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Utilization of Soluble Phospholipids for Studying the Phospholipid Metabolism of Microorganisms

Jei-Oh Yon*, Akinori Ohta[1]
Institute of Biotechnology, Yeungnam University, Gyongsan 712-749, Korea
[1]Department of Biotechnology, The University of Tokyo, 1-1-1, Bunkyo-ku, Tokyo 113-3657, Japan

How the membrane lipid content and lipid molecular species are maintained within a small fluctuation is an old but not yet fully answered question. To understand the intracellular movement and metabolism of membrane phospholipids, it is desirable to have a system in which externally added phospholipids are incorporated into the cells and can be analyzed their fate.

For the molecular genetic approach in studying intracellular phospholipid movement and metabolism, a yeast *Saccharomyces cerevisiae* is a suitable organism, because it can be easily cultured and has organelles common to higher eukaryotic cells, and its genetic manipulation system is very well developed. However, such analysis have been hampered because of the difficulty to let yeast cells to uptake extracellular phospholipids due to their tight cell wall structure.

S. cerevisiae cho1/pss mutants are defective in the synthesis of phosphatidylserine(PS), which is precursor of major nitrogen containing phospholipids, phosphatidylethanolamine(PE) and phosphatidylcholine(PC), but they can grow on medium containing the hydrophilic head group precursor ethanolamine or choline.

We tried to grow an *S. cerevisiae cho1/pss* null mutant in the presence of a group of phosphatidylcholine (PC) with short acyl residues[1]. We found that significant amount of PC with octanoic acid (diC8PC) or decanoic acid (diC10PC) residues were incorporated into the cells and that these molecules supported the growth of the *cho1/pss* mutant cells.

To determine how the short acyl chain PCs support the growth of the *cho1/pss* mutant we examined the effect of their water-soluble breakdown products, because it was plausible that the phospholipids were first decomposed by extracellular phospholipases. The soluble hydrolytic product of PC, phosphorylcholine and glycerophosphorylcholine did not correlated with the short acyl chain PC supported growth of the *cho1/pss* mutant cells at comparable concentration. Addition of 10 mM hemicholinium-3, a choline transport inhibitor, or disruption of *CTR* gene encoding choline transporter inhibited the growth of the *cho1/pss* mutant in the presence of choline, but not in the presence of 0.1mM diC8PC, suggesting choline -independent growth of the *cho1/pss* mutant in the diC8PC supplemented medium. These results suggest that observed growth-supporting effect of PCs with short acyl residues was not due to their water-soluble decomposition products.

Under the diC8PC-supported growth condition, octanoic acid was hardly detectable among the cellular phospholipids, but recovered in the culture medium as free acid, suggesting that most of the short acyl residues of the consumed diC8PC were released into the medium. These results raise the

possibility of the presence of deacylation and remodeling system of incorporated diC8PC in yeast cells.

Since yeast phospholipaseB (*PLB*) are potential candidates for remodeling enzymes which can convert short-chain- PCs to long-chain-cellular available PCs, we disrupt all known yeast *PLB* genes, *PLB1*, *PLB2* and *PLB3*. Despite the disruption of these *PLB* genes and *CHO1/PSS* gene, the yeast cells were still viable in the diC8PC supplemented medium.

Since eight or ten-carbon fatty acid residues are too short to maintain the structure and function of yeast membranes, it is likely that acyl substitution is a prerequisite for the growth-support ability of short chain PCs, and some remodeling enzyme activity , including phospholipase activity, will be exist in yeast cells.

For detecting such enzyme activity, we developed in vitro enzyme assay system by using the soluble form of fluorescence phospholipid (NBD-PC) as substrate.

PLB like activity still remained by using our assay system after triple disruption of *PLB* genes in yeast cells, although the other groups have reported that triple *plb* null mutant had no detectable activity[2]. We assume that our result is due to unnatural form of phospholipid substrate, which should be converted to natural form in yeast cells.

Genetic screening of mutants that can not utilize the diC8PC should provide a clue to identify and to isolate genes which are involved in phospholipid remodeling system.

[1.] Biochim Biophys Acta. 1998 Oct 2;1394(1):23-32.
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[2.] J Biol Chem. 1999 Oct 1;274(40):28121-7.
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