Biochimica et Biophysica Acta, 443 (1976) 157–168

BBB 77395

IN VIVO MODIFICATION OF PLANT MEMBRANE PHOSPHOLIPID COMPOSITION

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(Received January 12th, 1976)

SUMMARY

Tomato seedlings treated with ethanolamine showed altered phospholipid composition. The changes included altered acyl chain composition as well as changes in the relative amounts of the phospholipid classes. Specifically, there was an increase in phosphatidylethanolamine and phosphatidylserine with a concomitant decrease in phosphatidylcholine and no overall increase in phospholipids. Treatment with ethanolamine increased the relative amount of C_{18} acyl chains (especially 18 : 2) in phosphatidylethanolamine and phosphatidylcholine at the expense of 16 : 0 and 16 : 1. Acyl composition of other phospholipid classes were unchanged. Labeled ethanolamine was incorporated mostly into phosphatidylethanolamine and phosphatidylcholine. Ethanolamine-stimulated incorporation of labeled oleate was entirely into acyl chains and appeared only as 18 : 1 and 18 : 2. There was greater incorporation, but less conversion of 18 : 1 to 18 : 2 with choline. Stearate was incorporated but not desaturated.

INTRODUCTION

Differences in the physico-chemical properties of biological membranes are thought to be determined in large part by their amphopathic lipid composition. Differences in biological and physiological function are, in turn, consequences of these physico-chemical differences. Since the composition of membrane phospholipids can vary with respect to both polar head groups and acyl chains, experimental modification of these constituents can lead to a better understanding of their relative contribution to both membrane properties and function. Modification of membrane phospholipid can be achieved by genetic means, but this is not yet easily attained with eukaryotic organisms. An alternative to obtaining specific membrane mutants is chemical (metabolic) modification of phospholipid composition.

Because acyl chain composition of the membrane phospholipid can reflect in part the composition of the fatty acids available to the organism, exogenous supplementation of fatty acids or fatty acid derivatives [27] has been used extensively in modifying prokaryote membranes [2, 17], yeast [5], and cultured animal cell phos-

pholipids [25]. This technique is limited by the cytotoxicity of the high levels of fatty acid or fatty acid derivatives needed to modify the membrane phospholipids, especially when polyunsaturated fatty acids are used [25].

Modification of the polar head groups of amino base phospholipids (i.e. phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylmonomethylethanolamine, phosphatidyldimethylethanolamine) is more easily attainable. This category of lipid modification has been achieved by exposure of yeast [23], potato tuber slices [24], or animal tissue culture cells [9] to amino alcohols. The amino base in question is taken up by the cell and incorporated into the phospholipid in place of the normal amino base, i.e. choline or ethanolamine is replaced by the supplied amino alcohol derivative.

This report concerns the alteration of plant membrane phospholipid composition by treatment of tomato seedlings with a specific amino alcohol (ethanolamine) and a fatty acid derivative (Tween oleate). The focus of the study has been the modification of phospholipid polar head groups, coupled with an increase in specific polyunsaturated phospholipid acyl chains by addition of lipid precursors.

MATERIALS AND METHODS

Growth and treatment of seedlings

Tomato seeds (*Lycopersicon esculentum* cv. VF145-21-4, Petoseed Co.) were germinated in the dark in vermiculite at 25 °C. The plants were watered daily with tap water. Etiolated seedlings 3–5 days old were transplanted to blotter paper (20×15.5 cm, Anchor Paper Co.) and covered with two layers of Kimwipe tissue. The seedlings, sandwiched between the blotter paper and the Kimwipes, were then placed for support on a 20×15.5×0.32 cm sheet of Plexiglas (blotter paper first). This Plexiglas-blotter-seedling-Kimwipe assembly (slant-board) was then saturated with the test solution, which resulted in the firm adhesion of all the components. The slant-board, with 10 seedlings per board, was placed at a slight angle to the vertical in a shallow Plexiglas tray so that the bottom edge of the board was immersed in the test solution. The slant-board and tray were covered with a Plexiglas box to limit evaporation. This arrangement provided continuous exposure to the test solution and adequate aeration for growth. To assure that the entire seedling was maintained in contact with the test solution during the 5-day treatment, they were also sprayed with it daily.

Ethanolamine (Eastman Chemical Co.) was made up in distilled water/tap water (1 : 1, v/v), titrated to pH 7.0 with 5 M HCl and diluted to a final concentration of 5 mM. Tween oleate (polyoxyethylene sorbitan trioleate, Sigma Chemical Co.) was emulsified with a small volume of ethanolamine solution for 1 min in a glass homogenizer fitted with a Teflon pestle. The resulting emulsion was diluted with 5 mM ethanolamine solution to a final concentration of 0.005 % (v/v) Tween oleate. A similar procedure was used to prepare choline chloride/Tween oleate solutions (5 mM choline chloride/0.005 % Tween oleate, pH 7.0). Autoclaved 0.25 % (v/v) Hoagland's solution was substituted for tap water in some experiments.

Analysis of phospholipids

The tissue was killed by boiling for 5 min in isopropanol to halt possible enzymatic deacylation [12]. Extraction of total lipids was completed by homogenizing in

chloroform/methanol (2 : 1, v/v). The homogenate was filtered, washed with an additional volume of chloroform/methanol and partitioned with 0.9 % (w/v) NaCl [7]. The chloroform phase was diluted with an additional volume of chloroform, dried over anhydrous Na_2SO_4, and flash evaporated to a minimal volume.

Phospholipids were separated from free fatty acids, galactolipids, sulfolipids, and neutral lipids on 0.25 mm thick silica gel H (Adsorbosil-5, Applied Science Labs.) using acetone/acetic acid/water (100 : 2 : 1, v/v) [8]. The phospholipids were eluted from the gel with chloroform/methanol/ammonia (36 : 13 : 3, v/v), taken to dryness under N_2, and resuspended in benzene. Individual phospholipids were resolved on precoated plates (Quantum Q5) in chloroform/methanol/water (65 : 25 : 4, v/v). In some experiments, the lipids were run in a second direction with butanol/acetic acid/water (6 : 2 : 2, v/v) to resolve phosphatidylethanolamine from phosphatidylmonomethylethanolamine [9]. All plates were activated for 1 h at 110 °C and stored desiccated until used. Lipids were exposed to iodine vapor or visualized with 2',7'-dichlorofluorescein (0.1 % (w/v) in methanol) under ultraviolet light. Lipid phosphorus was estimated by the technique of Fiske and SubbaRow [6].

The phospholipid fatty acids were transesterified to form their methyl esters with 0.5 M sodium methoxide methanol. The samples in 0.5 ml of benzene were placed in culture tubes with 2 ml of sodium methoxide reagent. The vials were flushed with N_2, sealed with a Teflon lined screw cap and reacted at 80 °C for 30 min. The samples were then allowed to cool to room temperature. 2 ml of water were added and the fatty acid methyl esters recovered by extracting with three 2-ml portions of diethyl ether.

The methyl esters were then analyzed on a Varian Aerograph Model 1800 gas chromatograph fitted with a hydrogen flame detector and a 1.8 m glass column packed with Supelco SP-222-SPB on 100/120 mesh supelcoport. The injector was operated at 205 °C, the column at 200 °C, and the detector at 210 °C. Peak areas were measured by use of a Varian Aerograph Model 477 electronic digital integrator.

Treatment of tissue with radioactive substrates

Incorporation of exogenous ethanolamine was estimated by incubating 0.1 g (fresh weight) samples of excised whole cotyledons, apical hypocotyl segments, or basal hypocotyl segments for 2 h with 1 ml of a solution of [1,2-^{14}C]ethanolamine (6.3 Ci/mol, New England Nuclear) containing 2 μCi/ml. Incubation was terminated by rapidly washing the tissue with excess distilled water and dropping the tissue into boiling isopropanol. Lipid extraction and separation proceeded as described in the previous section. Used as reference standards were phosphatidylcholine, phosphatidylethanolamine, phosphatidyldimethylethanolamine, phosphatidylserine, and phosphatidylglycerol (Sigma Chemical Co.). Silica gel areas corresponding to the reference phospholipids were scraped from the plate, placed in vials containing Aquasol-2 (New England Nuclear), and counted in the Packard liquid scintillation spectrometer.

Tween [1-^{14}C]oleate (specific activity 13.2 Ci/mol) was synthesized by transesterification of [^{14}C]oleic acid with Tween acetate by the procedure of Williams et al. [25]. When *cis*-9-[1-^{14}C]oleic acid (50 Ci/mol, New England Nuclear) or [1-^{14}C]-stearic acid (4.7 Ci/mol, Malinckrodt Chemical Co.) was used in the incubation medium, it was first homogenized in Tween 85 or Tween acetate by the same procedure used for unlabeled treatments. The method used to determine the uptake and incorporation of [^{14}C]oleate and Tween [^{14}C]oleate was essentially the same as that

employed for [^{14}C]ethanolamine.

The distribution of radioactivity among various fatty acids was measured by gas chromatography of their methyl esters using a Wilkins Instrument and Research Aerograph A-90-p equipped with a thermal-conductivity detector and coupled directly to a Nuclear-Chicago Biospan 4998 proportional radiation detector and Model 877 integrator. Separation was achieved at 150 °C using a 3 m stainless-steel column containing 15 % Hi-eff-2BP (Applied Science Labs.) as the stationary phase.

Alternatively, in some experiments, the fatty acid methyl esters were separated on $AgNO_3$-impregnated silica gel H plates (7 % $AgNO_3$-silica gel H by weight). Unlabeled methyl stearate, methyl oleate and methyl linoleate were spotted over the radioactive sample and the methyl esters were separated with hexane/ether (9 : 1, v/v) [20]. Spots were visualized with ultraviolet light after being sprayed with 2',7'-dichlorofluorescein in methanol. Radioactivity was determined by scraping and counting as described above.

Free fatty acids accumulated in the tissue were methylated with BCl_3 [19]. Radioactivity incorporated into phospholipid, galactolipid and diacylglycerols was measured by separation on thin-layer chromatograms, followed by transesterification in sodium methoxide in methanol for gas-liquid chromatography.

Neutral lipids were separated on silica gel H using light petroleum/diethyl ether/acetic acid (80 : 20 : 1, v/v). In some experiments where noted, $^{14}CO_2$ released from treated tissues was measured by trapping on 10 % NaOH-impregnated glass fiber filter paper (Whatman GF/C) suspended in the gas phase of the reaction flask.

Paper chromatography of cytosol (aqueous fraction of the Folch partition) was carried out to determine the distribution of label in water-soluble components. Two solvent systems (diethyl ether/methanol/HCl/water (10 : 10 : 1 : 3, v/v) or *N*-butanol/acetic acid/water (8 : 2 : 2, v/v) were used in one-dimensional chromatography on Whatman No. 1 paper.

RESULTS

Effect of exogenous ethanolamine/Tween oleate on polar head group and fatty acyl chain composition of phospholipid of tomato seedlings

When growing tomato seedlings were exposed for 5 days to media containing ethanolamine and Tween oleate, the total amount of lipid phosphorus increased slightly over that of the controls. Examination of the amounts of individual phospholipids revealed that the samples treated with ethanolamine/Tween oleate had a significant increase in the amount of phosphatidylethanolamine (Table I). Phosphatidylserine also increased in the treated seedlings. In contrast to phosphatidylethanolamine and phosphatidylserine, phosphatidylcholine declined in material treated with ethanolamine/Tween oleate. The levels of other major phospholipids were essentially the same in control and treated seedlings.

When seedlings were grown in the presence of ethanolamine alone, there was consistently some increase in the amount of linoleate in the total phospholipids (Table II). Consistent and slightly greater increases in the amount of linoleic acid in the total phospholipids were produced by treatment of tomato seedling with ethanolamine applied in combination with Tween oleate (Table II). There were no substantial changes in any of the other major fatty acids found in the seedling phospholipids.

TABLE I

POLAR HEAD GROUP COMPOSITION OF THE PHOSPHOLIPIDS OF TOMATO SEEDLINGS TREATED WITH ETHANOLAMINE/TWEEN OLEATE

Results are expressed as the mean ±S.D. for three separate experiments. Tomato seedlings were exposed to ethanolamine/Tween oleate for a period of 5 days before extraction and analysis of lipids. The amounts of the different phospholipids were determined by lipid phosphorus or by quantitative gas-liquid chromatography of the fatty acid methyl esters with methyl heptadecanoate used as an internal standard. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PG, phosphatidylglycerol.

Treatment	nmol of phospholipid per g fresh wt.					Total phospholipid
	PC	PE	PI	PS	PG	
Control	418±44	227±15	46±9	38±2	28±6	757
Ethanolamine+ Tween oleate	390±37	292±22	45±5	53±3	23±9	803

TABLE II

THE FATTY ACID COMPOSITION OF THE TOTAL PHOSPHOLIPID FRACTION OF TOMATO SEEDLINGS TREATED WITH ETHANOLAMINE/TWEEN OLEATE

Seedlings were treated as described in Table I. The percent fatty acids for the control and the ethanolamine/Tween oleate treatment represent the mean ±S.D. for seven separate experiments, while the values for the ethanolamine alone are for only three experiments. Fatty acids comprising less than 1 % of the total detector response were omitted from the calculations.

Treatment	Percent of total fatty acid methyl esters					
	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3
Control	25±4	3±2	5±2	3±2	62±2	2±0.3
Ethanolamine	24±1	2±1	3±1	2±1	66±3	3±0.2
Ethanolamine+Tween oleate	25±2	1±0.6	4±1	1±0.3	67±1	2±1

Analysis of the major phospholipids of seedlings treated with ethanolamine/Tween oleate revealed significant differences in the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine (Table III). The amounts of linoleic acid in phosphatidylcholine and phosphatidylethanolamine were consistently higher in treated samples (Table III). Also, there was some increase in stearic acid in both of these phospholipids. In contrast, there were diminished amounts of palmitic and palmitoleic acids in the phosphatidylcholine and phosphatidylethanolamine from treated tissue. The fatty acid composition of other phospholipids was not altered by treatment with ethanolamine/Tween oleate.

TABLE III

THE EFFECT OF ETHANOLAMINE/TWEEN OLEATE TREATMENTS ON THE FATTY ACID COMPOSITION OF THE MAJOR INDIVIDUAL GLYCEROPHOSPHATIDES

Seedlings were treated as described in Table I. Values are means from two separate experiments in which absolute variation of fatty acid methyl ester percentages did not exceed ±1.5%. Zeros indicate samples where fatty acids could not be detected (less than 0.19% of total). Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine.

Fatty acid	Treatment	PC	PE	PI	PS
16 : 0	Control	30.6	34.2	40.0	64.3
	Ethanolamine+ Tween oleate	22.0	28.5	42.2	60.2
16 : 1	Control	2.3	0.7	0	0
	Ethanolamine+ Tween oleate	0.5	0.4	0	0
18 : 0	Control	0.6	1.6	2.0	0
	Ethanolamine+ Tween oleate	5.1	4.0	0.7	2.3
18 : 1	Control	2.5	0.5	0	0
	Ethanolamine+ Tween oleate	3.2	0.4	0.2	0
18 : 2	Control	57.5	61.2	58.0	35.7
	Ethanolamine+ Tween oleate	66.2	66.6	55.6	37.5
18 : 3	Control	1.2	1.9	0	0
	Ethanolamine+ Tween oleate	0.9	0.2	1.3	0

Uptake and incorporation of [^{14}C]ethanolamine and Tween [1-^{14}C]oleate into seedling phospholipids

To better assess the metabolic fate and extent of incorporation of exogenous ethanolamine and Tween oleate in different tissues, cotyledons, apical hypocotyl segments, and basal hypocotyl segments were pulse labeled with radioactive ethanolamine or Tween oleate. In all tissue segments examined, radioactive ethanolamine was taken up in the cytosol and incorporated into phospholipid or degraded to CO_2. In whole cotyledons and apical hypocotyl segments, approx. 20% of the absorbed radioactivity accumulated in the total phospholipid fraction (Table IV). The basal hypocotyl segments only accumulated 12% in phospholipids. The bulk of the absorbed radioactivity (70%) remained in the cytosol (aqueous fraction of Folch partition) in all tissue segments. Paper chromatography of the cytosol, in both acidic solvent systems employed, revealed that 91% of the radioactivity co-chromatographed with authentic ethanolamine. However, it was impossible to achieve complete resolution of ethanolamine and serine in either of the solvent systems used. The distribution of radioactivity on the chromatograms was consistent with the conclusion that the majority of the label resided in free ethanolamine, but did not exclude the possibility that there was some conversion to serine and subsequently to phosphatidylserine. This conversion could account for the small amount of labeling of this phospholipid (Table V). A lesser amount of the absorbed label was completely degraded to CO_2 by all tissue segments.

TABLE IV

[^{14}C]ETHANOLAMINE INCORPORATION INTO TOMATO SEEDLING TISSUE

Seedlings were treated as described in Table I, then 0.1 g fwt. of whole cotyledons, apical hypocotyl (1 cm) and basal hypocotyl (1 cm) segments from the seedlings were incubated for 2 h in 1 ml of medium containing 5 mM ethanolamine, 0.005% (v/v), Tween oleate and 2 μCi of radioisotope ([1,2-$^{14}C_2$]-ethanolamine, 6.3 Ci/mol) cpm are per 0.1 g of tissue and percentages are expressed as the percent of total cpm absorbed by the tissue. The cytosol represents the aqueous phase of the Folch partition from well-rinsed tissue.

Tissue	Total lipid		Phospholipid		Cytosol		CO_2	
	cpm	%	cpm	%	cpm	%	cpm	%
Cotyledon	6376	19.1	5556	16.6	25 238	75.4	1826	5.5
Apical hypocotyl segment	4343	23.1	4227	21.5	13 672	69.5	1454	7.4
Basal hypocotyl segment	2332	13.0	2262	12.6	13 359	74.5	2246	12.5

TABLE V

THE DISTRIBUTION OF [1, 2 $^{14}C_2$]ETHANOLAMINE IN THE PHOSPHOLIPID FRACTION

Seedlings were treated as described in Table I and then incubated with radioisotopes as described in Table IV. The total ^{14}C-labeled phospholipids were separated by thin-layer chromatography. Each spot was assayed for radioactivity by scraping the area from the chromatogram and counting in a liquid scintillation spectrophotometer. Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; PS, phosphatidylserine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine.

Tissue	Phospholipid									
	PE		PC		PS		PMME		PDME	
	cpm	%	cpm	%	cpm	%	cpm	%	cpm	%
Cotyledon	1817	60.6	780	26.1	182	6.1	104	3.4	114	3.8
Apical hypocotyl	1382	58.5	581	24.6	198	8.4	120	5.1	82	3.4

Separation of the total phospholipid fraction by thin-layer chromatography revealed that the label was distributed primarily in phosphatidylethanolamine and phosphatidylcholine in both cotyledons and hypocotyls (Table V). Two-thirds of the label was associated with phosphatidylethanolamine, and one-fourth was found in phosphatidylcholine. Phosphatidylserine had much less label, while the intermediates of the *N*-methyltransferase pathway from phosphatidylethanolamine to phosphatidylcholine were labeled slightly.

When seedling tissue was exposed to either Tween [1-^{14}C]oleate (data not shown) or [1-^{14}C]oleate together with unlabeled Tween oleate (Table VI), 50% of the label taken up by the tissue was incorporated into phospholipid during the 2 h

TABLE VI

THE UPTAKE AND INCORPORATION OF [1-^{14}C]OLEATE INTO TOMATO SEEDLING TISSUE

Seedlings were treated as described in Table I and the tissue incubated as described in Table IV with 2 μCi of radioisotope ([1-^{14}C]oleate, 50 Ci/mol). cpm are per 0.1 g of tissue, percentages are expressed as percent of total cpm absorbed by the tissue. Cytosol represents the aqueous phase of the Folch partition from well-rinsed tissue.

Tissue	Total lipid		Total phospholipid		Cytosol		CO_2	
	cpm	%	cpm	%	cpm	%	cpm	%
Cotyledon	256 535	93.7	158 155	57.7	6244	2.3	11 102	4.1
Apical hypocotyl	204 400	89.8	104 978	46.2	5488	2.4	17 584	7.8
Basal hypocotyl	164 512	72.3	69 757	30.7	5296	2.3	5 532	2.4

TABLE VII

THE INCORPORATION OF [1-^{14}C]OLEATE INTO THE TOTAL PHOSPHOLIPID FRACTION

Seedlings were treated as in Table I and tissue incubated as in Table IV with 0.1 μCi of radioisotope (Tween [1-^{14}C]oleate, 13.2 Ci/mol). cpm are for 0.1 g of tissue, percentages are expressed as percent of total phospholipid.

Tissue	Total phospholipid (cpm)	Percent ^{14}C distribution in fatty acids of phospholipids			
		18 : 1		18 : 2	
		cpm	%	cpm	%
Cotyledon	587	395	68.8	179	31.2
Hypocotyl	433	276	68.8	137	33.2

pulse. 29 % of the Tween [1-^{14}C[oleate taken up by the tissue was unmetabolized, and the remaining label was distributed among various lipid fractions (neutral lipids and galactolipids). Only minor amounts of both labeled substrates were metabolized to CO_2. All of the label incorporated into phospholipids was found in the acyl side chains (Table VII). Furthermore, gas-liquid chromatography revealed that this label was restricted to oleate and linoleate.

Label incorporated into neutral lipids was found in largest amounts in diacylglycerols (42 %) followed by triacylglycerols (29 %), free fatty acids (19 %) and monoacylglycerols (10 %). The proportion of label in linoleate was smaller in the neutral lipids (11 %) than in the phospholipids. In the cotyledonary tissue, some label (6 % of the total cpm in lipids) was incorporated into monogalactosyldiacylglycerol. This was the only galactolipid labeled and it was found labeled in detectable amounts only in the cotyledons.

Characterization of oleic acid desaturase activity

To better characterize the mechanism of desaturation of oleate to linoleate,

tomato hypocotyls were pulsed with different amino base supplements and substrates. When choline (Table VIII) was substituted for ethanolamine in the incubation medium, the proportion of [^{14}C]oleate converted to [^{14}C]linoleate declined significantly although more label was incorporated into phospholipid. When no amino alcohol was included in the incubation medium, there was less total incorporation of labeled oleate and less desaturation of exogenous oleate. When compared to tissue treated with Tween acetate/[1-^{14}C]oleate, not only was the incorporation of [1-^{14}C]-

TABLE VIII

THE SPECIFICITY OF DESATURATION OF EXOGENOUS [1-^{14}C]OLEATE INCORPORATED INTO PHOSPHOLIPID

Seedlings were treated as described in Table I. Tomato seedling hypocotyls were pulsed with 1 μCi of [1-^{14}C]stearate plus 0.005 % (v/v) Tween derivative and 5 mM of amino alcohol (i.e. ethanolamine or choline) as indicated for a 2 h interval. cpm are per 0.1 g of tissue.

Treatment	cpm in phospholipid fatty acids			^{14}C-labeled 18 : 2/^{14}C-labeled 18 : 1
	18 : 0	18 : 1	18 : 2	
5 mM ethanolamine+ Tween oleate+[^{14}C]oleate	0	21 608	13 432	0.62
5 mM choline+ Tween oleate+[^{14}C]oleate	0	49 360	12 920	0.26
Tween acetate+[^{14}C]oleate	0	20 337	4 057	0.20
5 mM ethanolamine+ Tween oleate+[^{14}C]stearate	35 912	0	0	0
5 mM ethanolamine+ Tween oleate+[^{14}C]oleate +N_2 (anaerobic)	0	19 644	984	0.05

TABLE IX

THE SPECIFICITY OF DESATURATION OF EXOGENOUS [1-^{14}C]OLEATE INCORPORATED INTO PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE

Seedlings were treated as in Table I and tissue incubated with radioisotope as described in Table VIII. cpm are per 0.1 g of tissue.

Phospholipid	cpm/0.1 g tissue			
	5 mM ethanolamine Tween oleate+[1-^{14}C]oleate		Tween acetate+ [1-^{14}C]oleate	
	18 : 1	18 : 2	18 : 1	18 : 2
Cotyledon				
Phosphatidylcholine	11 912	3796	10 312	2172
Phosphatidylethanolamine	8 360	4160	3 100	1116
Apical hypocotyl				
Phosphatidylcholine	5 560	2212	3 908	392
Phosphatidylethanolamine	3 364	2093	1 776	624

oleate into the tissue phospholipid stimulated by the presence of ethanolamine in the incubation medium, but more of the incorporated label was found in phosphatidylethanolamine (Table IX). Moreover, a higher proportion of the administered oleate was desaturated to linoleate especially in the phosphatidylethanolamine fraction.

The specificity of desaturation was further substantiated by substitution of [1-^{14}C]stearate for [1-^{14}C]oleate. Labeled stearate was readily incorporated into phospholipid, although analysis of the phospholipid fatty acid methyl esters indicated that the label was restricted to stearate (Table VIII).

Furthermore, the desaturation of 18 : 1 to 18 : 2 was dependent on oxygen, as indicated by incubation in the presence of N_2 during a 2 h pulse with ethanolamine/Tween oleate/[1-^{14}C]oleate.

DISCUSSION

The phospholipid composition of tomato seedlings can be modified in vivo by treatment with ethanolamine and Tween oleate at 25 °C. The phospholipid polar head groups can be drastically altered in composition by exposure to exogenous amino alcohols, similar to the situation in rats [13, 14], cultured animal cells [9], potato tuber slices [24], and yeast [23]. Unlike yeast [23], potato tuber slices [24], and rat liver in vivo [13], where intermediates of the *N*-methyl transferase pathway (phosphatidylmonomethylethanolamine and/or phosphatidyldimethylethanolamine accumulate when treated with amino base precursors (i.e. monomethylethanolamine, dimethylethanolamine)), tomato seedlings exposed to ethanolamine only accumulated significant amounts of phosphatidylethanolamine. However, there was substantial methylation of phosphatidylethanolamine to phosphatidylcholine, as reported for excised tomato root [26], rat lung [13] and spinach leaves [18].

The amount of linoleic acid also increased in the phospholipid acyl chains of tomato seedlings supplemented with ethanolamine/Tween oleate. Similar to exogenous radioactive ethanolamine, exogenous [^{14}C]oleate (derived from Tween [1-^{14}C]-oleate) was incorporated into phospholipid of rapidly developing tissue (cotyledons and apical hypocotyl segments). During a 2 h pulse, a substantial amount of the labeled oleic acid was desaturated to linoleic acid and incorporated into phospholipid. Although [^{14}C]oleic acid was incorporated into monogalactosyldiacylglycerol, diacylglycerol, and triacylglycerol, it was not desaturated to the same extent as in phospholipids.

The low level of desaturation of [^{14}C]oleate in diacylglycerol agrees with the results of Devor and Mudd [3, 4] who showed with spinach leaves that diacylglycerols, although able to serve as precursors of phospholipids, did not have as much unsaturation as did the cellular phospholipids. These results indicate that the extent of unsaturation in plant phospholipids may be altered either by acyl transfer or post-synthetic desaturation.

The similarity of the fatty acid composition of phosphatidylethanolamine and phosphatidylcholine in tissue treated with ethanolamine/Tween oleate, together with the degree of methylation of phosphatidylethanolamine to phosphatidylcholine, as shown by a 2 h pulse with [^{14}C]ethanolamine, suggests that substantial amounts of phosphatidylcholine originate from phosphatidylethanolamine. These results are in accord with the hypothesis that specific species of phosphatidylethanolamine may be

involved in the conversion of phosphatidylethanolamine to phosphatidylcholine [15, 16] and that the phospholipid polar head groups may dictate specific phospholipid acyl chain composition [3, 4, 11].

The exact mechanism of desaturation and incorporation of linoleic acid into phospholipid was not pursued in this investigation. Oleoyl-CoA desaturation followed by rapid transfer of the product (linoleoyl-CoA to phospholipid) has been reported for safflower [22] and potato tuber slices [1]. In other plant tissue [10], investigators have suggested that oleic acid is first incorporated into phospholipid and then desaturated. The existence of both oleoyl-CoA and dioleoylphosphatidylcholine desaturase activity was recently reported for the yeast *Candida lipolytica* [21]. Tomato seedling desaturation of exogenous [^{14}C]oleic acid was similar to all of the above mechanisms in that it was O_2 dependent, a characteristic of mixed function oxygenase-mediated systems. Furthermore, the ethanolamine-stimulated desaturation in tomato seedlings was specific for oleic acid, and although [^{14}C]stearate was transferred to phospholipid, it was not desaturated.

Increased unsaturation of phospholipids achieved by concurrent modification of phospholipid polar head groups and fatty acyl chains as described here for tomato seedlings provides an alternative to direct supplementation of polyunsaturated fatty acids. These modifications offer the possibility to assess their effects on various membrane-dependent biochemical and physiological phenomenon in plant tissues.

ACKNOWLEDGEMENTS

The authors wish to thank Louis Brannaman for his advice and instruction on the gas chromatography of the fatty acid methyl esters. We are grateful to Bill Vance of P. K. Stumpf's laboratory for use of the radiochemical gas chromatograph and for supplying the labeled stearic acid. The Tween acetate was synthesized and very kindly donated by Adam Dalziel. The technical assistance of Bill King in culture and treatment of tomato seedlings is gratefully acknowledged.

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