Biochimica et Biophysica Acta, 1023 (1990) 380–382
Elsevier

BBAMEM 74814

Phenylmethylsulfonyl fluoride protects L-lysine transport in *Schizosaccharomyces pombe* against inactivation by ammonium ions

Jaroslav Horák, Hana Sychrová and Arnošt Kotyk

Department of Membrane Transport, Institute of Physiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)

(Received 17 October 1989)

Key words: L-Lysine transport; Ammonium ion; Amino acid transport; (Yeast); (*S. pombe*)

Ammonium ions inactivate the basic amino acid transport system in *Schizosaccharomyces pombe* in an irreversible manner. The inactivation is accompanied by a 4-fold decrease of K_T of L-lysine transport, leaving its J_{max} unchanged; phenylmethylsulfonyl fluoride protects the system against inactivation. In contrast, two basic amino acid transport systems in a *gap1* mutant of *Saccharomyces cerevisiae* are influenced by NH_4^+ ions in such a way that only the J_{max} decreases while the K_T of L-lysine transport is unchanged. Phenylmethylsulfonyl fluoride does not act here as a protective agent.

1. Introduction

Ammonium ions interfere with amino acid transport in several lower eukaryotic microorganisms [1]. In *Saccharomyces cerevisiae* they negatively influence the activity (by inactivation) and/or synthesis (by nitrogen catabolite repression) of proteins of at least three transport systems, the general amino acid system (GAP1). the proline specific system (PUT4) and one of the acidic amino acid transport systems (for reviews, see Refs. 1 and 2). Moreover, the ability of ammonium ions to compete for a common source of metabolic energy was considered [3,4].

In *Schizosaccharomyces pombe*, the xanthine transport is irreversibly inactivated and repressed by ammonium ions and the inactivation (but not repression) was shown to be largely prevented in cells pretreated with the serine proteinase inhibitor, phenylmethylsulfonyl fluoride (PMSF) [5]. Likewise, pretreatment of *Schizosaccharomyces pombe* cells with ammonium ions decreases the initial rates of transport of basic and 'hydrophobic' amino acids, mediated by at least two different, high-affinity, nonconstitutive and relatively unstable systems to a considerable extent; this decrease is largely prevented by PMSF [6,7].

Correspondence: J. Horák, Department of Membrane Transport, Institute of Physiology, Czechoslovak Academy of Sciences, 142 20 Prague 4, Czechoslovakia.

Kinetic analysis of the negative effects of ammonium ions in the L-lysine transport via the basic amino acid system of *Schizosaccharomyces pombe* and protection of its activity against NH_4^+ by PMSF, are considered in the present paper. For comparison, analogous data on L-lysine transport in the *gap1* mutant of *Saccharomyces cerevisiae* Σ1278b are shown.

2. Materials and Methods

Phenylmethylsulfonyl fluoride was from Serva, F.R.G., cycloheximide was from Fluka, Switzerland. L-[U-^{14}C]Lysine (8 MBq/ml) was obtained from the Institute for Research, Production and Uses of Radioisotopes, Czechoslovakia. All other chemicals were purchased from commercial suppliers and were of analytical purity.

Schizosaccharomyces pombe 972h$^-$ and *Saccharomyces cerevisiae* Σ1278b *gap1* mutant (a kind gift of Dr. M. Grenson of Brussels) were grown in a YPD medium containing 1% yeast extract, 1% bactopeptone and 1% glucose, at pH 4.5. Cells from the early stationary growth phase (26–28 h for *S. pombe*, 20–22 h for *S. cerevisiae*) were harvested by centrifugation, washed twice with distilled water and suspended in distilled water.

Preincubation of the cells with glucose and measurement of the amino acid transport were carried out as described previously [6] using membrane filtration and liquid scintillation counting.

3. Results and Discussion

Schizosaccharomyces pombe 972h$^-$ cells harvested from the stationary growth phase do not practically take up L-lysine and their transport activity appears only after preincubation with a metabolizable substrate, D-glucose being most suitable [6]. The stimulatory effect of D-glucose is significantly suppressible by cycloheximide which blocks protein synthesis in eukaryotic microorganisms, or by ammonium ions (Table I). If both these inhibitors were added together the suppression was nearly additive and PMSF partly protected L-lysine uptake activity against both of them, when added separately or together (Table I).

TABLE I

Inhibition of initial rate of uptake of L-lysine (50 μmol/l) by ammonium ions and cycloheximide

Cells were preincubated for 60 min with 1% glucose and inhibitors were added to the suspension 30 min before labeled L-lysine. The values shown are the means of at least six separate experiments.

Inhibitor	Concentration (mmol/l)	Inhibition (%)
None	–	0
Ammonium sulfate	3.8	54.8
Cycloheximide	0.4	82.4
Ammonium sulfate + cycloheximide	3.8 + 0.4	93.4
Ammonium sulfate + PMSF	3.8 + 2.0	23.5
Cycloheximide + PMSF	0.4 + 2.0	62.4
Ammonium sulfate + cycloheximide + PMSF	3.8 + 0.4 + 2.0	72.6

TABLE II

Effects of ammonium ions (3.8 mmol/l) on the kinetic parameters of L-lysine transport without and with PMSF (2 mmol/l) in Schizosaccharomyces pombe (A) and Saccharomyces cerevisiae (B)

Cells were pretreated as described in the legend to Table I. The values are means of three separate experiments. n.t., not tested.

Inhibitor	A		B	
	K_T (μmol/l)	J_{max} (nmol/mg dry wt. per min)	K_T (μmol/l)	J_{max} (nmol/mg dry wt. per min)
None	28.1	5.2	80.3	1.02
Ammonium sulfate	117.3	5.1	81.2	0.16
Ammonium sulfate + PMSF	36.4	5.1	n.t.	n.t.

Both the negative effect of ammonium ions on the L-lysine transport system and the ability of PMSF to neutralize their influence may be interpreted most simply in terms of: (i) repression of L-lysine transport protein synthesis by ammonium ions that may be followed by its proteolytic degradation in a reaction prevented by PMSF, as a serine proteinase inhibitor; (ii) inactivation of the corresponding transport protein by ammonium ions, accompanied by its (conformational?) change to a less active form in a reaction, prevented by PMSF, as a group-specific modifier of serine hydroxyl groups of proteins [8]; (iii) combination of (i) and (ii).

To decide between these alternatives, the kinetic parameters of L-lysine transport were estimated in *Schizosaccharomyces pombe* pretreated with ammonium

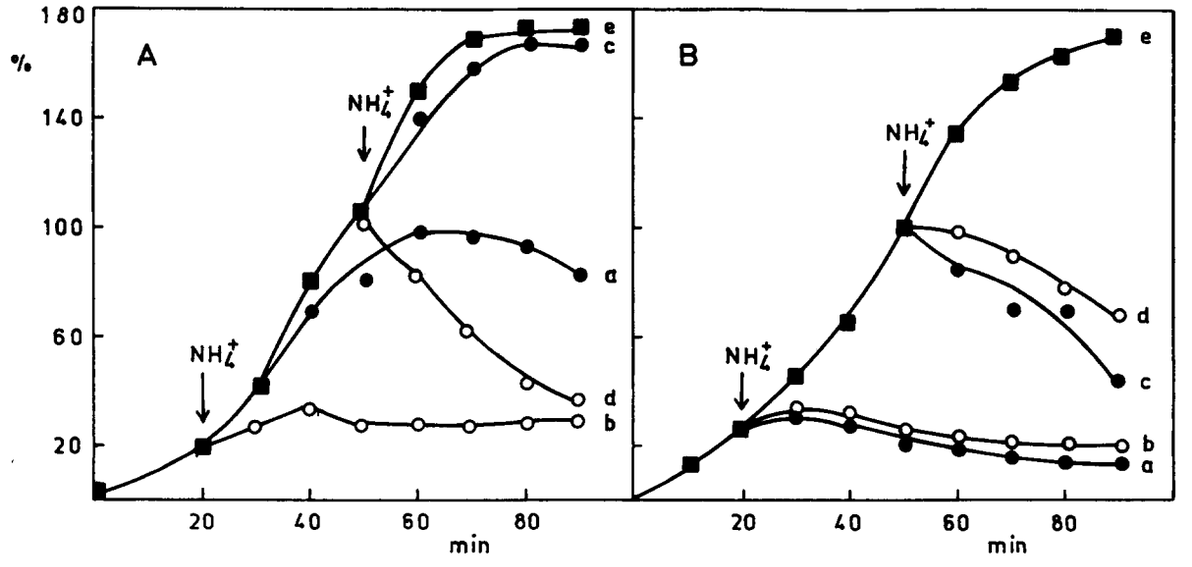

Fig. 1. Time course of L-lysine (50 μmol/l) transport inhibition by ammonium ions (3.8 mmol/l) added at 20 and 50 min of the preincubation period with (curves a, c) and without (b, d) PMSF (2 mmol/l) in *Schizosaccharomyces pombe* (A) and *Saccharomyces cerevisiae* (B). The initial transport rate after 50 min of incubation with 1% glucose is taken as 100%. Curves e correspond to untreated control.

ions alone and in combination with PMSF. As is shown in Table II, ammonium ions increase the K_T of L-lysine transport 4-fold, leaving the maximum rate of uptake J_{max} practically unchanged, while in cells pretreated with ammonium ions plus PMSF only a slight increase of the K_T value without J_{max} change may be observed. The J_{max} values of L-lysine transport increased a little with prolonged preincubation with glucose but at all times remained unaffected by either ammonium ions or ammonium plus PMSF. These kinetic data and the rapid decrease of initial rate of L-lysine transport caused by ammonium ions (but not in the presence of ammonium ions and PMSF; Fig. 1A) militates against possibilities (i) and (iii) because changes of J_{max} [case(i)] or K_T and J_{max} [case (iii)] would be expected here. Thus possibility (ii) remains the most probable mechanism.

To establish whether the competitive nature of interaction between ammonium ions and L-lysine transport is a general feature of basic amino acid transport systems in lower eukaryotes or whether it is a property restricted to the transport systems of *S. pombe*, analogous experiments were carried out with L-lysine transport in the *Saccharomyces cerevisiae* Σ1278b *gap1* mutant. In this strain L-lysine transport is mediated by only two constitutive, high-affinity systems, specific for arginine plus lysine, and for L-lysine, respectively [9,10]. As shown in Fig. 1B and Table II, two findings deserved more attention. (1) Pretreatment of *Saccharomyces cerevisiae* with ammonium ions decreases the initial rates of L-lysine transport, but here the decrease is not prevented by PMSF. (2) Ammonium ions decrease only the maximum rate of L-lysine transport, leaving its K_T value unchanged. Similar results were obtained with L-arginine, transported in the *gap1* mutant exclusively by the basic amino acid system (data not shown). Such results seem to be most plausibly explained by assuming that ammonium ions in *S. cerevisiae* act as trans inhibitor of amino acid transport.

Finally, to decide whether the inactivation of L-lysine uptake by ammonium ions in *Schizosaccharomyces pombe* is an irreversible process, cells were divided into three parts, preincubated with 1% glucose for 50 min and with ammonium ions (3.8 mmol/l) for subsequent 30 min. Then the cells were centrifuged, thoroughly washed in water and incubated with 1% glucose (tube A), glucose plus cycloheximide (tube B), and in water (tube C). At intervals, aliquots were taken from the tubes to measure the initial rate of L-lysine transport into cells. The results presented in Fig. 2 show that the 'inactivation' of L-lysine transport is not abolished by washing out the ammonium ions and that transport

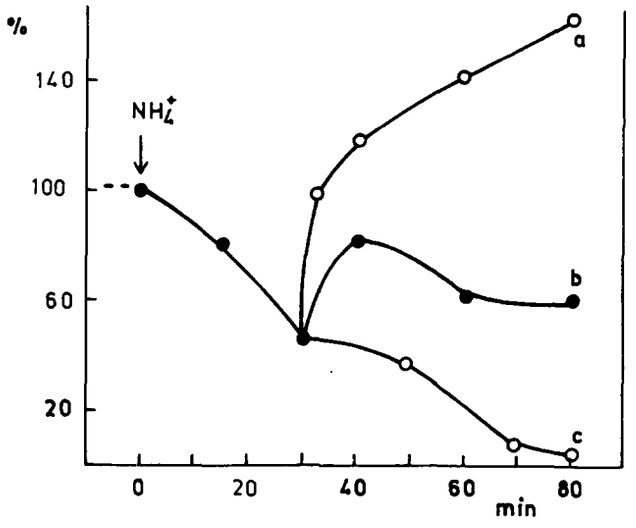

Fig. 2. Irreversible inactivation of L-lysine (50 μmol/l) transport in *Schizosaccharomyces pombe* by ammonium ions. After a 30-min preincubation with ammonium ions (3.8 mmol/l) the cells were centrifuged, washed with water, and L-lysine transport activity was estimated in the presence of 1% glucose (curve a), 1% glucose plus 0.4 mmol cycloheximide/l (curve b) and water (curve c). The initial transport rate after 50 min of preincubation with 1% glucose is taken as 100%.

activity only recovers when the cells are permitted to resume protein synthesis. The partial recovery of L-lysine transport may be explained in terms of residual (or mitochondrial?) proteosynthetic activity. L-Lysine transport mediated in *S. pombe* by the basic amino acid transport system is thus irreversibly inactivated in the presence of ammonium ions and only becomes regenerated through a de novo synthesis of the carrier protein in the presence of a suitable carbon source.

References

1 Horák, J. (1986) Biochim. Biophys. Acta 864, 223–256.
2 Cooper, T.G. (1982) in The Molecular Biology of the Yeast Saccharomyces; Metabolism and Gene Expression (Strathern, J.N., Jones, E.W. and Broach, J.R., eds.), Vol. 2, pp. 399–462, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
3 Roon, R.J., Meyer, G.M. and Larimore, F.S. (1977) Mol. Gen. Genet. 158, 185–191.
4 Roon, R.J., Levy, J.S. and Larimore, F.S. (1977) J. Biol. Chem. 252, 3599–3604.
5 Seipel, S. and Reichert, U. (1980) FEBS Lett. 115, 289–292.
6 Sychrová, H., Horák, J. and Kotyk, A. (1989) Biochim. Biophys. Acta 978, 203–208.
7 Sychrová, H., Horák, J. and Kotyk, A. (1989) Yeast 5, 199–207.
8 Hayashi, R., Bai, Y. and Hata, T. (1975) J. Biochem. (Tokyo) 77, 1313–1318.
9 Grenson, M., Mousset, M., Wiame, J.M. and Bechet, J. (1966) Biochim. Biophys. Acta 127, 325–338.
10 Grenson, M. (1966) Biochim. Biophys. Acta 127, 339–346.