INHIBITION OF PROTEIN SYNTHESIS AND SIMULATION OF PERMEASE TURNOVER IN YEAST

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Several authors recently reported that inhibition of protein synthesis by starvation for a required amino acid, by cycloheximide or by other inhibitors produces a rapid decay in specific transport activity of amino acids in Neurospora and animal tissues (Adamson, Langellutig and Anast, 1966; Elsas and Rosenberg, 1967; Wiley and Matchett, 1967; Yamada, Clark and Swendseid, 1967).

It was suggested in these papers, as one or as the sole interpretation of the results, that protein synthesis is an obligate requirement for the maintainance of carrier-proteins with short half-lives (i.e. rapid turnover).

The results presented here indicate that, in a similar situation analysed in yeast, this interpretation has to be eliminated. They suggest that the inhibition of amino acid transport activity is due to a pool of endogenous amino acids which accumulate as a consequence of the inhibition of protein synthesis.

Materials and methods :

The initial rate of uptake of ^{14}C-labelled amino acids was measured as described earlier, on isogenic strains derived from the haploid wild type strain Σ1278b of Saccharomyces cerevisiae and grown on medium M (Grenson, Mousset, Wiame and Béchet, 1966).

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Results and discussion :

Addition of cycloheximide (2 µg per ml) to a culture of yeast growing exponentially is rapidly followed by a strong decrease in arginine uptake activity (Fig. 1). This activity is almost completely lost after 6 hours of this treatment and 50 % of the activity has disappeared after 1 hour.

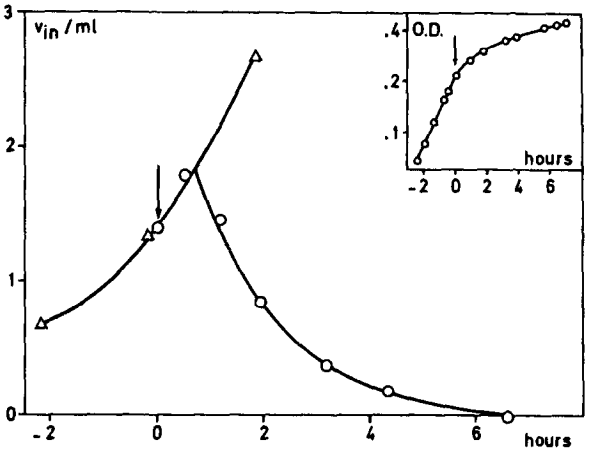

Fig. 1. Effect of cycloheximide on arginine uptake.
Ordinate : initial velocity of uptake of ^{14}C-uniformly labelled L-arginine (0.02 mM) in mµmoles x min^{-1} per ml of culture.
At the time shown by the arrow, cycloheximide (2 µg per ml) is added to a portion of the culture. Samples are taken from both subcultures at various times and incubated as such in the presence of ^{14}C-arginine for 2 minutes (one-ml samples removed every half minute) in order to measure the initial velocity of uptake.
Δ — Δ : control without cycloheximide; 0 — 0 : culture + cycloheximide;
o — o : growth curve. Strain : Σ1278b.
Note that the very presence of cycloheximide during the uptake test has no effect and that some time is needed for the inhibition to develop.

The same phenomenon was observed for leucine-, histidine-, and methionine uptake, but with different time constants (Fig. 2).

A similar loss of activity of the arginine-, methionine-, and leucine-uptake systems was observed as a result of histidine starvation in a histidine requiring mutant. The fact that, in this experiment, the histidine-uptake activity remained constant (Fig. 3) strongly suggests that the loss of uptake activity observed in the other cases (especially for histidine itself) is not due to permease destruction.

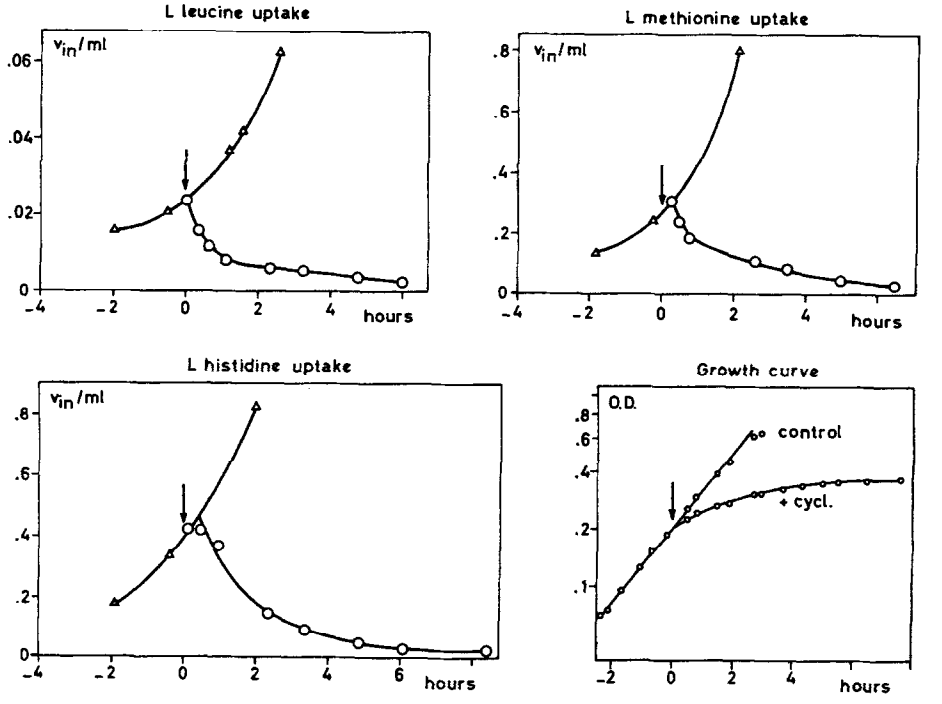

Fig. 2. Effect of cycloheximide on leucine-, methionine-, and histidine uptake. Details as in legend of Fig. 1. The three curves of uptake activity are obtained on the same culture of Σ1278b.

If the loss of amino acid-transport activity were due to the absence of reconstruction of very labile protein constituents, it should be induced as well by complete nitrogen starvation. That this is not the case is shown in Fig. 4 A, where it is apparent that nitrogen starvation has an opposite effect, the arginine-uptake activity showing a twofold increase. This observation suggests that the loss of amino acid-transport activity is a secondary result of the inhibition of protein synthesis, probably due to some accumulation of endogenous amino acids whose utilization is stopped. If this is the case, cycloheximide should have no effect when added to a nitrogen-starved culture. Such an experiment is presented in Fig. 4 B. Moreover, it can

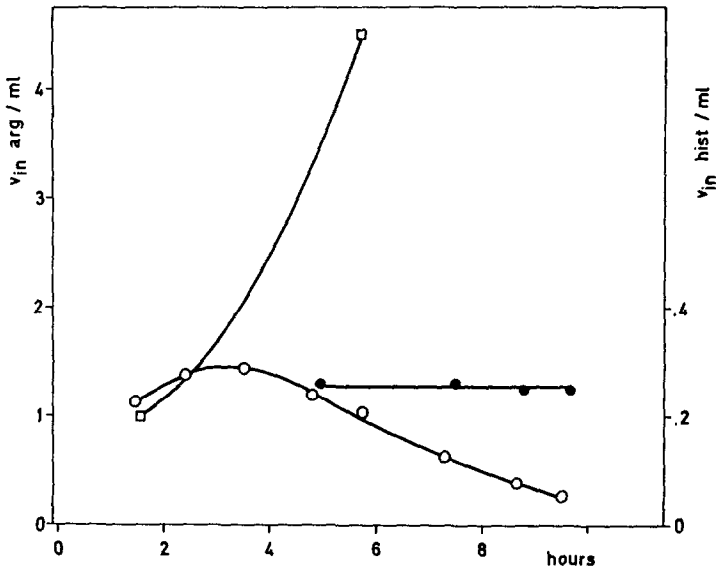

Fig. 3. Effect of histidine starvation on arginine- and histidine-uptake. Arginine uptake (O — O), and histidine uptake (● — ●) in strain MG 389 (histidine requiring) after removal of histidine from the medium at time zero. □—□ : control : arginine uptake in MG 389 during exponential phase of growth on minimal medium supplemented with L-histidine (25 µg/ml), in the presence of the culture medium. Details as in legend of Fig. 1.

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be seen in Fig. 4 that the arginine-transport system is very stable indeed, since no loss of activity is observed after 3 hours, and only 50 % loss after 21 hours of inhibition of protein synthesis.

We conclude from these experiments that the loss of amino acid-transport activity observed in yeast after cycloheximide treatment or histidine starvation is probably not due to the degradation of labile protein constituents, but rather to the accumulation of free amino acids as a result of the inhibition of protein synthesis.

The significance of this inhibition of amino acid transport by internally accumulated amino acids, as well as its implications with regard to the transport mechanisms, will be discussed elsewhere. It is probably related to the phenomenon described by Ring and Heinz (1966) and Heinz, Ring and Gross (1967) and referred to as feed-back control of amino acid transport.

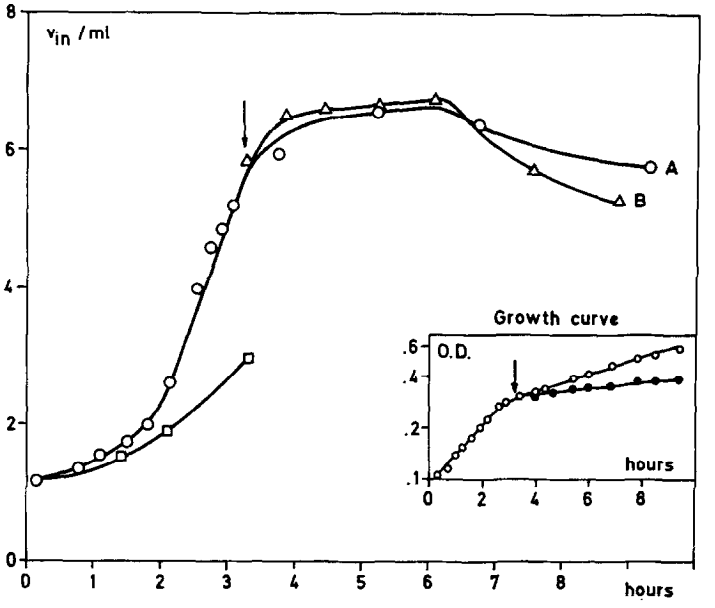

Fig. 4. Effect of nitrogen starvation (A), and of cycloheximide on a nitrogen-starved culture (B) on arginine uptake.

□—□ : control on complete medium; O — O : nitrogen-starved culture (curve A); Δ — Δ : nitrogen-starved culture with cycloheximide (2 µg/ml) added at the time indicated by the arrow (curve B).
Nitrogen starvation is obtained by transferring the cells, at time zero, in a medium with 20 times lower content in ammonium ions. See also legend of Fig. 1. Growth curve of the nitrogen-starved cultures corresponding to curve A (o — o) and curve B (● — ●).

From a practical point of view, this situation makes impossible any determination of amino acid uptake in yeast under conditions where protein synthesis is inhibited (except by nitrogen starvation) and forces one to adopt a special methodology in order to eliminate metabolic interferences in the study of transport mechanisms.

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