Alteration of Soybean Complex Lipid Biosynthesis by S-Ethyl Dipropylthiocarbamate

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ABSTRACT

Soybean (Glycine max [L.] Merr. var. Glabrous D62-7812) plants were grown in aerated Hoagland and Arnon mineral nutrient solution containing 0 or 2.6 μM S-ethyl dipropylthiocarbamate (EPTC) in a growth chamber. After 19 days exposure to EPTC, total leaf fresh weight was reduced 18% by 2.6 μM EPTC while total leaf fatty acid content was reduced 63%. Galactolipid content decreased while phospholipid content increased. Linolenic acid content decreased from 67.5% of the total fatty acid content to 31.5% with 2.6 μM EPTC treatment. Equivalent increases were observed in palmitic (+6.3%), stearic (+1.1%), oleic (14.4%), and linoleic (+13.9%) acids.

EPTC\(^1\) inhibits long chain fatty acid synthesis in germinating seeds (1), alkane synthesis in epicuticular waxes (9), incorporation of unsaturated fatty acids into leaf complex lipids (18), synthesis of fatty acids in chloroplasts and tubers (17, 18, 20), fatty acid desaturation (20), and root PL synthesis (8). Several other thiocarbamate analogs also inhibit chloroplast fatty acid synthesis (17–19). EPTC does not function, primarily as an inhibitor of photosynthesis or respiration (2). Therefore an evaluation of complex lipid class fatty acid contents from plants treated with EPTC was undertaken to study the influence of EPTC on: (a) galactolipid and phospholipid synthesis in leaves; and (b) fatty acid syntheses in leaves.

MATERIALS AND METHODS

Plant Growth. Uniform, large soybean (Glycine max [L.] Merr. cv. Glabrous D62-7812) seeds were planted in sand, watered with 1 × Hoagland and Arnon complete mineral nutrient solution (6), covered and placed in a growth chamber at 27 C. Eight days after planting, sand was washed from the seedlings, and the plants were transferred to 0.5 Hoagland and Arnon aerated nutrient solution (6) containing 0 or 2.6 μM EPTC and grown in a growth chamber at 276 μeinsteins m⁻² sec⁻¹ with 27 C and 15-hr photoperiods. After 19 days, the plants were harvested for fresh weights of mature leaflet blades, other leaflet blades, stems plus petioles, and roots. All samples were frozen in dry ice and maintained at −20 C.

Lipid Preparation. Lipids of three, mature trifoliate leaflet blades from each plant were extracted in redistilled chloroform-methanol (2:1) at 0 C for 15 min and washed by the method of Folch et al. (4). The chloroform was evaporated on a glass rotary evaporator without heat and the samples were transferred to Teflon-capped test tubes in chloroform-methanol (2:1) and stored at −20 C under N\(_2\). Lipids were separated according to class by one-dimensional TLC method of Pohl (10) using silica gel (0.5 mm) activated 30 min at 130 C and cooled in a desiccator. A 2-ml lipid aliquot was streaked on the plate. The developing solvent was aceton benzene-distilled H\(_2\)O (91:30:8) in a filter-paper-lined, saturated chamber. Separated bands were found according to Pohl (10) (i.e. neutral lipids I, II, MGDG, CL, DGDG, SL, PG, PE, PI, and PC). However, separation of PG, PE, and PI was not sufficient for accurate analyses. Therefore, PG, PE, and PI were analyzed together and combined with PC as PL herein. Separation of CL, PG, and PA was checked by TLC of pure standards. They did not overlap. After visualization of the lipid classes by rhodamine and UV (7), the individual lipid class bands were eluted with chloroform-methanol (2:1). Solvent was evaporated on a rotary evaporator and fatty acid methyl esters were prepared by the method of Karunen (8) utilizing 1 ml of 0.5 n NaOH in methanol, 65 C, 5 min, for saponification and 6 ml of 14% BF\(_3\)-MeOH, 65 C, 5 min, for esterification in Teflon-lined screw-capped 20-ml culture tubes. After cooling and the addition of 10 ml of distilled H\(_2\)O, fatty acid methyl esters were extracted in petroleum ether (b.p. 30 to 60 C), transferred to serum vials, solvent evaporated under N\(_2\), and the dry sample was stored at −20 C under N\(_2\) until analyzed by GLC. Quantitation was by the addition of 0.2 mg of heptadecanoic acid to each lipid class prior to saponification. GLC procedures were published previously (16).

Carbohydrate Content. Sucrose, reducing sugar, and total carbohydrate contents of ground lyophilized leaves were measured by a colorimetric determination based on a ferrocyanide-arsenomolybdate complex (15).

RESULTS

Although average plant fresh weight was reduced 18% by 2.6 μM EPTC, total leaf fatty acid content was decreased 63% (Table I). Quantitatively, the decrease in fatty acid content was seen in all components except oleic acid which was increased in quantity (Table I). Qualitatively, linolenic acid content was decreased from 67.4 to 31.5% of the total leaf fatty acids by 2.6 μM EPTC while palmitic, stearic, oleic, and linoleic acids were increased by a percentage equal to the decrease in linolenic acid. Extent of qualitative modification is shown by the ratios of unsaturated to saturated fatty acids (ΣU to ΣS) which dropped significantly and by the ratio of linolenic acid to oleic + linoleic acids which decreased drastically with EPTC treatment (Table I).

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\(^{1}\) Abbreviations: EPTC: S-ethyl dipropylthiocarbamate; MGDG: monogalactosyl diglyceride; DGDG: digalactosyl diglyceride; SL: sulfoquinovosyl diglyceride; CL: cardiolipids; PL: phospholipids; PC: phosphatidylincholine; PG: phosphatidylglycerol; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PA: phosphatidic acid.
Influence of EPTC on the total fatty acid content of the lipid classes varied. MGDG and CL fatty acid contents decreased while DGDG total fatty acid content remained unchanged, and SL and PL fatty acid contents were markedly increased (Table II). MGDG fatty acid composition was altered by reduced quantities of oleic, linoleic, and linolenic acids, while palmitic and stearic acids contents remained unaltered. Thus, the percentage of each component fatty acid present in MGDG was not changed appreciably while the ratio $\Sigma U/\Sigma S$ was altered significantly. Similar responses were found in SL. However, in DGDG the major changes observed were a decrease in linolenic acid content which was essentially equal to the increased contents of stearic, oleic, and linoleic acids. These “balanced” alterations resulted in a large change in the linolenic to oleic + linoleic acids ratio without major modification of the $\Sigma U/\Sigma S$ ratio. Additionally, the MGDG to DGDG ratio decreased from 0.94 to 0.64 while the total galactolipid to $\Sigma PL$ ratio decreased. SL fatty acid content was increased nearly 4-fold by 2.6 $\mu M$ EPTC.

Although the SL linolenic acid content was decreased by 2.6 $\mu M$ EPTC, the quantities of each of the other components were increased. Similar results were observed with the PL fraction (Table II). Additionally, the percentage of the total fatty acids present within each lipid class was altered. Percentages of MGDG, CL, and DGDG present in the total fatty acid contents were decreased by 2.6 $\mu M$ EPTC while SL and PL total fatty acid contents increased (Table II).

**DISCUSSION**

The EPTC-induced inhibition of leaf total fatty acid content (Table I) constitutes the first demonstration of a decrease in internal fatty acid content/g fresh weight of leaves by a thiocarbamate herbicide. Although inhibition of fatty acid synthesis/ plant by EPTC and other analogs has been documented (8, 9, 16–20), concomitant growth reductions and metabolic synthesis alterations usually preclude determination of primary activity for this group of compounds. Wheat root growth was less inhibited by EPTC than was PL synthesis (8). Since fatty acid synthesis is inhibited more than growth (Table I) and growth inhibitions by EPTC are reversed by concomitant applications of giberellic acid $A_2$ (5), the data presented herein substantiate the suggestion that fatty acid synthesis inhibition is a major mode of action for the thiocarbamate herbicides (17–20). Inhibition of fatty acyl elongation was suggested as the biochemical activity of EPTC (9). However, the synthesis of unsaturated fatty acids was stimulated by an EPTC antidote (20). The inhibition of linolenic acid synthesis (Table I) suggests that the inhibition of polyunsaturated fatty acid synthesis may be an important factor. These data do not discriminate between the two hypotheses of linolenic acid synthesis in leaves. However, the major decrease in linolenic acid content associated with the lack of accumulation of triple unsaturated acyl units shorter than C$_{18}$ and the increased percentage content of oleic and linoleic acids suggests the synthesis of linolenic acid by desaturation of linoleic acid as reported by Cherif et al. (3).

Alteration of the quantity of the major lipid classes by EPTC (Table II) and the $\Sigma GL$ to $\Sigma PL$ ratio reveals an inhibition of galactolipid synthesis with an accompanying increase in PL content. This accumulation of PL is consistent with the suggestion (11, 12) that acyl units are transferred from PC to the galactolipids. Whether the inhibition of galactolipid synthesis was due...
solely to fatty acid inhibition is questionable. Reducing sugar content was reduced 37%. Assuming an equivalent diminution of available galactose without any influence on fatty acyl availability, the \( \Sigma \)GL to \( \Sigma \)PL ratio would decrease from 1.40 to 0.50. This is a greater reduction in the \( \Sigma \)GL to \( \Sigma \)PL ratio than was found. Galactosylation is not specific for unsaturated diglycerides as is shown by the fatty acid content of MGDG and DGDG from EPTC-treated leaves (Table II).

**LITERATURE CITED**

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