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The inhibition of K^+ and phosphate uptake in yeast by cycloheximide

Cycloheximide (actidion) has been used extensively in inhibition studies of protein synthesis. It functions as a potent inhibitor in mammalian and yeast cells but not in bacteria. CLARKE-WALKER AND LINNANE[1] have shown that cycloheximide inhibits the growth of yeast cells. Though its exact site of action is not clear, the inhibition is said to affect the peptide bond formation step[2]. MAYO *et al.*[3] have reported that cycloheximide interferes with rRNA formation in *Saccharomyces carlsbergensis*. Other antibiotics of polyene nature inhibit glycolysis in the yeast cell and also affect membrane permeability, causing K^+ loss. Certain of the larger polyene compounds have also been shown to bring about the loss of P_i from the yeast cell[4].

In the present paper we report an effect by cycloheximide on K^+ and phosphate accumulation in yeast cells at concentrations which block protein synthesis according to the results of other workers[1,3].

Commercial baker's yeast (Hefefabriken AG Hindelbank) was starved under aeration for 15 h and washed 3 times with distilled water. The amount of cells used in each experiment was measured by a microcytocrit method. No correction was made for interstitial water (amounting to about 22 %) trapped between the packed cells[5].

When not otherwise stated, yeast cells were suspended in 33 mM potassium–disodium phosphate buffer (pH 6).

Phosphate uptake by yeast was measured by following changes in the phosphate content of the suspending medium. The phosphate was analysed by a modification of the Fiske–Subbarow method[6]. K^+ uptake was measured by following changes in the K^+ content of the suspending medium, using an EEL Flame Spectrophotometer.

CO_2 production and O_2 consumption were determined by the Warburg manometric technique.

Cycloheximide (actidion) was obtained from Fluka Chemical Co.

When starved yeast cells are suspended in phosphate buffer a small leakage of K^+ may take place, but the cells are able to reabsorb lost cations. The phosphate content of the cells does not change under these circumstances. In an unbuffered medium the losses are of the same order. As shown in Fig. 1, the addition of different amounts of cycloheximide to the suspension causes a permanent loss of K^+. This loss is increased by increasing the concentration of the cycloheximide. Addition of glucose to the buffered suspension after a 60-min pretreatment with cycloheximide produces the pattern shown in Fig. 2. Compared to the control, a marked reduction in the rate of K^+ uptake is observed, though total uptake in 60 min, even in the presence of 20 mM cycloheximide, reaches a level of more than 70 % of the control value.

Studies of CO_2 production and O_2 consumption during glucose metabolism show that cycloheximide inhibits to some extent respiration and fermentation rates. O_2 consumption is reduced to 78 % of the control value by cycloheximide concentrations ranging from 0.1 to 20.0 mM. However, under anaerobic conditions cycloheximide at 0.1 and 1.0 mM inhibited CO_2 production to 69 % and at 20.0 mM to 56 % of the controls.

It is clear from Figs. 2 and 3 that the effect of cycloheximide on phosphate differs from that on K^+. Phosphate uptake is inhibited 90 % by 20 mM cycloheximide

and approx. 10% by 0.1–0.01 mM inhibitor. These concentrations also extend the initial lag period observed in phosphate uptake. The lag period in the absence of cycloheximide seems to be related to the level of K^+ in the cell. When yeast was pretreated with K^+ and glucose this lag period disappeared, as has also been shown

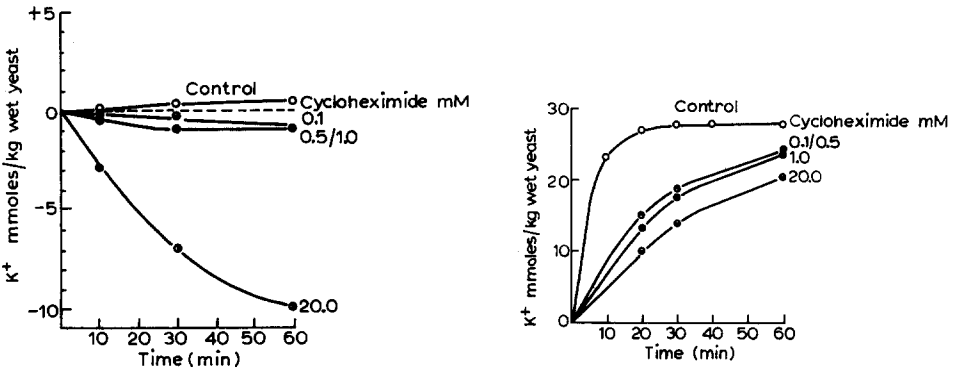

Fig. 1. The effect of cycloheximide on K^+ content of yeast suspended in water in the absence of substrate. Yeast, which had been starved for 15 h by suspending in water and bubbling with air, was washed and resuspended in water to a final concentration of 2% (w/v). Cycloheximide was added to the concentrations indicated and samples of the suspension taken for K^+ analysis after different time intervals.

Fig. 2. The effect of cycloheximide on K^+ uptake by yeast during metabolism of glucose. The cycloheximide was incorporated into a 2% (w/v) suspension of starved yeast in phosphate buffer (pH 6.0) and the suspension shaken in air at 37° for 1 h. Glucose, to a concentration of 100 mM, was added at the end of this period. The incubation was continued and samples taken for analysis at the times indicated.

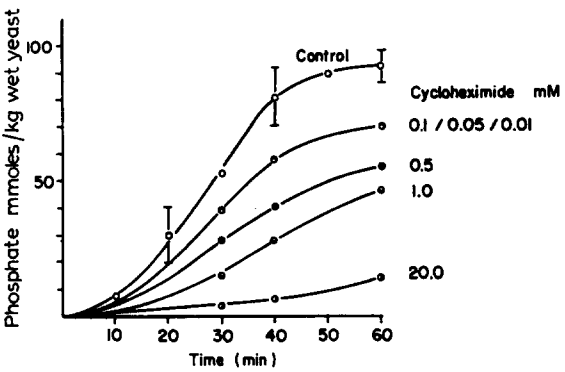

Fig. 3. The effect of cycloheximide on phosphate uptake by yeast from a medium containing glucose and phosphate buffer. Experimental conditions were identical with those described under Fig. 2.

by ROTHSTEIN *et al.*[7]. While some of the effects on phosphate may be attributed to changes in K^+ uptake, the data strongly suggest that the antibiotic also has a direct effect on phosphate uptake. Thus 0.1–20.0 mM cycloheximide concentrations are all found to inhibit K^+ uptake to about the same degree but have widely differing effects on phosphate. Also the amount of K^+ taken up at 1 h in 20 mM inhibitor is 80% of normal, but the rate of phosphate uptake in this time is very low. Moreover, as Table I shows, the time relationship of effects on K^+ and phosphate are quite different.

TABLE I

UPTAKE OF K^+ AND PHOSPHATE BY YEAST PRETREATED WITH CYCLOHEXIMIDE

Pretreatment consisted of suspending 2 g wet yeast in 100 ml phosphate buffer at pH 6.0 containing 0.1 mM cycloheximide. Uptake of K^+ and phosphate were measured by adding glucose to a final concentration of 100 mM to samples of the original suspension and taking aliquots for analysis at zero time and after a 60-min incubation, with shaking, at 37°.

Length of pretreatment (h)	*Uptake of K^+ (mmoles/kg wet yeast in 60 min)*	*Uptake of phosphate (mmoles/kg wet yeast in 60 min)*
1	26.0	57.0
3	18.0	46.5
20	8.0	42.0

The observations of the effects of prolonged pretreatment of yeast with cycloheximide are important, since the antibiotic has been used by some workers as an inhibitor of yeast growth over extended periods[1,8]. The results reported here indicate that the inhibition of growth might well be accounted for by inhibition of K^+ and phosphate uptake, both of which are essential for cell growth.

The effects of cycloheximide on the cell membrane should be taken into consideration when using this antibiotic as an inhibitor of protein synthesis in cells.

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