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Fatty acid profiles from the plasma membrane and detergent resistant membranes of two plant species

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ABSTRACT

It is essential to establish the composition of the plant plasma membrane in order to understand its organization and behavior under continually changing environments. Knowledge of the lipid phase, in particular the fatty acid (FA) complex repertoire, is important since FAs determine many of the physical–chemical membrane properties. FAs are constituents of the membrane glycerolipid and sphingolipid backbones and can also be linked to some sterols. In addition, FAs are components of complex lipids that can constitute membrane micro-domains, and the use of detergent-resistant membranes is a common approach to study their composition. The diversity and cellular allocation of the membrane lipids containing FAs are very diverse and the approaches to analyze them provide only general information. In this work, a detailed FA analysis was performed using highly purified plasma membranes from bean leaves and germinating maize embryos and their respective detergent-resistant membrane preparations. The analyses showed the presence of a significant amount of very long chain FAs (containing 28C, 30C and 32C), in both plasma membrane preparations from bean and maize, that have not been previously reported. Herein is demonstrated that a significant enrichment of very long chain saturated FAs and saturated FAs can occur in detergent-resistant membrane preparations, as compared to the plasma membranes from both plant species. Considering that a thorough analysis of FAs is rarely performed in purified plasma membranes and detergent-resistant membranes, this work provides qualitative and quantitative evidence on the contributions of the length and saturation of FAs to the organization of the plant plasma membrane and detergent-resistant membranes.

1. Introduction

The most abundant fatty acids (FAs) in biological systems are carboxylic acids with a hydrocarbon chain of ten or more carbons that can contain modifications such as hydroxylation and double bonds in different numbers and positions of the chain. As a result of the combination of these structural features, considerable chemical diversity can occur. FAs can exist in free forms at low levels in the cells, but the bulk is linked to other molecules to yield complex lipids with different functions. In plants, FAs make up a high percentage of: waxes located in the cuticle that cover the epidermal surfaces (Smith et al., 2013); storage lipids, these mainly being triglycerides allocated in oil bodies (Chapman and Ohlrogge, 2012); glycerolipids, sphingolipids, and some conjugated sterols that are the matrix of the plant membranes (Cacas et al., 2012). In the lipid bilayer, FAs influence membrane thickness, stability and fluidity. FAs with unsaturated and long acylic chains form fluid membranes and disordered lipid phases. In contrast, FAs with very long saturated chains prevail in rigid and ordered lipid phases (Niemelä et al., 2009; Quinn and Wolf, 2009). The differential enrichment of these FA species thus contributes to defining the ability of the plasma membrane (PM) to tolerate low environmental temperatures (Falcone et al., 2004; Román et al., 2012; Vaultier et al., 2006).

FA compositions from plant sources has been described for whole plant extracts or for fractions containing total cell mem-

Abbreviations: BHT, butylhydroxytoluene; DRM, detergent-resistant membranes; FA, fatty acid; FB1, fumonisin B1; LCB, long chain base; LCFA, long chain fatty acids; OPA, *o*-phthaldialdehyde; PEG, polyethylene glycol; PM, plasma membrane; SAT, sphinganine-*N*-acyltransferase; SFA, saturated fatty acids; TX100, triton X100; UFA, unsaturated fatty acids; VLCFA, very long chain fatty acids.

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branes (Bach et al., 2011; Raffaele et al., 2008; Welti et al., 2002). In these studies, inferences are made that the lipid species identified belong to the membrane system mainly because of their structural features. Yet, in fact, their cell location and specific distribution in the various anatomical regions of the plant, or in each specific cell membrane, have not really been determined (Bach et al., 2011; Markham et al., 2011; Welti et al., 2002). This is important, because available evidence indicates that every endomembrane in the plant cell contains a specific assortment of lipids to tailor the functional role in a particular bilayer, as in the case of the tonoplast (Ozolina et al., 2013; Yoshida and Uemura, 1986), photosynthetic membranes (Ivanov et al., 2012; Moellering and Benning, 2011) and PM (Cacas et al., 2012). In a few cases, FAs have been studied in isolated PMs (Bohn et al., 2001; Laloi et al., 2007; Mongrand et al., 2004; Yoshida and Uemura, 1986) and, depending on the plant species and on the analytical techniques, variations of the individual molecular species have been reported, indicating the complexity of the PM lipid composition. Therefore, it is fundamentally important to assess lipid distribution in order to understand the PM organization and behavior under different conditions in which plants are exposed to natural environments.

From all the FA-containing lipids, glycerolipids, sphingolipids and, to a minor extent, sterols, are the major classes of lipids composing the PM from plants (Bohn et al., 2001; Borner et al., 2005; Laloi et al., 2007; Lefebvre et al., 2007; Mongrand et al., 2004). In addition, the PM contains very low levels of di- and tri-acylglycerols and mono- and di-galactosyl diacylglycerols (Laloi et al., 2007; Lefebvre et al., 2007; Mongrand et al., 2004). However, every lipid class has a huge diversity and thanks to the development of more selective methods of lipid extraction and to more sensitive and exhaustive analytical methods (Cacas et al., 2012; Markham et al., 2006), the quantitative contribution of individual lipid species in the PM lipid set has been gradually established. For example, this is the case for polyphosphoinositides, important signaling components of the plant PM (Furt et al., 2010), which could not be assessed in studies in the past. Information concerning the qualitative and quantitative contributions of the FA species in PM and detergent-resistant membranes (DRM) composition has been obtained mainly from the glycerolipid class, especially the LCFAs, which are the more abundant forms (Laloi et al., 2007; Lefebvre et al., 2007; Mongrand et al., 2004).

Of recent interest in plant membranes is the existence of membrane microdomains, whose compositions have been assessed through isolation and analysis of DRMs (Borner et al., 2005; Buré et al., 2011; Cacas et al., 2012; Malinsky et al., 2013; Mongrand et al., 2004, 2010). Although some proteomic and lipidomic analyses have been performed using such preparations (Borner et al., 2005; Buré et al., 2011; Mongrand et al., 2004, 2010), an exhaustive scrutiny of their FA compositions are still lacking.

In this work, the FA compositions from highly pure PM and DRM fractions isolated from plant tissues were studied. These represented two taxonomic groups – monocot and dicot – and which also exemplified two different developmental stages. They were used as biological materials in order to determine specific and/or general trends in the distribution of particular FA forms. Maize (*Zea mays* L.) germinating embryos represent the non-photosynthetic tissue of a dicot species, and bean (*Phaseolus vulgaris*) leaves, the active photosynthetic tissue of a dicot species.

2. Results

2.1. FA composition of PM and DRM preparations

Total FA contents of the PM and DRM fractions from bean leaves and maize embryos were compared and the distribution of the main groups is shown in Table 1. It was clearly observed that PM and DRM from bean leaves showed very similar values in terms of total amounts of FA per mg of protein. The same trend was observed between the PM and DRM from maize embryos. However, the FA contents in PM and DRM from the latter source were 2-fold higher than those in bean leaves.

Five different LCFAs were detected in the bean PM, and 4 of those remained in the DRM. Concerning the VLCFAs, 10 species were determined in both the PM and DRM (Table 1). Ten saturated FA (SFA) types were also identified in bean leaves PM and 10 in the DRM, while 5 and 4 unsaturated FAs (UFA) were also respectively determined.

In the case of maize embryos, the LCFAs determined were 5 in the PM and 4 in the DRM, and 6 and 9 types in the VLCFAs from the corresponding sources (Table 1). The SFAs present in the PM were 6 and 9 in the DRM, while 5 UFAs were measured in the PM and 4 in the DRM.

In order to analyze the contribution of each FA to the overall FA profile of the PM and DRM from the two plant tissues, the relative value of an individual FA species was calculated in mol%. Two structural features in the FA acyl chains were considered, namely the length and presence of double bonds, as these are important structural determinants in the PM properties and in the configuration of membrane microdomains.

2.2. Analysis of PM and DRM FAs from bean leaves according to chain length

The FA profiles from PM and DRM of bean leaves were performed (Fig. 1A and B, for values, see Table S1). In the PM (Fig. 1A and B, light bars), 15 FAs were quantified. Of these, 5 LCFAs (C16:0, C16:1, C18:0, C18:1, C18:2) had the highest relative contribution (*ca.* 92 mol%) as the main acyl chains (Fig. 1A), while the 10 VLCFAs measured (C20:0, C20:1, C22:0, C22:1, C24:0, C25:0, C26:0, C28:0, C30:0, C32:0), were of low relative abundance (total < 8 mol%) (Fig. 1B), but nevertheless constituting significant PM components. The FA compositions of the DRM from bean leaves (Fig. 1A and B, dark bars) showed 14 different FAs, of which 4 LCFAs (C16:0, C18:0, C18:1, C18:2) were the more abundant (Fig. 1A), as compared to the 10 VLCFAs (C20:0, C20:1, C22:0, C22:1, C24:0, C25:0, C26:0, C28:0, C30:0, C32:0) (Fig. 1B). This distribution resembles the pattern found in the PM, although no C16:1 was found in the DRM. However, comparison among the relative amount of all FAs in the PM and the DRM showed several differences: while C16:0 was present in similar amounts in the PM and DRM, the C18:0 type was higher in the DRM as compared to the PM, and the C18:1 and C18:2 were also 4- and 5-fold more abundant, respectively, in the PM than in the DRM. Regarding the VLCFAs, a striking but consistent feature was found: all 10 VLCFA types were more highly represented in the DRM, as compared to the corresponding levels in the PM. This was very clear for the VLCFA C22:1, C28:0, C30:0, C32:0 species as 9- to 11-fold differences were observed.

2.3. Analysis of PM and DRM FA from maize embryos according to the chain length

The FA profiles of the PM and DRM from maize embryos were also analyzed (Fig. 2A and B, for values, see Table S2). The PM (Fig. 2A and B, light bars) contained 11 detectable FA types: 5 LCFA (C16:0, C16:1, C18:0, C18:1, C18:2) that contributed to most of the FA profile (about 99 mol%) (Fig. 2A) and 6 VLCFAs (C20:0, C20:1, C22:0, C22:1, C24:0, 26:0) that were present in minor levels (*ca.* 1 mol%) (Fig. 2B). Determination of FAs in maize DRM (Fig. 2A and B, dark bars) yielded 2 additional species that were undetected in the respective PM from which they were obtained, giving a total of 13 FA. Four of them, the more abundant, were LCFAs (C16:0, C18:0, C18:1, C18:2, no C16:1 was found) as shown in Fig. 2A, as