EVIDENCE FOR THREE GLUTAMIC ACID TRANSPORTING SYSTEMS WITH SPECIALIZED PHYSIOLOGICAL FUNCTIONS IN SACCHAROMYCES CEREVISIAE

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Summary : A set of three glutamate uptake systems with different specificities and regulatory properties allows the wild-type yeast Σ1278b to use external glutamate for different functions under different physiological conditions. One of these uptake systems is the general amino acid permease. The two other systems transport dicarboxylic amino acids with much higher specificity. One of them is constitutive in cells grown on NH_4^+ ; its activity is inhibited in washed cells coming from a growth medium containing glutamate and NH_4^+ simultaneously. The rate of synthesis of the third permease is strongly reduced in the presence of NH_4^+ , except in gdhA$^-$ mutants. In cells grown on glutamate, the formation of this system is not affected, but its activity is inhibited.

A detailed study of the uptake of glutamic acid and of its regulation has become urgent not only in view of the central role of glutamic acid in nitrogen metabolism, but furthermore since the observation that the gdhA$^-$ mutants of Saccharomyces cerevisiae (in which the structural gene for the anabolic glutamate dehydrogenase has been impaired) have lost the "ammonia inhibition" on several catabolic enzymes and permeases (1 - 4). This was shown to be true in Aspergillus nidulans as well (5, 6).

Glutamate was known to enter the cells of the wild-type yeast Σ1278b (grown on NH_4^+ as nitrogen source) by means of a permease which seemed specific for dicarboxylic amino acids (7). On the other hand, it was clear that glutamate competitively inhibits the uptake of

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amino acids by the general amino acid permease, but it could not be determined whether glutamate was taken up by this system (8).

Materials and Methods

Medium and growth conditions were previously described (9). Medium 149 supplemented with 3 % glucose and vitamins (biotin, thiamine, meso-inositol, Ca-pantothenate and pyridoxine) was used throughout. Minimal ammonium medium (M.am) contained 0.02 M NH_4^+ (as ammonium sulfate) as sole source of nitrogen ; minimal proline medium (M.pro) and minimal glutamate medium (M.glt) are identical to M.am, except that ammonia is replaced by L-proline and L-glutamate (1 mg/ml) respectively. M.pro + am contains ammonia and proline simultaneously, while M.glt + am contains ammonia and L-glutamate.

The uptake rate of ^{14}C-L-glutamate (Radiochemical Center, Amersham) was measured as previously (9). When the growth medium contained glutamate, the cells were filtered and resuspended in fresh medium without glutamate just before the uptake test. In such cases, the controls were treated in the same way.

Strains : The wild-type strain Σ1278b (9), and the gap mutant 2512c, which has lost the activity of the general amino acid permease (8), are isogenic.

Results

Two slopes are observed in a Lineweaver-Burk plot of glutamate uptake in cells of the wild-type strain Σ1278b grown on proline as sole nitrogen source (Fig. 1). One of them is absent in a gap mutant (Fig. 1) which has lost the activity of the general amino acid permease (8). The uptake activity corresponding to the second slope shows a higher affinity for glutamate. It can be measured in the

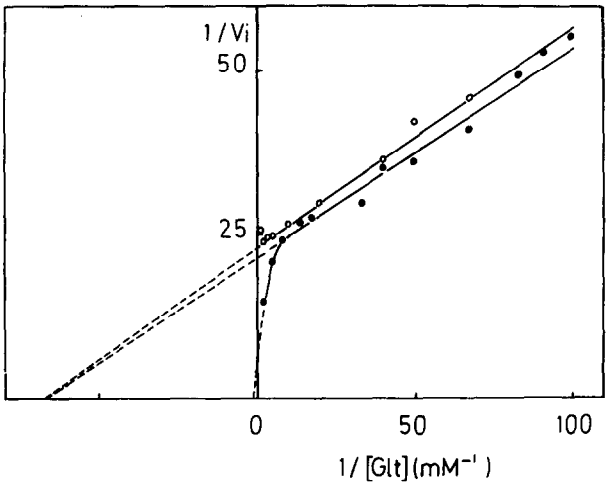

Fig. 1 : L-glutamic acid uptake into the wild-type strain Σ1278b and a gap mutant (2512c) : Lineweaver-Burk plot ; M.pro medium ; v_i in µmoles per mg protein per min. ● — ● : Σ1278b ; o — o : 2512c.

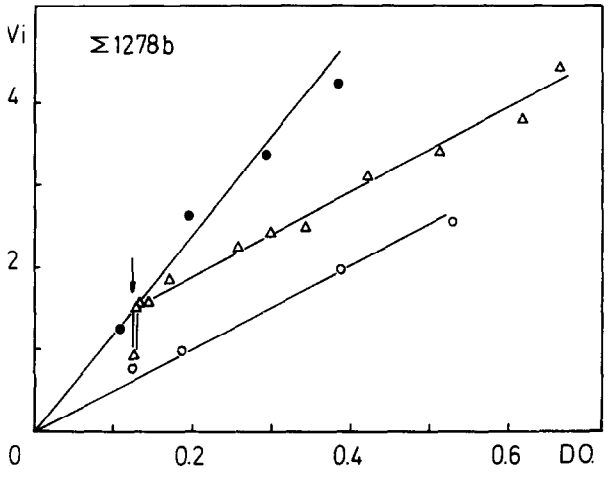

Fig. 2 : Differential rate of increase in L-glutamic acid uptake activity after addition of ammonium to the culture. Wild-type strain Σ1278b growing in M.pro medium : ● — ● ; and in M.pro + am medium : o — o . At the time shown by the arrow, 0.02 M NH_4^+ was added to a portion of the culture : Δ — Δ . L-glutamic acid uptake activity (initial rate of uptake of 0.02 mM ^{14}C L-glutamic acid in nmoles per min per ml of culture) was followed as a function of the increase in absorbance of the cultures.

wild-type strain without interference of the general amino acid uptake system provided that a low concentration of glutamate is used (usually 0.02 mM). This permease shows a narrow specificity for dicarboxylic amino acids and structurally closely related substances. Its rate of synthesis is strongly depressed in the presence of ammonia, which, given alone, does not inhibit its activity (Fig. 2). The addition of glutamate to the growth medium does not affect the rate of synthesis of this permease, but it results in a rapid inhibition of its activity as tested in washed cells (Fig. 3 A and B). The inhibitory effect of ammonia on the rate of synthesis of this system is suppressed in the *gdhA*$^-$ mutants (which have lost the activity of the NADP-linked glutamate dehydrogenase) ; the inhibitory effect of glutamate on the uptake activity remains unchanged in this mutant (to be published elsewhere).

The third glutamate uptake activity, which persists on M.glt as well as on M.am medium, is certainly not due to the general amino acid permease activity, since it transports dicarboxylic amino acids with a much narrower specificity, it is resistant to ammonia inhibition, and it is not affected in the *gap* mutants. It also seems to be distinct from the uptake system whose rate of synthesis is lowered in the presence of ammonia. Its kinetic properties as well as its mode of regulation are different ; its activity is inhibited when NH_4^+ and glutamate are present simultaneously. This view is based on the data shown in fig. 4, taking into account the intracellular concentrations of NH_4^+ and glutamate as determined previously (4). Additional arguments in favour of the individuality of this third glutamate uptake system will be presented elsewhere.

Conclusions

As suggested by their specificity and regulatory properties,

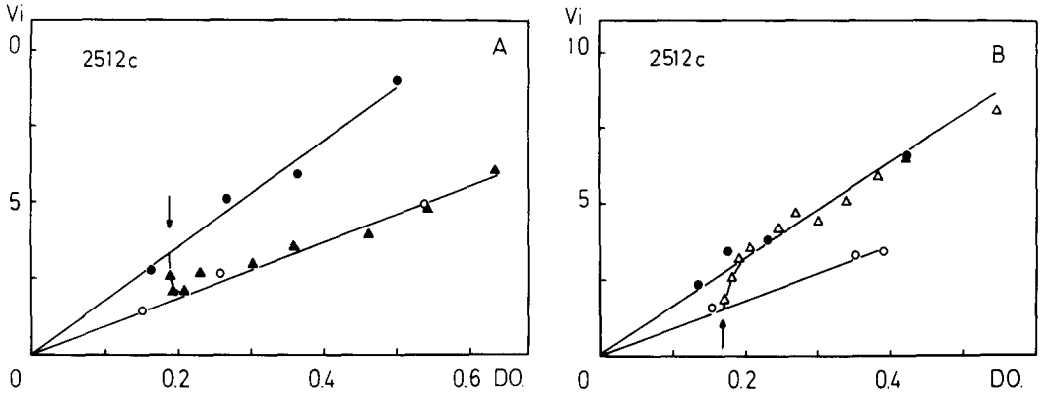

Fig. 3 : Differential rate of increase of L-glutamic acid uptake activity in a gap mutant (2512c) growing in M.pro medium : ● — ● ; in M.pro + glt medium : o — o . (A) At the time shown by the arrow, 6.8 mM L-glutamic acid was added to a portion of the culture growing in M.pro medium : ▲ — ▲ . (B) At the time shown by the arrow, a portion of the culture growing exponentially in the presence of glutamic acid was filtered, washed and transferred to M.pro medium : Δ — Δ. v_i : initial rate of uptake of 0.02 mM ^{14}C L-glutamic acid in nmoles per min per ml of culture, as measured after filtration (see Materials and Methods).

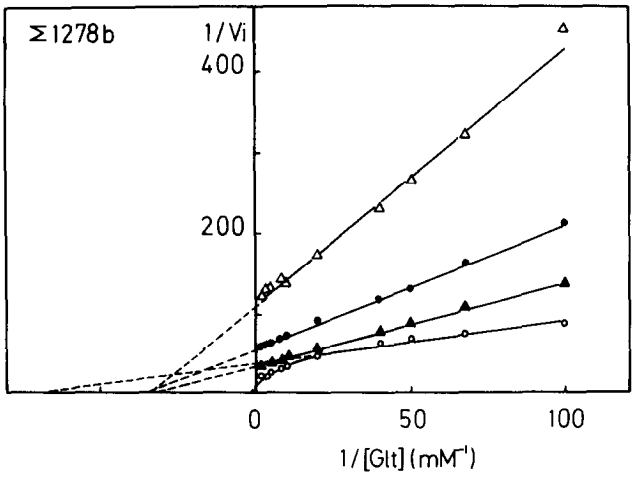

Fig. 4 : L-glutamic acid uptake into the wild-type strain Σ1278b : Lineweaver-Burk plot. Cells growing in M.am medium : ● — ● ; in M.glt + am medium : Δ — Δ ; in M.glt medium : ▲ — ▲ ; in M.pro medium : o — o . The uptake rate of 0.02 mM ^{14}C L-glutamic acid (v_i , in μmoles per mg protein per min) was measured after filtration as described under Materials and Methods.

these three glutamate uptake systems might be specialized physiologically. The "specific permease" which is synthesized in the presence of ammonia seems to be able to supply glutamate for anabolic purposes ; the second "specific permease", the rate of synthesis of which is strongly inhibited by NH_4^+ , might accumulate glutamate as a source of nitrogen, and the third "non specific permease" seems to function as uptake system of any amino acid used as a source of nitrogen.

Acknowledgements

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