Oleate Desaturation in Young Winter Wheat Root Tissue[1]

CLAUDE WILLEMOT AND JACQUES LABRECQUE
Agriculture Canada, Research Branch, Sainte-Foy, Quebec G1V 2J3 Canada

ABSTRACT

[1,2-¹⁴C]Acetate was incorporated into the lipids of young wheat (*Triticum aestivum* L. cv Kharkov 22 MC) root tissue, but predominantly into sterols. [1-¹⁴C]Ammonium oleate was initially incorporated mainly into phosphatidylcholine (PC), and later into triglycerides (TGs). Diglycerides (DGs) contained 16% of the lipid ¹⁴C after 5 minutes and 8% after 40 minutes. The proportion of the label of each lipid group incorporated into linoleate during an 80-minute incubation increased at similar rates for each group, and was always highest in PC. Radioactivity was detected in PC-linoleate earlier than in linoleate of the other groups. During a prolonged incubation after a 15-minute pulse labeling, the percentage of the lipid ¹⁴C incorporated into PC and DGs was high at the end of the pulse but decreased later, while that in TGs increased to 64% after 4 hours. The proportion of the label of each group recovered in linoleic acid peaked in all groups after 4 hours, except for the TGs where it increased slowly throughout the experiment. Only traces of radioactivity were detected in linolenate. The data are compatible with a pathway in which oleate is incorporated into PC, is desaturated to linoleate on PC, and where the linoleate-enriched DGs are transferred from PC to TGs.

The present study of polyunsaturated fatty acid biosynthesis in winter wheat root and crown at room temperature was an obligate preliminary to the investigation of the effect of temperature on fatty acid metabolism in these tissues. It was observed previously (3, 29) that the distribution of fatty acids shifted in winter wheat root and crown from 45% linoleate, 30% linolenate at 20°C to 30% linoleate, 50% linolenate within 2 weeks of frost hardening by exposure to 1°C. Similar observations were made with rye (2). The pathway leading to linolenic acid in wheat roots is not known. Most of the work on polyunsaturated fatty acid biosynthesis has been done with leaf tissue (5, 6, 9, 11, 12, 14–17, 20, 22, 24, 25, 28, 30–32) and shows that oleic acid, synthesized in the chloroplast, is incorporated into microsomal PC[2] where it is desaturated to linoleic acid. Linoleate is then desaturated to linolenic acid either on PC, probably in the microsomes, or on chloroplast MGDG after transfer of the linoleate-containing DGs from PC to MGDG, depending on the tissue and its stage of development. Desaturation of oleate on PC was demonstrated in developing seeds (23, 26). Desaturation of oleate and linoleate on PC was indicated in developing soybean cotyledons (27), while the results obtained by Murphy and Stumpf (15) with developing cucumber cotyledons indicated oleate desaturation on PC, and linoleate desaturation on

MGDG. In leaves of two gramineae, barley (25) and oats (16), the results indicated oleate desaturation on PC, and linoleate desaturation on PC for barley and on MGDG for oats. Wheat roots which do not contain chloroplasts but only proplastids must, however, be able to synthesize linolenic acid as indicated by the shift in fatty acid composition at low temperature. In this tissue, oleate and linoleate desaturation would likely occur on PC in the microsomes.

MATERIALS AND METHODS

Plant Material. Seeds of winter wheat (*Triticum aestivum* L. cv Kharkov 22 MC) were soaked overnight in aerated distilled H_2O, and allowed to germinate in the dark on cheese cloth dipping in Hoagland No. 1 solution, at a temperature cycle of 16 h at 20°C and 8 h at 15°C. After 3 d, the seedlings were exposed to light during the 16-h period (160 μE m^{-2} s^{-1}) in a growth cabinet (Controlled Environment, Winnipeg). One week after imbibition, the plants were removed from the nutrient solution and 5-mm segments were trimmed off the root tips into a tray of iced K-phosphate (0.1 M, pH 7.5, 10 μM $CaSO_4$) (25).

Feeding of Radioactive Precursors to Root Tips. Root tissue (0.1 g fresh weight) was weighed and placed into 20 × 100 mm culture tubes in 3 ml of phosphate buffer on ice. The tissue was infiltrated with the buffer in a vacuum desiccator. The buffer was replaced by 250 μl of the same buffer containing 5 μCi of [¹⁴C] ammonium oleate (final concentration, 0.425 mM). A stock solution of [¹⁴C]ammonium oleate was prepared by evaporating the ethanol off 250 μCi [¹⁴C]oleate (59.0 Ci/mol, New England Nuclear) under a jet of N_2, and adding 20 μl 7 M NH_4OH followed by 480 μl water. The tubes were shaken on a serological rotator (Tektator V, Evanston, IL; 3 oscillations/s) in darkness at 25°C. The incubation was stopped by replacing the medium with 3 ml of hexane:isopropanol (3:2, v/v) (8). When [1,2-¹⁴C]sodium acetate (56.5 Ci/mol, New England Nuclear) was used, 5 μCi were fed to the infiltrated roots (0.2 g fresh weight) in 250 μl of K-phosphate 50 mM, pH 5.0).

Lipid Analysis. Tissues were ground in a Ten Broek homogenizer in hexane:isopropanol (3:2, v/v). The homogenizer was rinsed twice with 1 ml of the same solvent. The extract was washed with 2 ml of 6.5% (w/v) Na_2SO_4. After centrifugation, the upper phase, containing the lipids, was collected. The lipid extract, including two washes of the interface with a small volume of hexane, was evaporated to dryness under nitrogen and redissolved in 0.5 ml chloroform. Aliquots of the extract were separated by TLC on silica gel G in hexane: diethyl ether:acetic acid (80:20:1, v/v/v) for neutral lipids and in chloroform:methanol:ammonia (65:25:2, v/v/v) for polar lipids. The lipids were detected by exposure to iodine vapor and identified by cochromatography with suitable reference compounds (Applied Science, State College, PA). Reference MGDG was purified from wheat leaves by silicic acid column chromatography followed by TLC in chloroform:methanol:acetone:acetic acid (80:15:5:1, by volume). The radioactive lipids were located by autoradiography with X-OMAT AR film (Kodak), and then scraped off the plates for measurement

of radioactivity or for transmethylation on the silica gel in 0.5 M NaOCH₃ in methanol (21). The fatty acid methyl esters were purified by TLC on silica gel G in the solvent for neutral lipids and were separated by TLC on silica gel G containing 5% (w/w) AgNO₃, in benzene. Radioactivity was measured on the gel in 0.5% (w/v) p-terphenyl in xylene:Triton X-100:water (6:3:1, v/v/v) (22).

Experiments were repeated at least twice, with good reproducibility.

RESULTS

Incorporation of [1,2-¹⁴C]Acetate into Lipids. When root segments were fed labeled acetate, the radioactivity was rapidly incorporated into the lipids. The bulk (75–90%) of the ¹⁴C was recovered in the neutral lipids at the solvent front after TLC in chloroform:methanol:ammonia (65:25:2, v/v/v). The little radioactivity incorporated into the polar lipids showed early labeling of PC and subsequent accumulation of ¹⁴C into PE and PG.

After separation of the neutral lipids by TLC in hexane:diethyl ether:acetic acid (80:20:1), only a small amount of the radioactivity was associated with the TGs while most of it was associated with the sterols. When labeled samples were transmethylated with 12.5% borontrifluoride in methanol (w/v; Applied Science, State College, PA), 67% of the lipid-soluble radioactivity migrated with the sterols and only 21% with the fatty acid methyl esters. When lipid samples were saponified in 0.5 M NaOH in methanol for 10 min at 90°C, the unsaponifiable fraction represented 74% of the total radioactivity and most of this radioactivity migrated with the sterols.

Short-Time Incorporation of [1-¹⁴C]Ammonium Oleate into Lipids. Five min after infiltration of 5 μCi [1-¹⁴C]ammonium oleate, 45% of the radioactivity was recovered in the lipid-soluble fraction of the tissue (Fig. 1). The radioactivity of this fraction peaked at 60% of the radioactivity provided, after 20 min of incubation. Most of this radioactivity was initially free oleate which was progressively incorporated into complex lipids: 5% of the lipid-soluble label after 5 min, and 40% after 80 min. Identical results were obtained for oleate, whether the radioactive areas were scraped from plates developed for separation of neutral or polar lipids.

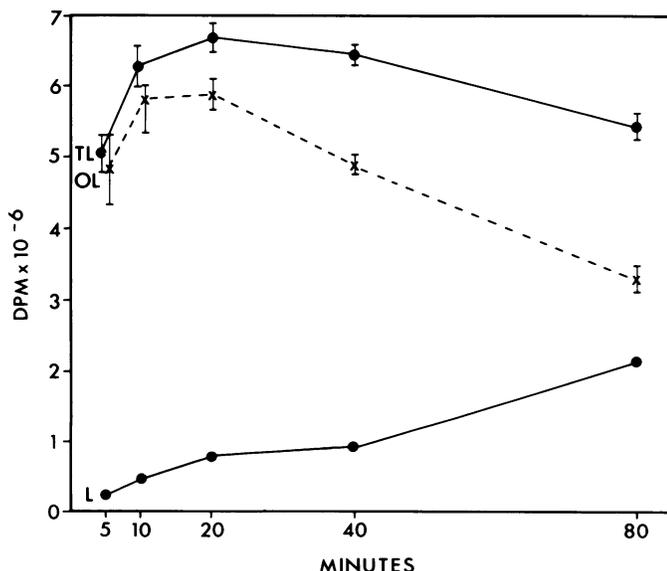The distribution of the label among various groups of complex

FIG. 1. Radioactivity from 5 μCi [¹⁴C]ammonium oleate recovered in total lipids (TL), oleate (OL), and complex lipids (L) of young wheat root tissue. (Average of three samples ± SD, where large enough).
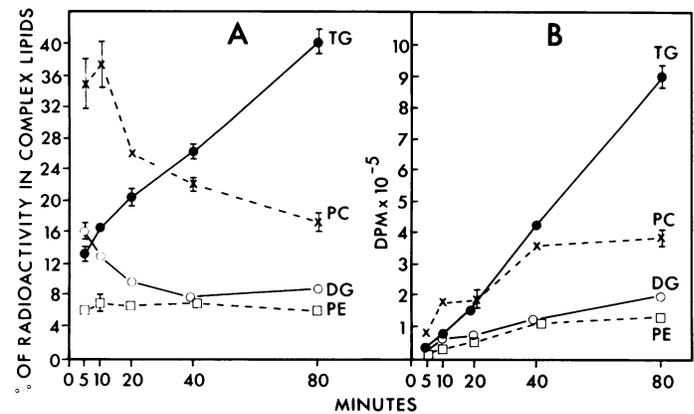

FIG. 2. Distribution of the label from 5 μCi [1-¹⁴C]ammonium oleate among lipids of young wheat root tissue. A, Percentage of radioactivity in complex lipids; B, dpm. (Average of three separate determinations ± SD).

lipids is shown as percentage of total complex lipids (Fig. 2A) and as dpm (Fig. 2B). After 5 min of incorporation, polar lipids contained 70% of the label incorporated into complex lipids from free oleate. This radioactivity was transferred progressively to the neutral lipids (40% after 80 min). PC labeling was initially high but decreased from 35% after 5 min to 17% after 80 min. DG labeling was also high after 5 min (16%) and decreased to 8% after 40 min. Most of this radioactivity was transferred to the TGs (13% after 5 min, 40% after 80 min). A small but constant proportion of the radioactivity was incorporated into PE. The autoradiograms of the TLC plates developed in chloroform:methanol:ammonia (65:25:2, v/v/v) showed in addition some radioactivity associated with PG and PI. Traces of radioactivity were detected at the origin after 5 min of incorporation. This label, probably in PA, was not measured but increased to significant levels after 20 min. No radioactivity was detected in MGDG, but two unknowns, accounting together for 10 to 15% of the label in complex lipids, migrated near MGDG. The data, when expressed as dpm, show that the bulk of the radioactivity was incorporated initially into PC and later into the TGs.

The distribution of the radioactivity among the fatty acids of the various groups of lipids showed that the proportion of the label of each group incorporated into linoleic acid (the product of oleate desaturase) increased at similar rates for each group (Fig. 3A). However, at each time of sampling, PC had the highest proportion followed by PE, DGs, and TGs. Radioactivity was present in PC-linoleate at the first sampling after 5 min of incorporation, but was not detected in the linoleic acid of the other groups before 20 min of incorporation. The percentage of the label in PC recovered in linoleate was greater than that in the polar lipid fraction. The proportion of the label of the combined two unknowns, migrating near MGDG, in linoleate was close to, but always higher than that in PC. Figure 3B shows that the bulk of the linoleate radioactivity was initially in PC and later also in TGs. No radioactivity was detected in linolenic acid in the short-time experiments.

Pulse Labeling of Root Lipids with [1-¹⁴C]Ammonium Oleate. To clarify the relationship existing between the fatty acids of different groups of lipids, especially PC, DGs, and TGs, a pulse labeling of wheat root lipids was carried out. The buffer infiltrated tissue was allowed to absorb [1-¹⁴C]oleate (0.425 mM) for 15 min and was then incubated for 21 h in the presence of 20 times as concentrated unlabeled substrate (8.5 mM). In these experiments, most of the [¹⁴C]oleate absorbed was metabolized. Some 45% of the labeled substrate was recovered in the lipid extract of the tissue at the end of the 15-min incorporation, and 90% of this label was free oleate. After 21 h of chase, only 10% of the radioactivity initially incorporated into lipids was left in this fraction, and 25%
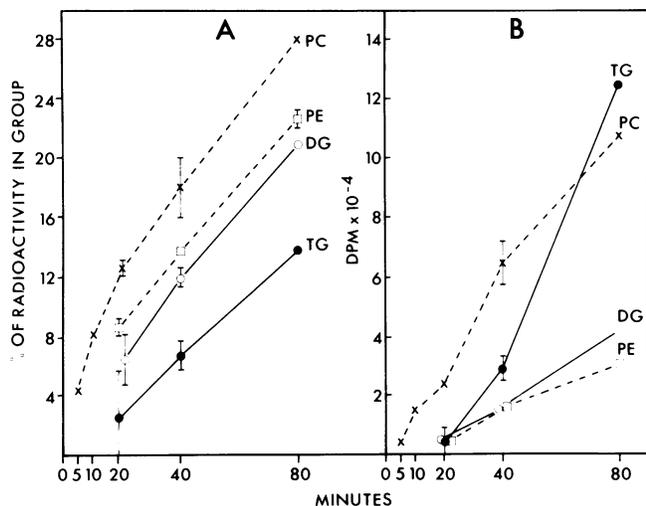
FIG. 3. Radioactivity from 5 μCi [1-¹⁴C]ammonium oleate incorporated into linoleate of TGs, PC, DGs, and PE of young wheat root tissue. A, Percentage of radioactivity in each group; B, dpm. (Average of three separate determinations ± SD).
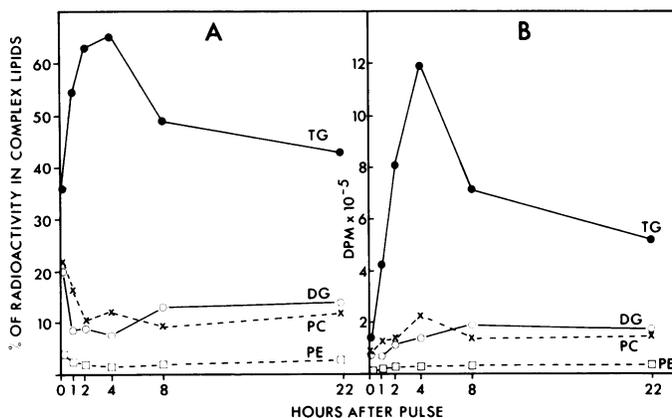

FIG. 4. Radioactivity from 5 μCi [1-¹⁴C]ammonium oleate incorporated into TGs, PC, DGs, and PE in young wheat root tissue during a 22-h chase after a 15-min pulse labeling. A, Percentage of radioactivity in complex lipids; B, dpm. (Average of two separate determinations).

of this was free oleate.

Sixty per cent of the radioactivity recovered in the complex lipids had been incorporated into the neutral lipids at the end of the pulse labeling, and this percentage increased to 78% after 4 h at the expense of the polar lipids. Most of the radioactivity of the neutral lipids was associated with the TGs which reached a peak of 65% of the complex lipid radioactivity after 4 h but decreased later (Fig. 4A). The DGs contained initially 20% of the complex lipid radioactivity, but this label fell to 8% within 1 h after the pulse, to equilibrate at 13% after 8 h. The main labeled polar lipid, PC, contained 22% of the acyl lipid radioactivity at the end of the pulse but only 8% after 8 h of chase. The dpm data (Fig. 4B) show the TGs rapidly accumulating radioactivity until 4 h after the pulse, but being extensively degraded later. PC also peaked after 4 h, but to a much lesser extent. After a rapid decrease from an initially high percentage value, DGs showed increasing radioactivity for 8 h. Labeling of PE remained relatively low throughout the experiment, with a slightly higher percentage of the radioactivity at the end of the pulse (time 0 on Fig. 4A).

The proportion of the label of each group recovered in linoleic acid increased most rapidly in PC, from 5% at the end of the pulse to 20% 4 h later, but fell sharply to 9% after 21 h of chase (Fig. 5A). The labeling in DG-linoleate followed much the same pat-
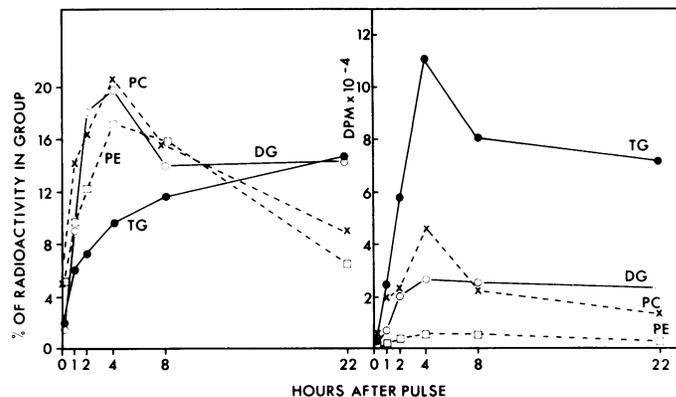

FIG. 5. Radioactivity from 5 μCi [1-¹⁴C]ammonium oleate incorporated into linoleate of TGs, PC, DGs, and PE of young wheat root tissue during a 22-h chase after a 15-min pulse labeling. A, Percentage of radioactivity in group. B, dpm. (Average of two separate determinations).

tern, although less abruptly. PE-linoleate radioactivity also peaked after 4 h but reached only 17%. TG-linoleate radioactivity on the other hand increased much more slowly from 4% at the end of the pulse to 15% after 21 h of chase. The dpm data (Fig. 5B) show TG- and PC-linoleate radioactivity peaking sharply after 4 h, while DG- and PE-linoleate reached at that time a maximum dpm which was maintained. The radioactivity in PE-linoleate was small as compared to that in DG- and PC-linoleate. TG-linoleate, which contained relatively little label at the end of the pulse, accumulated far more radioactivity than PC-linoleate within 1 h after the pulse. Some of the autoradiograms of the AgNO₃ plates showed definite evidence for traces of radioactivity, but never more than traces, in linolenic acid from total polar lipids, PC, PE, DGs, and TGs as early as 2 h after the pulse.

DISCUSSION

The data presented above are compatible with a pathway in which oleate is incorporated into PC before it is desaturated to linoleate, and in which linoleate-enriched DGs are transferred from PC to TGs. PC was highly labeled early in the incorporation (Fig. 2A), reached a maximum percentage of the radioactivity in the complex lipids after 20 min and rapidly released this radioactivity. The label apparently flowed into the TGs which were hardly labeled after 5 min but contained 40% of the complex lipid radioactivity after 80 min and more than 60% 2 h after a 15-min pulse labeling (Fig. 4A). DGs were the likely intermediates in this transfer. The proportion of the radioactivity incorporated into DGs was high at the beginning but decreased rapidly (Fig. 2A). DGs are present in only small amounts in this tissue (C. Willemot, unpublished). The labeling in DGs increased to 12% of the complex lipid radioactivity 8 h after the pulse when dynamic equilibrium was reached (Fig. 4A). These observations are indicative of important intermediates, present in small amounts, with high specific activity and high turnover. The central role of PC in this pathway is indicated by the fact that the bulk of the radioactivity in polar lipids was in PC, with much less label in PE, PG, and PI. The role of PC as substrate for oleic acid desaturase is indicated by the fact that at each sampling time PC showed a higher percentage of its radioactivity in linoleic acid than PE, DGs, and TGs (Fig. 3A). PC-linoleate was labeled after 5 min, while labeled linoleate could not be detected in PE, DG, and TG before 20 min. The data also indicate a transfer of labeled linoleate from PC to TGs. The percentage of the PC label recovered in linoleate peaked 4 h after the pulse, while that in TGs slowly accumulated to a high level 21 h after the pulse (Fig. 5A). When expressed as dpm, PC-linoleate radioactivity was much greater than that in linoleic acid of TGs, PE, and DGs during the first 40

min of incorporation (Fig. 3B) but became much less than that in TGs during the prolonged incorporation after a pulse labeling (Fig. 5B).

Whether PC is the only substrate for oleate desaturation in wheat roots cannot be ascertained at present. The lag in labeling of linoleate in PE, DGs, and TGs while these three groups contained labeled oleate after 5 min suggests direct incorporation of oleate in all groups but little, if any, desaturation of oleate outside of PC. The same conclusion is inferred from the increase in the percentage of radioactivity in linoleate of PC, PE, and DGs at the same rate during 80 min of incorporation (Fig. 3A). The percentage of the PE label recovered in linoleate, which was consistently higher than that for DGs, may be interpreted as some desaturation of oleate occurring on PE or as specificity of the 1,2-DG-ethanolamine phosphotransferase towards polyunsaturated DGs.

Although the large accumulation of linolenic acid in winter wheat roots at low temperature (3, 29) indicates that this tissue is able to synthesize linolenate, only traces of radioactivity were detected in linolenic acid after prolonged incubation of 2 h or more. No label was detected in MGDG. We cannot conclude from these data that MGDG is not involved in linoleate desaturation in this tissue, because linolenate synthesis may in some tissues depend on galactolipid synthesis (10, 18).

Although isopropanol used in the extraction is considered to inhibit the degradation of phospholipids by phospholipase D in plant tissue (1, 7), our data show evidence for extensive degradation. The radioactivity at the origin of the chromatograms for polar lipids was most probably associated with phosphatidic acid; it was very low at the beginning of the incubation, but markedly increased with time, indicating degradation of phospholipids during the incorporation; the two labeled unknowns with R_Fs close to that of MGDG were in the area where one would expect to find phosphatidylmethanol, a common product of phospholipase D action in the presence of methanol (4, 13, 19); when the tissue was boiled for 3 min before extraction, more radioactivity was recovered in PC and less in the two unknowns and in PA. That the unknowns were derived mainly from PC is further indicated by their combined ^{14}C fatty acid composition, which was very close to that of PC, although always somewhat richer in]^{14}C]linoleate. This is not surprising as, in wheat root tissue, the hydrolases which degrade the phospholipids showed a preference for less saturated species (C. Willemot, unpublished). The nature of the unknowns was not established.

Why most of the acetate-label was channeled into compounds tentatively indentified as sterols in this tissue is not known. This may be characteristic of young meristematic root tissue. Alternatively, the conditions of preparation and incubation of the tissue may have been unfavorable to de novo fatty acid synthesis. It should be stressed that the use of oleate (the product of de novo synthesis in the plastids, which is exported to the cytoplasm) instead of acetate as precursor of polyunsaturated fatty acids gives information mainly on the extra-plastidic events in fatty acid metabolism.

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