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AN INDUCIBLE PROLINE TRANSPORT SYSTEM IN *CANDIDA ALBICANS*

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Summary

1. When *Candida albicans* cells were preincubated with proline or grown in the presence of proline as the sole nitrogen source they exhibited a rapid increase in the influx of proline (the inducible transport system).

2. The induction appeared to be specific for proline and also demonstrated in other *Candida* species.

3. Both the inducible and constitutive proline uptake systems exhibited similar characteristic features.

4. The nature of the inducer for proline uptake in *C. albicans* appeared to be free proline.

5. The development of the inducible proline transport system was dependent on concomitant synthesis of RNA and protein and the induction was not affected by glucose or any other carbon sources used.

Introduction

The existence of well-defined inducible amino acid transport systems that are apparently under genetic control are reported in *Salmonella typhimurium* [1], *Pseudomonas aeruginosa* [2], *Escherichia coli* [3,4] and *Bacillus megaterium* [5]. However, evidences for the existence of such systems in yeast cells are very scarce, though there are few reports on the inducible glucose transport system in *Kluyveromyces lactis* [6] and inducible pentitols transport in *Rhodotorula gracilis* [7]. In addition, an inducible transport system for galactose [8], α-methyl-D-glucoside [9] and maltose [10] have also been reported in baker's yeast. It is demonstrated that the inducible transport systems are subject to catabolite repression or glucose effect [2,4,7,11]. As an extension of our earlier

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work on the characteristics of proline uptake in *Candida albicans* [12—14], it is observed in the present study that proline uptake is induced when cells are grown in presence of proline or preincubated with proline. Furthermore, it is observed that unlike other yeasts (baker's yeast), *C. albicans* cells do not exhibit transinhibition phenomenon [16—19].

Materials and Methods

Chemicals: ^{14}C-labelled amino acids (specific activity: 100 Ci/mol) were purchased from the BARC, Bombay, India. Cycloheximide, ethidium bromide and amino acids were purchased from Sigma Chemical Company, U.S.A. Other chemicals were of analytical grade.

Yeast and growth conditions: C. albicans 3100, a wild-type pathogenic yeast strain, *Candida utilis* 3336, *Candida lipolytica* 3229 and *Candida krusei* 3129 were obtained from the National Chemical Laboratory, Pune, India. In each instance, cells were transferred from a slant into minimal medium containing 0.3% (w/v) KH_2PO_4, 0.3% (w/v) $(NH_4)_2SO_4$, 0.025% (w/v) $CaCl_2$, 0.025% $MgSO_4$ and 0.001% (w/v) biotin with the addition of a carbon source 0.5% (w/v) glucose. Cells were grown at 30°C for 16—17 h and the inoculum was than transferred to the same medium. Cell growth was monitored turbidimetrically by reading the absorbance at 470 nm in a Bausch and Lomb Spectronic 20 spectrophotometer. For uptake measurements cells growing in mid-exponential phase were harvested by centrifugation (1500 × *g* for 10 min), washed three times with sterile distilled water and suspended in the same.

Preincubation of cells: It involved the addition of various amino acids (2 mM) to the exponentially growing cells (500 μg protein/ml of cell suspension). Cells were harvested after an hour of preincubation and used for transport assay. When proline was used as the only nitrogen source, the $(NH_4)_2SO_4$ in the medium was replaced by 0.3% proline (proline-grown cells).

Measurement of [^{14}C]proline uptake: A reaction mixture containing normal or preincubated or proline-grown cells (160—180 μg protein/ml) were incubated at 30°C for 10 min after the addition of cycloheximide (final concentration 200 μg/ml) to inhibit protein synthesis. The reaction was initiated by the addition of ^{14}C-labelled L-proline (1 mM, 5 μCi/ml). At indicated time intervals 0.1 ml aliquots were removed with an Eppendorf pipette and immediately diluted in 5 ml chilled distilled water, or in 5 ml chilled phosphate buffer (0.05 M, pH 7.0). The diluted suspension was rapidly filtered through a 0.45 μm Millipore filter and radioactivity retained was counted in a Packard scintillation counter using a toluene-based scintillation fluid. Protein was estimated according to Lowry et al. [15].

Results

When proline-preincubated *C. albicans* cells were tested for their ability to accumulate proline, they exhibited a significant enhancement in the rate as well as in the level of its total accumulation. A similar pattern was observed in proline-grown cells, however, the extent of enhancement was more pronounced (Fig. 1A). Other strains of *Candida* such as *C. utilis*, *C. lipolytica* and *C. krusei*

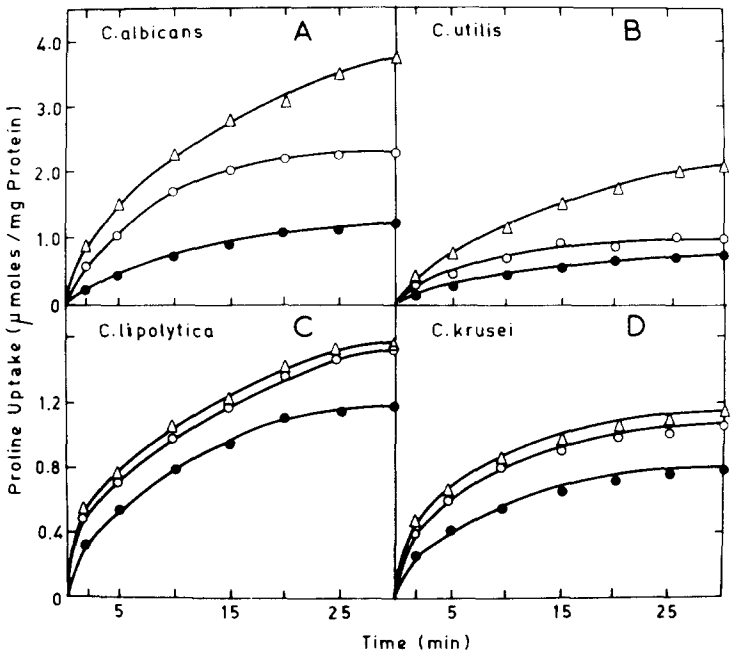

Fig. 1. Uptake of proline in various *Candida* species. Preincubation and proline uptake were done as described in Materials and Methods. ●———●, control; ○———○, preincubated; △———△, proline-grown cells.

have also demonstrated a stimulated rate of proline uptake, but the extent of stimulation in these strains was variable (Fig. 1B—D). The kinetic analysis showed that the apparent K_m for proline uptake in preincubated or proline-grown *C. albicans* cells was similar to normal cells. In contrast, the V values of proline-grown or preincubated cells were significantly higher than the normal cells (Fig. 2; normal cells 0.3 μmol/min per mg protein; proline-preincubated cells 2 μmol/min per mg protein; proline-grown cells 4 μmol/min per mg protein). Other characteristics (sensitivity to various respiratory inhibitors, and ionophores, optimum pH, etc.) of stimulated proline uptake were similar to

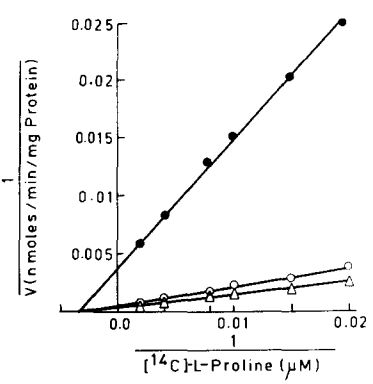

Fig. 2. Lineweaver-Burk plot of proline uptake in *C. albicans* cells. ●———●, control; ○———○, preincubated; △———△, proline-grown cells.

constitutive uptake (data not shown). In order to determine the minimum concentration of proline required to elicit the maximum stimulated rate of proline uptake, different concentrations of proline were used for preincubation of *C. albicans* cells. The results demonstrated that the enhanced rate and level of proline uptake was independent of proline concentration in the range above 0.25 mM. When proline was replaced by other amino acids for preincubation, it was found that out of twenty amino acids only five amino acids, e.g. histidine, threonine, alanine, arginine and cystine had a stimulatory effect on proline uptake (data not shown). It is pertinent to mention here that these five amino acids also compete with the proline uptake in normal cells [12].

In order to ascertain whether the transport of other amino acids can also be amplified, the cells were preincubated with different amino acids and the uptake of their respective ^{14}C-labelled L-amino acids was then followed. As shown in Table I the preincubation of cells with Met, Lys, Ser, Phe, Glu or Leu did not influence the rate as well as the total level of accumulation of these amino acids. The preincubation of cells with proline also had no effect on the uptake of these amino acids (Table I).

The maximum enhancement of proline uptake was achieved within 1 h with an initial lag of 10 min (Fig. 3A). The observed lag for the stimulated rate of proline uptake may be due to the synthesis of a component involved in proline transport. The addition of cycloheximide or ethidium bromide during the preincubation of cells with proline prevented the increased rate and level of accumulation (Fig. 3B). This suggests that synthesis of a new protein may be a prerequisite for an inducible proline transport.

When the preincubation of proline was done in the presence of different carbon sources, namely glucose, fructose, galactose, succinate and *N*-acetylglucosamine the magnitude of inducible proline uptake remained unaffected irrespective of the carbon sources used (Table II). The induction system appears to be unspecific for a particular carbon source.

TABLE I

UPTAKE OF VARIOUS AMINO ACIDS AFTER PREINCUBATING THE CELLS WITH THE SAME UNLABELLED AMINO ACIDS OR WITH PROLINE (2 mM)

The cells were preincubated with indicated amino acids or with proline (2 mM) for 1 h and then the uptake of methionine (1.5 mM), lysine (1.66 mM), serine (0.25 mM), phenylalanine (2 mM), glutamic acid (0.83 mM) and leucine (2 mM) were followed as described in Materials and Methods.

Amino acids	μmol/mg protein per 10 min		
	Control	Preincubation with same amino acids	Preincubation with proline
Methionine	1.13	1.02	1.02
Lysine	1.74	1.65	1.65
Serine	1.50	1.50	1.50
Phenylalanine	2.32	2.35	2.35
Glutamic acid	0.78	0.81	0.78
Leucine	3.10	3.10	3.10

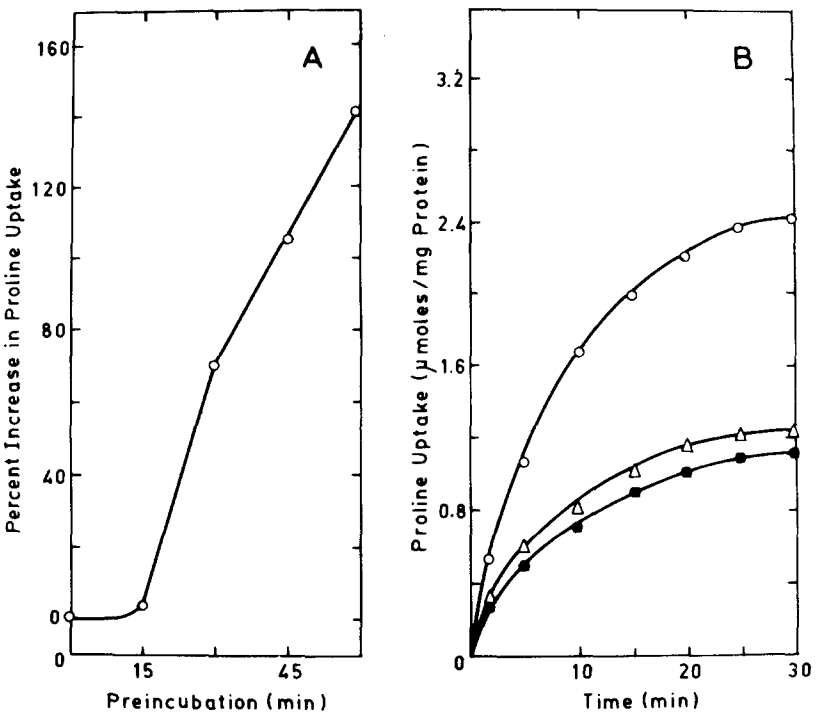

Fig. 3. (A) Proline uptake after preincubating *C. albicans* cells with unlabelled proline (2 mM). Percent increase of proline uptake was calculated as given by

$$100 \times \left(\frac{\text{preincubated} - \text{control}}{\text{control}}\right)$$

(B) Effect of ethidium bromide (140 μM) or cycloheximide (200 μg/ml) on induction. Cycloheximide or ethidium bromide was added along with proline to the growing cells. ○———○, preincubated; preincubation in presence of ethidium bromide (△———△) or cycloheximide (■———■).

Discussion

Several microorganisms, apart from their constitutive transport systems, have been shown to possess inducible transport systems for the uptake of various amino acids [1—5]. A constitutive transport system for the uptake of proline

TABLE II

EFFECT OF VARIOUS CARBON SOURCES ON INDUCIBLE PROLINE UPTAKE

Cells growing in minimal medium containing different carbon sources were induced for 1 h with 2 mM proline and then proline uptake was followed as described in Materials and Methods.

Carbon sources	μmol/mg protein per 10 min	
	Control	Preincubated
Glucose	0.66	1.55
Succinate	0.60	1.20
N-Acetylglucosamine	0.71	1.42
Galactose	0.34	0.68
Fructose	0.63	1.53

was earlier demonstrated in *C. albicans* cells [12—14]. When *C. albicans* cells are grown in the presence of proline as the sole nitrogen source or preincubated with proline under growing conditions, a more efficient system for the influx of proline is induced. The inducible proline uptake was also observed in few other *Candida* species. The results are in contrast to earlier reports [16—21] where the preincubation of various amino acids in *Saccharomyces cerevisiae* and in other microorganisms leads to transinhibition of their subsequent uptake. The transinhibition phenomenon has been referred as a mechanism employed by cells to arrest the indefinite accumulation of various solutes.

The apparent K_m values for proline uptake in both induced and uninduced cells were identical (normal cells 250 μM, induced cells 250 μM). However, the rate of influx of proline (V) in the induced cells was 6—12 fold higher than the uninduced cells. The preincubation of *C. albicans* cells with several other amino acids, e.g. Met, Lys, Ser, Phe, Glu and Leu did not influence their subsequent uptake. This could suggest a specificity for the induction of proline permease. The free form of proline might exert this effect since 90% of the accumulated proline could be recovered as free proline (data not shown). The abolition of stimulated proline uptake by the addition of cycloheximide or ethidium bromide suggests that synthesis of a new protein may be required to exhibit the induction. It appears from the present data that there are two transport systems for proline in *C. albicans* cells namely a 'constitutive' and an 'inducible'. The literature shows paucity in reports where an inducible amino acid transport system has been demonstrated for yeast cells; however, there are several reports to show the repression and derepression of various amino acids permeases [22—26]. NH_4^+ are known to repress the transport of proline, sarcosine and several other amino acids [22—24]. Such repression could be reversed (derepression) either by starving the cells in glucose or galactose-containing media or by growing in a medium containing poor nitrogen source (proline) [22—26]. In contrast to such reports the proline uptake in *C. albicans* cells was neither repressed by NH_4^+ nor there was any derepression when cells grown on NH_4^+ were starved for nitrogen source in a medium containing glucose. All the studied amino acid uptake systems of *C. albicans* did not exhibit any repression by NH_4^+. *C. albicans* cells exhibited a rather specific induction of proline uptake when cells are either grown in proline or preincubated in a medium containing proline. Under similar growing or preincubating conditions, none of the other studied amino acids show any induction. It is pertinent to mention the work of Grenson and Kotyk's group in *S. cerevisiae* [16,17] where the preincubation or preloading of the cells with respective unlabelled amino acids resulted in transinhibition.

It has been previously demonstrated in *S. cerevisiae* that the addition of glucose inactivates the maltose and galactose uptake systems [27,28]. The inducible synthesis of pentitols carrier in *R. gracilis* is also sensitive to catabolite repression [7]. Unlike Reizer and Grossowicz observations [29], our results demonstrate that glucose or any other carbon sources used, could not affect the inducible proline uptake. The specific development of inducible proline uptake upon proline preincubation might constitute an important regulatory difference between baker's yeast and *C. albicans*. The fact that preloading of *C. albicans* cells with unlabelled proline does not inhibit its subsequent uptake argues against a regulation by specific transinhibition.

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References

1 Ratzkin, B., Grabnar, M. and Roth, J. (1978) J. Bacteriol. 133, 737–743
2 Kay, W.W. and Gronlund, A.F. (1969) Biochim. Biophys. Acta 193, 444–455
3 Boezi, J.A. and Demoss, R.D. (1961) Biochim. Biophys. Acta 49, 471–484
4 Burrous, S.E. and Demoss, R.D. (1963) Biochim. Biophys. Acta 73, 623–637
5 Bouknight, R.R. and Sadoff, H.L. (1975) J. Bacteriol. 121, 65–69
6 Royt, P.W. and Macquillan, A.M. (1976) Biochim. Biophys. Acta 426, 302–316
7 Hofer, M., Danle, P. and Kloppel, R. (1975) Biochem. Soc. Trans. 3, 1083–1085
8 Kuo, S.C., Christensen, M.S. and Cirillo, V.P. (1970) J. Bacteriol. 103, 671–678
9 Okada, H. and Halvorson, H.O. (1964) Biochim. Biophys. Acta 82, 538–546
10 De la Fuente, G. and Sols, A. (1962) Biochim. Biophys. Acta 56, 49–62
11 Baxter, L., Mckillen, M. and Syms, M. (1975) Biochem. Soc. Trans. 3, 1205–1208
12 Jayakumar, A. and Prasad, R. (1977) in Proceedings of the National Symposium on Biological Membrane and Model Systems (Talekar, S.V., Balaram, P., Poddar, S.K. and Khetrapal, C.L., eds.), pp. 141–146, Phonexis Press, India
13 Jayakumar, A., Singh, M. and Prasad, R. (1978) Biochim. Biophys. Acta 514, 348–355
14 Singh, M., Jayakumar, A. and Prasad, R. (1978) Arch. Biochem. Biophys. 191, 680–686
15 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
16 Crabeel, M. and Grenson, M. (1970) Eur. J. Biochem. 14, 197–204
17 Kotyk, A. and Rihova, L. (1972) Biochim. Biophys. Acta 288, 380–389
18 Chan, P.Y. and Cossins, E.A. (1976) Plant Cell Physiol. 17, 341–353
19 Morrison, C.E. and Lichstein, H.C. (1976) J. Bacteriol. 125, 864–871
20 Ring, K., Gross, W. and Heins, E. (1970) Arch. Biochem. Biophys. 137, 243–253
21 Pall, M.L. and Kelly, K.A. (1971) Biochem. Biophys. Res. Commun. 42, 940–947
22 Kuznar, J., Schwencke, J. and Magana-Schwencke, N. (1973) Biochim. Biophys. Acta 318, 273–280
23 Schwencke, J. and Magan-Schwencke, N. (1969) Biochim. Biophys. Acta 173, 302–312
24 Grenson, M., Hou, C. and Crabeel, M. (1970) J. Bacteriol. 103, 770–777
25 Seaston, A., Inkson, C. and Eddy, A.A. (1973) Biochem. J. 134, 1031–1043
26 Indge, A., Seaston, A. and Eddy, A.A. (1977) J. Gen. Microbiol. 99, 243–255
27 Gorts, C.P.M. (1969) Biochim. Biophys. Acta 184, 299–305
28 Matern, H. and Holzer, H. (1977) J. Biol. Chem. 252, 6399–6402
29 R[illegible]zer, J. and Grossowicz, N. (1974) J. Bacteriol. 118, 414–424