CHAPTER II

EFFECT OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYL-
ETHANOLAMINE ENRICHMENT ON THE STRUCTURE AND
FUNCTION OF YEAST MEMBRANE

The lipid composition of yeast plasma membrane can be manipulated in a predictable manner by varying the nature and composition of the environment (Waechter and Lester, 1971; Hunter and Rose, 1972; Ratcliffe et al., 1973; Jigami et al., 1979; Beaven et al., 1982). The same can also be achieved by using mutants unable to synthesize specific membrane lipids (Atkinson et al., 1980a, b; Nikawa and Yamashita, 1981, 1982). Similar to various procaryotic and eucaryotic systems (Fourcans and Jain, 1974; Silbert, 1975; Cronan, 1978; Hawrot and Kennedy, 1978; Raetz, 1978; Green et al., 1981), the altered lipid composition of yeast may
help in elucidating the physiological role of lipids. The transport phenomenon in yeast is well characterized (Seaston et al., 1973, 1976; Eddy et al., 1977; Larimore and Roon, 1978; Stepanni and Ramos, 1978; Jayakumar et al., 1979; Merkel et al., 1980; Theuvenet and Bindels, 1980; Ohsumi and Amraku, 1981), however, the role of lipid in its cellular permeability is less documented.

Yeast mutants auxotrophic for the phospholipid precursors, fatty acids (Resnick and Mortimer, 1966; Schweizer and Bolling, 1970; Henry and Fogel, 1971), inositol (Culbertson and Henry, 1975; Donahue, 1979; Greenberg et al., 1981; Nikawa and Yamashita, 1982), choline/ethanolamine (Letts and Dawes, 1979; Atkinson et al., 1980a,b; Kovac et al., 1980) and temperature-sensitive for total phospholipid synthesis (Letts, 1980) have been described. However, in studies pertaining to membrane properties, the yeast mutants have not been exploited to the same degree as have bacterial mutants (Keith et al., 1973; Silbert, 1975; Raetz, 1978).

Saccharomyces cerevisiae Cho mutants (Appendix D), defective in a single step of phospholipid
biosynthesis, have been utilized in our present work to elucidate the involvement of membrane phospholipid components in its structure and function. These mutants, which have earlier been isolated (Atkinson et al., 1980b), permit the specific manipulation of PC and PE levels of yeast membrane. The effect of such an exclusive phospholipid change on nutrient (amino acids) uptake is investigated. A fluorescent probe, ANS, is also used to monitor structural changes resulted due to altered phospholipid composition.

It has also been shown earlier by Hossak et al. (1977) and others (Waechter and Lester, 1971, 1973; Hunter and Rose, 1971, 1972) that the growth of \textit{S. cerevisiae} cells in high concentrations of choline- or ethanolamine-supplemented medium led to an enrichment of PC and PE levels, respectively. Therefore, besides the above auxotrophic strains, we have used \textit{S. cerevisiae} (3059) cells and grown it in choline- or ethanolamine-supplemented medium to ascertain if the percentage increase in PC and PE contents by two different ways have similar effects on structure and function of yeast membrane.
RESULTS

Growth Characteristics of the Mutants

Similar to wild-type cells (ade 5α), the mutants were also able to grow well in YEPD medium (Appendix A) without any additional supplements. However, the auxotrophs grown on SCM (Appendix A) required the supplementation of 1 mM choline or 1 mM ethanolamine (Figs. 13A and B). In the absence of any supplement, mutants did not grow after one generation (Atkinson et al., 1980b). The initial lag phase of 1-4 h was common to both type of cells, however, the final growth achieved by wild-type cells was 2- to 3-times more than either of these mutants. (Figs. 13A and B).

Growth Characteristics of S. cerevisiae (3059)

Cells were grown in SGM (Appendix A) with the supplementation of varying concentrations of choline or ethanolamine (Figs. 14A and B) to check the maximum possible enrichment of respective phospholipid. Phospholipid composition revealed that 20 mM concentration of either choline or ethanolamine resulted in the maximum enrichment of respective phospholipid.
Fig. 13: Growth rates of: (A) wild-type (○——○), cho1 grown in (△——△) choline- and in (●——●) ethanolamine-containing medium; (B) wild-type (○——○), Cho⁺ grown in (△——△) choline- and in (●——●) ethanolamine-containing medium.
Fig. 13.
Fig. 14: Growth rates of *Saccharomyces cerevisiae* (3059) with varying concentrations of: (A) non-supplemented (Δ—Δ), 1 mM (○—○), 10 mM (▲—▲) and 20 mM (●—●) choline-supplemented medium; (B) non-supplemented (Δ—Δ), 1 mM (○—○), 10 mM (▲—▲) and 20 mM (●—●) ethanolamine-supplemented medium.
Fig. 14.
It should be pointed out, however, that the overall growth was not affected significantly between 10 and 20 mM supplementation of choline or ethanolamine. Higher concentrations of these precursors led to severe alkanity and ultimate cell death. The inhibitory effects of higher concentration (20 mM) were overcome by adjusting the pH of the medium to 6.5.

Lipid Composition of Crude Plasma Membrane of Mutants and \textit{S. cerevisiae} (3059)

A. Total lipid and phospholipid contents

There was no significant change in the total lipid contents between the mutants and the wild-type cells (Table 11). However, as compared to wild-type cells, the total phospholipid contents were decreased in Cho* cells which were between 30% and 40%.

The supplementation of high concentration of choline or ethanolamine did not result in any noticeable change in total lipid and phospholipid levels of \textit{S. cerevisiae} (3059) (Table 11).

B. Ergosterol and triglyceride contents

Similar to total lipid and phospholipid contents of crude plasma membrane, no major difference was found in the total ergosterol levels (Table 12). A
TABLE II
TOTAL LIPID AND PHOSPHOLIPID CONTENTS OF SACCHAROMYCES CEREVISIAE CRUDE PLASMA MEMBRANE

Lipid extraction and estimation were done as described in 'Materials and Methods'. All values are an average of two to three separate determinations.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Medium supplemented</th>
<th>_ _ _ Lipid composition _ _ _ _ _ _</th>
<th>Total lipid (mg/g dry wt)</th>
<th>Phospholipid (mg/gm total lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ade 5α</td>
<td>none</td>
<td></td>
<td>120</td>
<td>50</td>
</tr>
<tr>
<td>KA101</td>
<td>(cho1) choline</td>
<td></td>
<td>115</td>
<td>43</td>
</tr>
<tr>
<td>KA101</td>
<td>(cho1) ethanolamine</td>
<td></td>
<td>110</td>
<td>49</td>
</tr>
<tr>
<td>MC13</td>
<td>(Cho+) choline</td>
<td></td>
<td>110</td>
<td>32</td>
</tr>
<tr>
<td>MC13</td>
<td>(Cho+) ethanolamine</td>
<td></td>
<td>110</td>
<td>36</td>
</tr>
<tr>
<td>3059</td>
<td>none</td>
<td></td>
<td>108</td>
<td>41</td>
</tr>
<tr>
<td>3059</td>
<td>choline</td>
<td></td>
<td>108</td>
<td>35</td>
</tr>
<tr>
<td>3059</td>
<td>ethanolamine</td>
<td></td>
<td>110</td>
<td>39</td>
</tr>
</tbody>
</table>
TABLE 12

ERGOSTEROL AND TRIGLYCERIDE CONTENTS OF SACCHAROMYCES CEREVISIAE CRUDE PLASMA MEMBRANE

Ergosterol and triglyceride contents were estimated as described in 'Materials and Methods'. All values are an average of two to three separate determinations.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Medium supplemented</th>
<th>Lipid composition (mg/gm of total lipid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ergosterol</td>
</tr>
<tr>
<td>Ade 5α</td>
<td>none</td>
<td>20</td>
</tr>
<tr>
<td>KA101</td>
<td>choline</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>ethanolamine</td>
<td>18</td>
</tr>
<tr>
<td>MC13</td>
<td>choline</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>ethanolamine</td>
<td>20</td>
</tr>
<tr>
<td>3059</td>
<td>none</td>
<td>39</td>
</tr>
<tr>
<td>3059</td>
<td>choline</td>
<td>31</td>
</tr>
<tr>
<td>3059</td>
<td>ethanolamine</td>
<td>34</td>
</tr>
</tbody>
</table>
noticeable decrease in total triglyceride contents was observed in ethanolamine-supplemented cells. The total ergosterol and triglyceride contents of crude plasma membrane fraction of \textit{S. cerevisiae} (3059) did not change significantly when the cells were grown in choline- or ethanolamine-supplemented medium (Table 12).

C. \textbf{Phospholipid composition}

The two auxotrophic mutants, choi and Cho\(^+\), have already been well characterized (Atkinson \textit{et al.}, 1980a,b). Cho mutant appears to use almost exclusively the alternate pathway as described by Kennedy and Weiss (1956) (Fig. 2) for the production of PE and PC, bypassing PS as an intermediate. However, the activity of methyltransferase(s) in the auxotroph Cho\(^+\) is probably altered (Atkinson \textit{et al.}, 1980a). These mutant cells, depleted of two phospholipid bases, were unable to grow in medium deficient in phospholipid bases but remained viable for sometime. Due to the differences in phospholipid synthetic pathway of these mutants, the phospholipid composition of non-supplemented cells was different. For example, the levels of PC and PE were very low in choi due to non-supplementation of exogenous bases (data not shown).
Therefore, in the present work the phospholipid composition and amino acids transport in choline- or ethanolamine-grown cells has been compared with their parent wild-type strain (ade 5α).

The phospholipid composition of wild-type, choi and Cho⁺ strains was examined under different growth conditions. Crude plasma membrane fraction, isolated from different cells, was used for phospholipid extraction and analysis. As can be seen from Table 13 both choi and Cho⁺ when grown in media containing choline chloride (1 mM) resulted in PC accumulation. As compared to the wild-type strain, the increase in PC content was about 15% in both the auxotrophs. However, when these auxotrophs were grown in media containing ethanolamine (1 mM), the increase in total PE content was between 30% and 55%. The significant decrease in PC level of Cho⁺ cells grown in media containing ethanolamine is probably due to its defective methyl-transferase(s) activity (Atkinson et al., 1980a). It is pertinent to mention here that the addition of either choline or ethanolamine to the growth media of wild-type strain did not change the level of PC or PE (Atkinson et al., 1980a).
**TABLE 13**  
**PHOSPHOLIPID COMPOSITION OF CRUDE PLASMA MEMBRANE OF SACCHAROMYCES CEREVISIAE**

Determination of lipid phosphorus and identification of different phospholipids were done as described under 'Materials and Methods'. Phosphorus contents were multiplied by 25 to give the total phospholipid contents. All the values are an average of three to five separate determinations.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Media supplemented</th>
<th>PI+PS</th>
<th>PC</th>
<th>PE</th>
<th>CL</th>
<th>PC/PRa</th>
</tr>
</thead>
<tbody>
<tr>
<td>ade 5α</td>
<td>none</td>
<td>23.9</td>
<td>48.5</td>
<td>19.1</td>
<td>8.5</td>
<td>2.6</td>
</tr>
<tr>
<td>KA101</td>
<td>choline</td>
<td>25.8</td>
<td>56.5</td>
<td>14.5</td>
<td>3.3</td>
<td>3.9</td>
</tr>
<tr>
<td>KA101</td>
<td>ethanolamine</td>
<td>26.6</td>
<td>44.7</td>
<td>24.7</td>
<td>4.0</td>
<td>1.8</td>
</tr>
<tr>
<td>MC13</td>
<td>choline</td>
<td>24.8</td>
<td>55.5</td>
<td>12.6</td>
<td>7.1</td>
<td>4.4</td>
</tr>
<tr>
<td>MC13</td>
<td>ethanolamine</td>
<td>24.9</td>
<td>36.2</td>
<td>29.8</td>
<td>9.1</td>
<td>1.3</td>
</tr>
<tr>
<td>3059</td>
<td>none</td>
<td>20.6</td>
<td>42.9</td>
<td>16.7</td>
<td>4.2</td>
<td>2.6</td>
</tr>
<tr>
<td>3059</td>
<td>choline</td>
<td>20.0</td>
<td>60.0</td>
<td>14.0</td>
<td>3.0</td>
<td>4.3</td>
</tr>
<tr>
<td>3059</td>
<td>ethanolamine</td>
<td>20.0</td>
<td>37.5</td>
<td>25.0</td>
<td>7.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

a. Ratio  
b. The strain has been shown to lack any detectable phosphatidylserine  
(Atkinson et al., 1980a,b)
In addition to the auxotrophic strains, we have also used a wild-type strain, *S. cerevisiae* (3059) cells, grown in 20 mM choline- and 20 mM ethanolamine-supplemented media (Steiner and Lester, 1972). This was done to ascertain if the percentage increase in PC and PE contents by two different ways would have a similar effect on yeast membrane. Table 13 demonstrated that there was about 40-50% increase in PC and PE contents in cells grown on choline- or ethanolamine-supplemented media, respectively. As compared to both the auxotrophic strains, the percentage increase in PC content was more in choline-supplemented cells (Table 13). The contents of other phospholipids were not much affected except in the case of cho1 membranes, where CL was 50-60% less as compared to wild-type. It became evident from both sets of studies that the phospholipid composition of yeast plasma membrane can be influenced predictably by manipulating the growth conditions. As a consequence of supplementation, the phospholipid composition of mutant cells and of 3059 cells exhibited enrichment of PC or PE content (depending upon the nature of supplementation).
D. **Fatty acid composition**

Fatty acid analysis of choline- or ethanolamine-supplemented crude plasma membranes of *S. cerevisiae* (3059) revealed a significant decrease in the ratio of unsaturated to saturated fatty acids (Table 14). This was observed mainly due to a significant decrease in the level of palmitoleic acid (16: 1) and oleic acid (18: 1) and due to an increase in saturated fatty acids (palmitic acid, 16: 0; stearic acid, 18: 0). Fatty acids composition of the two auxotrophs could not be quantitated because of the limited material, however, the preliminary analysis of fatty acid composition revealed changes similar to *S. cerevisiae* (3059) membranes (data not shown). Earlier observations have shown that the supplementation of growth media of *E. coli* (Ingram et al., 1980) with ethanol and *S. cerevisiae* (Kanoh, 1969; Kovac et al., 1980) with choline or ethanolamine also resulted in altered fatty acid composition.

**Amino Acids Transport in Choline and Ethanolamine Auxotrophs**

Figs. 15A and B show the effect of PC and PE enrichment on the transport of Phe, Met, Ser, Leu, Gly, Lys, Glu and Pro. The observed reduction in
TABLE 14

FATTY ACID COMPOSITION OF SACCHAROMYCES CEREVISIAE (3059) CRUDE PLASMA MEMBRANE

Fatty acid esters are prepared and resolved as described under 'Materials and Methods'.

<table>
<thead>
<tr>
<th>Medium Supplemented</th>
<th>14:0</th>
<th>16:0</th>
<th>16:1</th>
<th>16:2</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:0</th>
<th>U/S*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.3</td>
<td>13.4</td>
<td>12.2</td>
<td>4.6</td>
<td>13.8</td>
<td>22.0</td>
<td>17.7</td>
<td>8.5</td>
<td>1.6</td>
</tr>
<tr>
<td>Choline</td>
<td>4.0</td>
<td>20.6</td>
<td>6.8</td>
<td>6.7</td>
<td>14.8</td>
<td>13.6</td>
<td>12.7</td>
<td>5.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>1.3</td>
<td>22.5</td>
<td>6.8</td>
<td>6.7</td>
<td>19.0</td>
<td>14.5</td>
<td>17.2</td>
<td>2.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Abbreviations: S, saturated fatty acid; U, unsaturated fatty acid.
Fig. 15: Uptake of amino acids in wild-type and in choi 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 L-(14C) labelled 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, 0.25 mM) to the assay mixture. The amino acids' concentrations were two to three times higher than their respective $K_m$ values of cells without supplemented medium. At indicated time intervals, 0.1 ml aliquots were taken out, diluted (50-fold with chilled distilled water), filtered and radioactivity retained was determined. All the values are an average of three separate determinations. Wild-type cells (○—○), choi cells grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium.
Fig. 15 A.
Fig. 15B.
the accumulation of all amino acids was between 12% and 70% in both choline- and ethanolamine-grown cells of choi. In general, the pattern was the same when Cho<sup>+</sup> cells were grown in choline- or ethanolamine-containing medium, where the reduction in the uptake of amino acids was between 10% and 40% (Figs. 16A and B). It is apparent that the extent of reduction in the total accumulation of Gly, Leu, Ser and Pro was significantly different between the two auxotrophic strains.

**Kinetics of Amino Acids Uptake in Choline and Ethanolamine Auxotrophs**

The enrichment of PC or PE in these two auxotrophs which resulted in the reduced levels of accumulation of several amino acids, may be due to a change in the affinity of the carrier(s) for these amino acids. The kinetic data revealed that the apparent $K_m$ values of all the amino acids in choi and Cho<sup>+</sup> auxotrophs, grown in choline or ethanolamine-containing medium, were increased as compared to wild-type. The $V_{max}$ values, however, were not much affected in these cells. This indicated a decrease in the affinities of the carrier(s) as a consequence of PC or PE enrichment (Figs. 17A and B; 18A and B).
Fig. 16: Uptake of amino acids in wild-type cells (○——○), Cho+ cells grown in (Δ——Δ) choline- and in (●——●) ethanolamine-containing medium. Assay conditions were similar to those described for Fig. 15.
Fig. 16A.
Fig. 16B.
Fig. 17: Lineweaver-Burk plots of amino acids in wild-type cells (○—○), cho1 cells grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium.

Assay conditions were similar to those described for Fig. 15. The reaction was terminated after 30 sec.
Fig. 17A.
Fig. 17B.
Fig. 18: Lineweaver-Burk plots of amino acids in wild-type cells (○—○), Cho+ cells grown in (△—△) choline- and in (●—●) ethanolamine-containing medium. Assay conditions were similar to those described for Fig. 15. The reaction was terminated after 30 sec.
Fig. 18A.
Fig. 18B.
Amino Acids Transport in Choline- and Ethanolamine- Supplemented Cells

When *S. cerevisiae* (3059) cells were grown in presence of 20 mM choline- or 20 mM ethanolamine-supplemented medium, their membrane phospholipids became enriched with PC or PE, respectively (Table 13). It is known that 10-20 mM concentrations of choline or ethanolamine give maximum enrichment of PC or PE, respectively (Waechter *et al.*, 1969; Steiner and Lester, 1972). As compared to non-supplemented cells, PC or PE enrichment in choline- or ethanolamine-supplemented cells resulted in the reduction of transport of all the eight amino acids (Figs. 19A and B). The extent of reduction in amino acids transport among auxotrophic strains and supplemented cells was significantly variable. For instance, the reduction in the uptake of Lys was 45-70% in choline- or ethanolamine-supplemented cells and was only 10-18% in auxotrophic strains. On the other hand, the reduction in Gly accumulation in auxotrophic strains was more pronounced as compared to the supplemented cells (Figs. 15B; 16B; 17B).
Fig. 19: Uptake of amino acids in (○—○) non-supplemented, (△—△) choline- and (●—●) ethanolamine-supplemented cells of *Saccharomyces cerevisiae* (3059). Assay conditions were similar as that of Fig. 15.
Fig. 19A.
Fig. 19B.
Kinetics of Amino Acids Uptake in Choline- and Ethanolamine-Supplemented Cells

Kinetic data revealed that the apparent $K_m$ values for all these amino acids were higher as compared to $K_m$ values of amino acids in non-supplemented cells (Figs. 20A and B). As in auxotrophic strains, the increase in PC or PE level in choline- or ethanolamine-supplemented cells resulted in decreased affinities (high $K_m$ values) for respective amino acids.

ANS Response in PC- or PE-Enriched Spheroplasts

The conformational changes due to PC or PE enrichment in membrane were monitored by using a fluorescent probe, ANS (Slavik, 1982). It was observed that ANS did not give a detectable response when intact cells of various strains were used. Therefore, the fluorescence of ANS was monitored in the spheroplasts of these cells where fluorescence was detectable and reproducible. The lack of response of ANS dye in intact cells may be due to the presence of cell wall which may affect the permeability and binding of the dye.
Fig. 20: Lineweaver-Burk plots of amino acids in (○—○) non-supplemented, (△—△) choline- and (●—●) ethanolamine-supplemented cells of *Saccharomyces cerevisiae* (3059). Assay conditions were similar to those described for Fig. 15. The reaction was terminated after 30 sec.
Fig. 20A.
Fig. 20B.
There was a decrease in the relative fluorescence intensities of bound ANS in both choline- and ethanolamine-grown auxotrophs. However, the decrease in fluorescence was more pronounced (~40%) in ethanolamine-grown auxotrophs (Figs. 21A and B). Similar pattern was observed when ANS fluorescence was measured in spheroplasts of choline- or ethanolamine-supplemented cells (Fig. 21C). While analyzing the fluorescence data, it was noted that the phospholipid composition of various strains also revealed a higher percentage of increase in PE level (30-35%) in ethanolamine-grown auxotrophs and ethanolamine-supplemented spheroplasts (Table 13). The difference in PE vs. PC level is reflected in the relative fluorescence intensities of ANS which could directly be correlated to the phospholipid composition.

**Binding Parameters of ANS in PC- or PE-Enriched Spheroplasts**

The observed decrease in ANS fluorescence, monitored in PC- or PE-enriched spheroplasts, may be due to a decrease in the binding of dye molecules to the membrane components, a decreased affinity of dye to the membrane and/or a change in relative quantum yield (Prasad et al., 1975a).
Fig. 21: Emission spectra of ANS. The reaction mixture contained 50 mM Tris-acetate buffer (pH 7.2), 33.3 μM ANS and the concentration of spheroplasts (protein) was 100 μg/ml in each case. Other details were as described under 'Materials and Methods'. (A) Spheroplasts of wild-type (○—○); of choi grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium. (B) Spheroplasts of wild-type (○—○); of Cho+ grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium. (C) Saccharomyces cerevisiae (3059): spheroplasts of (○—○) non-supplemented, (Δ—Δ) choline- and (●—●) ethanolamine-supplemented medium.
The maximum fluorescence of ANS, $F_{\text{max}}$ (in arbitrary units), was determined by double reciprocal plot (Azzi, 1975) between observed fluorescence and varying membrane (protein) concentrations (Figs. 22A and B; 24A). The $F_{\text{max}}$ values of PC- or PE-enriched spheroplasts were reduced between 10% and 30% (Table 15). The affinity of ANS to PC- or PE-enriched membrane was determined by double reciprocal plot (Prasad et al., 1975a; Azzi, 1975) between observed fluorescence and varying ANS concentrations (Figs. 23A and B; 24B). The data show the decreased fluorescence even at increasing amount of ANS in various spheroplasts which could be due to less availability/affinity of ANS to membrane enriched with PC or PE.

In order to ascertain if the affinity for ANS changed following PC or PE enrichment of membrane, the apparent dissociation constant ($K_d$) was determined by Scatchard plots (Figs. 25A and B; 26). It was observed that $K_d$ was slightly increased due to PC or PE enrichment of spheroplasts (Table 15). The numbers of binding site for ANS, determined by Scatchard plots, were decreased from 105 to 81-77 nmol ANS/mg protein in auxotrophic strains, grown in
Fig. 22: ANS response at varying concentrations of spheroplasts (protein) of: (A) wild-type (○—○), choi grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium; (B) wild-type (○—○), Cho¹ grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium. The reaction mixture was same as that of Fig. 21.
Fig. 23: ANS response at varying concentrations of ANS with spheroplasts of: (A) wild-type (○—○), choi grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium; (B) wild-type (○—○), Cho⁺ grown in (Δ—Δ) choline- and in (●—●) ethanolamine-containing medium. The reaction mixture was same as that of Fig. 21.
Fig. 24: ANS response in *Saccharomyces cerevisiae* (3059) at varying concentrations of: (A) spheroplasts (protein) of (○—○) non-supplemented, (Δ—Δ) choline- and (●—●) ethanolamine-supplemented medium; (B) ANS with spheroplasts of (○—○) non-supplemented, (Δ—Δ) choline- and (●—●) ethanolamine-supplemented medium. The reaction mixture was same as that of Fig. 21.
Fig. 24.
Fig. 25: Scatchard plots of ANS binding to spheroplasts of: (A) wild-type (○—○), choi grown in (△—△) choline- and in (●—●) ethanolamine-containing medium; (B) wild-type (○—○), Cho⁺ grown in (△—△) choline- and in (●—●) ethanolamine-containing medium. The reaction mixture was same as that of Fig. 21.
Fig. 26: Scatchard plots of ANS binding to spheroplasts of *Saccharomyces cerevisiae* (3059): (○—○) non-supplemented, (△—△) choline- and (●—●) ethanolamine-supplemented medium. The reaction mixture was same as that of Fig. 21.
Fig. 26.
**TABLE 15**

**RELATIVE NUMBER OF BINDING SITES (n) AND APPARENT DISSOCIATION CONSTANT (Kd) OF ANS BOUND TO SPHEROPLASTS OF SACCHAROMYCES CEREVISIAE**

Arbitrary unit, $F_{\text{max}}$, was determined by double reciprocal plot of the fluorescence intensity versus varying membrane (protein) concentration. Reaction mixture contained, in a final volume of 3 ml, 50 mM Tris-acetate (pH 7.2), 33.3 μM ANS and varying concentration of membrane (protein).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Media supplemented</th>
<th>$F_{\text{max}}$ (n mole/mg protein)</th>
<th>n</th>
<th>Kd (μM)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ade 5α</td>
<td>None</td>
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<td>36.84</td>
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*'n' and 'Kd' of ANS bound to various membranes were determined by Scatchard plots (Azzi, 1975).
presence of choline or ethanolamine (Figs. 25A and B). Similarly, the binding sites of ANS were reduced in choline- and ethanolamine-supplemented spheroplasts (Fig. 26). The decrease in ANS fluorescence in PC- or PE-enriched membrane may also partly be due to the changed hydrophobic environment around the embedded dye.

DISCUSSION

In this part of study, an attempt had been made to specifically alter the phospholipid composition of yeast plasma membrane to ascertain the physiological role of various membrane phospholipids especially PC and PE.

The yeast choI mutant has been shown to be impaired in the synthesis of PS from Ser both in vitro and in vivo (Atkinson et al., 1980a). The diminished affinity of the synthesizing system for PS is presumed to be due to lowered activity of the enzyme; CDP-diglyceride: serine phosphatidyltransferase (EC 2.7.8.8), caused either by a structural or a regulatory mutation (Atkinson et al., 1980a). This defect in PS synthesis was corroborated by the fact that no detectable PS was observed while analyzing the phospholipid composition
of choi crude plasma membrane. The supplementation conditions which led to the dramatic accumulation of methylated intermediates in Cho⁺ strain has indicated that some aspect of the regulation or activity of the methyltransferase(s) (EC 2.1.1.17) is altered in the mutant (Atkinson et al., 1980a). Due to the alteration in methyltransferase(s) activity, the conversion of PE to PC was not as rapid in Cho⁺ cells as that of choi cells. As a result, PC level was lower in ethanolamine-grown Cho⁺ cells whereas choi cells well maintained the PC level in ethanolamine-supplemented medium.

In growth medium, non-supplemented with lipid precursors, the deficiency in PS synthetase has hampere d the synthesis not only of PS but also of PE and PC as the latter two species arise in yeast sequentially from PS by decarboxylation and stepwise methylation (Hubscher, 1962; Waechter and Lester, 1973) (Fig. 2). In choline- or ethanolamine-supplemented medium, the mutant cells could grow continually as did wild-type cells. Apparently, the mutant cells were able to grow as long as PC or PE could be formed at the expense of diminishing PS. Choi mutant, on the other hand,
appeared to be slightly leaky for the defect in PS synthesis. In yeast (Steiner and Lester, 1972), as in bacteria (Kanfer and Kennedy, 1963, 1964), PS is believed to be synthesized from free serine and CDP-diglyceride and may be due to base exchange reactions (Steiner and Lester, 1972; Waechter and Lester, 1973). A conclusion may be drawn from the properties of the yeast choi mutant that the presence of PS is not indispensable for continuing vegetative growth and division of this eucaryotic organism. Such a study on bacterial mutant with a temperature-sensitive defect in PS synthetase demonstrates the cessation of growth at the nonpermissive temperature (Raetz, 1978).

The observed increase in PI level of choi cells could be due to its rapid synthesis (Atkinson et al., 1980b). The increased level of PI may be required to maintain the overall charge of the membrane surface. The maintenance of lipid net charges has been observed in the abnormal lipid metabolism of yeast (Becker and Lester, 1977) and Neurospora crassa (Hubbard and Brody, 1975). It has been shown that under physiological pH, molecules of PS and PI carry negative charges and PE is also slightly negative whereas zwitterionic PC is
neutral (Papahadjopoulos and Weiss, 1969; Kolber and Haynes, 1979). Thus, it appears since PS and PI carry the same net negative charge, the increased production of PI may reflect regulation of net lipid charge in the absence of PS synthesis. Some flexibility in membrane charge is, however, allowed (Hubbard and Brody, 1975; Hawrot and Kennedy, 1978).

The effect of PC or PE enrichment on Pro, Lys, Phe, Gly, Met, Ser, Leu and Glu uptake has been studied. The results indicated that the uptake of all the amino acids, in general, was reduced as a consequence of PC or PE enrichment. The differences in Ser uptake between the two auxotrophic strains need special mention. Choi mutant completely lacks PS since the mutant is deficient in its synthesis (Atkinson et al., 1980a,b). The existence of an alternate biosynthetic pathway, utilizing free ethanolamine or choline permits yeast, to circumvent the requirement for PS as a precursor for other lipids (Atkinson et al., 1980b). As a consequence of this, Ser uptake is much reduced in choi cells (Van Thienen and Postma, 1973). The reduction in amino acids transport was observed for both auxotrophic strains as well as for cells grown in choline- or ethanolamine-
supplemented medium. However, the extent of reduction in amino acids accumulation was variable. It has been reported that PC synthesized by Kennedy pathway contains a greater proportion of saturated fatty acid residues as compared to the methylation pathway (Kanoh, 1969; Waechter and Lester, 1973). The difference in PC/PE ratio and a possible difference in the molecular species of phospholipids of these strains, may explain the observed variation in the accumulation of various amino acids between the two auxotrophs.

It is interesting to note that both PC or PE could influence the uptake of several amino acids. Since choi lacks PS in its phospholipid composition, the level of PS is predicted to be dispensable for various physiological functions of S. cerevisiae. The fact that PS is absent from choi phospholipid constitutes a major difference between choi and Cho⁺ phospholipid composition. It would mean that a critical concentration or level of each phospholipid is a prerequisite demand for proper functioning of yeast membrane.

The observations from fluorescence studies on PC- or PE-enriched membranes indicated that the
enrichment lead to the conformational changes. Studies with the fluorescent probe, ANS, revealed that the decrease in relative fluorescence intensity was more pronounced in PE-enriched membranes, obtained either by growing choi or Cho* in ethanolamine or by supplementing S. cerevisiae (3059) cells with ethanolamine. While attempting to explain the reason(s) for the decrease in ANS fluorescence, one has to consider the phospholipid composition of PC- or PE-enriched membrane.

Under physiological pH, PE is reported to be slightly negatively charged as compared to PC which is neutral and exists as zwitterion (Kovac et al., 1980). Demel et al. (1972, 1977) and Becker and Lester (1977) reported that the bulkier zwitterion of PC, compared with PE, kept the fatty acyl chains further apart which in turn could lead to increased instability in membrane. In addition, Phillips and co-workers (1972) discovered from X-ray analysis of PC dispersions that the preferred orientation of the PC zwitterion is normal to the bilayer, whereas, negatively charged PE tends to lie tangentially to the bilayer. Therefore, the binding of ANS, which itself is anionic
at pH 7, would be decreased in membrane having more PE. The reduced ANS fluorescence upon PC or PE enrichment could be attributed to a decrease in number of irreversible or slowly reversible binding sites because of the orientation of charges over membrane. It was observed that number of binding sites for ANS was decreased from 105 to 81-77 nmol ANS/mg protein in auxotrophic strains. Similarly the binding sites of ANS were reduced in ethanolamine-supplemented spheroplasts (Table 15).

It can be concluded that yeast tolerates some variations in phospholipid composition of its membrane but certain requirement should be met with to ensure normal growth and functioning of the cell. It may be concluded that PC or PE enrichment of yeast plasma membrane results in the alteration of both structure and function. It is also apparent that a critical concentration of these phospholipids is a prerequisite demand for proper functioning of the membrane. Yeast provides a very useful model system for the role of phospholipid composition in the structure and function of eucaryotic microorganisms.