HYDROGENATION OF THE PHOSPHATIDYLCHOLINE OF *Helianthus annuus*

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Unsaturated fatty acids, especially the essential fatty acids, present in phospholipids should apparently increase their physiological value, but the oxidation and polymerization of these acids lower the physiological activity of the phospholipids. Attempts have been made to perform the partial or complete catalytic saturation of the phospholipids [1], and these have also been hydrogenated for preparative purposes [2].

We have performed the hydrogenation of a homogeneous phosphatidylcholine with a Pd/Al catalyst in order to confirm the length of the hydrocarbon radicals of the fatty acids present in its molecule and for other analytical purposes. A pure fraction of 1,2-diacyl-sn-glyceryl-3-phosphorylcholine was obtained by solvent fractionation and the use of a column of silica gel [3]. The purity of the product obtained was confirmed by chemical and physical methods, and the fatty-acid composition [4] and the location of the acyl groups in its molecule have been determined [5].

The phosphatidylcholine was hydrogenated in a hydrogenation vessel constructed in such a way that it was possible to regulate the temperature of the process and to measure the amount of hydrogen absorbed [6]. As the catalyst we used finely disperse palladium obtained by the precipitation of the metal from an aqueous solution of Na_2PdCl_4 by displacing it with metallic aluminum powder [7]. The sample was saturated until the iodine number was zero. The amount of hydrogen absorbed was measured every minute at first and then, when the rate of hydrogenation had fallen considerably, every 5 min.

The volume of hydrogen (V_0) consumed for the saturation of the sample taken to zero iodine number was calculated from the formula [8]

$$V_0 = \frac{112 \cdot (\text{I. No.}) \cdot a}{127}$$

where a is the weight of the sample, g; and I. No. is the iodine number, % I_2.

In our case, the iodine number of the phosphatidylcholine was 127.34% of I_2. For the exhaustive hydrogenation of a 0.5-g sample it was necessary to use

$$V_0 = \frac{112 \cdot 127.34 \cdot 0.5}{127} = 56 \text{ ml of hydrogen}$$

(under standard conditions: 0°C, 760 mm Hg).

Under the conditions of the experiment

$$V = V_0 \frac{1}{k} = 56 \frac{1}{0.83} = 67.4 \text{ ml of hydrogen}$$

(where k is the coefficient for bringing the volume of hydrogen under the experimental conditions, V, to the volume under standard conditions, V_0).

We give information on the absorption of hydrogen with time during the hydrogenation of the phosphatidyl-

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choline: after 1 min, 20 ml of hydrogen had been absorbed, after 3 min, 40 ml; 5 – 46; 7 – 52; 9 – 55; 15 – 58.5; 25 – 61.5; 35 – 64; 45 – 65; and 50 – 65. The hydrogenate obtained was separated from the catalyst by filtration and, after the elimination of the solvent, it was analyzed. The hydrogenated phosphatidylcholine consisted of a white powder and had the following indices: P 3.97%; $\overset{+}{N}$ 1.72%; $RCOO^-$ 2.05 μeq. IR spectrum (cm^{-1}): weak band in the 3450 region (OH); 2950, 2915, 2850, 1475, 1375, 830 and 770 (CH, CH_2, CH_3); 1745, 1180 (COOR); 1100, 1060, 960 (P–O–C); 1250 (P=O); and 975 [$\overset{+}{N}(CH_3)_3$]. The absorption bands of the ethylenic bonds of unsaturated acids in the 1670-1650 cm^{-1} region and the bands at 3015 cm^{-1} corresponding to the stretching vibrations of cis-CH = CH bonds were absent. An absorption band at 975 cm^{-1} in the hydrogenated phosphatidylcholine is characteristic for this phospholipid [9].

The fatty-acid composition (%) of the hydrogenated phosphatidylcholine was determined and was compared with the native composition [5, 10]:

Fatty acid	12 : 0	14 : 0	16 : 0	18 : 0
Initial	1.0	1.2	12.0	85.8
Position 1	1.1	1.1	20.5	77.3
Position 2	0	0	3.5	96.5

The results obtained showed the complete saturation of the double bonds and confirmed that the main acids of sunflower lecithin are acids of the C_{16} and C_{18} groups.

We checked the possibility of enzymatic hydrolysis of the hydrogenated phosphatidylcholine at pH 10.15 with 0.1 M tris buffer. As the source of phospholipase A we used the venom of the Azerbaidzhan kufi. The enzymatic hydrolysis of the hydrogenated phosphatidylcholine took place at the same rate as that of native phosphatidylcholines, provided the temperature of the reaction mixture was raised to 30-34°C.

The chromatographic mobility of the hydrogenated phosphatidylcholine and the products of its enzymatic hydrolysis in a thin layer of silica gel in the chloroform–methanol–water (65 : 25 : 4) solvent system was characterized by the following R_f values: for the fatty acids 0.85; for the phosphatidylcholine 0.40; for the lysophosphatidylcholine 0.16; i.e., the same as for the native substances [5]. The products of enzymatic hydrolysis were separated in the way described previously [5, 10].

The molar ratios of the components of the 1-acyl-sn-glyceryl-3-phosphorylcholine $RCOO^-$: P : N were 1 : 1 : 1. The fatty acids split off from the lysophosphatidylcholine and regenerated were methylated with diazomethane and chromatographed on GLC (the results are given below). They agree with those for the unhydrogenated sample and show that the specificity of the action of the phospholipase of kufi venom is retained for completely hydrogenated phosphatidylcholine which, in its turn, confirms literature information that phospholipase A specifically splits the fatty acids in position 2 of a phospholipid regardless of the degree of their saturation [11].

Our investigations show the possibility of performing the enzymatic hydrolysis of native [5] and of completely hydrogenated phosphatidylcholine by means of kufi venom phospholipase A at a high pH value at which the specificity and activity of the enzyme are retained.

EXPERIMENTAL

For column and thin-layer chromatography we used silica gel with a grain size of 100-150 and 150-200 mesh, respectively, washed with hydrochloric acid, with water, and with organic solvents. For TLC we used a fixed layer (with 5% of gypsum). The composition of the fatty acids was determined by GLC on a UKh-2 chromatograph at 200°C with a column 2.5 m long filled with INZ-600 brick (40-60 mesh) impregnated with 15% of PEGS; the carrier gas was helium.

Hydrogenation of the Phosphatidylcholine. The catalyst was prepared immediately before hydrogenation. The process was performed at 29°C and atmospheric pressure (726 mm Hg). The phosphatidylcholine (500 mg) was hydrogenated in 10 ml of methanol with 0.5% Pd/Al. First, 50 mg of aluminum powder was added to the hydrogenation vessel and then a solution of sodium tetrachloropalladate, and the whole system was purged with 500-600 ml of hydrogen to eliminate oxygen and to reduce the catalyst to metallic palladium. After this, a solution of the phosphatidylcholine in methanol was added and it was saturated completely with hydrogen. The catalyst was separated off by filtration through a paper filter of medium density and was washed with 50 ml of diethyl ether; the filtrate was additionally purified on a column containing 5 g of silica gel (100-150 mesh).

The solvent was driven off under vacuum, and the homogeneity of the hydrogenate was checked by chromatography in a thin layer of silica gel.

Analysis of the Hydrogenate. The IR spectrum was taken in the form of a film on a UR-10 instrument. For the 680-200 cm^{-1} region NaCl prisms were used, and for the 2000-4000 cm^{-1} region LiF. The nitrogen content was determined by the micro Kjeldahl method and the phosphorus from an aliquot of the sample by Tevekelov's method [12]. The number of ester groups was determined by the method of Stern and Shapiro [13] in our modification [14] and the colorimetric reaction mixture was brought to 6.0 ml by the addition of a mixture of ethanol and ether (3 : 1) before its optical density was measured.

The enzymatic hydrolysis of the hydrogenated phosphatidylcholine and separation of the products obtained were performed by methods that we have described previously [5], but the reaction was carried out at 30-34°C (to improve the dissolution of the hydrogenate). The lysophosphatidylcholine obtained was analyzed as described above for the hydrogenate.

The fatty acids split off (from position 2) were methylated with diazomethane and the esters were subjected to GLC. The fatty acids from position 1 were determined after their regeneration from the lysophosphatidylcholine by alkaline hydrolysis (0.5 N solution of KOH in methanol, 70°C, 90 min).

SUMMARY

1. The optimum conditions have been found for the exhaustive hydrogenation of chromatographically pure phosphatidylcholine of plant origin on a Pd/Al catalyst.

2. It has been shown that under the conditions of the enzymatic hydrolysis of the hydrogenated phosphatidylcholine by kufi venom phospholipase A at pH 10.15 the specificity and activity of the enzyme are retained.

3. The main physical and chemical indices of the hydrogenated phosphatidylcholine and of the products of its hydrolysis are given.

LITERATURE CITED

1. N. L. Melamud, G. V. Chebotareva, T. A. Danilova, L. T. Smirnova, and D. M. Maiorov, Trudy Vsesoyuzn. N.-I. In-ta Zhirov, No.26, 295 (1967).
2. D. J. Hanahan, J. Biol. Chem., 211, 321 (1954).
3. L. A. Shustanova, A. U. Umarov, and A. L. Markman, Khim. Prirodn. Soedin., 518 (1971).
4. L. A. Shustenova, A. U. Umarov, and A. L. Markman, Khim. Prirodn. Soedin., 3 (1971).
5. L. A. Shustanova, A. U. Umarov, and A. L. Markman, Khim. Prirodn. Soedin., 137 (1971).
6. A. L. Markman, Zh. Obshch. Khim., 23, 1622 (1953).
7. I. P. Nazarova, A. L. Markman, and A. I. Glaushenkova, Maslozhir, Prom., No. 8, 13 (1969).
8. L. S. Golodova and D. V. Sokol'skii, Maslozhir. Prom., No. 8, 10 (1965).
9. Handbook on Methods of Investigation and the Technical Control and Accounting of Production in the Oils and Fats Industry [in Russian], Book 1, VNIIZh (1967), p. 467.
10. L. A. Shustanova, An Investigation of the Phospholipids of the seed kernels of the sunflower Helianthus annuus L.," Author's Abstract of Candiate's Dissertation, Tashkent (1972).
11. D. J. Hanahan, H. Brockerhaff, and E. J. Barron, J. Biol. Chem., 235, 1917 (1960); L. L. M. van Deenen, J. Am. Chem. Soc., 43, 296 (1966).
12. D. T. Terekelov, Izv. In-ta po Khranen, Bolg. AN, 7, 21 (1968).
13. J. Stern and V. Shapiro, J. Clin. Path., 6, 158 (1953).
14. L. A. Shustanova, Khim. Prirodn. Soedin., 655 (1974).