Incorporation of [1-^{14}C] Acetate into Phospholipids by Soybean Seedlings

T. NEGISHI, Department of Agricultural Chemistry,
Obihiro Zootechnical University, Obihiro, Hokkaido, Japan

ABSTRACT

Incorporation of [1-^{14}C] acetate into lipids by slices of soybean seedlings was studied. The results were as follows: (a) the greatest amount of radioactivity was detected in the phospholipid fraction prepared from the main axis; (b) in the cotyledons, radioactivity was about the same in pigment, phosphatidylethanolamine, and phosphatidylglycerol; (c) phosphatidylcholine was the main phospholipid labeled in the axis; (d) the distribution of radioactive fatty acids in the axis suggested that this tissue has the capacity for both phospholipid synthesis and fatty acid desaturation.

INTRODUCTION

The storage lipid provides the main source of energy for the germination process in oil bearing seeds. Many investigators (1-5) have reported on the changes in the lipids of seedlings, such as flax, wheat, and castor bean. However, most of these studies have shown only rate of change of oil and moisture contents and fatty acid composition of total lipid during germination.

Changes in fatty acid composition in soybean seedlings have been reported by Brown et al. (6) and Joshi et al. (7). They both showed a decrease in oleic acid content during germination in the dark. Recently it was demonstrated that the decrease in oleic acid was primarily due to rapid decreases in the phospholipid (PL) fraction. The nonpolar lipid (NPL) fraction remained essentially unchanged (8). In the present work, I report on the metabolic feature of fatty acid synthesis in soybean seedlings grown in the light.

MATERIALS AND METHODS

'Okuhara,' an early variety of soybean, was obtained from the Experimental Farm of Obihiro Zootechnical University. The seeds were germinated in a petri plate (without cover) containing cotton wool wetted with distilled water. The plate was placed in an incubator at 20 C in indirect sunlight. Six days following germination, seedlings were weighed, excised into cotyledon and axis, and sliced into sections ca. 1 mm thick with a razor blade. Slices (10 g) were incubated with 20 μCi of [1-^{14}C] acetate in 10 ml of 0.1M potassium phosphate:0.1M potassium bicarbonate buffer (pH 6.5) for 3 hr at 20 C under the light. The light source consisted of two 20W daylight lamps suspended ca. 10 cm above the flasks.

The tissues were removed and ground to a fine homogenate with sea sand in a mortar. The lipids then were extracted five times with 30 ml of chloroform:methanol (1:1) and the extracts combined and washed twice with 50 ml of water. The chloroform layer was dried under vacuo and chloroform added to a fixed volume.

Identification of the lipid casses was performed by thin layer chromatography (TLC) using chromatoplates coated with Wako Gel B-5 (Wako Pure Chemical Ind. Co. Tokyo, Japan) by standard procedures (9).

Radioactivity in the lipid bands was detected by radio thin layer chromatoscanner (Nippon Musen Co., Type 11-B, Tokyo, Japan). The total lipid was developed initially in chloroform:methanol (95:12) and scanned. Three major radioactive lipids, NPL, PL, and glycolipid fractions, were separated, the bands eluted with chloroform:methanol (1:1), and each fraction rechromatographed -- the NPL with petroleum:ether:acetic acid (90:10:1) and PL with chloroform:methanol:water (65:25:4). When separation of glycolipid and PL into lipid classes was unsatisfactory, these fractions were again rechromatographed with chloroform:methanol:acetic acid:water (85:15:10:3) (10). Radioactivity in the purified lipid classes was analyzed by liquid scintillation spectrophotometer (Horiba Instrument Inc., Kyoto, Japan) with 0.2% diphenyloxazole and diphenyloxacole-benzene in toluene solution.

Each radioactive lipid was trans-esterified with 5 ml of 5% hydrochloric acid in methanol for 3 hr at 100 C and extracted with 5 ml of hexane and water. The fatty acid methyl esters were analyzed by gas chromatography on column (0.4 x 100 cm) packed with 10% diethylene glycol succinate on chromosorb. Operating conditions: oven temperature, 180 C; detector temperature, 210 C; and carrier gas flow rate, 20 ml/min nitrogen (Hitachi Instrument Inc., Model 063-5018 Radio-gas chromatograph equipped with a G-M counter and oven).

RESULTS AND DISCUSSION

Radioactivity of Lipid Fractions

The percent of radioactivity incorporated into each lipid

TABLE I

Percent of Radioactivity Incorporated into Each Major Lipid Class[a]

Lipid fraction	Cotyledon[b]	Axis[c]
Nonpolar lipid	35.2	18.8
Polar lipid	64.8	81.2
Glycolipid	ND[d]	ND

[a]Expressed as percent of area (calculated from peak wt) of total radioactivity incorporated by radio thin layer chromatography scanning.

[b]Radioactivity of total lipid = 1,201 x 10^3 cpm (8.45% of precursor).

[c]Radioactivity of total lipid = 1,205 x 10^3 cpm (8.48% of precursor).

[d]ND = Not detectable.

TABLE II

Percent of Radioactivity Incorporated into Individual Lipids[a]

Lipid class[b]	Cotyledon	Axis
TG	tr[c]	7.1
FFA	9.8	11.7
Pigment[d]	25.4	tr
PE	25.3	14.9
PG	18.6	8.5
PC	14.2	46.4
PI	6.7	11.3

[a]Calculated as cpm (lipid class)/total cpm (lipid classes).

[b]TG = Triglyceride, FFA = free fatty acid, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PC = phosphatidylcholine, PI = phosphatidylinositol.

[c]tr = Trace, $P < 0.5\%$.

TABLE III

Radioactivity of Fatty Acids of Lipid Classes in the Soybean Cotyledons[a]

Lipid fraction[b]		Fatty acid (%)						
		12:0-16:0	16:0	16:1	18:0	18:1	18:2	18:3
TG	Mass	tr[c]	14.2	2.1	4.4	36.1	36.4	6.8
	% of label	ND	ND	ND	ND	ND	ND	ND
FFA	Mass	0.5	16.4	2.8	5.5	25.6	41.5	7.7
	% of label	tr	tr	tr	tr	tr	tr	tr
PE	Mass	tr	26.2	1.5	6.2	7.6	43.8	14.8
	% of label	3.0	50.3	18.8	9.7	18.2	tr	ND
PG	Mass	1.4	48.1	2.4	6.3	5.4	24.3	12.1
	% of label	tr	75.1	9.3	9.1	5.2	1.3	ND
PC	Mass	1.0	24.7	1.9	8.9	10.3	46.0	7.1
	% of label	7.1	44.1	7.5	16.4	23.8	1.1	ND
PI	Mass	tr	39.1	3.1	10.0	8.5	36.8	2.6
	% of label	tr	44.7	9.5	23.1	22.8	tr	ND

[a]Expressed as percent of area (calculated from peak wt) of total radioactivity incorporated by radio thin layer chromatography scanning.

[b]TG = Triglyceride, FFA = free fatty acid, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PC = phosphatidylcholine, PI = phosphatidylinositol.

[c]tr = Trace, P< 0.5%.

[d]ND = Not detectable.

TABLE IV

Radioactivity of Fatty Acids of Lipid Classes in the Soybean Axis[a]

Lipid fraction[b]		Fatty acid (%)						
		12:0-16:0	16:0	16:1	18:0	18:1	18:2	18:3
TG	Mass	0.8	14.3	1.7	4.8	8.1	30.7	19.2
	% of total	8.0	12.8	18.4	16.8	28.4	15.6	tr
FFA	Mass	3.2	24.9	1.6	14.6	17.5	16.5	23.6
	% of total	6.8	24.4	7.6	13.9	25.0	14.7	7.6
PE	Mass	1.9	26.2	2.7	19.1	25.8	14.4	10.0
	% of total	5.5	33.9	18.0	9.7	19.0	10.3	5.8
PG	Mass	tr	85.7	tr	10.1	tr	4.2	tr
	% of total	4.0	57.4	15.7	3.0	14.1	5.5	tr
PC	Mass	tr	28.9	1.7	6.6	3.5	34.1	25.2
	% of total	tr	27.6	4.5	6.2	38.4	23.4	tr
PI	Mass	tr	43.7	2.6	6.4	3.7	24.2	19.3
	% of total	3.3	55.6	5.8	24.3	9.2	2.0	tr

[a]Expressed as percent of area (calculated from peak wt) of total radioactivity incorporated by radio thin layer chromatography scanning.

[b]TG = Triglyceride, FFA = free fatty acid, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PC = phosphatidylcholine, PI = phosphatidylinositol.

[c]tr = Trace, P< 0.5%.

fraction is shown in Table I. The pattern of incorporation differed in the cotyledons and the axis. Relatively more label was incorporated into the PL of the axis. It was noteworthy that even though the cotyledons and upper portions of the axis contained large quantities of chloroplasts, little label was incorporated into the glycolipid.

Radioactivity of Lipid Classes

The percent of radioactivity incorporated into individual lipids is shown in Table II. In the cotyledons, the most highly labeled lipids were pigments, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and phosphatidylcholine (PC). Only trace amounts of label was detected in the triglyceride (TG). On the other hand, in the axis, PC was the most highly labeled phospholipid and TG picked up 7.1% of the total label. Zimmerman and Klosterman (1) emphasized that TG was catabolized in one tissue and resynthesized in another tissue of the seedlings (flax) simultaneously. This apparently is also occurring here.

Radioactivity in Fatty Acids of Lipid Classes in the Cotyledons

The distribution of mass and radioactivity of fatty acids in the cotyledon lipids is shown in Table III. The TG and free fatty acid (FFA) classes revealed no clear incorporation in the component fatty acids. It is possible that, in the TGs, endogenous TG dilutes the newly synthesized fatty material to the extent that the radioactivity cannot be detected. This is not the case in the FFA fraction, however, because endogenous FFA is unlikely to be large enough to dilute the radioactivity excessively. A probable explanation is that radioactive FFA composes shorter chain fatty acid than endogenous FFA.

Radioactivity of Fatty Acids of Lipid Classes in the Axis

The distribution of mass and radioactivity of fatty acids in the axis is shown in Table IV. As compared to cotyledon, high radioactivity was detected in 18:2 and 18:3 of the axis. Because the mass of 18:2 was lower in the axis, the specific activity of this fatty acid was considerably higher in the axis tissue.

The results indicate that a desaturase system is present in the axis. Stumpf et al. (11) reported that 18:3 was synthesized from 12:3 and not from 18:2 (in spinach leaf). In these experiments, some label was incorporated in 18:3, but the substrate was not determined.

In conclusion, both cotyledon and axis incorporated [1-^{14}C] acetate into phospholipids, mainly PE in the cotyledon and PC in the axis. The distribution of mass and radioactivity in the PL suggested rearrangement of fatty acid composition in the lipids, i.e., differences between mass and incorporation data, during seedling growth.

ACKNOWLEDGMENT

Dr. R.P.A. Sims, Research Branch, Agriculture Canada, provided advice and suggestions.

REFERENCES

1. Zimmerman, D.C., and J.H. Klosterman, JAOCS 42:58 (1964).
2. De La Roche, I.A., C.J. Andrews, M.K. Pomeroy, P. Weinberger, and M. Kates, Can. J. Bot. 50:2401 (1972).
3. Yamada, Y., and P.K. Stumpf, Plant Physiol. 40:635 (1965).
4. Yamada, Y., and P.K. Stumpf, Ibid. 40:659 (1965).
5. Huton, D., and P.K. Stumpf, Ibid. 44:508 (1969).
6. Brown, B.E., E.M. Meade, and J.R. Butterfield, JAOCS 39:327 (1961).
7. Joshi, A.C., B.K. Chopra, L.C. Collins, and V.M. Doctor, Ibid. 50:282 (1973).
8. Negishi, T., Res. Bull. Obihiro Zootech. Univ., Series 1, 9:211 (1974).
9. Noda, M. in "Lipid," Vol. 1, Edited by S. Funahashi, Kyoritsu Shuppan Inc., Tokyo, Japan, 1970, p. 503.
10. Akino, T., and T. Arai, Biochim. Biophys. Acta 248:274 (1971).
11. Kannangara, C.B., B.S. Jacobson, and P.K. Stumpf, Biochem. Biophys. Res. Commun. 52:648 (1973).

[Received June 6, 1975]