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Lipid profiles of detergent resistant fractions of the plasma membrane in oat and rye in association with cold acclimation and freezing tolerance

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ABSTRACT

Cold acclimation (CA) results in alteration of the plasma membrane (PM) lipid composition in plants, which plays a crucial role in the acquisition of freezing tolerance via membrane stabilization. Recent studies have indicated that PM structure is consistent with the fluid mosaic model but is laterally non-homogenous and contains microdomains enriched in sterols, sphingolipids and specific proteins. In plant cells, the function of these microdomains in relation to CA and freezing tolerance is not yet fully understood. The present study aimed to investigate the lipid compositions of detergent resistant fractions of the PM (DRM) which are considered to represent microdomains. They were prepared from leaves of low-freezing tolerant oat and high-freezing tolerant rye. The DRMs contained higher proportions of sterols, sphingolipids and saturated phospholipids than the PM. In particular, one of the sterol lipid classes, acylated sterylglycoside, was the predominant sterol in oat DRM while rye DRM contained free sterol as the major sterol. Oat and rye showed different patterns (or changes) of sterols and 2-hydroxy fatty acids of sphingolipids of DRM lipids during CA. Taken together, these results suggest that CA-induced changes of lipid classes and molecular species in DRMs are associated with changes in the thermodynamic properties and physiological functions of microdomains during CA and hence, influence plant freezing tolerance.

1. Introduction

Cold acclimation (CA) is one of the most important processes for adaptation to freezing temperatures in plants. During this process, the plasma membrane (PM) composition changes dynamically, which is crucial for acquisition of freezing tolerance. The PM is composed of a variety of proteins and lipids which are considered to be distributed homogeneously and to move relatively freely [40]. However, recent studies have raised the possibility that PM contains microdomains in which components are restricted in their movement. Subsequent biochemical studies proposed that microdomains can be extracted as detergent resistant membrane fractions (DRM) and suggested that DRM-associated proteins and lipids are important for determining the structure and function of microdomains [38,15,54,8,26]. In fact, previous studies, using proteomics techniques, identified DRM-enriched proteins in oat and rye PM and revealed their dynamic responses to CA [42,43]. The results suggested that some microdomain proteins associated with

Abbreviations: CA, cold acclimation; NA, non-acclimation; PM, plasma membrane; DRM, detergent-resistant membrane fraction of the plasma membrane; PC, phosphatidylcholine; TLC, thin layer chromatography; FS, free sterol; SG, sterylglycoside; ASG, acylated sterylglycoside; GlcCer, glucocerebroside; PL, phospholipid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PG, phosphatidylglycerol; PA, phosphatidic acid; PS, phosphatidylserine; PI, phosphatidylinositol; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; LysoPE, lyso-phosphatidylethanolamine; LysoPC, lyso-phosphatidylcholine; LysoPG, lyso-phosphatidylglycerol; H_{II}, hexagonal II; DOPC, dioleoyl-PC; DOPE, dioleoyl-PE; UGT, UDP-glucose: sterol glucosyltransferases; FAH, fatty acid 2-hydroxylase; PLD, phospholipase D; d18:1, 8-sphingenine; d18:2, 4,8-sphingadienine; t18:1, 4-hydroxy-8-sphingenine; ANOVA, analysis of variance.

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CA in the two plant species contribute to their vastly different freezing tolerance.

In addition to proteins, many studies have suggested that lipids are necessary for microdomain functions. Simons and Ikonen considered the lateral organization of raft-like lipid microdomains to be due to preferential packing of sphingolipids and cholesterol in specific small regions [38,40]. Sphingolipids, in fact, form nanodomains in phosphatidylcholine (PC)-based lipid bilayers and cholesterol fills the intermolecular spaces of sphingolipids [35,37]. Thus, microdomain formation and stabilization may be driven by lipid–lipid and lipid–protein interactions in addition to protein–protein interactions [11,27,38,39]. In plants, some studies have examined the significance of lipids in microdomains. Borner et al. [4] and Mongrand et al. [31] first quantified lipid class and fatty acid composition using DRM as microdomain-enriched fractions. In the same way, the DRM lipid compositions of maize embryos and bean leaves have been characterized [6,7]. Furt et al. [12] reported that phosphatidylinositol-4,5-bisphosphate forms a cluster-like structure that is not influenced by sterol depletion, and that phosphoinositide metabolism-related enzyme activities in DRMs are higher than in the PM.

Lipid changes in DRMs were reported in *Arabidopsis* leaves during CA [30]. Although free sterol proportions changed in DRMs of the *Arabidopsis* PM during CA [30], the authors performed only lipid composition measurements at the lipid class level, and did not examine the molecular species in each lipid class. In the present study, we took biochemical and lipidomic approaches to determine the lipid compositions in oat and rye DRMs during CA. Considering a previous study that dealt with PM lipid compositions in oat and rye during CA [44], we aimed to (a) compare lipid compositions between the PM and DRMs, (b) investigate compositional changes of PM and DRM lipids during CA and (c) compare lipid composition and CA-induced changes between low freezing-tolerant oat and high freezing-tolerant rye. The physical properties of membrane lipids influence their stability and are important for the acquisition of plant freezing tolerance during CA [13,41,46]. Therefore, both the physical properties of the PM and microdomains and the biochemical functions of microdomains during CA will be discussed by comparing oat and rye.

2. Materials and methods

2.1. Plant materials and isolation of DRM fractions

Non-acclimated and cold-acclimated oat (*Avena sativa* L. cv. New almighty) and rye (*Secale cereale* cv. Maskateer) were grown under conditions reported by Takahashi et al. [42]. PM and DRM isolation were performed in accordance with Takahashi et al. [42].

2.2. Total lipid extraction and thin layer chromatography

Extraction of total lipids from PM and DRM fractions was carried out according to the method of Bligh and Dyer [3]. Isopropanol (2.5 mL) was added to 1 mL of PM or DRM suspensions. Subsequently, 1.25 mL of chloroform was added twice and samples in test tubes were mixed well. After adding 1.25 mL of 0.9% (w/v) NaCl, samples were incubated for 15 min at room temperature. Test tubes were then centrifuged at 196× *g* for 5 min to induce phase separation and the lower phase was collected in a new test tube. To re-extract lipids, chloroform (1.5 mL) was added to the remaining upper phase, test tubes were then centrifuged (784× *g* for 10 min) and the lower phase was combined with the previously collected lower phase. After adding 2 mL of chloroform/isopropanol/0.9% (w/v) NaCl (3:47:48, v/v/v) to the combined lower phase, samples were centrifuged at 196× *g* for 5 min and the lower phase was collected in a new test tube. The lower phase was then dried at 40 °C under N_2 gas flow and after adding an aliquot of chloroform, it was stored at −20 °C under N_2 gas until use.

Thin layer chromatography (TLC) analysis of total lipids was conducted using silica gel plates (Silica gel 60, 0.25-mm thickness, Merck, Darmstadt, Germany) with chloroform/methanol/water (65:25:4, v/v/v) as the developing solvent. Subsequently, solvent on thin layer plates was removed completely and lipids were visualized by spraying with 0.1% (w/v) primuline in acetone (Sigma–Aldrich, St Louis, MO, USA).

2.3. Quantification of sterols, glucocerebrosides and phospholipids

Silica gel of the spot corresponding to free sterol (FS), sterylglycoside (SG), acylated sterylglycoside (ASG), glucocerebrosides (GlcCer) and phospholipids (PLs) were collected and subjected to quantification of each lipid class.

Sterol quantification was carried out according to the method of Zlatkis and Zak (1969) with a slight modification [55]. Briefly, 1.1 mL of acetic acid was added to the silica powder in sample tubes and sonicated. An aliquot (1 mL) of *o*-phthalaldehyde in acetic acid (0.1%, w/v) and 1 mL of concentrated sulfuric acid were added successively and mixed well. These additions generated heat. After cooling the mixture at room temperature for 15 min, sample tubes were centrifuged at 196× *g* for 10 min to precipitate silica powder. Absorbance of supernatants at 550 nm was measured. Standard curves where generated from absorbances of cholesterol standards in acetic acid in the range of 0–0.15 μM.

GlcCer quantification was performed as sugar determination according to the method of DuBois et al. (1956) [9]. Briefly, 1 mL of water was added to silica powder in sample tubes and then sonicated. Subsequently, 0.5 mL of 5% (w/v) phenol and 2.5 mL of concentrated sulfuric acid were successively added and mixed well. These additions generated heat. After cooling the mixture at room temperature for 15 min, sample tubes were centrifuged at 196× *g* for 10 min. Supernatants were collected and absorbance at 485 nm was measured. Standard curves where generated from absorbances of glucose standards in the range of 0–0.15 μM.

PL was determined as phosphate according to the method of Marinetti (1962) [29]. Briefly, 50 μL of water and 0.5 mL of 70% perchloric acid were added to sample tubes containing silica gel and mixed well. Subsequently, tubes were incubated at 200 °C for 30 min. After cooling to room temperature, 3 mL of water and 0.5 mL of 2.5% (w/v) ammonium molybdate, 0.2 mL of Fiske-SubbaRow reagent (30 g of sodium hydrogen sulfite, 1 g of sodium sulfite and 1 g of 1-amino-2-naphthol-4-sulfonic acid in 200 mL of water) were added in sequence and mixed well. Tubes were boiled for 7 min, cooled to room temperature and centrifuged at 196× *g* for 10 min. Supernatants were collected and absorbance at 700 nm was measured. Standard curves where generated from absorbances of KH_2PO_4 standards in the range of 0–0.25 μM.

2.4. Determination of molecular species of sterols, glucocerebrosides and phospholipids

Total lipid fractions obtained from 50 μg of PM protein were separated into neutral lipid (e.g. FS), glycolipid (SG, ASG and GlcCer), and PLs using silica gel column chromatography according to the method of Lynch and Steponkus [28]. Each separated fraction was dried by blowing N_2 gas, dissolved in 1 mL chloroform/acetic acid (100:1, v/v) and transferred to a Sep-Pak Silica Classic Cartridge (Waters, Milford, MA, USA) coupled to a grass syringe barrel. Neutral lipids were eluted with 10 mL chloroform/acetic acid (100:1, v/v). Glycolipids were then eluted by sequential addition of 5 mL acetone and 5 mL acetone/acetic acid (100:1, v/v).

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