Simultaneous ^{32}P- and ^{14}C-labeling of phospholipids by germinating soybeans

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SUMMARY Doubly labeled (^{32}P and ^{14}C) phospholipids of high specific activity are obtained by incorporating the labels during germination of soybeans.

KEY WORDS phospholipids · double labeling · soybean · germination

STUDY OF THE intestinal absorption of phospholipids with the aid of singly labeled compounds (1, 2) is hampered by their partial hydrolysis in the small intestine with subsequent resynthesis after absorption (3). We have therefore devised a method for the labeling of natural phospholipids simultaneously with ^{32}P and ^{14}C. Earlier studies showed that acetate-1-^{14}C (4) and phosphate-^{32}P (5) are well incorporated during the germination of the soybean. They were therefore used for the double labeling.

Procedure. Soybeans, 50 g, are moistened with 5 mc of phosphate-^{32}P and 0.5 mc of acetate-^{14}C in 5 ml of boiled water, pH 7. After the solution is completely absorbed, the seeds are allowed to germinate in the dark at 35°C for 48 hr, since at this time the absolute phospholipid content is at a maximum. Enzymic degradation is avoided by quickly freezing the seeds and lyophilizing them.

Triglycerides are extracted by homogenizing the beans 3 times for 30 min with dry acetone at 2°C and centrifuging. Phospholipids are extracted from the residue by homogenization, 3 times for 30 min with chloroform–methanol 1:1 at 30, 40, and 50°C respectively and centrifugation. The solutions are combined, mixed with one-half their volume of chloroform, and shaken with water. The lower layer is taken to dryness at a temperature below 35°C and dissolved in 10 ml of ether. Acetone, 120 ml, is added to this solution with stirring (magnetic stirrer) and the mixture is left for 12 hr at −15°C for complete precipitation.

The precipitated phospholipids are resolved into their individual components on a column of alumina (6) or silicic acid 100–200 mesh, by the method of Ansell and Hawthorne (3). After paper chomatographic separation (7) of the phospholipids, the ^{14}C and ^{32}P activities are determined directly on the paper in a liquid scintillation counter (Packard TriCarb): channel A for ^{14}C (gain 20%, window 70–400), channel B for ^{32}P (gain 1.8%, window 300–1000) in toluene containing 5 g of 2,5-diphenyloxazole and 500 mg of 1,4-bis[2-(5-phenyloxazolyl)]-benzene per liter.

TABLE 1 SPECIFIC ACTIVITY OF PHOSPHOLIPIDS AND PERCENTAGE INCORPORATION OF PHOSPHATE-^{32}P AND ACETATE-1-^{14}C INTO SOYBEAN LIPIDS

	^{32}P		^{14}C	
	dpm/μg P	%	dpm/μg P	%
Phosphatidyl choline	31,900	1.44	12,570	5.55
Phosphatidyl ethanolamine	21,300	0.76	7,660	2.44
Monophosphoinositide	14,200	0.53	17,800	2.78
Phosphatidic acid	15,200	0.07	18,300	0.78
Phosphatidyl glycerol	45,300	0.47	71,800	4.08
Diphosphatidyl glycerol	12,900	0.14	15,050	0.95
Total		3.41		16.58
Triglycerides				3.16

5 mc of phosphate-^{32}P and 0.5 mc of acetate-1-^{14}C were incubated with 50 g of soybeans.

Since the phospholipids are not eluted by the scintillation fluid, the P content of the spots on the paper can be measured after ashing (7).

Products. The ^{32}P and ^{14}C specific activities are shown in Table 1. As in previous studies (5), the highest values were found in phosphatidyl glycerol. Phosphatidyl choline and phosphatidyl ethanolamine had the highest ^{32}P specific activities, while the ^{14}C activities of all the phospholipids were about equal. The percentage incorporation of both ^{14}C and ^{32}P was highest for phosphatidyl choline.

Of the added activities 3.4% of phosphate-^{32}P and 16.6% of acetate-^{14}C were incorporated into the phospholipids, in contrast to only 3.2% incorporation of acetate-^{14}C into triglycerides, which represent over 20% of the soybean dry weight. Earlier studies have shown that the triglyceride content does not change at the beginning of germination (8); these compounds apparently turn over more slowly than phospholipids.

The β-fatty acids were removed with phospholipase A (*Crotalus adamanteus*) and the ^{14}C activity was measured in the lyso compounds. The lysophosphatidyl choline and lysophosphatidyl ethanolamine were found to contain 50% less radioactivity than the original phospholipids. Since, furthermore, glyceryl phosphoryl choline and glyceryl phosphoryl ethanolamine were not labeled, one can conclude that the radioactivity was equally distributed between the α- and β-fatty acids. Gas–liquid chromatography of the fatty acids showed that no detectable oxidation of the phospholipid fatty acids occurred during germination as a result of the action of lipoxidase.

From 50 g of soybeans we obtained 120 mg of phosphatidyl choline with 69 μc of ^{32}P and 27 μc of ^{14}C, and 40 mg of phosphatidyl ethanolamine with activities of 15.4 μc of ^{32}P and 5.5 μc of ^{14}C.

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