Preparation of [^{32}P]Phosphatidylcholine and [^{32}P]Lysophosphatidylcholine by using Soya Beans

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This method describes a procedure that can be carried out easily to obtain large amounts of [^{32}P]phosphatidylcholine and [^{32}P]lysophosphatidylcholine. The method involves germinating soya beans in the presence of [^{32}P]P_i. The yield was 0.58 % for [^{32}P]phosphatidylcholine and 0.52 % for [^{32}P]lysophosphatidylcholine, and the specific radioactivity of both was 10⁷ d.p.m./ μmol .

To study resorption, transport, distribution and metabolism of phospholipids in animals, it is necessary to use radioactively labelled compounds. Because of the distribution in the whole body, it is not possible to obtain a sufficiently high value for specific radioactivity by feeding radioactively labelled precursors. Since phospholipids of soya beans show an increased metabolism during germination (Wagner *et al.*, 1963) they are suitable experimental compounds for the production of ^{32}P -labelled phospholipids with high specific radioactivity.

Experimental

Preparation of [^{32}P]phosphatidylcholine by using soya beans

To 30 g of dried soya beans in a beaker was added 10 ml of an aqueous solution containing 20 ± 2 mCi of [^{32}P]P_i (sp. radioactivity 50 mCi/mmol; Amersham/Buchler, Braunschweig, Germany). The beans were moistened uniformly by vigorous shaking of the beaker. When the water was consumed a further 20 ml portion of water was added to allow swelling and the beans were germinated in an incubator at 37°C. After 30 h, the germinated seedlings were homogenized in a mixer and freeze-dried.

The removal of the neutral lipids from the dried material was carried out mainly by extraction with four portions of 500 ml of acetone for 1 h each time. Thereafter the soya-bean phospholipids were extracted from the residue with three portions of 500 ml of chloroform/methanol (1:1, v/v), the soya-bean homogenate being incubated with the chloroform/methanol mixture at 40°C for 1 h with vigorous stirring. The combined chloroform/methanol ex-

tracts were evaporated to dryness in a rotary evaporator. The water-soluble impurities of the extract were removed by suspending the residue in 375 ml of chloroform and extraction with 70 ml of water. The lower chloroform phase containing the phospholipids was then evaporated to dryness in a rotary evaporator.

[^{32}P]Phosphatidylcholine was separated from the soya-bean phospholipids by chromatography on Al₂O₃ (Rhodes & Lea, 1957). Al₂O₃ (10 g) was mixed with sufficient chloroform/methanol (1:1, v/v), poured into a column (2 cm $\times$ 16 cm) and washed extensively with the same solvent. The phospholipid extract in chloroform/methanol (1:1, v/v) was applied to the column directly and the [^{32}P]phosphatidylcholine was eluted with 750 ml of chloroform/methanol (1:1, v/v).

After concentration of the eluate to dryness the lipids were purified by removing the remaining neutral lipids on a silicic acid column (Merck); a slurry of silicic acid (10 g) in 12 ml of chloroform/methanol (1:1, v/v) plus 7 ml of 25 % ammonia was poured into a column and washed with chloroform extensively. The soya-bean phospholipid fraction in chloroform was applied to the column and the neutral lipids were removed by elution with 500 ml of chloroform. The elution of [^{32}P]phosphatidylcholine was carried out with 400 ml of chloroform/methanol (4:1, v/v) containing 1.5 % water. This eluate contained only [^{32}P]phosphatidylcholine and was dried by evaporation.

Hydrolysis of soya-bean [^{32}P]phosphatidylcholine to [^{32}P]lysophosphatidylcholine

Hydrolysis of soya-bean [^{32}P]phosphatidylcholine to [^{32}P]lysophosphatidylcholine (Klenk *et al.*, 1962) was carried out by using phospholipase A₂ (EC 3.1.1.4) of snake (*Crotalus terrificus terrificus*) venom (Boehringer).

To a solution of 10 mg of [^{32}P]phosphatidylcholine

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in 10ml of diethyl ether were added 0.05ml of phospholipase A₂ solution and 0.025ml of 1M-CaCl₂. After stoppering the glass and intensive shaking the sample was stored for 18h at room temperature (22°C). The diethyl ether was removed by evaporation and the remaining viscous residue was subjected to lipid extraction with chloroform/methanol (2:1, v/v) twice. After the extract was evaporated to dryness the [³²P]lysophosphatidylcholine was precipitated by addition of acetone. [³²P]Lysophosphatidylcholine appeared as a white greasy pellet and was dried *in vacuo*.

Results

Thin-layer chromatograms of the soya-bean [³²P]-phosphatidylcholine and [³²P]lysophosphatidylcholine preparations showed, on staining with I₂, intense yellow bands with *R_F* values corresponding to pure phosphatidylcholine and lysophosphatidylcholine respectively and a light-yellow front. T.l.c. was carried out on silica-gel plates (Merck) with chloroform/methanol/water (14:6:1, by vol.) as solvent.

The thin-layer chromatogram was scanned by

scraping off bands of 1 cm from the origin up to the front and measuring their radioactivities in a liquid-scintillation counter. For [³²P]phosphatidylcholine 1% of the radioactivity was detected at the origin in the [³²P]phosphatidylcholine preparation. In the [³²P]lysophosphatidylcholine preparation 1% of the [³²P]lysophosphatidylcholine radioactivity was at the origin and 2% corresponded to the *R_F* value for phosphatidylcholine.

The yields were 174 mg of [³²P]phosphatidylcholine and 156 mg of [³²P]lysophosphatidylcholine, both with specific radioactivities of 10⁷ d.p.m./μmol (Fiske & SubbaRow, 1925).

References

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