Phosphatidylserine Synthesis Required for the Maximal Tryptophan Transport Activity in Saccharomyces cerevisiae

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Saccharomyces cerevisiae cho1/pss mutants, which are severely impaired in phosphatidylserine (PS) synthesis, do not have detectable amounts of PS in their lipid fractions. Their derivatives with mutations that cause defects in tryptophan synthesis grew poorly in a medium containing 5 μg/ml of L-tryptophan, a concentration that met the requirements of tryptophan-auxotrophic CHO1/PSS strains. The rates of tryptophan uptake of trp1 cho1/pss mutants were low at low tryptophan concentrations. This defect in the use of tryptophan was restored either by expression of CHO1/PSS or by introduction of a gene encoding tryptophan transporter, TAT1 or TAT2. These results indicate that PS synthesis is required for the maximal tryptophan-transporting activity of S. cerevisiae at low tryptophan concentrations.

Key words: phosphatidylserine synthesis; phospholipid; Saccharomyces cerevisiae; tryptophan transporter

Membrane phospholipids form stable bilayer structures and provide many membrane-associated proteins proper circumstances for their activities. In general, roles of the phospholipids in biomembrane seem to be passive ones, but some kinds of phospholipids are more actively involved in the functions of membrane enzymes.1-3 However, only a limited amount of information is available about the roles of the individual phospholipid species on the membrane-associated protein activities.

In Saccharomyces cerevisiae, phosphatidylserine (PS) is synthesized from l-serine and CDP-diacylglycerol by PS synthase that is encoded by the CHO1/PSS gene.4,5 A cho1/pss null mutant was viable when ethanolamine or choline was present in the medium, though they grew more slowly than the wild type CHO1/PSS strains. Since the cho1/pss null mutant had neither detectable PS synthase activity nor PS,6 investigation of its properties will indicate the role of PS in yeast cells. In a preceding study, Hamamatsu et al. showed that the cho1/pss disruptant had aberrant vacuolar functions, suggesting that some proteins involved in vacuolar functions require PS for their activities.7

S. cerevisiae takes up exogenous amino acids by amino acid transporters that reside in the plasma membrane.8-13 S. cerevisiae has two kinds of amino acid transporters. One is the general amino acid permease, which can transport more than ten kinds of amino acids, the activity of which is controlled by cellular content of nitrogen source.14 The other is specific transporters to individual amino acids. In general, S. cerevisiae seems to have more than one transporters for each kind of amino acid, which are different in capacity and affinity for their substrates.

S. cerevisiae internalizes exogenous tryptophan by the general amino acid permease and specific transporters for tryptophan. S. cerevisiae has two specific transporters for tryptophan; one, encoded by TAT1, is of low capacity and the other, encoded by TAT2, is of high capacity. The former transporter is actually a tyrosine transporter.15

In this report, we present another example of sensitive alteration of tryptophan transporting activity in response to the change of membrane lipid synthesis. We found a higher tryptophan requirement of a cho1/pss trp1 double mutant of S. cerevisiae and demonstrated that the tryptophan transport activity of S. cerevisiae was dependent on the cellular PS synthesis.

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Abbreviation: PS, phosphatidylserine
Materials and Methods

Chemicals. L-[14C]tryptophan and other 14C-labelled amino acids were purchased from ARC (St. Louis, MO). Constituents of media were from Difco. Other chemicals used were of reagent grade.

Growth conditions, strains, and plasmids. S. cerevisiae SY18A (MATa cho1/pss), a cho1/pss mutant auxotrophic for ethanolamine, was isolated from mutagenized colonies of strain X2180-1A (MATa gal2 SUC2) and purified by crossing twice with X2180-1B (MATa gal2 SUC2). SY18A was crossed with C5558-2B (MATα trp1 ura3) and SY188 (MATa cho1/pss trp1 ura3) was examined. The strain SY18A was also crossed with strains S178B (MATα trp3 ura1 ura2) and KYc56 (MATα trp3 his6 leu1 pho8) to obtain strains SY18T3 (MATa cho1/pss trp3) and SY18T4 (MATa cho1/pss trp4), respectively. Strains S178B and KYc56 were from S. Harashima (Osaka University). Construction of strain EY132 (MATα cho1::LEU2 trp1 ura3) was described previously.

Construction of plasmid Ycps50GPSS bearing URA3 and CHO1/PSS of which expression was under the control of GAL7 promoter was described previously. Minimal synthetic medium, SD, for yeast growth was prepared as described previously. When necessary, 2% d-glucose in SD was replaced with 2% d-galactose and 0.2% d-glucose for induction of GAL7 promoter (SDG medium). Tryptophan was added after filter sterilization. Yeast cells were transformed using the alkali cation treatment method. Escherichia coli strain JA221 (F− recA1 leuB6 trpDE5 hisDR− hisDM+ lacY) was used as a general bacterial host for plasmids.

Phospholipid analysis. Yeast phospholipids were labeled with [32P]Pi for at least five generations at 30°C, extracted, separated, and measured as described previously.

Analysis of tryptophan uptake. Yeast cells were grown to the exponential phase (OD600 = 0.6–0.8) in SD medium containing 100 µg/ml of L-tryptophan and harvested by centrifugation. Cells were washed with the same medium without tryptophan and suspended in fresh SD medium to a concentration of 1.0×10^6 cells/ml. Samples (480 µl each) were withdrawn and mixed with 20 µl of 125 µg/ml of L-[14C]tryptophan and incubated at 30°C for indicated periods. Cells were then collected rapidly on glass-fiber filters (Whatman GF/C) and immediately washed 5 times with 5 ml of SD medium containing 100 µg/ml of tryptophan. Filters were transferred to scintillation vials, dried, and counted for radioactivity with 5 ml of scintillator Aquasol-2 (NEN). Cell numbers were counted by hemocytometer.

Results

cho1/pss trp double mutants require high concentrations of tryptophan

The cho1/pss mutant SY18A was defective in the PS synthase activity and did not contain PS at a detectable level (Table 1). This strain grew when ethanolamine or choline was present in the medium. We crossed it with the strain C5558-2B to introduce trp1 marker. Interestingly, all cho1/pss trp1 double mutants grew very poorly on YPD medium. They grew normally when tryptophan or indole were added to the YPD medium at concentrations of more than 50 µg/ml.

We examined the tryptophan-dependent growth of one such trp1 cho1 strain, SY188, in a liquid medium (Fig. 1A). When the concentration of tryptophan in the medium was 5 µg/ml, this strain grew at a slow rate and reached stationary phase at a density lower than that of the strain C5558-2B. The same SY188 grew as well as C5558-2B when the concentration of tryptophan was 100 µg/ml. Though the extent of the dependence on the high concentrations of trypto-

<table>
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<tr>
<th>Strain</th>
<th>% of total phospholipids</th>
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<tr>
<td></td>
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<tr>
<td>C5558-2B</td>
<td>36.8</td>
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<tr>
<td>SY188</td>
<td>43.7</td>
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<td>EY132/Ycps50</td>
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<td>EY132/Ycps50GPSS(Gal)</td>
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Mediation used for cultivation: YPD, SD containing 1 mM ethanolamine and 10 µM tryptophan; SDG containing 1 mM ethanolamine and 100 µM tryptophan. Abbreviations are: PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

Fig. 1. Growth of the cho1/pss trp Double Mutants in the Medium Containing Various Concentrations of Tryptophan.

Cells were grown in SD medium with 100 µg/ml (circle) or 5 µg/ml (triangle) of tryptophan or without tryptophan (square) at 30°C. (A) C5558-2B (CHO1/PSS trp1, open symbols) and SY18A (cho1/pss trp1, closed symbols). (B) KYc56 (CHO1/PSS trp4, open symbols) and SY18T4 (cho1/pss trp4, closed symbols).
phosph was different from that of the strain SY188, ch01/pss mutants with trp3 or trp4 mutation also required high concentrations of tryptophan for their growth, suggesting that any mutations in the tryptophan-synthetic pathway should cause similar defects in tryptophan use. Figure 1B shows that a ch01/pss trp4 mutant did not grow at all when the concentration of tryptophan was 5 µg/ml. Based on these results, we assumed that the ability of exogenous tryptophan use was impaired in the ch01/pss mutants.

To confirm further the relationship between the PS synthesis and the tryptophan uptake, we introduced a centromere plasmid YCp50GPSS, which bears a CHO1/PSS gene of which promoter was replaced with that of the GAL7 gene, into a ch01 disruptant EY132. In a medium containing glucose and 5 µg/ml of tryptophan, neither the strain EY132 with YCp50GPSS (EY132/YCp50GPSS) nor the strain EY132 with the vector YCp50 (EY132/YCp50) formed colonies, while they grew on a medium containing 120 µg/ml of tryptophan (Fig. 2, right two panels). When the carbon source was changed to galactose, the strain EY132/YCp50GPSS, which grows well without ethanolamine or choline because of the induced PS synthase activity, grew well on the medium containing 5 µg/ml of tryptophan, but the strain EY132/YCp50 did not (Fig. 2, left two panels).

**Rate of tryptophan uptake of the ch01/pss disruptant**

Under conditions in which the tryptophan concentration was adjusted to 120 µg/ml, the strain EY132/YCp50GPSS that was grown in a medium containing galactose (SDG) took up tryptophan at a similar rate to that of the same strain grown in the glucose-containing medium (SD) (Fig. 3A). When the rate of tryptophan uptake was measured with the tryptophan concentration adjusted to 5 µg/ml, the strain EY132/YCp50GPSS that was grown in the SDG medium took up tryptophan at an about 5 times higher rate than that of the same strain grown in a SD medium (Fig. 3B). The strain EY132/YCp50 grown on either carbon source took up tryptophan at similar rates to that of the strain EY132/YCp50GPSS grown in the SD medium. The PS content of the latter strain was severely reduced in the SD medium (Table 1). Thus, it is clear that the defect in PS synthesis caused a decrease in tryptophan-uptake activity and that this is a likely reason why the ch01/pss trp1 mutants required high concentrations of tryptophan for their growth.

**Introduction of TAT1 or TAT2 and restored growth of the ch01/pss trp1 mutant at the low concentration of tryptophan**

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**Fig. 2.** Tryptophan-dependent Growth of the ch01/pss Disruptant.

The strain EY132 which contained the plasmid YCp50GPSS or YCp50 were grown for 2 days at 30°C on SD or SDG agar plates containing 120 µg/ml or 5 µg/ml of tryptophan.

**Fig. 3.** Tryptophan Uptake by the Strain EY132/YCp50GPSS.

Cells were grown in the medium containing glucose or galactose and measured uptake of L-[14C]tryptophan in the same medium as those used for cultivation, but containing 120 µg/ml (4.4 × 10^4 cpm/nmol) (A) or 5 µg/ml (1.8 × 10^5 cpm/nmol) (B) of tryptophan, respectively.
We introduced the tryptophan transporter genes \textit{TAT1} or \textit{TAT2} into the strain EY132 by YCp plasmids, YCplac33TAT1 or YCplac33TAT2, and obtained transformants EY132/YCplac33TAT1 or EY132/YCplac33TAT2, respectively. They grew on a low-tryptophan medium, while a strain that carried the vector YCplac33 did not (Fig. 4). The strain EY132/YCplac33TAT2 took up tryptophan even at a higher rate than the strain EY132/YCp50GPSS grown in the galactose-containing SDG medium (Fig. 5). These results suggest that the requirement for a high concentration of tryptophan by \textit{chol/pss trp1} mutant is due to a reduced number or impaired function of the tryptophan transporters, which is caused by the deficiency of PS synthesis.

\textbf{Transport activities of leucine and lysine were not affected by the deficiency of PS synthesis.}

To examine relationships between the PS synthesis and other amino acid transporters, we measured uptake rates of the leucine and lysine by the strain.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Introduction of \textit{TAT} Gene Restored the Growth of the \textit{chol/pss trp1} Mutant at the Low Concentration of Tryptophan.}
\end{figure}

The strains EY132 with the indicated plasmids were grown at 30°C for 2 days on the SD medium containing 5 \(\mu\)g/ml (A) or 120 \(\mu\)g/ml (B) of tryptophan.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5.png}
\caption{Effects of the Introduction of \textit{TAT2} Gene on the Tryptophan Uptake by the Strain EY132.}
\end{figure}

With the plasmid YCp50GPSS (circle) or the plasmid YCplac33TAT2 (square) in SD (open symbols) or in SDG (closed symbols) containing 5 \(\mu\)g/ml of tryptophan.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Uptake of Leucine or Lysine by the Strain EY132/YCp50GPSS.}
\end{figure}

Cells were grown in the medium containing glucose (open circles) or galactose (closed circles). Reaction media were the same as those used for cultivation except for the presence of 25 mM of \[^{14}\text{C}\]-L-leucine (A) or 25 mM of \[^{14}\text{C}\]-L-lysine (B).
Maximal Tryptophan Transport Activity Requires PS Synthesis

EY132/YCp50GPSS. When the concentration of leucine or lysine was adjusted to 25 \( \mu \)M, the strain EY132/YCp50GPSS that was grown on the SD medium took up leucine or lysine at similar rates to those of the same strain grown in the galactose-containing SDG medium (Fig. 6), suggesting that transporters for these amino acids were not affected by the deficiency of PS synthesis.

Discussion

The observed poor growths of the \( \text{cho}1/pss \text{ trp}1 \) double mutants in the low-tryptophan medium are due to the reduced tryptophan transport activity under the conditions where PS synthesis was defective. This conclusion is derived from the following lines of evidence. First, the expression of the \( \text{CHO}1/PSS \) gene, which restored cell growth of the \( \text{cho}1/pss \text{ trp}1 \) strain in the low-tryptophan medium, increased the rate of tryptophan uptake. Second, \( \text{cho}1/pss \) mutants with other \( \text{trp} \) mutations also required high concentrations of tryptophan, indicating that \( \text{trp}1 \) mutation itself is not specifically involved in this phenomenon. Third, introduction of either gene encoding the tryptophan transporter Tat1p or Tat2p restored tryptophan uptake and growth of the \( \text{cho}1/pss \text{ trp}1 \) strain in the low-tryptophan medium. This last evidence also suggests that the reduced tryptophan transport activity of the \( \text{cho}1/pss \) strain is due to the reduced number or the impaired activity of the tryptophan transporters in the PS-deficient cells. Since the transporter genes were introduced on a low-copy-number vector, only a 2 to 3-fold quantitative increase of tryptophan transporters seems to be sufficient for supplying the required tryptophan by \( \text{cho}1/pss \text{ trp}1 \) cells. We suppose that yeast cells have only a limited number of the tryptophan transporters that are not efficient at low concentrations of tryptophan. Therefore, their reduction in number or in activity, which is somehow caused by the defective PS synthesis in the \( \text{cho}1/pss \) mutants, resulted in very diminished use of tryptophan in a medium containing 5 \( \mu \)g/ml of tryptophan. This concentration is close to the lower limit that can support the growth of \( \text{trp} \) mutants, since a \( \text{CHO}1/PSS \text{ trp}1 \) strain did not grow at all in a medium containing 1 \( \mu \)g/ml of tryptophan (data not shown). Which transporter of Tat1p or Tat2p was more susceptible to PS deficiency is not clear. However, Tat2p is a high-capacity transporter and hence, it is likely that the function of Tat2p was more affected than that of Tat1p.

The content of PS is very high in the plasma membrane (30% of total phospholipids) in comparison with those in other membranes (4-8% of total phospholipids). It is very likely, therefore, that proteins that function in the plasma membrane are influenced by the deficiency of PS. In the case of ergosterol, which is also concentrated in the plasma membrane, Gaber \textit{et al.} reported that the rate of tryptophan uptake was low in an \textit{erg6} disruptant which was defective in ergosterol synthesis. We suppose that PS as well as ergosterol contributes to the proper environment for the activities of tryptophan transporters in the plasma membrane.

Neither a \( \text{cho}1/pss \text{ his}3 \) mutant nor a \( \text{cho}1/pss \text{ ura3} \) mutant required high concentrations of histidine or uracil, respectively (data not shown), and the transport activities of leucine and lysine were not affected by the deficiency of PS synthesis, although the strain used in the experiment was not auotrophic for these amino acids. Thus, tryptophan transporters seem to be different from other transporters and especially susceptible to the change of membrane lipids. The observations that the \( \text{trp}1 \) mutants often had a cold-sensitivity defect and this phenotype was suppressed by genes encoding amino acid permeases and that sphingolipid long chain bases inhibited tryptophan transport support this speculation.

However, there are other possibilities how the loss of PS synthesis leads to the decreased tryptophan transporter activity. An immunosuppressant drug FK506 produced a similar change in tryptophan transport activity to that by the deficiency of PS. Though this drug is lipophilic and might bind to the plasma membrane and produce a similar effect on tryptophan transporters to the deficiency of PS synthesis, inhibition of protein phosphatases by FK506 might somehow decrease the activity of tryptophan transporters. Similarly, the deficiency of PS which activates protein kinase C or deficiency of ergosterol which has a cell-cycle-sparking effect might also somehow produce inhibitory effect on tryptophan transporters.

Our results presented here show the importance of PS or PS synthesis for the proper function of tryptophan transporting system in yeast. We expect that future analyses on the tryptophan transporters at the molecular level will reveal how this peculiar phospholipid functions in the cellular process.

Acknowledgments

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References