Effect of Ozone on Sterols and Sterol Derivatives in Bean Leaves

Harley Tomlinson and Saul Rich

Assistant Plant Pathologist and Senior Plant Pathologist, respectively, Department of Plant Pathology and Botany, The Connecticut Agricultural Experiment Station, New Haven 06504.

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ABSTRACT

Acylated sterol glycosides (ASG) and sterol glycosides were found to increase consistently as the free sterols decrease in bean leaves exposed to ozone. The following fatty acids were detected in bean leaf ASG analyzed by separating the methyl esters by gas-liquid chromatography: palmitic, stearic, oleic, linoleic, and linolenic. All these fatty acid components increase in the ASG of ozonated leaves, with a major increase in linolenic acid. Ozone increases cell permeability. Sterols and their derivatives are important components of cellular membranes, and these changes may be one step in ozone toxicity. Phytopathology 61: 1404-1405.

Additional key words: Phaseolus vulgaris L.

Toxic doses of ozone change the structure and function of cellular membranes (1, 8). Recently, sterols and sterol derivatives have been found to be associated with all membrane-containing fractions of tobacco leaves (3). To understand how ozone alters membranes, we have been studying the changes in the sterols of ozonated bean leaves. We now report that in the leaves of beans exposed to ozone, free sterols decrease with a simultaneous increase of sterol glycosides (SG) and acylated sterol glycosides (ASG).

Pinto bean plants (*Phaseolus vulgaris* L.) 10-14 days old were placed in a lighted plastic chamber and exposed to ozone (0.25 μliter/liter) for 2.5-3 hr when cells in the primary leaves begin to leak. Control plants were exposed to ambient air for the same length of time. Leaf discs (3 cm diam) were cut from the leaves and extracted with chloroform:methanol (1:1). Lipids in the concentrated extract were resolved in bands on silica-gel thin-layer chromatography (TLC) plates in chloroform-methanol (9:1). We located sterols and sterol derivatives as purple bands by spraying all or part of the plate with 50% sulfuric acid and heating to 70 C. The average R_f values were SG 0.2 and ASG 0.4, and sterols were 0.8. The lipid concentrations were determined by visual comparison with known compounds, and by the Lieberman-Burchard reaction after elution (4). Concentrations of ASG were also determined by measuring peak areas of fatty acid esters analyzed by gas-liquid chromatography.

Acylated sterol glycosides eluted from TLC plates were purified by washing with aqueous sodium carbonate, acidifying the residue with hydrochloric acid, and rechromatographing. The purified ASG were eluted, and the fatty acids were treated with acidic methanol in screw-cap test tubes at 70 C for 4 hr. The resulting methyl esters were extracted in chloroform, purified by TLC in benzene, and recovered for gas-liquid chromatography. Recovered esters were separated through a 6-ft stainless steel tube containing chromosorb W 80/100 mesh and 15% diethylene glycol succinate at 210 C in a Perkin Elmer Model 810 instrument. Comparisons were made with known pure esters, detected by a dual, hydrogen flame ionizer.

By visual comparison with known amounts of sterol, SG, and ASG chromatographed on TLC plates, it was estimated that ozonated leaves contained 25% less free sterol, 100-150% more ASG, and 50% more SG. In six different experiments, these lipids were eluted from TLC plates and measured by using the Lieberman-Burchard reaction (4). The μmoles found per 10 leaf discs for free sterols, SG, and ASG were 0.93 ± 0.01, 0.25 ± 0.01, and 0.16 ± 0.01, respectively. After ozonation, free sterols had decreased to 0.73 ± 0.03 μmoles, whereas SG and ASG had increased to 0.33 ± 0.03 and 0.27 ± 0.03 μmoles respectively. In three separate experiments, the sum of the peak areas of fatty acids from ASG of control plants were 75, 122, and 148 mm^2 as compared to areas of 202, 232, and 297 mm^2 from the ozonated plants.

The principal fatty acids found in bean leaf ASG were palmitic, stearic, oleic, linoleic, and linolenic. The major components were palmitic and linolenic acids. Palmitic acid made up 40% of the total both before and after ozonation, whereas the linolenic composition increased from 25% before ozonation to 39% of the total after ozonation. In the ASG from control plants, the ratio of linolenic to palmitic was 0.62. After ozonation, this ratio increased to 1.0.

As sterols and sterol derivatives are found in most living cells and are associated with cellular membranes of tobacco leaves, Grunwald (2) suggested that sterols may control the permeability of plasma membranes in all plants. Ongun & Mudd (7) reported evidence that SG and ASG are normally formed at the expense of free sterols in nonozonated plants. An initial symptom of ozone toxicity to plants is the leaking of cellular liquids into the intercellular spaces. Therefore, the loss of free sterols by conversion to SG and ASG in ozonated bean leaves may be associated with the changes in cellular permeability.

The first known report of ASG was β-sitosteryl-D-glucoside monopalmitate isolated from opium cake in 1962 (6). Since then, the composition, structure, and chromatographic properties of several ASG from plants have been established (5). Our analyses of the fatty acids of the ASG from nonozonated bean leaves agreed in general with analyses of ASG from other plant material. However, following ozonation the linolenic acid content of bean leaf ASG is uncommonly high. Since linolenic acid is the major fatty acid component of lipids in leaf cell membranes, it may be suggested that SG and ASG are also synthesized at the expense of essential diglycerides in ozonated bean leaves. Support for the suggestion

comes from the evidence that ozone inhibits galactosyldiglyceride synthesis by chloroplast suspensions (J. B. Mudd, *personal communication*). The metabolic increase of ASG in ozonated bean leaves clearly involves intact linolenic acid. Because of our finding that there is no evidence of lipid peroxidation in bean leaves immediately after ozonation (9), we suggest that important metabolic changes in lipid synthesis occur as a direct result of ozonation, and that lipid peroxidation is a secondary effect.

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