METHOD OF PURIFYING THE TOTAL PHOSPHOLIPIDS FROM CARBOHYDRATES

Kh. S. Mukhamedova and S. T. Akramov

UDC 547.953.665.37

It is known that when the total phospholipids from plant raw materials after defatting are extracted with a mixture of chloroform and methanol (2:1) [1, 2], nonlipid components — carbohydrates, amino acids, and other hydrophilic compounds, among which the carbohydrates amount to 35-50% of the total — pass into the extracts in addition to the lipids. These nonlipid substances have a considerable influence on the composition, properties, and consistency of the phospholipids: the tendency of the phospholipids to form a powder is due to the considerable amount of sugars in them.

There are several methods of purifying the total phospholipids from water-soluble impurities: the chloroform–methanolic extracts are washed with water [1-5] or with dilute solutions of NaCl [1, 5-7] or $CaCl_2$ [8]. The free sugars can also be separated from the phospholipids in petroleum-ether solutions by means of 50% ethanol [9]. However, with the various methods of washing nonlipid impurities out of the extracts, considerable losses of the individual phospholipids are observed and also inadequate purification of the phospholipids from carbohydrates [10, 11]. It has been shown [12] that when the extracts are washed with water or salt solutions the amount of substances extracted is increased by the presence of phosphatidycholines, phosphatidylethanolamines, and polyglycerophosphatides. We have confirmed these results for the case of the purification of the total phospholipids of the seeds of cotton plants of varieties 108-F, Tashkent-1, Tashkent-3, etc. Column chromatography on cellulose [8, 13-15], on DEAE-cellulose [16, 17] and on Sephadex G-25 [18-20] has also been used for freeing phospholipids from nonlipid components. Sephadex is the most promising adsorbent for the purification of phospholipids from carbohydrates.

To purify the total phospholipids we selected silica gel. The experiments were performed in two directions:

1. The total material was adsorbed on silica gel and the carbohydrates were eluted with distilled water, while the phospholipids were eluted first with chloroform–methanol (2:1) and then with methanol.

2. The combined phospholipids in the form of a 1% solution in chloroform–methanol–water (90:10:1) [20] were passed through a column of silica gel. The bulk of the pure phospholipids emerged with the solvent and the remainder (7-8%) was eluted by a fairly large amount of the same mixture.

The carbohydrates can be eluted from the column with water, methanol, or aqueous methanol. The desorption of the phospholipids from silica gel requires more solvent than from Sephadex.

The purified total phospholipids obtained by the methods mentioned contain no carbohydrates (TLC) and show no change in their qualitative and quantitative composition.

EXPERIMENTAL

The experiments were performed with type KSK silica gel (100 μ for thin layers and 160-250 μ for columns) washed free from cations and activated at 110-115°C for 7-9 h.

The completeness of the freeing of the total phospholipids from carbohydrates was

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR.
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 570-572, September-October, 1975. Original article submitted June 25, 1974.

checked by thin-layer chromatography in the chloroform–ethanol–water (65:35:5) and chloroform–methanol–25% ammonia (65:35:5) systems. Phospholipids were revealed with the Vas'Kovskii and Kostetskii reagents and the carbohydrates with aniline phthalate and 10% sulfuric acid in methanol.

Experiment I. A solution of 0.76 g of total phospholipids of the seeds of the cotton plant of variety 108-F [2] in 100 ml of chloroform was used to form a suspension containing 38 g of silica gel. After 5-10 min, the solution was filtered through a Schott funnel, and the silica gel was washed with another 50 ml of chloroform. The filtrate formed a colorless clear solution containing 20 mg of dissolved matter (neutral lipids). Then the silica gel was washed with distilled water (500 ml). The water was evaporated in a rotary evaporator. Yield 0.3 g (white crystals of sugars). The silica gel was transferred to a round-bottomed flask and, after the addition of a small amount of acetone, it was dried in vacuum. On elution with acetone, 300 ml of filtrate yielded 90 mg of a mixture of phospholipids and steroids (violet spots on treatment with sulfuric acid).

The total phospholipids were eluted from the silica gel with chloroform–methanol (2:1) and with pure methanol. The solvent was distilled off in vacuum in a current of nitrogen. Yield 0.32 g.

Experiment II. A solution of 0.5 g of the combined phospholipids in 50 ml of chloroform–methanol–water (90:10:1) was passed through a column (1.8 × 60 cm) containing 12.5 g of silica gel. The mixture was distilled in vacuum under a current of nitrogen (weight 0.18 g). Then the column was eluted with 600-700 ml of the same mixture (weight 0.07 g). The yield of phospholipids freed from carbohydrates was 0.25 g.

SUMMARY

A method is proposed for freeing plant phospholipids from carbohydrates; silica gel has been used as adsorbent.

LITERATURE CITED

1. J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
2. Kh. S. Mukhamedova and S. T. Akramov, Khim. Prirodn. Soedin., (1972), p. 663.
3. M. Lepage, J. Chromatogr., 13, 99 (1964).
4. L. A. Shustanova, A. U. Umarov, and A. L. Markman, Khim. Prirodn. Soedin., 292 (1970).
5. G. N. Novozhilova, S. S. Mkhitaryan, N. A. Bogoslovskii, et al., Prikl. Biokhim. i Mikrobiol., 5, 111 (1969).
6. I. A. Shabanova, Biokhimiya, 32, 1155 (1967).
7. V. Ya. Dvorkin, D. A. Chekverikova, and A. Shmelev, Biokhimiya, 28, 475 (1963).
8. J. N. Hawthorne and G. Hübsher, Biochem. J., 71, 195 (1959).
9. Handbook. Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], Leningrad, III (1964), p. 57.
10. D. G. Therriault, J. Amer. Oil Chemists' Soc., 40, 395 (1963).
11. H. Jongh and J. G. Pelt, J. Lipid Res., 3, 385 (1962).
12. W. Bartley, G. S. Getz, M. Brenda, and N. Renshaw, Biochem. J., 82, 540 (1962).
13. C. H. Lea and D. N. Rhodes, Biochem. J., 54, 467 (1953).
14. R. H. Smith, Biochem. J., 57, 130 (1954).
15. L. Svennerholm, Nature, 177, 521 (1956).
16. G. Rouser, A. J. Bauma, G. Kritchevsky, D. Heller and J. S. O'Brien, J. Amer. Oil. Chemists' Soc., 38, 544 (1961).
17. G. Rouser, G. Kritchevsky, D. Heller, and E. Lieber, J. Amer. Oil Chemists' Soc., 40, 425 (1963).
18. M. A. Wells and J. C. Dittmer, Biochem., 2, 1259 (1963).
19. A. N. Siakotos and G. Rouser, J. Amer. Oil Chemists' Soc., 42, 913 (1965).
20. M. E. McKillican and J. A. G. Larose, J. Amer. Oil. Chemists' Soc., 47, 256 (1970).