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Effect of the chronic ethanol action on the activity of the general amino-acid permease from *Saccharomyces cerevisiae* var. *ellipsoideus*

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The presence of ethanol and cycloheximide during growth were found to inhibit the function of the general amino-acid permease of *Saccharomyces cerevisiae* var. *ellipsoideus*. Contrary to cycloheximide, the effect of ethanol upon growth in alcohol-free medium was reversible. The effect of both inhibitors could be explained in terms of reduction of the number of active carrier molecules located in the plasma membrane.

It has been suggested that the plasma membrane lipid composition governs the sensitivity of *Saccharomyces cerevisiae* to ethanol [1,2]. However, since ethanol-sensitive mutants do not show significant differences in their plasma membrane lipid composition [3], it appears that the targets for the action of ethanol during growth are still far from being fully elucidated.

Recently, it has been shown that the acute administration of alkanols inhibited the function of the general amino-acid permease (GAP) in resting cells (Ref. 4; see Ref. 5 for an excellent review on amino-acid transport in yeast). In the present work we report on the chronic action of ethanol on the activity of the GAP in growing cultures of *Saccharomyces cerevisiae* var. *ellipsoideus*.

S. cerevisiae var. *ellipsoideus* CIFI 266 obtained from Dr. V. Arroyo at the Instituto de Fermentaciones Industriales (CSIC, Madrid, Spain) was used throughout this work. Cells were maintained, grown and harvested as previously reported [6,7]. The culture medium was 2% (w/v) yeast extract and 2% (w/v) D-glucose in type I water, conditions in which repression of the GAP by ammonium are negligible [5].

The experimental approach was as follows: the cells were grown in the absence or the presence of the inhibitor either ethanol or cycloheximide, then they were isolated, suspended in inhibitor-free buffer and assayed for GAP activity.

To assay the activity of the GAP we measured the uptake of L-[^{3}H]valine in 500-μl aliquots of yeast suspension in 0.1 M phosphate buffer (pH 5) containing 50 mM D-glucose and 1 mM L-[^{3}H]valine (spec. act. 0.525 Ci/mol) that were incubated for 10 min at 28°C in a rotary shaker under aerobic conditions. Uptake was alted by dilution of 200 μl of the suspension with 2.5 ml of cold 0.1 M phosphate buffer (pH 6) and evaluated by filtration as previously described [6].

Under these conditions and with this yeast strain, the amount of tracer incorporated into proteins was less than 15% of total tracer taken up [6] and the radioactivity derived from L-[^{3}H]valine by metabolic degradation was negligible (over 95% of the acid-soluble radioactivity was L-valine as judged by paper chromatography; data not shown). Therefore, the radioactivity taken up essentially mean incorporation of L-valine to the acid-soluble intracellular pool of amino acid.

As illustrated in Fig. 1A, the exposure of growing cells to either ethanol or cycloheximide for variable times lead to a deactivation of the GAP system measured as uptake of 1 mM L-[^{3}H]valine for 10 min at 28°C.

The exposure of growing cells to cycloheximide, a typical inhibitor of protein synthesis that acts specifically on eukaryotic systems [8] and that was reported as promoter of protein degradation in logarithmically growing cells [9], induced the same decline as ethanol. The effects of both inhibitors on the growth corre-

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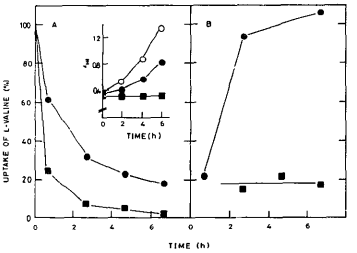

Fig. 1. Decay and recovery of the specific rate of uptake of L-valine transport system in *S. cerevisiae* var. *ellipsoideus*. Cells from the exponential phase were divided into three batches, each reciving: none (control); 5% (v/v) ethanol (●); or 0.5 mM cycloheximide (■). They were then allowed to grow for the times indicated in panel A (decay). Following this the cells were isolated, washed, suspended in buffer and assayed for L-valine uptake as described in the text. The inset in panel A represents the effects of both inhibitors on growth corresponding to the experiment of panel A: ○, control; ●, 5% (v/v) ethanol; ■, 0.5 mM cycloheximide. In panel B (recovery), three cell batches containing none (control cells), 5% (v/v) ethanol (●) or 0.5 mM cycloheximide (■) were incubated for 100 min. The cells were then isolated, washed and suspended in inhibitor-free medium. At the indicated times the cells were processed as in panel A. Results are presented as percentage of control values for each time. 100% represents in panel A 0.60, 0.77, 0.76 and 1.00 $\mu mol \cdot (g\ cells)^{-1} \cdot min^{-1}$ for 40, 160, 280 and 400 min, respectively, and in panel B 1.3, 1.8 and 1.0 $\mu mol \cdot (g\ cells)^{-1} \cdot min^{-1}$ for 40, 160 and 400 min, respectively. All data of cells are given as dry weight.

sponding to the experiment of Fig. 1A are illustrated in the inset of Fig. 1A.

5% ethanol induced a moderate inhibitory action on growth lower than the exerced over the GAP function (Fig. 1A) while cycloheximide, as expected, fully blocked the growth.

To answer whether the inhibitory action of ethanol on the GAP system was irreversible or not, cells previously exposed either to ethanol or to cycloheximide were isolated, washed and suspended in an alcohol or antibiotic-free medium and then the activity of the GAP system assayed at the indicated times.

As illustrated in Fig. 1B while ethanol-treated cells fully recovered their ability to transport L-valine by incubation in an alcohol-free medium, cycloheximide-treated cells did not, this being probably due to their strong binding to the ribosomes contrary to what would be expected from ethanol.

Ethanol-dependent reduction of L-valine uptake observed in cells growing in the presence of the alcohol could be due to different effects such as the inhibition of the GAP synthesis, its removal from the plasma membrane or an inactivation process by physical interaction between ethanol and GAP. Alternatively a membrane damage based on the disturbance of the protonmotive force could explain the observed effect of ethanol, but since the effect of the alcohol on the growth was much lower than on the GAP function this seems rather improbable. To gain more inshight on these posibilities we studied the kinetic parameters for the GAP system in the yeast cells.

Using a broad L-valine concentration range, the GAP was found to exhibit two forms for L-valine uptake with apparent K_m values of 12.51 and 1.02 mM and V_{max} values of 6.1 and 2.5 $\mu mol \cdot g^{-1} \cdot min^{-1}$, respectively (Fig. 2). To the best of our knowledge no previous reports have described the presence of these two forms for L-valine uptake in yeast.

Cell growth in the presence of 5% (v/v) ethanol induced the decrease in the V_{max} of the two forms leaving the K_m unchanged (Fig. 2).

To exclude the posibility of the existence of at least two transport systems for L-valine in this yeast, one unspecific as GAP and other specific for L-valine, L-phenylalanine which is exclusively transported by the GAP system in *S. cerevisiae* [4,5] was assayed for competition with L-valine uptake. L-Phenylalanine tested

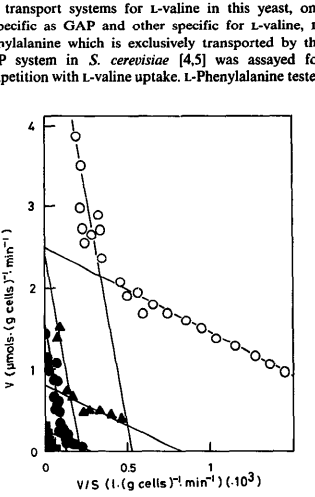

Fig. 2. Eadie-Hofstee plots for L-valine uptake by *S. cerevisiae* var. *ellipsoideus*. Cells from the exponential phase were treated as in Fig. 1A for 2 h. They were then isolated, washed and suspended in 0.1 M sodium phosphate (pH 5) buffer and L-valine uptake was assayed for different concentrations of labelled amino-acid, as described in the text. ○, control cells; ●, 5% (v/v) ethanol-pretreated cells; ■, 0.5 mM cycloheximide-pretreated cells. The results of an experiment of competition of L-valine uptake (range 0.5–20 mM) with L-phenylalanine (at twice the L-valine concentration for each L-valine concentration) is also shown (▲).

at only twice the concentration of L-valine in each experimental point (L-valine range 0.5–20 mM) strongly inhibits the transport system (near 65%; the Eadie-Hofstee plot for this experiment is shown in Fig. 2). This indicates that in our conditions and yeast strain the transport of L-valine is carried out essentially by the GAP system, as indicated previously for other strains of *S. cerevisiae* [5] specially in the absence of reppresion by ammonium.

A typical eukaryotic protein synthesis specific inhibitor such as cycloheximide had the same effect as ethanol on the GAP function (Fig. 1). This action of cycloheximide agrees with previous results obtained on the yeast sugar transport systems when the cells are exposed to protein synthesis inhibitors [10]. On the other hand, recently it has been reported that this antibiotic efficiently inhibits both the synthesis and intracellular degradation of insulin receptors, but does not inhibit the translocation of the receptor through the plasma membrane; these abilities are shared by other protein synthesis inhibitors [11]. This should lead to a reduction of the number active carrier molecules in the plasma membrane. Assuming a similar routing of plasma membrane proteins for yeast cells as for higher eukaryotes, and since ethanol was reported as a direct inhibitor of protein synthesis in this yeast [6,7], our results could be explained in terms of either a blockade in the supply of active carrier molecules by inhibition of protein synthesis (more probable in our opinion), or an increased deactivation or removal of active carriers from the plasma membrane, without apparent changes of the affinity of the molecules remaining at the plasma membrane.

An action of ethanol similar to that proposed here has been postulated in cultured rat hepatocytes [12]. In that study, 100 mM ethanol reduced the A and N amino-acid transport systems of cultured hepatocytes by 40–70%. The authors favoured the hypothesis that the effect was mediated by the inhibition of the synthesis of the carrier components by the alcohol, according to the finding that very low concentrations of ethanol inhibited the bulk protein synthesis in isolated rat liver cells [13,14]. Furthermore, it has been shown that ethanol inhibits the syntnesis of rat liver plasma membrane proteins [15] and export protein synthesis in isolated rat liver cells [16], both kinds of proteins being intracellular traffic-dependent and membrane assembly-dependent.

The effect of ethanol on the activity of general amino-acid permease of growing yeast might reduce the intracellular availability of most amino-acids and hence probably other metabolites derived from. This effect, together with the direct arrest of protein synthesis caused by the presence of ethanol [6,7], could merely reflect the existence of priority, important and different targets for the action of ethanol during growth and fermentation of this ethanol-tolerant yeast.

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References

1 Thomas, D.S., Hossack, J.A. and Rose, A.H. (1978) Arch. Microbiol. 117, 239–245.
2 Beavan, M.J., Charpenter, C. and Rose, A.H. (1982) J. Gen. Microbiol. 128, 1445–1447.
3 Aguilera, A. and Benitez, T. (1986) Arch. Microbiol. 143, 337–344.
4 Leao, C. and Van Uden, N. (1984) Biotech. Bioeng. 26, 403–405.
5 Eddy, A.A. (1982) Adv. Microb. Physiol. 24, 1–78.
6 Girbés, T. and Parrilla, R. (1983) Cell. Mol. Biol. 29, 443–449.
7 Girbés, T. and Parrilla, R. (1986) Cell. Mol. Biol. 32, 545–549.
8 Vázquez, D. (1974) FEBS Lett. 40, S63–S84.
9 Wolf, D.H. (1980) Adv. Microb. Physiol. 21, 267–338.
10 Busturia, A. and Lagunas, R. (1985) Biochim. Biophys. Acta 820, 324–326.
11 Knutson, V., Ronnett, G. and Lane, D. (1985) J. Biol. Chem. 260, 14180–14188.
12 Dorio, R.J., Hoek, J.B. and Rubin, E. (1984) J. Biol. Chem. 259, 11430–11435.
13 Morland, J. and Bessesen, A. (1977) Biochim. Biophys. Acta 474, 2312–2320.
14 Girbés, T., Susin, A., Ayuso, M.S. and Parrilla, R. (1983) Arch. Biochem. Biophys. 266, 37–49.
15 Mailliard, M.E., Sorell, M.F., Volen..., G.D. and Tuma, D.J. (1984) Biochem. Biophys. Res. Commun. 123, 951–958.
16 Girbés, T., Ferreras, J.M., Muñoz, R. and Alonso, P. (1989) Toxic. Vitro, in press.