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Changes in Membrane Polar Lipids Associated with Bud Break in Apple Induced by Nitroguanidines

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Abstract. The predominant lipids in membranes obtained from apple buds were galacto- and phospholipids. The major galactolipid components in apple bud were monogalactosyl diglyceride (MGDG) and digalactosyl diglyceride (DGDG). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were the major phospholipids in the apple buds. α-Linolenic acid (C 18:3) was the major fatty acid in MGDG, DGDG, and PC. Phosphatidylglycerol (PG) is the only lipid to contain significant amounts of palmitic acid (C 16:0) in the dormant buds. An increase in the galacto- and phospholipids and the ratio of the unsaturated fatty acids to the corresponding saturated fatty acids of the buds occurred as a result of induction by 1-(3,5-dichlorophenyl)-3-nitroguanidine or 1-(α-ethylbenzyl)-3-nitroguanidine during bud break. The identities of fatty acids in apple buds were confirmed by gas chromatography–mass spectrometry.

Biological membranes are the fundamental active sites for many specific enzyme activities, transport of ions, and hormonal receptors (Brenner 1984). Changes in acyl-lipid saturation of membranes often occur in response to external stimuli (light, temperature, and chemicals) (Bishop et al. 1979, Bladocha and Benveniste 1983, Gardner and Stowe 1979, Kuiper 1985). The physical state of membrane lipids is important in determining the physiological function of plant tissue (Brenner 1984, Raison and Chapman 1976, Wang 1982). The

Abbreviations: CI, chemical ionization; DGDG, digalactosyl diglyceride; DMSO, dimethyl sulfoxide; EI, electron impact; FAME, fatty acyl methyl esters; FID, flame ionization detector; GC-MS, gas chromatography–mass spectrometry; MDGD, monogalactosyl diglyceride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; TLC, thin-layer chromatography.

composition of membrane lipids may also be a factor determining major biological properties of membranes that in turn may influence biological changes, such as dormancy or resumption of growth in plants. The plant bioregulant, thidiazuron (N-phenyl-N′-1,2,3-thidiazol-5-ylurea), releases lateral buds from dormancy and is correlated with an increase in unsaturated polar membrane fatty acids (Wang and Faust 1988). A new class of synthetic cytokinins, known as benzylnitroguanidines, induce a sequence of changes in the sterol composition associated with bud break and bud development (Wang and Faust 1989). A decrease in the percentage of sitosterol and sitosteryl ester was accompanied by an increase in campesterol and stigmasterol at the beginning of rapid growth. A decrease in the ratio of free sterols to phospholipids and an increase in the ratio of campesterol + stigmasterol to sitosterol occurred upon breaking of dormancy in apple buds induced by these compounds (Wang and Faust 1988). The purpose of this study was to determine the changes of galacto- and phospholipid and their fatty acid composition in buds during bud break and bud growth induced by nitroguanidines.

Materials and Methods

Plant Material and Treatments

Dormant apple seedling buds (*Malus domestica* Borkh, cv. York Imperial) treated with 1-(3,5-dichlorophenyl)-3-nitroguanidine, or 1-(α-ethylbenzyl)-3-nitroguanidine, were used in this study (Wang and Faust 1989). Treatments were applied to first five buds. All solutions were prepared in 2.5% DMSO plus 0.5% Tween-20 and applied directly to the buds with a brush until runoff. Five buds were harvested from each plant. Triplicate bud samples of 0.5 g fresh weight were collected at 5-day intervals over a 25-day period after treatment with nitroguanidines.

Extraction, Fractionation, and Analysis of Polar Lipids

The methods of extraction, fractionation, and analysis of lipids in apple buds have been reported (Wang and Faust 1989). Purified lipids were separated into neutral, glycolipid, and phospholipid fractions by silicic-acid column chromatography on 100- to 200-mesh BioSil A (BioRad Laboratories, Richmond, CA, USA). The glycolipid and phospholipid fractions were further separated by TLC on 20 × 20 cm glass plates precoated with a 250-μm thickness of silica gel 60 (EM Reagents, Darmstadt, FRG) using acetone–acetic acid–water (100:2:1, v/v) and chloroform–methanol–acetic acid–water (85:15:10:3.5, v/v), respectively. Individual galactolipids and phospholipids were identified by cochromatography with authentic standards (Sigma Chemical Co., St. Louis, MO, and Supelco, Bellefonte, PA, USA) and by detection with spray reagents specific for hexose sugars (Christie 1973) or phosphate (Dittmer and Lester 1964). Individual lipid bands were scraped and eluted in chloroform–methanol (2:1, v/v) followed by a Folch wash (Folch et al. 1957). Total fatty

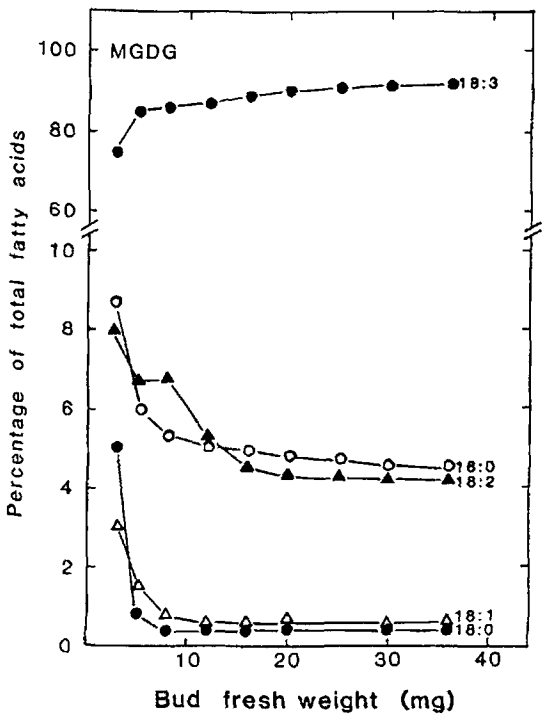

Fig. 1. Fatty acid composition (weight % of total) of MGDG in apple bud tissue during bud break induced by nitroguanidines. Data are pooled from various treatments with 1-(3,5-dichlorophenyl)-3-nitroguanidine or 1-(α-ethylbenzyl)-3-nitroguanidine, based on fresh weight of the buds. The first data point is for the dormant bud. LSD (5%) for 16:0 = 2.11; 18:0 = 5.73; 18:1 = 2.74; 18:2 = 2.19; 18:3 = 8.32.

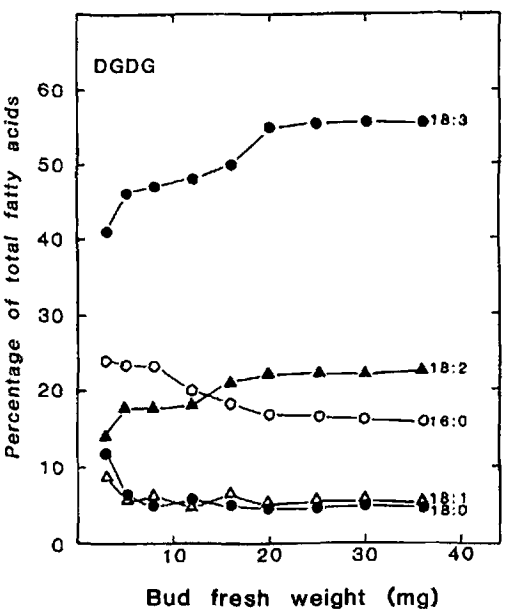

Fig. 2. Fatty acid composition (weight % of total) of DGDG in apple bud tissue during bud break induced by nitroguanidines. For details see Fig. 1. LSD (5%) for 16:0 = 2.88; 18:0 = 4.02; 18:1 = 2.08; 18:2 = 1.20; 18:3 = 4.36.

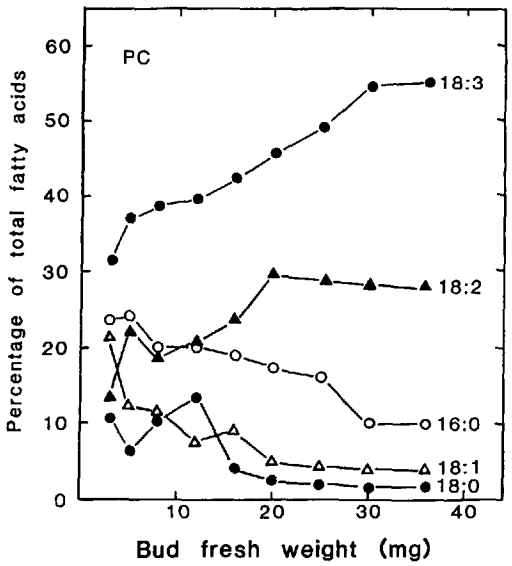

Fig. 3. Fatty acid composition (weight % of total) of PC in apple bud tissue during bud break induced by nitroguanidines. For details see Fig. 1. LSD (5%) for 16:0 = 2.01; 18:0 = 3.84; 18:1 = 5.12; 18:2 = 3.84; 18:3 = 5.76.

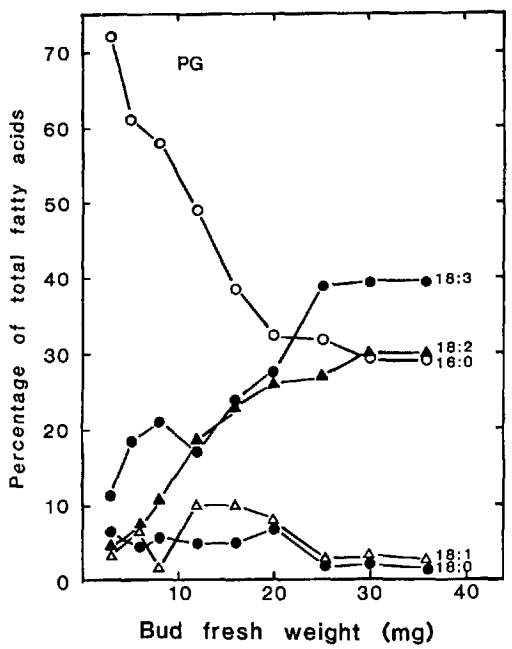

Fig. 4. Fatty acid composition (weight % of total) of PG in apple bud during bud break induced by nitroguanidines. For details see Fig. 1. LSD (5%) for 16:0 = 8.32; 18:0 = 1.47; 18:1 = 3.52; 18:2 = 5.13; 18:3 = 5.72.

acids esterified to polar lipids were derivatized to fatty acyl methyl esters (FAMEs) (Kates 1972) for FID-GC analysis (Wang and Faust 1988). n-Heptadecanoic acid was included in all samples as an internal standard, and methyl heptadecanoate was used as an external standard. Individual FAMEs were identified by comparison of retention times with those of authentic standards

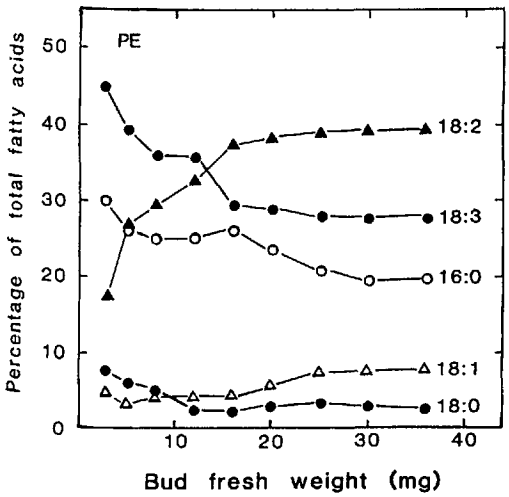

Fig. 5. Fatty acid composition (weight % of total) of PE in apple bud tissue during bud break induced by nitroguanidines. For details see Fig. 1. LSD (5%) for 16:0 = 3.75; 18:0 = 1.97; 18:1 = 1.98; 18:2 = 4.59; 18:3 = 4.75.

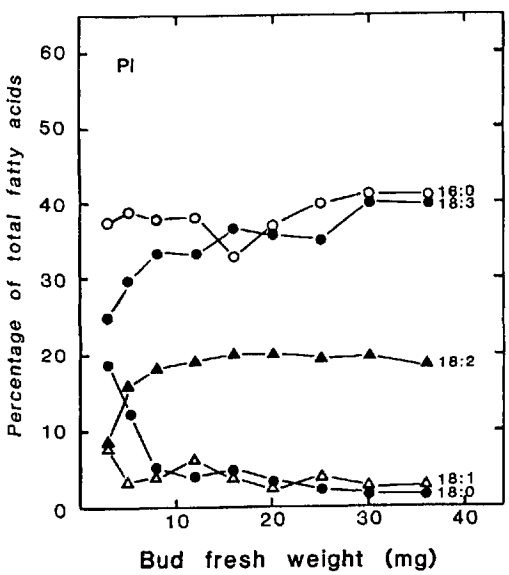

Fig. 6. Fatty acid composition (weight % of total) of PI in apple bud tissue during bud break induced by nitroguanidines. For details see Fig. 1. LSD (5%) for 16:0 = 2.72; 18:0 = 5.83; 18:1 = 2.17; 18:2 = 3.83; 18:3 = 2.19.

(Supelco). This tentative identification of the major polar lipid fatty acids was corroborated by further analysis of the FAMEs by gas chromatography–mass spectrometry (GC-MS).

Confirmation of Fatty Acids by GC-MS

A Perkin-Elmer Sigma 3B gas chromatograph equipped with a fused silica capillary column coated with a 0.25 μM film of DB-Wax (15 m × 0.32 mm i.d., J &

W Scientific, Inc. Folsom, CA, USA) and an Extrel ELTQ-400-3 mass spectrometer were used to confirm individual FAMEs. Electron energies were 70 eV for EI and 300 eV for CI. Isobutane CI was used to obtain very intense quasimolecular ions $(M + 1)^+$. The isobutane pressure, and ion source conditions were adjusted to obtain an ion intensity ratio of 10 to 1 for CI reagent ions of m/z 57 to 43. Column temperature was held at 150°C for 3 min and then programmed from 150–200°C at 5°C/min. Ultrapure helium was used as a carrier gas, and the head pressures of the helium carrier were 5 and 10 PSIG for EI and CI, respectively. The injector port and GC-MS interface line temperature was 250°C.

Results and Discussion

Palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and α-linolenic (C18:3) acids occurred in galacto- and phospholipids in apple bud tissue membranes (Figs. 1–6). Palmitic acid, linoleic acid, and α-linolenic acid were predominate fatty acids. The identification of these fatty acid esters was established by GC-MS. The full EI mass spectra of fatty acid esters was similar to the authentic standards, as previously reported (Heller and Milne 1978). The EI mass spectra of these compounds have weak molecular ions M^+. Isobutane CI has very intense quasimolecular ions $(M + 1)^+$. The FAMEs of palmitate, stearate, oleate, linoleate, and linolenate derived from extracted galacto- and phospholipids were also confirmed by selected ion-monitoring using the molecular ions M^+ and quasimolecular ions $(M + 1)^+$ in EI and CI, respectively. The molecular ions M^+ in EI of palmitate, stearate, oleate, linolate, and linoleate were 270, 298, 296, 294, and 292, respectively. The quasimolecular ions $(M + 1)^+$ in CI of palmitate, stearate, oleate, linolate, and linoleate were 271, 299, 297, 295, and 293, respectively. The GC-MS relative retention times of palmitate (5.93 min), stearate (9.52 min), oleate (9.80 min), linoleate (10.53), and α-linolenate (11.63) were identical to the relative retention times of authentic samples.

The lipid composition of the membrane obtained from apple buds was dominated by galacto- and phospholipids. PC, MGDG, and PE represented a higher percentage of the total lipids (Fig. 7). The galactolipid (MGDG and DGDG) and phospholipid (PC, PE, PG, and PI) changes and their fatty acid composition during bud break and bud growth induced by 1-(3,5-dichlorophenyl)-3-nitroguanidine were similar to those induced by 1-(α-ethylbenzyl)-3-nitroguanidine. Therefore, the changes of galacto- and phospholipids in apple buds induced by nitroguanidines were expressed based on the fresh weight of the buds. The effect of nitroguanidine, 1-(3,5-dichlorophenyl)-3-nitroguanidine, or 1-(α-ethylbenzyl)-3-nitroguanidine on bud break and bud growth has been reported (Wang and Faust 1989).

An increase in the galacto- and phospholipid of the buds occurred during bud break induced by nitroguanidines. The rate of increase was different for each lipid. The increase was 3.75-fold for MGDG and 2.28-fold for DGDG. The increase in phospholipid also paralleled the increase of bud weight during bud development. The major galactolipid components in apple bud tissue were

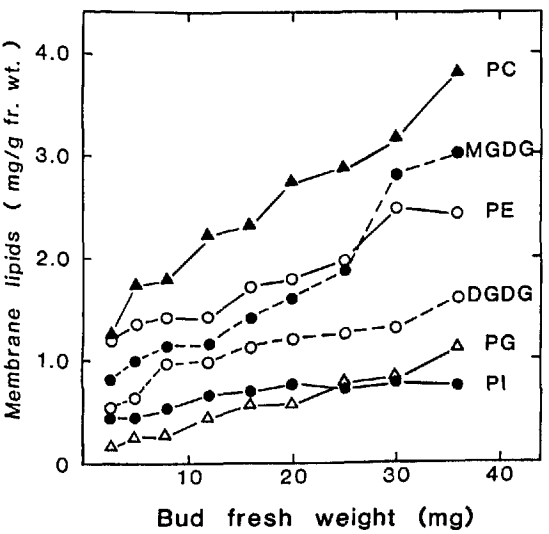

Fig. 7. Changes in membrane polar lipids associated with bud break and resumption of growth in apple bud tissue induced by nitroguanidines. For details see Fig. 1. LSD (5%) for MGDG = 0.32; DGDG = 0.27; PC = 0.43; PE = 0.19; PG = 0.35; PI = 0.21.

MGDG and DGDG. Linolenic (18:3) was the major acid in MGDG and DGDG (Figs. 1, 2). MGDG and DGDG are the major chloroplast thylakoid lipid (Williams et al. 1983). Chloroplast development is accompanied by an increased synthesis of galactosyl diglycerides and linolenic acid. They play an essential role in the maintenance of the electron transport system (Williams et al. 1983).

PC, PE, PG, and PI contain mainly palmitic, linoleic, and linolenic acid, which is typical for these phospholipids from higher plants. PC and PE were the major class of phospholipids in apple buds, comprising 76.5% of the total phospholipids (Fig. 7). PC is the major phospholipid constituent of almost every plant tissue (Galliard 1973). PC contained highly unsaturated fatty acids, mainly 18:3 (Fig. 3). PG was the only lipid to contain a significant amount of palmitic acid (16:0) in dormant buds (Fig. 4).

The relative percentages of 18:3 and 18:2 in PC and PG increased upon bud break, along with decreases in the relative percentage of 16:0 (Figs. 3, 4). The proportion of 18:2 in PE also increased at the expense of 18:3 and 16:0 during bud break (Fig. 5). Phosphatidic acid was detected but was less than 1% of total phospholipids (data not shown). PG and PI accounted for less than 25% of total phospholipids. The proportions of these two phospholipids also increased during bud break (Fig. 7). The changes of fatty acid in PI were less pronounced than in other phospholipids. However, the relative percentage of 18:3 and 18:2 also increased upon bud break and bud growth (Fig. 6).

The ratio of the unsaturated fatty acids to the corresponding saturated fatty acids increased in galacto- and phospholipids in apple bud tissue upon bud break and bud growth after induction by 1-(3,5-dichlorophenyl)-3-nitroguanidine or 1-(α-ethylbenzyl)-3-nitroguanidine (Fig. 8). Among major lipid components, MGDG contained the highest proportion of polyunsaturated fatty acids, and PC had the highest ratio of unsaturated to saturated among all phospholipids. The fatty acids of PG and PI were less unsaturated (Fig. 8). Inter- and intramolecular mixing of different acyl chains affect the physical properties of

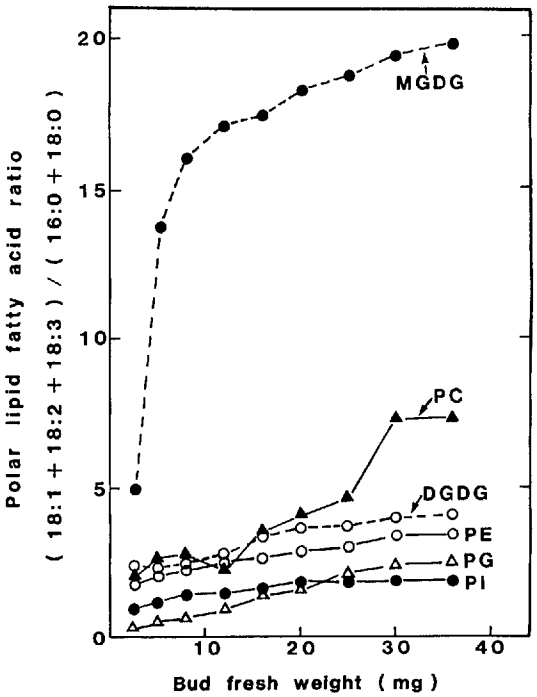

Fig. 8. Fatty acid saturation of apple bud tissue polar membrane lipids during bud break induced by nitroguanidines. For details see Fig. 1. LSD (5%) for MGDG = 0.97; DGDG = 0.58; PC = 0.34; PE = 0.26; PG = 0.32; PI = 0.20.

lipids (Phillips et al. 1970, 1972). The lipid composition of the various membranes of plant cells determines the fluidity of their lipid matrix. Increased ratio of unsaturated/saturated enhances fluidity, facilitates the efflux of water, and affects the physical state and functional properties of membranes (Brockerhof 1974, Oldfield and Chapman 1972). Changes in membrane permeability could affect both the movement of stimulus and the transport of metabolites in the membranes during bud break and bud growth.

Based on the results presented here, the ratio of unsaturated/saturated fatty acids in galacto- and phospholipids increased during bud break. Whether changes in membrane lipids associated with bud break and bud growth induced by nitroguanidines are due to modification in the rate of their synthesis, or are the result of degradation or conversion to other products, remains to be determined.

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