Can digalactosyldiacylglycerol substitute for phosphatidylcholine upon phosphate deprivation in leaves and roots of Arabidopsis?

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Abstract
To explore the role of digalactosyldiacylglycerol (DGDG) in plants the dgdl mutant of Arabidopsis thaliana was grown in the presence and absence of inorganic phosphate. Phosphate deficiency in the dgdl mutant causes a strong decrease in all phospholipids accompanied by an increase in DGDG and sulfolipid. Moreover, a significant DGDG accumulation was found in roots upon phosphate deprivation as well. Our data indicate that DGDG accumulation upon phosphate deprivation is due to the activation of a specific eukaryotic dgdl-independent biosynthetic pathway. We propose that DGDG may substitute for phosphatidylcholine upon phosphate deprivation.

Introduction
Phosphate deficiency is a common nutritional problem faced by plants in many habitats worldwide. To cope with low phosphate availability plants evolved numerous mechanisms [1]. It has been shown that phosphate deficiency during growth strongly affects the membrane lipid composition by reducing the relative proportions of the phospholipids phosphatidylglycerol (PG), phosphatidylcholine (PC) and phosphatidylethanolamine in both photosynthetic bacteria [2,3] and in Arabidopsis thaliana [4,5]. Previous experiments in our lab focused on two different mutants of A. thaliana, dgdl and phol. The dgdl mutant has a 90% reduction in digalactosyldiacylglycerol (DGDG) content due to an inactivation of the DGDG synthase [6,7]. In the phol mutant of A. thaliana, a defect in phosphate translocation from roots to shoots results in phosphate deprivation of leaves [8], which in turn causes a significant increase in the relative amount of sulphoquinovosyldiacylglycerol (SQDG) and DGDG [4,5]. The finding that the sum of the anionic phospholipid PG and SQDG remained almost constant with changing phosphate availability has led to the hypothesis that the negatively charged SQDG can substitute for the negatively charged PG under phosphate deprivation [9]. However, the SQDG-PG substitution hypothesis does not explain the possible role of the increasing DGDG content upon phosphate deprivation. Therefore, in an attempt to unravel the function of DGDG in this process, the response of the dgdl mutant to phosphate limitation in the growth medium was investigated.

Materials and methods
Plants were grown in a growth chamber (AR-75L, Percival Scientific, Boone, IA, U.S.A.) at light of 75 μmol of photons·m⁻²·s⁻¹. A 14-h light/10-h dark (23/18 °C) regime was applied. Surface-sterilized seeds of A. thaliana wild type (ecotype Columbia 2) and the dgdl and act1 mutants were germinated on 0.8% (w/v) agar-solified Murashige-Skoog medium [10] supplemented with 1%

(w/v) sucrose. For phosphate-limitation experiments, a sterile mineral solution was used as described in [11], but at half concentration and containing 0.8% agarose, 1% sucrose and 20 mM Mes (pH 6.0), and KH$_2$PO$_4$ as indicated. Plants were grown for 10 days on 0.8% (w/v) agar-solified Murashige–Skoog medium supplemented with 1% (w/v) sucrose and then transferred to the agar plates containing the phosphate-limited medium. Total content of inorganic phosphate in leaf and root tissues was determined as described in [4]. Lipids were extracted and analysed as described previously [6].

Results and discussion

To investigate the role of DGDG upon phosphate deprivation, A. thaliana wild type and the dgd1 mutant were grown in the presence (1 mM) and absence of inorganic phosphate. Similar reductions in the endogenous amounts of inorganic phosphate to about 7% (leaves) and 13% (roots) were found in the absence of inorganic phosphate in the growth medium in both wild type and dgd1 mutant, respectively. Growth was reduced to the same extent in wild type and dgd1 mutant in the absence of inorganic phosphate; however, on a fresh-weight basis the total amount of lipids was found to be unchanged as compared with plants grown in the presence of inorganic phosphate (results not shown).

Upon phosphate deprivation, all phospholipids in leaves were found to be reduced, whereas the relative amounts of the non-phosphorous SQDG and DGDG increased in both wild type and dgd1 mutant (Figure 1). There is a 9-fold accumulation of DGDG in the phosphat-deprived dgd1 mutant, whereas DGDG in the wild type increased 2.1-fold. The relative proportion of MGDG (monogalactosyldiacylglycerol), the precursor for DGDG biosynthesis, remained constant. Regardless of the differences in DGDG, both phosphate-deprived wild type and dgd1 mutant show an inverse relationship in the relative amounts of PG and SQDG, providing corroborating evidence that changes in the two anionic lipids may be directly related [4,5,9]. Given that in the dgd1 mutant a TAA (stop) codon within the dgd1

Figure 1
Proportions of lipids from leaves of wild type and dgd1 and act1 mutants of A. thaliana grown in the presence (+) and absence (−) of 1 mM P$_i$

The means of three experiments (mol%) are shown. Lipids with no numbers: < 1 mol%. PI, phosphatidylinositol; PE, phosphatidylethanolamine; MGDG, monogalactosyldiacylglycerol.

Figure 2
Proportions of lipids from roots of wild type and dgd1 mutant of A. thaliana grown in the presence (+) and absence (−) of 1 mM P$_i$

The means of three experiments (mol%) are shown. Lipids with no numbers: < 1 mol%. PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; MGDG, monogalactosyldiacylglycerol.
Table 1

Degree of saturation of fatty acids in DGDG from leaves of wild type and dgdl mutant grown in the presence (+) and absence (−) of 1 mM Pi. The means of three experiments (mol%) are shown. The standard error was less than 15% n.d., not detectable.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Fatty acid</th>
<th>Degree of saturation (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>16:1</td>
<td>16:2</td>
</tr>
<tr>
<td>+ Wild type</td>
<td>19.4</td>
<td>2.7</td>
</tr>
<tr>
<td>− Wild type</td>
<td>26.4</td>
<td>2.2</td>
</tr>
<tr>
<td>+ dgdl</td>
<td>27.4</td>
<td>10.4</td>
</tr>
<tr>
<td>− dgdl</td>
<td>36.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Table 2

Degree of saturation of fatty acids in DGDG in comparison with phospholipids from leaves of the dgdl mutant grown in the absence of 1 mM Pi. The means of three experiments (mol%) are shown. The standard error was less than 15% n.d., not detectable; PE, phosphatidylethanolamine.

<table>
<thead>
<tr>
<th>Lipid</th>
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<tbody>
<tr>
<td>16:0</td>
<td>16:1</td>
<td>16:2</td>
</tr>
<tr>
<td>DGDG</td>
<td>36.5</td>
<td>3.0</td>
</tr>
<tr>
<td>PC</td>
<td>28.4</td>
<td>1.4</td>
</tr>
<tr>
<td>PE</td>
<td>37.6</td>
<td>3.0</td>
</tr>
<tr>
<td>PG</td>
<td>35.6</td>
<td>21.3</td>
</tr>
</tbody>
</table>

allele is in agreement with complete inactivation of the encoded DGDG synthase [7], the DGDG accumulation in dgdl upon phosphate deprivation is a surprising result. It suggests that a dgdl-independent pathways exists for DGDG biosynthesis, which is activated upon phosphate deprivation. Increasing the phosphate content to up to 30 mM did not further reduce the DGDG level in dgdl, excluding the possibility that the remaining low level of DGDG in dgdl is a consequence of a limited phosphate availability. Leaves of plants grown under sulphate-limiting conditions showed a decrease in SQDG associated with an increase in PG, but not of DGDG (results not shown), suggesting a mutual substitution of the negatively charged lipids PG and SQDG. Likewise, transgenic A. thaliana plants with almost no detectable amounts of SQDG showed a corresponding increase in the proportion of PG, with no effect on DGDG (Härtel and Benning, unpublished results).

The act1 mutant of A. thaliana is deficient in the activity of the chloroplast acyl-acyl carrier protein-1-m-glycerol-3-phosphate acyltransferase, which results in an almost complete block of the prokaryotic pathway [12]. Growing the mutant on the same phosphate-deprived medium as the wild type yields a similar ≈2-fold increase in the DGDG content (Figure 1), indicating that DGDG synthesized under conditions of phosphate limitation is independent of the prokaryotic pathway.

In phosphate-deprived roots, virtually no difference in the lipid pattern between wild type and dgdl is evident, yielding a similar ≈9-fold DGDG increase (Figure 2). Thus, phosphate-induced changes in the lipid composition are not restricted to leaves, but extend also to non-photosynthetic tissues.

Information about the origin of galactolipid molecules can be derived from the fatty acid composition. The fatty acid composition of DGDG reveals striking differences between wild type and the dgdl mutant (Table 1). An increase in 16:0-carbon fatty acids is accompanied by a strong decline in 18:3-carbon fatty acids. Notably, the
composition of DGDG in phosphate-deprived dgdl mutant leaves with its higher proportion of 16-carbon fatty acid strongly resembles the fatty acid composition in roots grown without phosphate (results not shown).

Due to the structural similarities of the fatty acid composition of PC and DGDG accumulating in the dgdl mutant (Table 2) and further supported by the fact that the sum of DGDG and PC remains largely constant (Figures 1 and 2), it is tempting to assume that DGDG synthesized upon phosphate limitation is compensating for the decrease in PC. Given the fact that both DGDG and PC are neutral lipids with bilayer-forming characteristics, whereas PG is negatively charged and phosphatidylethanolamine has non-bilayer-forming characteristics, the hypothesis that DGDG is substituting for PC upon phosphate deprivation seems to be further supported. This notion would also imply that at least part of DGDG synthesized upon phosphate deprivation is located outside of the chloroplast, for which evidence is accumulating [13].

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The multigenic family of monogalactosyl diacylglycerol synthases

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Abstract

Because the synthesis of monogalactosyldiacylglycerol (MGDG) is unique to plants, identified as an important marker of the plastid envelope, involved in a key step of plastid biogenesis and is the most abundant lipid on earth, MGDG synthase activity was extensively analysed at the biochemical and physiological levels. In the present paper, we present our current knowledge on the MGDG synthase's function, structure and topology in envelope membranes, and discuss possible roles in plant cell glycerolipid metabolism. The recent discovery of a multigenic family of MGDG synthases raised the possibility that multiple isoenzymes might carry out MGDG synthesis in various tissues and developmental stages.

Introduction

Galactolipids are the major constituents of plastid membranes since they represent up to 80% of thylakoid membrane glycerolipids, of which monogalactosyldiacylglycerol (MGDG) constitutes the main part (50%; for review, [1]). Higher-plant MGDG consists of two main molecular

References


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