Fatty Acid Profile Analysis of Membrane Phospholipids of Isolated Soybean Lipid Bodies

T.D. Simpson
Northern Regional Research Center, Agricultural Research Center, U.S. Department of Agriculture, Peoria, IL 61604

Phospholipids in isolated soybean lipid bodies at 30°C underwent degradation by acyl ester and phosphodiester hydrolysis and by phosphatidyl transfer. Under conditions that minimized oxidation, preferential loss of phosphatidylethanolamine and polyunsaturated fatty acids occurred, but acyl ester compositions of all phospholipids reflected enrichment of saturated and/or monounsaturated acids. Such fatty acid changes in phosphatidylinositol, which was degraded less than phosphatidylcholine or phosphatidylethanolamine, and in phosphatidic acid, which accumulated, also suggest that transesterification occurred extensively in lipid bodies. The specific temporal changes in phospholipid and acyl chain composition suggest that several enzymes remained active in isolated lipid bodies.

Earlier work revealed a systematic deterioration of membrane phospholipids (1) in lipid bodies isolated from soybean seeds. Enzymatic activity produced significant losses of phosphatidylcholine (PC) and phosphatidylethanolamine (PE), quantitative increases in phosphatidic acid (PA) and cardiolipin (CL), and little change in phosphatidylinositol (PI) and phosphatidylglycerol (PG). Variation of results with time or dispersion of the oleosomes in buffer suggested that several enzymes may remain active in isolated lipid bodies.

This report records specific temporal changes in the acyl chain composition of lipid body phospholipids, which associate acyl hydrolase activities, and possibly others, with isolated lipid bodies.

EXPERIMENTAL PROCEDURES

The isolation of soybean lipid bodies, phospholipid extraction and separation of individual phospholipids were performed as before (1,2). Soybeans, Century variety, 1986 harvest, were used. The only adaptation to the methodology was the use of butylated hydroxytoluene (BHT, 0.005%) in chloroform throughout the extraction and separation of the phospholipids.

Methanolysis. Phospholipids were methanolized with 12% (w/v) boron trifluoride in methanol (Aldrich Chem. Co.) without extraction from silica gel, according to the method of Morrison *et al.* (3). Nonadecanoic acid was an internal standard. Esters were analyzed in a Spectra-Physics SP7100 gas chromatograph with an open tubular capillary column coated with 007 CPS-2 from J and S Scientific (Crystal Lake, IL). Phospholipid concentrations were calculated from the fatty ester analysis data for each phospholipid.

RESULTS AND DISCUSSION

Perhaps by keeping in mind general knowledge of lipid-rich tissues (4), we were unable to obtain consistent lipid body preparations even though much effort was devoted to optimizing the precision of the isolation procedure. Unfortunately, little is known about how subtle variations in methodology affect lipid body composition and properties. For example, the lipid body isolate used for set 2 (Table 1) was visibly different from that of set 1; i.e., the viscosity of the set 2 preparation was greater. Greater viscosity has been cited as an indication of significant membrane deterioration in microsomal membranes from soybean axes (5), but it remains to be seen if lipid body membranes behave similarly. The set 2 preparation was comparatively rich in unsaturated fatty acids, which, as discussed below, is typical of unaged lipid bodies. Differences in phospholipid composition might also account for the greater viscosity of set 2 lipid bodies, but again the phospholipid profile for set 2 was more typical of that for unaged lipid bodies (1).

Compared to the phospholipid changes seen in earlier work (1) and in set 1, set 2 exhibited very modest change in PA and PC, which suggests that differences in non-lipid constituents, perhaps protein, might account for the different physical properties of the two lipid body preparations.

As observed repeatedly, the PE concentration was severely reduced regardless of lipid body preparation. This affirms suspicion that substrate specificity is a factor in the degradation of lipid body phospholipids. It implies a phosphatidylethanolamine-specific phospholipase D.

In contrast, acyl ester hydrolysis appeared non-specific with respect to phospholipid type.

The acyl chain populations of major lipid body phospholipids were analyzed immediately following isolation of the lipid bodies and after incubation at 30°C—set 1, after six days, set 2, after 19 days. Each set consisted of five individual samples taken from a corresponding lipid body preparation. Table 2 gives the results of these analyses for acids that were each present in an abundance greater than one percent of total phospholipid weight. PG and CL were present in both sets, but were quantitated only in set 2.

In previous work (1), CL was observed after 18 days, at 30°C. In this work, CL was present in fresh lipid bodies, but it essentially disappeared during incubation. It would seem that the method for preparing lipid bodies

TABLE 1

Phospholipid Concentrations of Soybean Lipid Bodies Before and After Incubation at 30°C[a]

Days	PA	PC	PE	PI
0	11.2 ± 0.6	59.7 ± 2.4	18.9 ± 1.7	10.2 ± 0.4
6	31.8 ± 3.3	44.1 ± 1.3	10.7 ± 1.7	13.5 ± 1.1
0	3.4 ± 0.5	68.8 ± 2.6	13.5 ± 2.3	14.4 ± 1.6
19	8.5 ± 4.0	66.1 ± 2.5	3.6 ± 0.6	21.9 ± 2.4

[a]Abbreviations are PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol. Values are per cent phospholipid by weight given as means ± S.D.

TABLE 2

Acyl Chain Composition of Soybean Lipid Body Phospholipids Before and After Incubation at 30°C[a]

Days	Acyl moiety	PA	PC	PE	PI	PG	CL
0	Palmitic	11.0 ± 1.1	13.1 ± 0.2	17.0 ± 1.4	24.3 ± 2.2	ND[b]	ND
	Stearic	2.8 ± 0.3	3.8 ± 0.1	2.8 ± 0.1	9.7 ± 0.5	ND	ND
	Oleic	16.2 ± 0.7	17.5 ± 0.2	15.8 ± 0.3	12.2 ± 0.5	ND	ND
	Linoleic	65.4 ± 0.7	61.7 ± 0.1	60.6 ± 0.9	49.6 ± 1.2	ND	ND
	Linolenic	4.5 ± 0.1	3.9 ± 0.3	4.0 ± 0.1	4.3 ± 0.2	ND	ND
6	Palmitic	12.2 ± 0.8	13.7 ± 0.1	16.8 ± 1.2	24.7 ± 1.1	ND	ND
	Stearic	2.9 ± 0.2	4.4 ± 0.2	3.6 ± 0.2	9.7 ± 0.4	ND	ND
	Oleic	16.3 ± 0.2	18.6 ± 0.1	16.9 ± 0.4	12.5 ± 0.3	ND	ND
	Linoleic	64.4 ± 0.7	60.2 ± 0.2	59.1 ± 0.6	49.0 ± 0.6	ND	ND
	Linolenic	4.2 ± 0.1	3.1 ± 0.1	3.6 ± 0.3	4.1 ± 0.1	ND	ND
0	Palmitic	8.1 ± 1.7	12.1 ± 0.3	17.2 ± 1.7	24.4 ± 2.5	13.7 ± 4.3	13.3 ± 3.0
	Stearic	3.9 ± 0.5	4.3 ± 0.1	3.1 ± 0.1	9.6 ± 0.7	5.1 ± 0.6	3.5 ± 0.4
	Oleic	9.7 ± 0.5	10.8 ± 0.1	9.2 ± 0.3	8.1 ± 0.3	10.9 ± 2.2	10.1 ± 0.5
	Linoleic	70.5 ± 1.4	66.4 ± 0.3	64.2 ± 1.3	51.4 ± 1.5	64.6 ± 2.6	66.7 ± 2.2
	Linolenic	7.7 ± 0.3	6.4 ± 0.1	6.3 ± 0.1	6.5 ± 0.2	5.7 ± 0.3	6.5 ± 0.3
19	Palmitic	11.6 ± 1.4	13.9 ± 0.7	15.0 ± 3.4	25.8 ± 1.1	17.1 ± 5.6	ND
	Stearic	4.8 ± 0.8	5.4 ± 0.2	4.7 ± 0.7	10.1 ± 0.2	6.9 ± 1.3	ND
	Oleic	12.2 ± 0.5	12.8 ± 0.2	11.4 ± 0.7	8.9 ± 0.1	11.5 ± 0.5	ND
	Linoleic	66.1 ± 0.8	63.8 ± 0.4	64.3 ± 2.3	50.2 ± 0.8	60.1 ± 3.8	ND
	Linolenic	5.3 ± 0.3	4.1 ± 0.2	4.5 ± 0.2	5.0 ± 0.2	3.8 ± 0.3	ND

[a]Abbreviations are PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; CL, cardiolipin. Values are percent acyl chain composition given as means ± S.D.

[b]ND, not determined as phospholipid was less than 1% of total recovery.

TABLE 3

Changes in Phospholipid Acyl Chain Composition During 30°C Incubation[a]

Experiment	Acyl moiety	PA	PC	PE	PI	PG
1	Palmitic	(+1.2)	+0.6	(−0.2)	(+0.4)	ND[b]
	Stearic	(+0.1)	+0.6	+0.8	(0.0)	ND
	Oleic	(+0.1)	+1.1	+1.1	(+0.3)	ND
	Linoleic	(−1.0)	−1.5	(−1.5)	−0.6	ND
	Linolenic	−0.3	−0.8	−0.4	−0.2	ND
2	Palmitic	+3.5	+1.8	(−2.2)	(+1.4)	(+3.4)
	Stearic	(+0.9)	+1.1	+1.6	(+0.5)	(+1.8)
	Oleic	+2.5	+2.0	+2.2	+0.8	+0.6
	Linoleic	−4.4	−2.6	(+0.1)	(−1.2)	(−4.5)
	Linolenic	−2.5	−2.3	−1.8	−1.5	−1.9

[a]Abbreviations are PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol. Values are the increases and decreases in the mean percent values of acyl chain composition for the incubation period. Values in parentheses are not statistically significant.

[b]ND, not determined.

can deliver preparations that are reasonably consistent in terms of major phospholipids and fatty acids, but inconsistent in terms of effect on factors responsible for CL.

As shown in Table 3, in terms of observed change of a given acyl chain type in each phospholipid, the concentrations of linoleic and linolenic chains decreased while those of oleic, stearic, and palmitic chains increased. Several values are not statistically significant, but they are still consistent with significant values, and may yet be real.

A preferential loss of the polyunsaturated acids, linoleic and linolenic, was quite obvious. Both decreased in percentage during the incubation periods except for the linoleic of PE in set 2. A small portion of the unsaturated acid decrease may be due to oxidation (6), but autoxidation was not considered a major factor in these analyses because of the low magnitude of unsaturation loss. Autoxidation was extensive in samples where phospholipids were extracted without BHT.

Percentage increases in oleic and stearic chains were expected as a result of preferential loss of polyunsaturates. However, with exception of PE, the loss of linoleic and linolenic from a given phospholipid species exceeded the gain in oleic and stearic. This is consistent with earlier

work (1) which found that membrane phospholipid content decreased about 24% during incubation. The overall effect of incubation on the 18-carbon acyl chain profile thus points to preferential hydrolysis of unsaturated phosphatides with polyunsaturated acyl esters being hydrolyzed to a greater extent than those of saturated acids. The selective removal of unsaturated acids produced noticeable enrichment of palmitic acid in most of the phospholipids. PE was the exception. It appeared that in PE the hydrolysis of palmitate occurred to a much greater extent than the hydrolysis of stearate. Again, either different enzymes degraded PE or the same enzyme treated PC and PE differently.

Increases in PA levels during incubation (Table 1) and temporal changes in PA acids were as expected from the action of phospholipase D on PC and PE and from the action of relatively non-specific lipase on all phospholipids.

Phosphatidylinositol (PI) was earlier shown to remain quantitatively constant throughout an 18-day incubation (1). The PI enrichment seen here (Table 1) was consistent with that result. The acyl chains of PI here, however, demonstrated the same pattern of change as PC and PA. For levels of PI to remain unchanged while PI acid compositon changes requires a mechanism for PI turnover: most likely, transesterification by non-specific lipase. It is also conceivable that as PA levels increase in the incubated lipid bodies, PI might be formed by phosphatidyl transfers. Phospholipase D is capable of phosphatidyl transfer (4). Such action may be the source of PG and CL seen in these studies and previous work.

One reason for examining isolated lipid bodies was to assess their suitability for use in studies of events that lead to destabilization of stored seed. The results reported both here and previously identify patterns that follow trends emerging from studies of seed viability and aging. For example, Pukacka and Kuiper (7) have examined seeds of Norway maples stored at varying degrees of water content and temperature. During storage, they observed losses in phospholipid content and decreasing levels of linoleic and linolenic acids derived from the phospholipids. Their results were much like those obtained in these studies with isolated lipid bodies. Pukacka and Kuiper suggested that phospholipid degradation and peroxidation and concurrent deterioration of cellular membranes were the chief contributors to seed senescence and viability loss. Francis and Coolbear (8), in contrast, observed phospholipid loss in aging tomato seeds, but concluded that such loss was likely coincidental with decreasing seed viability for a given tomato variety. In another example, Senaratna *et al.* treated the axis of soybean seeds *in vitro* with xanthine-xanthine oxidase to show that desiccated damaged beans can result from free radical mechanisms (5). Their results revealed phospholipid loss which was accompanied by an increase in the microviscosity of microsomal membranes. There was no discernible decrease in unsaturation of polyunsaturated acyl chains.

Phospholipid degradation in soybean lipid bodies bears striking similarity to the overall deteriorative changes in whole seed. The results suggest that examination of isolated intact lipid bodies will be a valid method of elucidating lipid body membrane physiology.

ACKNOWLEDGMENT

The author wishes to thank John A. Rothfus for much consultation and support.

REFERENCES

1. Simpson, T.D., and L.K. Nakamura, *J. Am. Oil Chem. Soc.*, in press.
2. Bligh, E.G., and W.J. Dyer, *Can. J. Biochem. 37*:911 (1959).
3. Morrison, W.R., S.L. Tan and K.D. Hargin, *J. Sci. Food Agric. 31*:329 (1980).
4. Galliard, T., in the *Biochemistry of Plants, Vol. 4*, edited by P.K. Stumpf and E.E. Conn, Academic Press, New York, 1980, p. 85.
5. Senaratna, T., B.D. McKersie and A. Barochov, *J. Exp. Bot. 38*:2005 (1987).
6. Roberts, E.N., and R.H. Ellis, in *The Physiology and Biochemistry of Seed Development, Dormancy and Germination*, edited by A.A. Khan, Elsevier Biomedical Press, New York, 1982, p. 465.
7. Pukacka, S., and P.J.C. Kuiper, *Physiol. Plant. 72*:89 (1988).
8. Francis, A., and P. Coolbear, *Ann. Bot. 59*:167 (1987).

[Received February 2, 1989; accepted April 19, 1989]
[J5646]