The Effect of Mineral Deficiency on the Photosynthetic Apparatus in Maize. I. The Role of Chloroplast Sulfolipid[1]

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Abstract

Galacto- and sulfolipid content has been determined in sulfur and nitrogen-deficient maize leaves. Only slight variations in monogalactosyl diglyceride and digalactosyl diglyceride levels were detected in S-deficient plants compared to normal maize leaves but there was a 50% decrease of both these lipids in N-deficient plants. The greatest difference was found in the sulfolipid content in either S- or N-deficiencies—the sulfolipid was reduced by 30 to 50%. Since decreased sulfolipid content seemed to be associated with increased Photosystem II activity and the occurrence of larger chloroplast grana stacks, it is proposed that sulfolipid may play a role in grana stacking along with other structural chloroplast lipids. At least a correlation between decreased sulfolipid and galactolipid content is associated with the occurrence of larger chloroplast grana stacks and increased Photosystem II activity.

Introduction

In our studies of photosynthetic activity of chloroplasts from nitrogen-, potassium-, phosphorus-, sulfur-, magnesium-, and calcium-deficient plants we found an increased Photosystem II activity in the chloroplasts from S- and N-deficient plants (3). Since increased rates for PS II activity were observed in 2 separate assay systems (water $\rightarrow$ indophenol dye and diphenylcarbazide $\rightarrow$ indophenol dye) and were correlated with increased grana stacking in studies of the fine structure of mineral-deficient chloroplasts by electron microscopy (9), it became important to investigate the role of sulfolipids in S- and N-deficient chloroplasts in relation to normal maize sulfolipid content.

Nichols and James (13) have reviewed the literature on chloroplast lipid composition while Haines has surveyed the chemical information on sulfolipids (8). Plant sulfolipids have been localized in the chloroplast and assayed in several species of plants (1, 2, 6, 14, 16). Previous studies on the composition of chloroplast lipids consider sulfolipid a minor constitutent ($<10\%$ present) and assign a structural role to it. Our discovery of reduced sulfolipid content in S- and N-deficient plants both of which exhibit higher than normal PS II activity points to the importance of sulfolipid not only as a passive structural element but also as something critical for proper enzymatic activity of chloroplast grana stacks.

[1]Supported by N.S.F. Grant #GB-27501.

Materials and Methods

Methods for nutrient culture of various mineral-deficient maize plants have been described (5). The galacto- and sulfolipid content of S- and N-deficient plants was determined as follows: 5-g quantities of fresh or frozen maize leaves were homogenized in 90% acetone in a Waring blender and strained through a sintered glass filter. The residue was rewashed with several aliquots of acetone. Then the total acetone extract was transferred to a separatory funnel containing an equal amount of petroleum ether (B.P. 40-60°C). Water-soluble components were removed by water washes and discarded. The green epiphase containing pigments and lipids was washed several times with increasing concentrations of methanol-water (50:50; 75:25; 90:10). The combined methanol-water extracts were evaporated to dryness in a rotary evaporator at 30°C to yield a total lipid fraction. Evaporation was slow and required the addition of several portions of acetone. The final dry lipid mixture was suspended in diethyl ether and used for thin layer chromatography. Silica gel G plates were developed in water, glacial acetic acid, methanol and chloroform (4:10:15:75) according to Nichols (12) or in water, benzene, acetone (8:30:91) by Pohl, Glasl and Wagner's method (15). Monogalactosyl diglyceride, digalactosyl diglyceride and sulfolipid were identified by spraying with 0.0035% rhodamine G in 0.5% KOH solution and observing fluorescent bands under U.V. light. After scraping the desired bands the various lipids were eluted from the silica gel with diethyl ether and evaporated to dryness under vacuum from an aspirator. If plates were developed in mixtures containing glacial acetic acid as in (12), the lipid bands were located by spraying one side of the chromatogram with reduced methylene blue spray according to Barr and Crane (4).

Monogalactosyl diglyceride, digalactosyl diglyceride and sulfolipid were assayed quantitatively by the phenol-sulfuric acid method as described by Roughan and Batt (16). Sulfolipid was also determined as inorganic sulfate after charring and acid hydrolysis in sealed glass tubes. The spectrophotometric analysis of sulfate with barium chloranilate reagent was performed according to Dittmer and Wells (7).

Results

The visible symptoms of sulfur- and notrogen-deficient maize leaves compared to normal maize are shown in Figure 1 (A). This figure also shows a thin layer chromatrogram (B) where the sulfolipid spot is visible as a light charred area in the control but not at all in S-deficient plants in which the sulfolipid content is reduced by about half. Figure 1 (C) shows the results of the phenol-sulfuric acid test for sugars by which monogalactosyl diglyceride and digalactosyl diglyceride can be identified by their galactose moiety and sulfolipid by its quinovose moiety. By using spectrophotometric comparisons to galactose standards, the sulfolipid content of normal maize leaves is higher than that of S-deficient leaves.

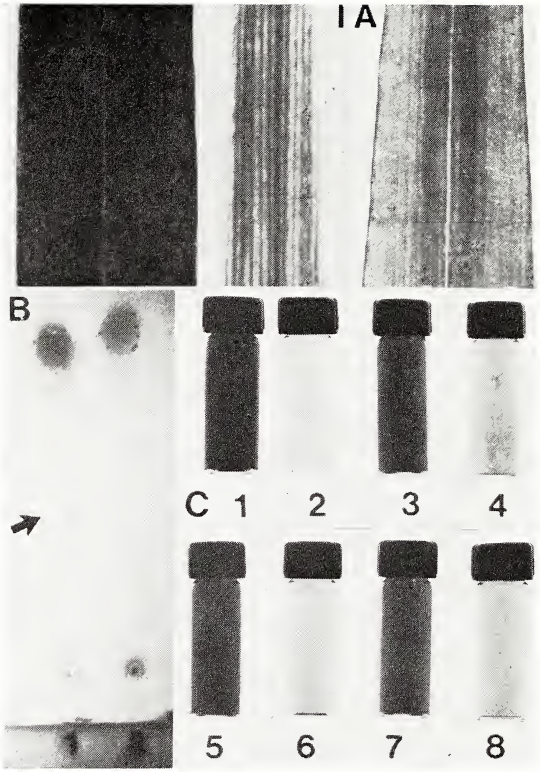

FIGURE 1. A. *Control, S-deficient and N-deficient maize leaves from left to right in the order indicated.* B. *Thin-layer chromatography of sulfolipid, (arrow) from 1) control and 2) S-deficient maize leaves (level below limit of detection by TLC).* C. *Results of phenol-sulfuric acid test with control and S-deficient maize leaves: 1-4, control; 1-MGD, 2-plate blank, 3-DGD, 4-sulfolipid; 5-8, S-deficient; 5-MGD, 6-plate bland, 7-DGD, 8-sulfolipid.*

TABLE 1. *A comparison of galactolipid and sulfolipid levels in normal* versus *S-deficient maize leaves.*[1]

Designation	By Sugar Analysis		
	MGD	DGD	SL
	μmoles/g Fresh Weight		
Control	2.95	1.11	0.85
-S	2.23	1.41	0.35
-N	0.96	0.30	0.48
	By Sulfur Analysis		
Control	—	—	0.84
-S	—	—	0.50

[1]Plants grown in nutrient culture in the greenhouse during July and August at high temperatures (25-30°C).

Table 1 summarizes the results of chloroplast lipid analysis. In S- and N-deficient leaves the sulfolipid content is reported from either of 2 separate assays: 1) by analysis of the sugar moiety using the phenol-sulfuric acid test and 2) by sulfur analysis with the barium chloranilate test for inorganic sulfur. The results of both tests indicate a reduced sulfolipid content of S- and N-deficient leaves in contrast to normal maize while differences in galactolipid—monogalactosyl diglyceride and digalactosyl diglyceride—levels are not marked for S-deficient plants but are sharply reduced in N-deficient plants in relation to the control.

Figures 2 and 3 show electron micrographs of normal and S- or N-deficient chloroplasts at the age of 8 weeks. It can be seen that both deficiencies result in larger grana stacks and less stroma lamellae than normal maize plants. The size of osmiophilic globules is increased in chloroplasts from N-deficient plants (Fig. 3, B).

Discussion

From our studies of PS I and II activities in six types of mineral deficiencies (N, P, K, S, Mg, Ca) in maize, we find increased PS II activity in S- and N-deficiencies. Sulphur- and N-deficient plants contain chloroplasts with larger grana stacks and a proportionally larger amount of grana lamelle in relation to stroma lamellae (3, 9). This led us to investigate the lipid composition of S- and N-deficient plants reported here (Table 1), since chloroplast lipids have been implicated as structural components of chloroplast membrane systems. As this table shows we found only slight differences in the two major chloroplast lipids, monogalactosyl diglyceride and digalactosyl diglyceride, in S-deficient versus normal maize leaves but a striking reduction in the amount of galactolipids present in N-deficient plants. The sulfolipid content was also reduced up to 50% in the leaves from S- and N-deficient plants. Thus sulfolipid, although present in lesser amounts than chloroplast galactolipids, may function in the control of grana stacking. Since only about a third to a half of the normal amount of sulfolipid is present in S- and N-deficient plants while the proportion of grana to stroma lamellae is increased, it may be inferred that in the absence of the normal amount of sulfolipid stroma lamellae stick together more easily to form abnormally large grana stacks. Thus sulfolipid appears to control grana stacking in S- and N-deficient maize chloroplasts.

Other agents which alter the control of grana stacking include light and the concentration of cations. In bright light grana stacks are dissociated in *Amaranthus* chloroplasts while in plants grown in dim light abundant grana stacking is present (10). In isolated chloroplasts grana can be dissociated into single lamellae but with the addition of cations reassociation occurs (11). Both of these factors may operate in conjunction with the lipid composition in controlling grana stack formation.

FIGURE 2. *Normal maize chloroplast at the age of 8 weeks. Glutaraldehyde and* OsO_4 *fixation with uranyl acetate and lead citrate stains. X 25,000*

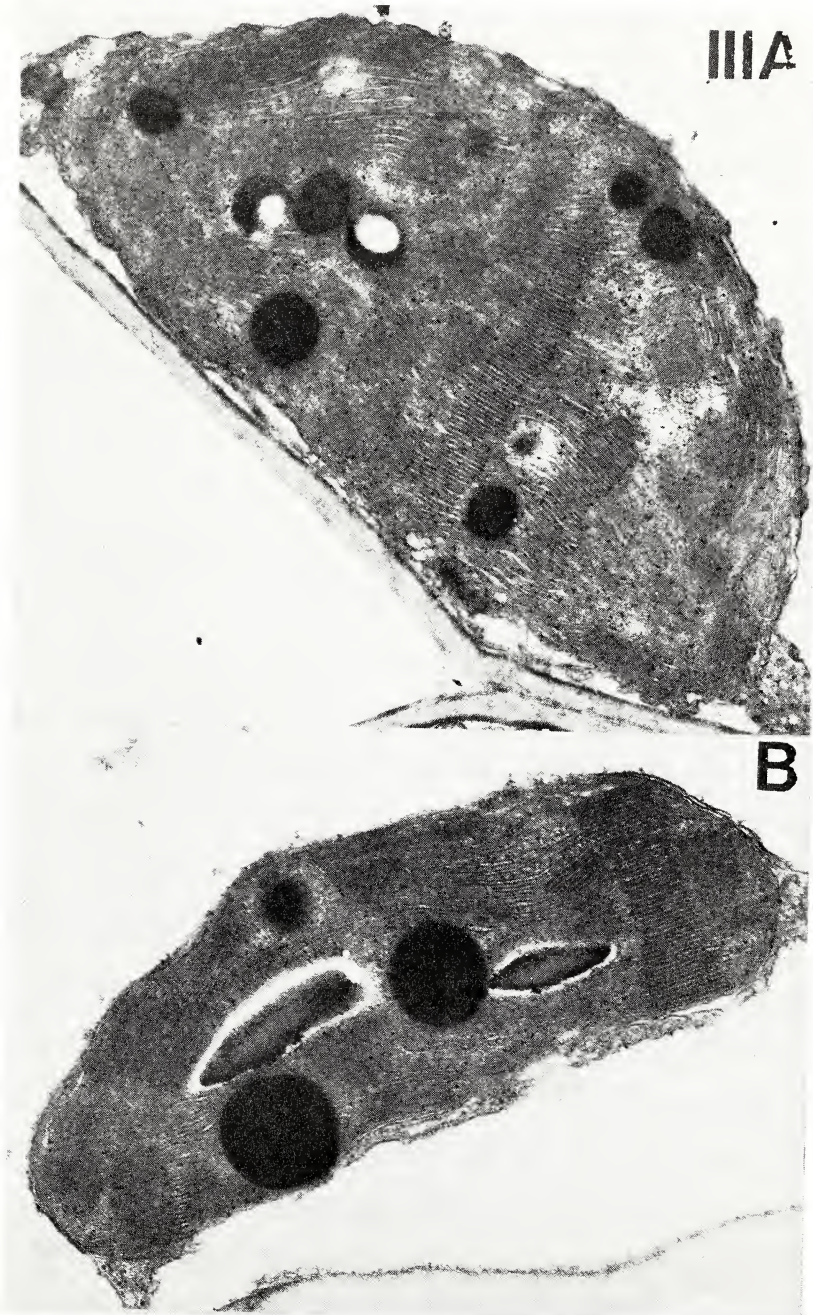

FIGURE 3. A. *Sulfur-deficient chloroplast at the age of 8 weeks. Fixation and staining as for Fig. 2. X 25,000.* B. *Nitrogen-deficient chloroplast at the age of 8 weeks. Fixation and staining as above. X 35,000*

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