Quantification and Fatty Acid Profiles of Sulfolipids in Two Halophytes and a Glycophyte Grown under Different Salt Concentrations

Balasubramanian Ramania, Holger Zornb, and Jutta Papenbrocka,*

a Institut für Botanik, Universität Hannover, Herrenhäuserstr. 2, D-30419 Hannover, Germany. Fax: +49 511 762 3992. E-mail: Jutta.Papenbrock@botanik.uni-hannover.de
b Institut für Lebensmittelchemie, Universität Hannover, Wunstorferstr. 14, D-30453 Hannover, Germany

* Author for correspondence and reprint requests

Z. Naturforsch. 59c, 835–842 (2004); received June 22/July 23, 2004

This study was aimed at understanding the role of sulfolipids in salt tolerance mechanisms of the halophytes Aster tripolium L., Compositae, and Sesuvium portulacastrum L., Aizoaceae, and of the glycophyte Arabidopsis thaliana (L.) Heynh., Brassicaceae. In Aster and Sesuvium the sulfolipid contents increased significantly under salt stress conditions (517 mm or 864 mm). In Arabidopsis, changes in sulfolipid contents were not observed (NaCl up to 100 mm). The fatty acid profile of sulfoquinovosyldiacylglycerol (SQDG) in Aster was modified with increasing NaCl concentrations. LC-MS analyses of sulfolipids from Aster and Sesuvium revealed the presence of 18:3/18:3 and 16:0/18:3 molecules. Obviously, the function of sulfolipids during salt stress differs between halophytic species and between halophytes and glycophytes where sulfolipid accumulation was not observed.

Key words: Fatty Acids, Salt Tolerance, Sulfolipids

Introduction

Benson and co-workers (1963) reported a new sulfur-containing lipid in plants and named it sulfoquinovosyldiacylglycerol (SQDG). Since then, SQDG has been found in numerous photosynthetic plants, algae, cyanobacteria, purple sulfur and non-sulfur bacteria (e.g. Harwood, 1980; Barber and Gounaris, 1986), and also in substantial amounts in the non-photosynthetic bacterium Rhizobium meliloti (Cedergreen and Hollingsworth, 1994). Recently, the entire biosynthetic pathway was elucidated for higher plants (Yu et al., 2002). In higher plants the glycolipid SQDG is localized in the thylakoid membranes of chloroplasts (7 mol% of all lipids in the spinach membrane) (Block et al., 1983). The quantities of saturated SQDG (16:0/18:0) range from 26% (Triticum aestivum L.) to 62% (Carica papaya L.) (Kenrick and Bishop, 1986).

It has been shown previously that environmental and nutritional factors like water deficit (Muller and Santarius, 1978), temperature (Quartacci et al., 1995; Sato et al., 2003), sulfate deprivation (DeKok et al., 1997), and phosphate starvation (Essigmann et al., 1998; Yu et al., 2002) influence the synthesis of SQDG. It has also been suggested that SQDG stabilizes protein complexes in chloroplast membranes such as photosystem II (PSII) (Minoda et al., 2003) and CF0–CF1 ATPase (Pick et al., 1985). Supporting evidence was given by the fact that ATPase activity was inhibited when SQDG tightly bound to the CF0–CF1 complex of chloroplast ATPase was removed (Barber and Gounaris, 1986).

The sea aster Aster tripolium L., Asteraceae, grows in diverse environments especially in the salt marshes along the coastlines in temperate regions but also in other inland places with saline soils (Wagenvoort et al., 1989). Sesuvium portulacastrum L. belongs to the Aizoaceae family and is located naturally in the tropical and subtropical brackish areas around the world. These two halophytes along with the glycophyte Arabidopsis thaliana (L.) Heynh. (ecotype Wassilewska), Brassicaceae, were chosen for this study. Salt tolerance studies in Aster and Sesuvium are vital because both are potential vegetables for the future. The objective of this study was to know the effect of salt stress on sulfolipids and the possible contribution of unsaturated fatty acids in SQDG towards salt tolerance in these halophytes.
Material and Methods

Biological material

Seeds of Aster tripolium (Dollart area, North Sea, Germany), Sesuvium portulacastrum (collected close to Dakhla, Morocco), and Arabidopsis thaliana (ecotype Wassilewskaya) were germinated on substrate TKS1 (Floragard, Oldenburg, Germany). Aster seeds needed a stratification period of 14 d at 4 °C on moistened TKS1. The seedlings were transplanted in TKS2 substrate (Floragard) into pots after about 30 d in the case of Aster and 20 d in the case of Arabidopsis. At the beginning of the salt treatments the Aster plants were about 40 d old having about 5 leaves and the Arabidopsis plants were 30 d old having 8 leaves. Sesuvium plants were propagated by cuttings. The cuttings developed roots in about 7 d. 15 d after making cuttings the salt treatment began. At this state the plants possessed about 8 leaves. The plants were watered daily with nutrient-supplemented tap water without or containing the respective concentrations of NaCl: for the treatment of

Thin layer chromatography (TLC) of SQDG

For SQDG analysis the leaves were ground with a mortar and pestle to a fine powder in liquid nitrogen. 100 mg of the plant material was suspended in 300 µl of methanol/chloroform/water (65:25:4 v/v), mixed and centrifuged for 10 min at 16,000 × g at 4 °C. After centrifugation the lower phase (green) was transferred to a new tube and 300 µl of methanol/chloroform/water (258 mm and 517 mm, for Sesuvium plants 428 mm and 856 mm; for Arabidopsis plants 50, 75, and 100 mm). Conditions in the greenhouse were a 16 h light/8 h dark rhythm at temperatures of 23 °C/21 °C.

Assignment of the fatty acids was performed by comparison to the retention indices of authentic reference substances and on the basis of their respective mass spectra.

Mass spectrometry of SQDG

Sample application was performed by loop injection (200 µl min⁻¹, CH₃CN/H₂O 1:1 v/v) from a Waters Alliance (Milford, Massachusetts, USA) HPLC system. Mass spectra were recorded in the negative electrospray mode (cone voltage 30 V) by an LCT time of flight mass analyser (Micro-
mass, Manchester, UK), equipped with a LockSpray™ unit for exact mass measurement.

Miscellaneous

The salt treatment experiments were repeated three times independently. For the analysis four plants were combined at each time point. The determination of SQDG was done three times for each experiment. The mean and the standard error are given for each analysis.

Results and Discussion

Effect of salt treatment on the phenotype of experimental plants

It was reported that Aster plants are able to grow at salt concentrations up to 500 mM (Kerstiens et al., 2002). Sesuvium tolerates up to 1000 mM (Mssedi et al., 2000), and Arabidopsis up to 200 mM (Shi et al., 2003). In pre-tests NaCl concentrations were determined, which caused comparable effects on growth rates retardation and reduction in the fresh weight increase (data not shown). All experimental plants showed reduced growth after 10 d of salt treatment with the NaCl concentrations indicated. Aster was able to grow on NaCl levels between 0 and 517 mM, close to seawater salinity of 599 mM (Fig. 1). The succulent plant Sesuvium proved to be more tolerant to NaCl and could grow up to 856 mM NaCl concentration with a similar reduction in growth in comparison to the Aster plants. In the glycophyte Arabidopsis (ecotype Wassilewska) growth was severely affected already at 100 mM NaCl. At higher concentrations Arabidopsis plants showed necrotic cell death at the tips and margins of the older leaves (data not shown). The NaCl-stressed plants treated with the NaCl concentrations mentioned above could recover from stress after about 2 weeks. However, after treatment with higher NaCl concentrations (about 900 mM in the case of Aster and 1200 mM in the case of Sesuvium) for more than 10 d the effects of salt stress were not reversible any more.

Effect of NaCl on the SQDG contents

In the halophytes Aster and Sesuvium the SQDG contents increased in comparison to the control plants watered without NaCl (Fig. 2A, B). In Aster the differences in SQDG contents between the control and 258 and 517 mM NaCl in the watering solution were already substantiated after 1 d and did not change during the duration of the experiment. After a 10-day-treatment with 856 mM NaCl in the watering solution the SQDG contents in Sesuvium were about twice as high as in the untreated control. In the glycophyte Arabidopsis no significant differences could be observed between control and salt-treated plants during the 10-day-exposure (Fig. 2C). The amount of SQDG in the plants investigated ranged from 140 to 580 µg per g fresh weight (FW).

Fatty acid patterns of SQDG under salt stress

The fatty acid profiles of SQDG from Aster and Sesuvium were analysed by means of GC-MS. Sesuvium and Aster were grown for 7 and 10 d, respectively, with and without salt as described in Material and Methods. The results of the fatty acid composition of SQDG are summarized in Table I. SQDG contained predominantly 16:0 (palmitic acid) and 18:3 (γ-linolenic acid), and trace amounts of 18:2 (linoleic acid). In Aster the degree of unsaturation slightly decreased after 1 d of salt treatment in comparison to control plants based on 18:3 and 18:2 species. During longer salt treatment (10 d) the degree of unsaturation slightly increased having less 16:0 and almost two-fold 18:2 species in comparison to the controls. In Sesuvium after 7 d of salt treatment the 18:2 species clearly increased in comparison to the control in a similar
way as in Aster. To prove the reliability of the method for the determination of the fatty acid profiles the fatty acid composition of the SQDG from Nicotiana tabacum L. was determined and compared to published data (Bishop et al., 1985) which are in agreement with our data (data not shown).

**LC-MS of the SQDG pool**

We were not only interested in knowing the percentage of fatty acids in all SQDG molecules in the halophytes, but also wanted to elucidate the fatty acid composition at sn-1 and sn-2 position. For the commercially available SQDG standard molecular ions were registered at 812.8796 Da and 834.8245 Da corresponding to C_{43}H_{73}O_{12}S and to C_{43}H_{71}O_{12}S (data not shown). In the SQDG isolated from Aster, ions were registered at 815.4979 Da and 837.4823 Da corresponding to elemental compositions of C_{43}H_{75}O_{12}S (0.1 ppm) and of C_{45}H_{73}O_{12}S (−0.2 ppm), respectively (Fig. 3A, B). The molecular mass of 815.5008 Da corresponding to C_{43}H_{75}O_{12}S is in good agreement with a 16:0/18:3 SQDG and the mass of 837.4813 Da (C_{45}H_{73}O_{12}S) matches the elemental composition of an 18:3/18:3 SQDG.

**Physiological implications of the results obtained**

The halophytes Aster and Sesuvium both contained increased SQDG contents when treated with salt in comparison to control plants. But the glycophyte Arabidopsis did not show any significant differences in its SQDG contents with respect to the salt treatment. Although both halophytes Aster and Sesuvium contained higher SQDG contents when treated with NaCl, their fatty acid composition differed based on their unsaturation capacity. Several reports describe the effect of salinity on the SQDG contents in glycophytes (Kuiper et al., 1974; Pearcy, 1978; Stuiver et al., 1981; Kettunen et al., 1996). For example, when NaCl was added to the external medium of seedlings of the glycophyte Hordeum vulgare L., SQDG contents remained unchanged in comparison to the control (Muller and Santarius, 1978). Also in salt-sensitive lines of Beta vulgaris L. NaCl did not alter the sulfolipid contents, but in a salt-tolerant genotype of Beta vulgaris the SQDG content increased during NaCl treatment (Kuiper and Kylin, 1981; Stuiver et al., 1981). Thus the formation of SQDG may be one important aspect of strategies involved in salt tolerance of halophytes.

Until now, the fatty acid composition of SQDG was predominantly analysed in glycophytes, namely spinach, tobacco, wheat, and cucumber (Bishop et al., 1985). Also the characterization of lipids from the chloroplast envelope in glycophytes has been well investigated (Heinz, 1973; Siebertz et al., 1979). The positional distribution of
Table I. Fatty acid patterns of SQDG in the leaves of *Aster tripolium* (top) and *Sesuvium portulacastrum* (bottom) watered with different concentrations of NaCl (*Aster*, 0 mm, 258 mm, and 517 mm; *Sesuvium*, 0 mm, 428 mm, and 856 mm NaCl). The composition is given in mol%. The values are the means of three independent experiments.

<table>
<thead>
<tr>
<th>(NaCl)</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32.4 ± 6.6</td>
<td>10.1 ± 1.9</td>
<td>11.0 ± 4.0</td>
<td>17.1 ± 6.1</td>
<td>29.8 ± 5.1</td>
</tr>
<tr>
<td>258</td>
<td>30.0 ± 4.6</td>
<td>13.1 ± 2.2</td>
<td>13.3 ± 3.8</td>
<td>17.4 ± 1.7</td>
<td>25.5 ± 4.7</td>
</tr>
<tr>
<td>517</td>
<td>32.6 ± 7.8</td>
<td>8.3 ± 1.7</td>
<td>9.8 ± 2.2</td>
<td>25.5 ± 7.3</td>
<td>23.4 ± 3.9</td>
</tr>
<tr>
<td>0</td>
<td>34.5 ± 4.0</td>
<td>6.0 ± 1.2</td>
<td>11.4 ± 2.4</td>
<td>14.4 ± 3.2</td>
<td>38.4 ± 5.4</td>
</tr>
<tr>
<td>258</td>
<td>34.9 ± 4.7</td>
<td>5.2 ± 2.5</td>
<td>9.8 ± 0.7</td>
<td>25.2 ± 2.4</td>
<td>24.9 ± 4.1</td>
</tr>
<tr>
<td>517</td>
<td>31.1 ± 8.5</td>
<td>5.7 ± 1.5</td>
<td>9.8 ± 2.8</td>
<td>29.9 ± 6.6</td>
<td>23.1 ± 3.0</td>
</tr>
<tr>
<td>0</td>
<td>33.1 ± 1.1</td>
<td>6.1 ± 0.5</td>
<td>20.4 ± 0.05</td>
<td>25.7 ± 1.8</td>
<td>15.1 ± 2.9</td>
</tr>
<tr>
<td>428</td>
<td>38.3 ± 6.3</td>
<td>7.6 ± 1.3</td>
<td>18.4 ± 3.0</td>
<td>23.3 ± 8.1</td>
<td>14.3 ± 3.3</td>
</tr>
<tr>
<td>856</td>
<td>39.8 ± 5.7</td>
<td>6.4 ± 0.5</td>
<td>15.9 ± 6.6</td>
<td>25.3 ± 6.6</td>
<td>12.3 ± 2.3</td>
</tr>
<tr>
<td>0</td>
<td>35.9 ± 2.8</td>
<td>8.7 ± 1.8</td>
<td>19.3 ± 0.8</td>
<td>23.3 ± 2.2</td>
<td>12.8 ± 0.4</td>
</tr>
<tr>
<td>428</td>
<td>37.8 ± 4.1</td>
<td>7.5 ± 2.5</td>
<td>18.6 ± 1.7</td>
<td>21.7 ± 4.4</td>
<td>12.4 ± 1.7</td>
</tr>
<tr>
<td>856</td>
<td>33.1 ± 4.8</td>
<td>4.5 ± 0.9</td>
<td>17.2 ± 0.5</td>
<td>30.9 ± 5.6</td>
<td>13.9 ± 2.8</td>
</tr>
</tbody>
</table>

Fig. 3. LC-MS spectra of SQDG extracted from *Aster tripolium* showing two different molecular ion peaks at (A) 837 Da and (B) 815 Da, in the complete mass spectrum with 815 and 837 Da peaks. Single mass analysis with 0.1 and – 2.0 ppm tolerance for 815 and 837 Da, respectively. Mass spectra were recorded in the negative electrospray mode (cone voltage 30 V) by an LCT time of flight mass analyser (Micromass, Manchester, UK), equipped with a LockSpray™ unit for exact mass measurement.

Fatty acids on sn-1 and sn-2 was investigated by Bishop et al. (1985). All samples of SQDG isolated from different glycohytic species contained higher contents of 16:0 than of the corresponding glycolipids occurring in plant membranes monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). The positional distribution of 16:0 demonstrated that in the 16:3-plants significant amounts of these acids are present at both the sn-1 and sn-2 positions, demonstrating...
that both prokaryotic and eukaryotic synthesis mechanisms for DAG production contribute to the biosynthesis of SQDG. In the 18:3-plants 16:0 acids are predominantly located in the sn-1 position of SQDG, again demonstrating the origin of the DAG as being the eukaryotic pathway. It is not known to which group of plants Aster and Sesuvium belong to. To our knowledge this is the first report of a clear increase of SQDG in halophytes due to salt stress and 16:0/18:3 and 18:3/18:3 SQDGs in the halophytes Aster and Sesuvium. The effect of low temperatures on the lipid content and composition of the thylakoid membrane was investigated in the glycophyte Pisum sativum L. (Chapman et al., 1983). Both, relative amounts of lipid classes and degree of saturation, were not changed significantly during growth at cold and warm conditions. However, it was demonstrated that in cold-grown plants there were slightly higher 18:3 and lower 18:2 contents for all three glycolipids, MGDG, DGDG, and SQDG. In our experiments temperature effects can be excluded, as temperature in the greenhouse was controlled. Therefore direct effects of the salt treatment on the SQDG contents can be assumed.

It was hypothesized that SQDG is involved in stabilization of ATPase complexes and PSII (Pick et al., 1985). This hypothesis seems to match our experimental results on F-ATPase activities. Aster F-ATPase activity increased with increasing NaCl concentration in the medium of growth (Ramani, 2004). Recently, Minoda et al. (2002, 2003) confirmed the role of SQDG in the functionality of PSII in more detail. The authors demonstrated that a Chlamydomonas reinhardtii mutant deficient in SQDG biosynthesis showed almost 40% reduction in PSII activity. The activity could be restored when SQDG was supplemented; furthermore chemical modifications of SQDG impaired the activity of PSII. Taken together SQDG clearly contributes to maintain the conformation and efficiency of PSII complexes in Chlamydomonas. Similar effects were reported for a Chlamydomonas SOD1 mutant. This genetically well characterized mutant showed increased sensitivity to a diuron herbicide, a herbicide binding to the Qb receptor site of PSII. PSII activity was blocked in the SOD1 mutant suggesting a role for SQDG in photosynthesis (Riekhof et al., 2003).

The nutritional status of the plants also influences the SQDG content, mainly sulfur and phosphorus: Sulfur deprivation has a decisive impact on lipid metabolism in Brassica oleracea (DeKok et al., 1997). There was a substantial decrease in the levels of SQDG in both roots and shoots when expressed in a total lipid basis. A major role of SQDG in glycophyles might be the exchange of phospholipids to SQDG during phosphate starvation. Under normal growth conditions about 30% of Pi are bound in phospholipids which might be released under phosphate deficiency when increasing amounts of SQDG take over the role of phospholipids, at least in Arabidopsis (Essigmann et al., 1998). This mechanism still remains to be proved in halophytes.

The increasing amounts of SQDG during salt treatment might also play a role in signalling processes. It was shown that spinach annexin binds in the presence of 100 μM Ca²⁺ not only to envelope membranes of purified chloroplasts, but also to vesicles consisting of phosphatidylcholine (PC) and SQDG (Seigneurin-Berny et al., 2000). However, only SQDG and not PC was accessible in the asymmetric outer envelope membrane and might communicate any signals via annexin to the cytoplasm or vice versa.

Based on the experimental results of SQDG quantification and fatty acids analysis in Aster and Sesuvium, it might be suggested that in some halophytes SQDG plays a protecting role during salt stress. To follow this hypothesis we need to construct and analyse halophytic mutants. It would be very useful and interesting to start a lipomics project. This project aims at investigating the abundance of all lipids and their fatty acids in different membranes of the cell during application of various abiotic stresses. That kind of analysis might help to reveal the role of SQDG in the plastids during salt stress.

Acknowledgements

We are grateful to Dr. G. Dräger, Institute for Organic Chemistry, University of Hannover, for performing the MS verification of SQDG. We would like to thank Prof. Dr. H. Lieth for providing Aster tripolium seeds, Dr. S. Daoud, Morocco, for sending Sesuvium portulacastrum seeds, and Prof. Dr. C. Benning for providing the seeds of the Arabidopsis thaliana of the ecotype Wassilewskia. We are grateful for an excellent technical assistance by Julia Volker and Imke Pillukat. We appreciate the valuable work of our gardeners in the greenhouse. The project was supported by a grant from the Land Niedersachsen and by the Graduiertenförderprogramm from the University of Hannover.


