on the H^+ permeability of plasma membranes is time dependent and an appreciable effect is noted only after about 30 min of pretreatment. A somewhat similar effect is observed when DCCD is incorporated in a system where yeast cells are at steady state during D-xylose transport. The results are shown in Fig. 2.

Incorporation of DCCD led initially to a transient increase in uptake, but, gradually, efflux started and finally the level of sugar in the cells, though tending to reach diffusion equilibrium, was always higher. As tested with control cells, the transient increase in sugar uptake is due to the effect of ethanol, used as a solvent for DCCD. The delayed efflux of sugar is, probably, due to an alcohol effect together with the slower rate of DCCD penetration in *Rhodotorula* [10]. The pattern of DCCD action on the efflux of sugar is unaltered upon the addition of an uncoupler, CCCP, which causes rapid efflux of sugar (Fig. 2, inset). It is well known that such protonophoric uncouplers cause a rapid lowering of membrane potential [14]. It is, therefore, logical to infer that prior to CCCP action, the membrane of *Rhodotorula* is depolarized, otherwise the rate of sugar efflux should increase on its incorporation. The decrease in intracellular D-xylose in control cells is due either to an effort by cells to equilibrate with the system after the alcohol effect is abolished or an effect of D-xylose catabolism [15].

H^+/sugar symport, characteristic of energized sugar transport in *R. glutinis* [13], was studied with cells treated with DCCD and the results are presented in Fig. 3.

In contrast to control cells, DCCD-treated cells lose the property of H^+ translocation when sugar

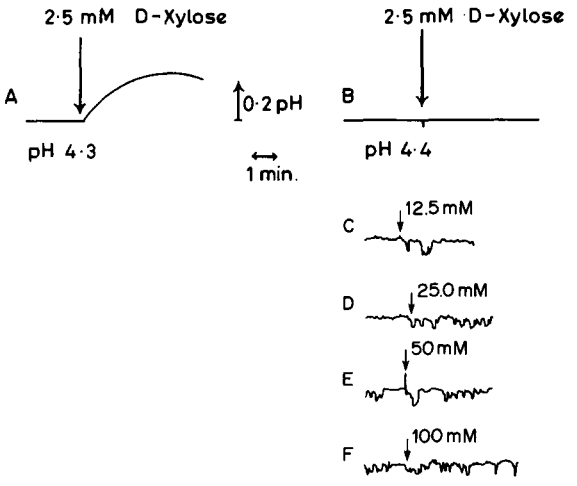

Fig. 3. H^+ uptake induced by sugar transport in *R. glutinis*. Unbuffered aqueous yeast suspensions (about 8 mg dry wt./ml) were incubated at 30°C for 60 min with ethanol (A) and 0.5 mM DCCD, separately. Incorporation of D-xylose registered an increase in pH by more than 0.2 pH units in A which served as control. No increase in pH was oberved with DCCD-treated cells at various sugar concentrations; B (2.5 mM), C (12.5 mM), D (25.0 mM), E (50.0 mM) and F (100.0 mM)

is incorporated in the system. This indicates that H^+/sugar symport is absent in these cells. It has been reported earlier [10] that DCCD, unlike CCCP and 2,4-dinitrophenol, does not disrupt the pH gradient across the plasma membrane of *Rhodotorula* and that its respiration rate remains almost unaffected. These observations, although not unequivocally demonstrative, are convincingly suggestive that DCCD, besides having other effects, depolarizes the membrane potential and thus inhibits sugar transport and its retention in *R. glutinis* cells. Since one of the properties of DCCD action is to inhibit H^+ translocation, the use of lipid-soluble cations in determining membrane potential under these conditions is, therefore, a matter of speculation. The inhibition of membrane potential generation by DCCD in *Rhodospirillum rubrum* has recently been reported [16].

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