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EFFECT OF PHOSPHATIDYLSERINE ENRICHMENT ON AMINO ACID TRANSPORT IN YEAST

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A 1.5- to 3.5-fold accumulation of phosphatidylserine was observed when *Candida albicans* and *Saccharomyces cerevisiae* cells were grown in the presence of hydroxylamine, a known inhibitor of phosphatidylserine decarboxylase. However, as compared to *S. cerevisiae* cells, the levels of phosphatidylcholine and phosphatidylethanolamine were much lower in *C. albicans* cells. The enrichment of phosphatidylserine selectively affected the transport of several amino acids.

Introduction

Membranes have multiple sites for a large number of metabolic processes occurring in biological systems and are essential for cell survival. The lipid composition of membranes, therefore, must be precisely and specifically regulated [1–4]. In our laboratory, several approaches have been used to determine the precise role of lipids in cellular permeability of yeast cells [5–7]. In one approach, the lipids of yeast have been manipulated by varying the nature and composition of the environment [5,6] and in another approach, by using the choline or ethanolamine auxotroph [7]. It is observed that altered lipid composition selectively influences the transport of several amino acids [5–7].

In the present study, the level of phosphatidylserine (PS) in *Candida albicans* and *Saccharomyces cerevisiae* membranes is specifically manipulated with hydroxylamine, a drug known to block phosphatidylserine decarboxylase (EC 4.1.1.65) activity [8–10]. The effect of phosphatidylserine enrichment on the uptake of various amino acids is explored.

Materials and Methods

C. albicans (3100) and *S. cerevisiae* (3059) were obtained from the National Chemical Laboratory, Pune, India. The cultures were stored and grown for transport studies as described previously [5,7]. The method of Folch et al. [11] was used for total extraction of lipids. Phospholipids were separated and analyzed by the two-dimensional thin-layer chromatography using silica gel-G as an adsorbant [12,13]. Proteins were determined by the method of Lowry et al. [14].

Results and Discussion

Fig. 1 shows the growth of *S. cerevisiae* and *C. albicans* in liquid synthetic complete medium in the absence and presence of various amounts of hydroxylamine, added at an early logarithmic phase (6 h). An inhibition of growth rates was observed at all the concentrations of hydroxylamine; however, the viabilities of cells were not affected at these concentrations (data not shown).

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Abbreviations: PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine.

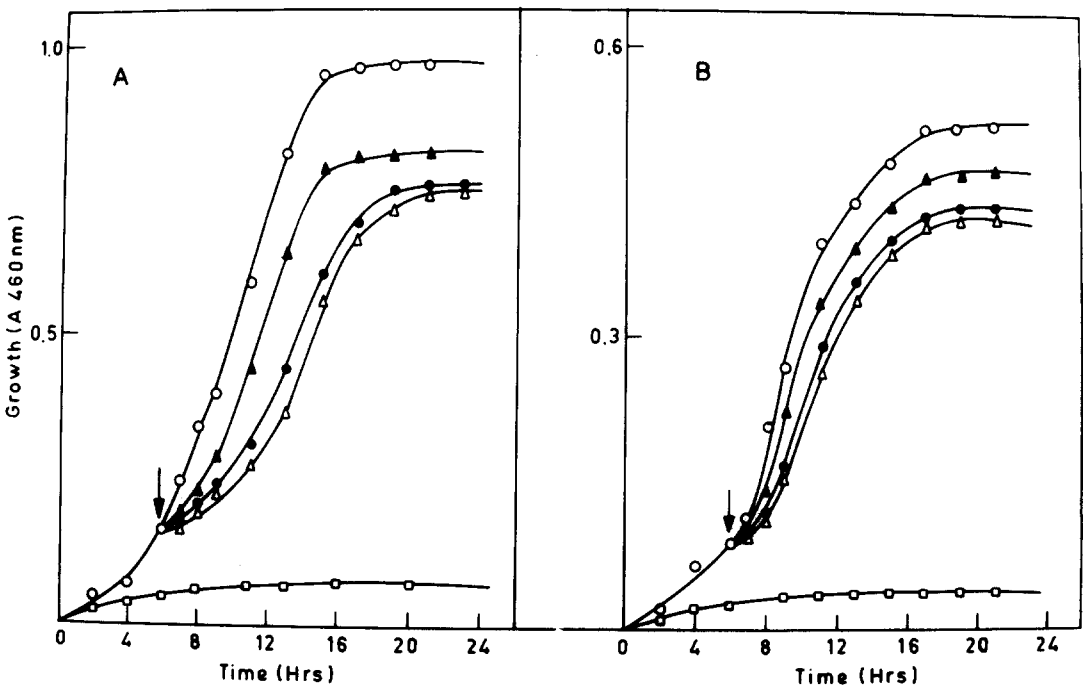

Fig. 1. Growth rate of (A) *Saccharomyces cerevisiae* and (B) *Candida albicans* with varying concentrations of hydroxylamine. Cells were grown in synthetic growth media at 30°C and hydroxylamine was added at the time indicated by an arrow (early logarithmic phase). Final concentrations of hydroxylamine added were: 1 mM (▲———▲); 10 mM (●———●); 20 mM (△———△); none (○———○) in early log phase and 20 mM (□———□) added from the beginning.

If hydroxylamine were added to the medium from the beginning, the same concentration was found to be growth inhibitory (Fig. 1). It seems that hydroxylamine delays the cell growth at an early stage. Since a maximum inhibition in growth was observed at 20 mM concentration of hydroxylamine, this concentration was used for further studies.

Phosphatidylserine is decarboxylated almost as rapidly as it is formed in yeast cells and is present only in traces (6–8% of total phospholipids) [15]. The steady-state phospholipid compositions were examined with and without hydroxylamine (20 mM) under different growth conditions (Tables I and II). It was observed that hydroxylamine-grown cells accumulated 1.5- to 3.5-fold more PS than that in normal cells grown without hydroxylamine. The enrichment in the PS level was maximum when the concentration of this drug was 20 mM (data not shown). The increase in PS level was associated with a decrease in phosphatidylethanolamine content (Tables I and II) as was to be expected from the biosynthetic pathway [15]. This suggests that phosphatidylserine decarboxylase is one of the rate-limiting steps for PE synthesis. Moreover, PS is strikingly different from PE due to an additional net negative charge at neutral pH [16]. The presence of such high levels of PS, therefore, may affect vital functions more severely than the presence of other phospholipids.

An alternative pathway has already been reported for the production of PE and PC to compensate any change in phospholipid composition in *S. cerevisiae* [17]. However, a noticeable decrease in PE and PC levels in *C. albicans* suggests that the alternative pathway may not be as active as reported for higher yeasts. The level of PE and PC in *C. albicans* was about 25–40% lower than that in *S. cerevisiae* (Tables I and II). This would mean that in the absence of an active phosphatidylserine decarboxylase, PE and PC could not be actively synthesized via the Kennedy pathway in *C. albicans* [17]. It is pertinent to mention that prokaryotic cells do not have the Kennedy path-

TABLE I

PHOSPHOLIPID COMPOSITION OF *CANDIDA ALBICANS* IN PRESENCE OF HYDROXYLAMINE

Determination of lipid phosphorus and identification of different phospholipids were done as described earlier [12,13]. Phosphorus contents were multiplied by 25 to give the total phospholipid contents. All the values are an average of three to five separate determinations.

Phospholipid	Total phospholipid (%) of cells grown					
	Without hydroxylamine (h)			With hydroxylamine (h)		
	8	10	12	8	10	12
Phosphatidylinositol	17.4	16.5	14.1	13.9	14.6	12.8
Phosphatidylserine	8.6	7.5	6.6	15.5	17.6	22.4
Phosphatidylcholine	43.1	40.0	39.3	40.5	31.5	24.8
Phosphatidylethanolamine	23.8	24.2	22.5	21.8	20.4	17.0
Cardiolipin + uncharacterized	7.1	12.2	18.0	8.3	15.9	23.0

TABLE II

PHOSPHOLIPID COMPOSITION OF *SACCHAROMYCES CEREVISIAE* IN PRESENCE OF HYDROXYLAMINE

Determination of lipid phosphorus and identification of different phospholipids were done as described for Table I. All the values are an average of three to five separate determinations.

Phospholipid	Total phospholipid (%) of cells grown					
	Without hydroxylamine (h)			With hydroxylamine (h)		
	8	10	12	8	10	12
Phosphatidylinositol	11.8	11.1	12.1	17.2	13.0	17.4
Phosphatidylserine	7.4	6.5	5.5	10.9	14.1	18.0
Phosphatidylcholine	42.7	46.0	44.1	42.8	44.3	38.1
Phosphatidylethanolamine	26.8	29.6	27.6	22.6	22.5	23.8
Cardiolipin + uncharacterized	11.3	7.0	10.7	6.6	6.1	2.7

way and depend solely on the interconversion of PS into PE [2,3,18].

In addition to the accumulation of phosphatidylserine, there was also an increase in the level of cardiolipin in *C. albicans*. An increase in the ratio of cardiolipin to phosphatidylglycerol has also been reported in phosphatidylserine decarboxylase mutant and in hydroxylamine-grown cells of *Escherichia coli* [10,16]. The increase in cardiolipin may be an expression of general response to growth-limiting conditions [16]. There was, however, no apparent change in the total phospholipid levels in either type of yeast (data not shown).

It was of interest to determine whether PS enrichment would in any way influence the cellular permeability of these cells. Accordingly, the transport of phenylalanine, methionine, serine, leucine, glycine, lysine, glutamic acid and proline was investigated. The transport of these amino acids had earlier been demonstrated to be a carrier-mediated, energy-dependent process in these two species of yeast [5,19,20]. As a consequence of PS enrichment, the accumulation of various amino acids was reduced to different levels in both the yeasts (Figs. 2 and 3). The observed reduction in the accumulation of methionine, phenylalanine, glutamic acid, serine and lysine was between 40% and 55% in *S. cerevisiae*, while the uptake of glycine, leucine and proline remained unaffected (Fig. 2). As a result of PS enrichment in *C. al-*

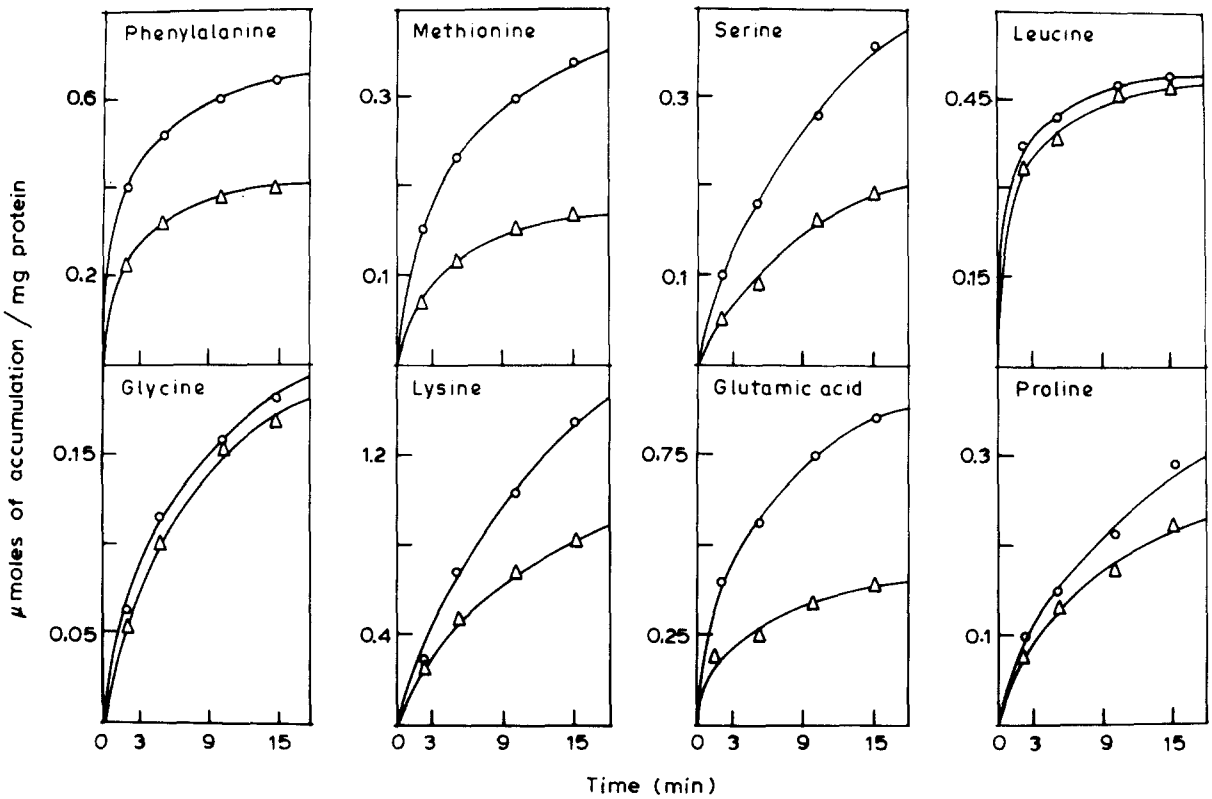

Fig. 2. Uptake of various amino acids in *Saccharomyces cerevisiae* cells. Cells (150–200 μg protein/ml) were preincubated for 10 min with cycloheximide (200 μg/ml) and the uptake was initiated by the addition of ^{14}C-labeled L-amino acids (lysine 1.66 mM; proline 1 mM; glutamic acid 0.83 mM; glycine 0.55 mM; phenylalanine 2 mM; leucine 2 mM; methionine 1.5 mM and serine 1.5 mM) to the assay mixture. The amino acid concentrations were 2- to 3-times their respective K_m values for normal cells. At indicated time intervals, 0.1 ml aliquots were taken out and diluted and filtered, and the retained radioactivity was determined. All the values are an average of three separate determinations. Normal (○——○); and hydroxylamine-incubated (△——△) cells.

bicans cells, the extent of reduction in methionine, phenylalanine, glutamic acid, serine, glycine and leucine accumulation was between 40% and 90%. However, the uptake of lysine and proline was unaffected (Fig. 3).

It is apparent that the extent of reduction in the total accumulation of amino acids is significantly variable in both yeasts. For instance, the reduction in the uptake of lysine was 40% in *S. cerevisiae*, while it was unaffected in *C. albicans* cells. On the other hand, the reduction in glycine and leucine uptake was more pronounced in *C. albicans* as compared to *S. cerevisiae* cells (Figs. 2 and 3). These variations could be due to the differences in strains and phospholipid compositions.

This constitutes another report in this series [10,12] to demonstrate the importance of phospholipids in yeast membrane structure and function. A heterogeneous effect of PS enrichment on transport activities has earlier been reported [10,16]. It is possible that the activities of functional proteins related to each transport system might be activated or suppressed because of their conformational changes due to altered phospholipid composition. The inability of *C. albicans* to make up the loss of PE and PC levels due to the blockade of phosphatidylserine decarboxylase is an interesting area for further research. Our preliminary results indicate that, unlike *S. cerevisiae*, the supplementation of *C. albicans* cells with choline or ethanolamine did not result in the enrichment of their respective phospholipids. The significance of these results is being further investigated.

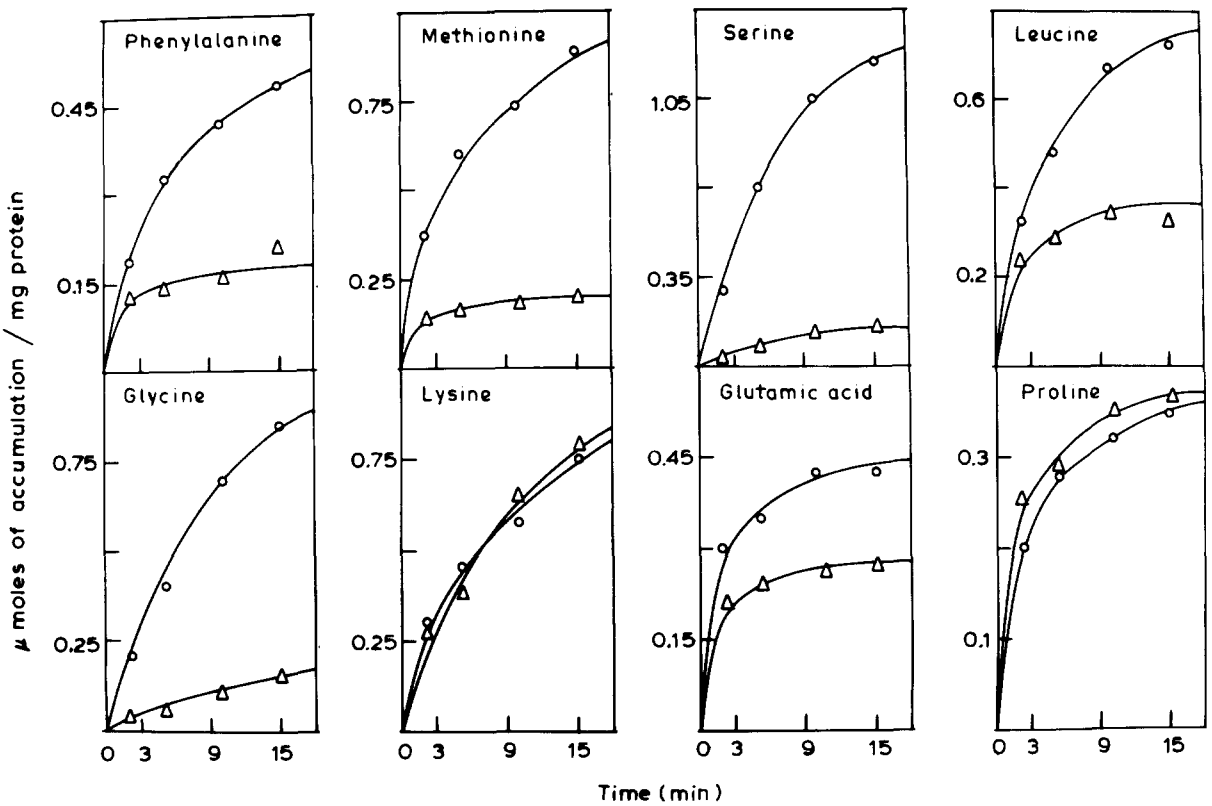

Fig. 3. Uptake of various amino acids in *Candida albicans*. Assay conditions were similar to those described in Fig. 2. Normal (○——○); and hydroxylamine-incubated (△——△) cells.

Acknowledgements

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