REGULATION OF URIC ACID UPTAKE IN THE YEAST *PICHIA GUILLIERMONDII*

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1. Introduction

Ammonium ions regulate the transport of a number of amino acids in fungi. Schwenchke and Magaña-Schwencke [1] found derepression of the proline uptake system in *Saccharomyces chevalieri* when the cells were incubated in an ammonia-free medium. Ammonium ions are known to be involved in the regulation of the general amino acid permease of *S. cerevisiae* [2], of the amino acid permeases of filamentous fungi [3, 4], and of the ureidosuccinate uptake system of *S. cerevisiae* [5].

The regulation of purine transport has been investigated only in bacteria [6, 7]. While studying purine uptake in the yeast *Candida utilis*, inhibition of uric acid transport by NH_4^+ ions has been shown [8, 9], yet the mechanism of this phenomenon has not been investigated. In a preliminary communication we have shown the dependence of purine uptake by cells of *Pichia guilliermondii* on protein synthesis [10]. The present report describes the results of further investigation of purine transport regulation, using uric acid.

2. Experimental

Yeast *P. gulliermondii* Wickerham, strain VKM Y-1257 was used. The cells were grown in a Burkholder medium [11] for the early stationary phase (48 hr) on a shaker (200 rpm) at 30°. Usually $(NH_4)_2SO_4$ was used as a nitrogen source. The cells were harvested by centrifugation, washed with phosphate buffer, pH 5.5, and stored at 4° until used.

For derepression of uric acid transport system equal volumes of cell suspension (1.6 mg dry weight per ml) in 25 mM K-Na phosphate buffer, pH 5.5, and of the incubation medium which contained 25 mM K-Na phosphate buffer, pH 5.5, 120 mM sucrose and 2 mM $MgSO_4$ were mixed. The incubation was carried out at 30° with shaking.

To determine transport rates, 2 ml aliquots of this suspension were removed after appropriate intervals and mixed with 2 ml of a reaction mixture which usually contained 25 mM K-Na phosphate buffer, pH 5.5, 60 mM sucrose, 1 mM $MgSO_4$, uric acid (about 0.2 mM), and cycloheximide (20 μg/ml). For control experiments the cells were incubated in the same medium but without purine. The reaction was carried out at 30° with shaking. Transport was stopped by filtering the suspensions through membrane filters. The uric acid uptake was determined spectrophotometrically by the change of filtrate absorbance. The velocity of uric acid uptake was expressed as nmoles of purine taken up by 1 mg (dry weight) cells per 1 min.

Uric acid uptake kinetics were determined in a manner similar to that of the derepression of the transport system, with the difference that the incubation mixture contained uric acid (0.1 mM final conc.).

3. Results

Uric acid uptake by cells previously grown in a medium with a high content of $(NH_4)_2SO_4$ (3 g/ℓ) was characterized by increasing velocity with time of incubation. In the presence of cycloheximide (20 μg/ml) such an increase did not occur; the observed

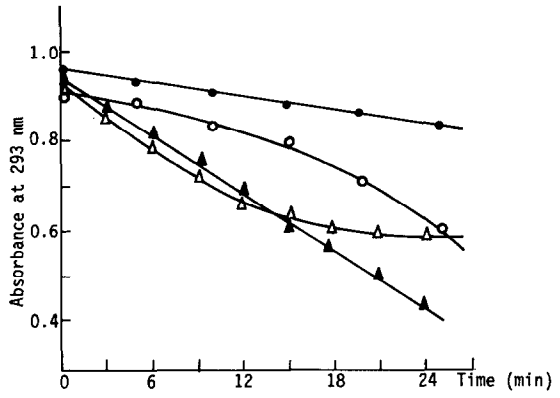

Fig. 1. Kinetics of uric acid uptake by yeast. (○–○–○) Cells (0.3 mg dry weight/ml) incubated without cycloheximide; (●–●–●) cells (0.8 mg dry weight/ml) incubated with cycloheximide (20 μg/ml); (▲–▲–▲) cells (0.1 mg dry weight/ml) preincubated in ammonia-free medium for 2 hr and then incubated with cycloheximide (20 μg/ml); (△–△–△) cells (0.1 mg dry weight/ml) preincubated in ammonia-free medium for 2 hr and then incubated with cycloheximide (20 μg/ml) and $(NH_4)_2SO_4$ (50 mM).

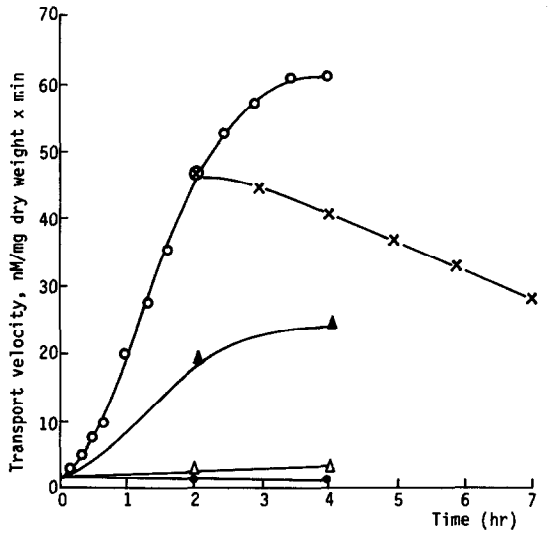

Fig. 2. The effect of different inhibitors on the derepression of uric acid uptake in nitrogen-free medium. (○–○–○) Without inhibitors; (●–●–●) cycloheximide (20 μg/ml) added at zero time; (X–X–X) cycloheximide added after 2 hr of incubation; (△–△–△) euflavine (100 μg/ml) added at zero time; (▲–▲–▲) rubomycin (200 μg/ml) added to cells 1 hr before zero time. For the determination of transport activity in the presence of euflavine or rubomycin the cells were filtered, washed with phosphate buffer, pH 5.5, resuspended in nitrogen-free medium without inhibitor, and uptake velocity determined as described in Experimental.

rate in this case remained the same as during the first minutes of incubation in the medium without antibiotic (fig. 1). When the cells were preliminarily incubated in a medium without nitrogen source, uric uptake in the presence of cycloheximide was linear but at a considerably increased rate. When such cells were incubated with cycloheximide and $(NH_4)_2SO_4$ the transport rate began to decrease after a certain period of time.

The linearity of uric acid uptake in the presence of cycloheximide (at least during the first minutes of incubation) permitted the calculation of the rate of transport per time unit; it was used for further experiments.

The kinetics of the increase of uric acid transport velocity (derepression) in cells incubated in a nitrogen-free medium was studied. Transport velocity for this purine was observed to increase with time and after 3.5 hr of incubation reached its maximum (fig. 2). When the cells were incubated in a nitrogen-free medium in the presence of cycloheximide (20 μg/ml) added at zero time, the rate of uric acid transport did not increase: rather a decrease of transport activity was observed. Derepression of the uric acid transport system was also prevented by rubomycin (an antibiotic apparently similar to daunomycin [12]) and by euflavine (3,6-diamino-10-methylacridine); substances known as inhibitors of nucleic acid biosynthesis.

It is interesting to note that the cells grown in a medium with low amount of $(NH_4)_2SO_4$ (0.8 g/ℓ) contained a derepressed transport system for uric acid. Partial derepression of permease was observed also when yeasts were grown in a medium containing L-glutamate.

When cycloheximide was added to the cells after 2 hr derepression a further increase of uptake velocity did not occur (fig. 2); on the contrary, a decrease of transport rate was observed. After 5 hr incubation in a medium with cycloheximide the rate of transport was diminished by about 40%. This result suggests degradation of uric acid permease when translation is absent.

After adding ammonium ions to derepressed cells the rate of uric acid uptake rapidly diminished (fig. 3). If this decrease is plotted on a semilogarithmical scale

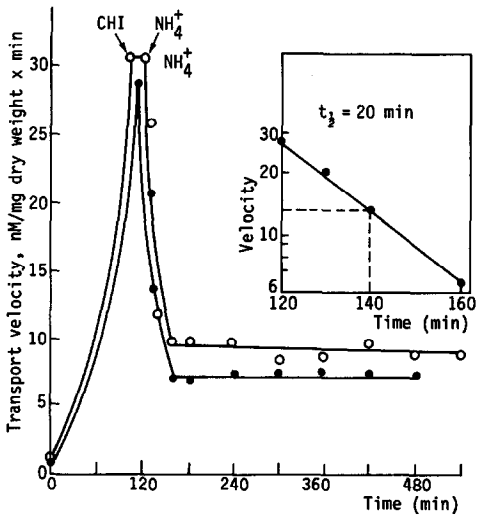

Fig. 3. The effect of NH_4^+ on the rate of uric acid uptake by the derepressed cells. (•–•–•) After 120 min of derepression $(NH_4)_2SO_4$ (50 mM) was added; (o–o–o) after 110 min of derepression cycloheximide (20 μg/ml) was added and after further 10 min, $(NH_4)_2SO_4$ (50 mM). The arrows indicate addition of cycloheximide (CHI) or $(NH_4)_2SO_4$ (NH_4^+). *Insert:* semilogarithmic plot of the inactivation in the presence of $(NH_4)_2SO_4$.

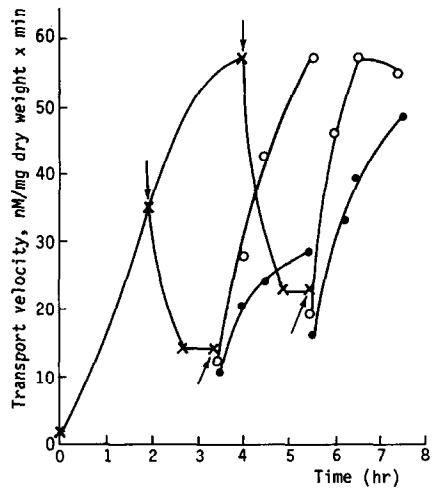

Fig. 4. The recovery of uptake activity after washing the cells from NH_4^+. (o–o–o) The cells washed from NH_4^+ were resuspended in nitrogen-free medium; (•–•–•) the same *plus* cycloheximide (20 μg/ml). The arrows directed downwards indicate addition of $(NH_4)_2SO_4$, the arrows directed upwards indicate washing from NH_4^+.

against time, it is possible to calculate that the period necessary for halving the transport activity under these conditions is 20 min. NH_4^+ did not inhibit the initial rate of uptake and apparently did not compete with uric acid for the acceptor site of permease. Preliminary addition of cycloheximide did not prevent the inhibitory action of ammonium ions; hence, NH_4^+ is not an inducer of some inactivation system of uric acid permease.

After transfer of cells whose transport activity was inhibited by ammonium into a nitrogen-free medium, an increasing rate of transport even in the presence of cycloheximide was observed (fig. 4). This indicates that NH_4^+ does not cause degradation of the permease but acts in another way.

4. Discussion

The data of the present communication allow one to suggest that the regulation of uric acid transport in *P. guilliermondii* is by two different mechanisms – repression–derepression and inactivation.

Although the exact nature of the effector is not determined, such a role may be played by ammonium ions.

The phenomenon of derepression of the uric acid uptake reported here is similar to the derepression of proline transport in *S. chevalieri* [1]. The increase of activity of general amino acid permease in *S. cerevisiae* after transferrring the cells into a medium with a low concentration of ammonium ions [2] can also be explained by the synthesis of transport protein(s).

The mechanism of uric acid permease inactivation in the presence of NH_4^+ ions remains obscure. One may suggest that the ammonium ion acts as a cofactor of a constitutive system of reversible permease inactivation.

Proceeding from our experimental data and from results of the works cited it is possible to make some generalizations about the general features of the regulation of purine, amino acid, and probably other permeases in fungi whose function is to supply the cells with nitrogen sources. After transferring the cells from a medium with a high concentration of NH_4^+ into a medium without nitrogen (or with a low concentration of ammonium ions) derepression of the synthesis

of permeases occurs. When afterwards NH_4^+ is added into the medium repression of transport protein synthesis begins; but as it is too slow a mechanism, the cells use also a more rapid process: an NH_4^+-dependent inactivation of permeases. Since the established steady-state level of activity is still high, further decrease of activity proceeds by means of the repression mechanism. The teleonomic significance of the existence of these two control mechanisms is obvious.

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