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Maintenance of membrane homeostasis by the remodeling of acyl residues in phospholipids

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Research aims

Biological membranes are composed of a wide variety of phospholipids diverse in their hydrophilic head moieties and hydrophobic acyl residues. The remodeling of acyl residues in phospholipids contributes to the generation of the diversity and the variation of phospholipid molecular species. In addition, improper or oxidized acyl residues in phospholipid molecules are supposed to be exchanged with appropriate ones by remodeling reaction to maintain membrane homeostasis. In higher eukaryotic cells, polyunsaturated fatty acids are introduced into phospholipids by remodeling reaction. Despite the importance of the remodeling of phospholipid acyl residues for the structure and function of biological membranes, its molecular mechanisms remain to be established. Recently, several lysophospholipid acyltransferases belonging to the membrane bound *O*-acyltransferase (MBOAT) family were reported to be involved in the remodeling of acyl residues at *sn*-2 position of various phospholipids.[1)]

In order to address the mechanism of metabolism of phospholipids, it is effective to establish a system, in which an exogenous phospholipid or a phospholipid analog is incorporated into the cells, and to monitor its metabolism in the cells. We constructed a system to analyze the remodeling of acyl residues of phosphatidylcholine (PC), one of the most abundant and essential phospholipids, in *Saccharomyces cerevisiae*. In this system, exogenous PC containing short acyl residues, which are more accessible to the plasma membrane of yeast cells due to their higher solubility in water than phospholipids containing longer acyl residues, is incorporated into yeast mutants defective in PC synthesis, and the remodeling is evaluated by observing the growth of the mutants and more directly by analyzing the metabolism of incorporated phospholipids by mass spectrometry.[2), 3)] In this study, we constructed a system to evaluate the remodeling of phosphatidylethanolamine (PE), an essential phospholipid with some unique functions, and analyzed the mechanism of the remodeling at its *sn*-2 position. In addition, we synthesized new PC analogs and constructed a system to specifically analyze the introduction of acyl residues at *sn*-1 position.

Methods

S. cerevisiae strains TKY12Ga (*MAT*a *his3 leu2 trp1 ade2 ura3 psd1*D::*kanMX4 psd2*D::*LEU2* P_{GAL1}-*ECT1*::*HIS3*) and PCY12G (*MAT*a *his3 leu2 trp1 ade2 ura3 pem1*::*HIS3 pem2*::*hph* P_{GAL1}-*CCT1*::*nat1*) were derived from the strain W3031A (*MAT*a *his3 leu2 trp1 ade2 ura3*).

Phospholipids were extracted by the method of Brigh and Dyer. MS/MS spectra were obtained on API3000 triple quadrupole instrument (Applied Biosystem). Phospholipids were dissolved in acetonitrile/methanol/water (4 : 4 : 1) containing 0.1% ammonium formate and infused into ESI source with a Harvard syringe pump at the flow rate of 10 ml/min. The ion spray voltage was set at 5.0 kV for positive scans and −3.8 kV for negative scans. Experiments were conducted with nitrogen as a collision gas and collision energy of 25~60 Vdc. For each spectrum, 100~200 scans were summed.

Results

Remodeling of PE containing short acyl residues

We constructed a yeast strain TKY12Ga, the endogenous PE synthesis of which was controllable, by deletion of both *PSD1* and *PSD2* encoding phosphatidylserine (PS) decarboxylases and replacement of the promoter of chromosomal *ECT1* encoding CTP:phosphoethanolamine cytidylyltransferase with the *GAL1* promoter.[4)] In a glucose-containing medium, PE synthesis in TKY12G was repressed and its growth was arrested. However, addition of PE containing decanoyl residues (diC10PE) supported the growth of this strain on glucose.

We synthesized diC10PE, in which an ethanolamine moiety was labeled with deuterium, to analyze its metabolism. TKY12Ga was incubated with deuterium-labeled diC10PE. Lipids were extracted and subjected to electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis.[5)] Deuterium-labeled diC10PE was rapidly converted to PE species containing C16 or C18 acyl residues. PE containing decanoic acid and C16 or C18 fatty acids as acyl residues, which are probably remodeling intermediates, were also detected. During growth in the presence of diC10PE, a substantial amount of decanoic acid was released into the culture medium. These results suggest that diC10PE was remodeled to PE species containing longer acyl residues and used as membrane components (Fig. 1).

We further investigated the mechanism of the remodeling of diC10PE. We constructed deletion mutants of genes en-

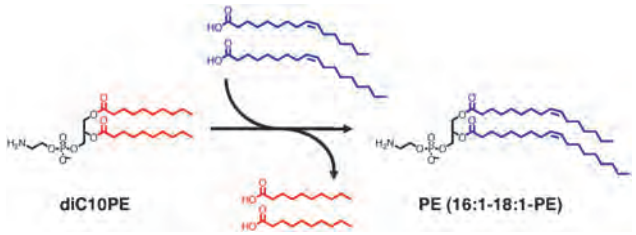

Fig. 1. Remodeling of PE containing short acyl residues.
PE containing decanoyl residues is remodeled to those with acyl residues of physiological length and used in *S. cerevisiae*.

coding various acyltransferases in TKY12Ga background, and analyzed the remodeling of diC10PE in them. The deletion mutants for *ALE1* encoding yeast MBOAT family acyltransferase and *SLC1* encoding lysophosphatidic acid acyltransferase exhibited the growth defects in the medium containing diC10PE. The analysis of the remodeling of deuterium-labeled diC10PE revealed that the amount of the remodeling products and the remodeling intermediates, particularly the remodeling intermediates containing a longer acyl residue at *sn*-2 position were decreased in these mutants. When deuterium-labeled diC10PE was incubated with the extract of the wild-type cells, the remodeling intermediates, i.e., the 10:0–16:1-PE and 10:0–18:1-PE were detected. In contrast, the amount of these remodeling intermediates was decreased in the reaction with the extract of the deletion mutant of *ALE1* or *SLC1*. These results imply the involvement of Ale1p and Slc1p in the remodeling of acyl residues at *sn*-2 position of diC10PE.

Remodeling of acyl residue at *sn*-1 position of PC

2-Acyl-lysophospholipids are less stable than 1-acyl-lysophospholipids, and acyl residues at *sn*-2 positions of 2-acyl-lysophospholipids produced by the reaction catalyzed by phospholipase A1 can be easily transacylated to *sn*-1 positions. This interferes with the accurate assessment of deacylation and reacylation at *sn*-1 position of phospholipids. To analyze the mechanism of the remodeling at *sn*-1 position of PC, we synthesized three PC analogs as substrates, namely, 1-palmitoyl-2-hexadecyl-PC (PHPC), 1-myristoyl-2-hexadecyl-PC (MHPC), and 1-hydroxyl-2-hexadecyl-PC (2-alkyl-lysoPC, ALPC), in which a hexadecyl residue is placed at *sn*-2 position through an ether linkage to prevent intramolecular transacylation.

We constructed a yeast strain PCY12G, the endogenous PC synthesis of which can be controlled, by deletion of both *PEM1* and *PEM2* encoding PE methyltransferases and the replacement of the promoter of chromosomal *CCT1* encoding CTP:phosphocholine cytidylyltransferase with the *GAL1* promoter. In a glucose-containing medium, the PC synthesis of PCY12G was repressed and its growth was arrested. However, addition of ALPC to the medium supported its growth on glucose. Analysis of the metabolism of ALPC using ESI-MS/MS suggested that ALPC was converted to the PC species containing C16 or C18 acyl residue at its *sn*-1 position. These results imply that incorporated ALPC was acylated at *sn*-1 position and used instead of endogenous PC.

Conclusion

Our results suggest the presence of a mechanism to maintain the physiological length of acyl residues of PE in yeast. In addition, it was suggested that Ale1p and Slc1p are involved in the remodeling of acyl residues at *sn*-2 position of PE. We also successfully synthesized PC analogs and constructed a system to specifically evaluate the remodeling at *sn*-1 position of PC. This system will be applicable to the identification and characterization of enzymes involved in the remodeling at *sn*-1 position of PC.

References

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