

STRUCTURAL CHANGES IN PHOSPHATIDYLCHOLINES DURING THEIR CHROMATOGRAPHY ON ALUMINA

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No structural changes take place in phosphatidylcholine on silica gel. On columns with the adsorbents neutral Al_2O_3 (according to Brockmann) and Al_2O_3^* treated with ethyl acetate, at constant times of 4, 24, and 48 h a marked decrease in yield and, at the same time, a rise in the amount of deacylated phosphatidylcholine take place. The amounts of unsaturated fatty acids present in both the first and second positions of the phosphatidylcholines also decrease sharply. The amount of the main molecular species remains unchanged, but the molecular composition of the phosphatidylcholines increases on prolonged contact, a nonspecific migration of the acyl radicals in the phosphatidylcholine taking place.

A number of adsorbents are used for the column chromatography of polar lipids: silica gel and alumina (most frequently) and also various Sephadexes and DEAE-cellulose. Various authors have reported a superiority of one adsorbent over others and at the same time defects in them have appeared.

The capacity of Al_2O_3 for causing the hydrolysis of esters and the isomerization of the acyls in mono-, di-, and triglycerides, and also in phospholipids, is well known [1-3].

Jenson and Marks [4] consider that only basic Al_2O_3 possesses a disrupting capacity, but the treatment of Al_2O_3 with ethyl acetate eliminates the catalytic centers on the surface of the adsorbent [5]. In a review [6], to obtain accurate results it is proposed to use only Al_2O_3 treated with ethyl acetate.

The aim of our investigation was to study the influence of Al_2O_3 and of silica gel on the structure of a phosphatidylcholine (PC) during column chromatography.

We used type KSK silica gel (treatment described in the Experimental part). For column chromatography, the silica gel was kept in anhydrous chloroform, and for TLC it was kept in glass jars. For TLC we also used type LS 5/40 silica gel (Czechoslovakia). We used alumina, Al_2O_3 (according to Brockmann) (Hungary), and also Al_2O_3^* treated with ethyl acetate as described by Meakins and Swindells [5]. The phosphatidylcholine was isolated from the total phospholipids (PLs) obtained from cotton seeds by repeated treatment with ethanol and subsequent freeing from accompanying substances [7]. As the eluents we used chloroform-methanol (1:1) and methanol. In view of the fact that acidified alcohols subject PLs to alcoholysis [8], for final elution we used the chloroform-methanol-25% NH_4OH (70:30:2) system. The preparation of the column and the elution of the PCs from the adsorbent were performed in the usual way [7].

The contact of the PC with the adsorbent was performed in a thin layer of silica gel and in columns filled with silica gel, Al_2O_3 , and Al_2O_3^* for 4, 24, and 48 h.

The source of phospholipase A_2 for enzymatic hydrolysis was snake venom (*Vipera lebetina* L.) [9].

The compositions and position distributions of the fatty acid radicals in the PC and lyso-PCs are given in Table 1. In view of the fact that the compositions and position distributions of the fatty acid radicals in the molecules were identical in the case of the contact of the PCs with silica gel for 4, 24, and 48 h, we have given the results only for 48 h. The same situation was observed in the case of TLC on the KSK and LS 5/40 silica gels.

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TABLE 1. Compositions and Position Distributions of the Fatty Acid Radicals in the Phosphatidylcholines after Contact with Adsorbents

Phosphatidylcholine	Acid														ΣS	ΣU
	10:0	11:0	12:0	14:0	15:0	16:0	16:1	18:0	18:1	18:2	X	e-18:0				
Initial phosphatidylcholine																
Total	0.4	—	0.4	0.2	—	17.4	0.6	3.6	27.8	48.8	0.8	—	22.8	77.2		
1	—	—	0.3	0.1	—	30.7	0.6	7.2	25.7	35.0	0.4	—	38.7	61.3		
2	0.8	—	0.5	0.3	—	4.1	0.6	—	29.9	62.6	1.2	—	6.9	93.1		
In a thin layer of silica gel (TLC)																
1	0.4	—	0.3	—	—	29.2	0.2	7.2	26.2	35.7	0.7	—	37.8	62.2		
2	0.4	—	0.5	0.4	—	5.6	0.1	—	29.4	61.9	0.9	—	7.8	92.2		
On silica gel																
1	0.5	—	0.2	0.1	—	29.6	—	7.2	26.4	35.4	0.6	—	38.2	61.8		
2	0.3	—	0.6	0.3	—	5.2	1.2	—	29.2	62.2	1.0	—	7.4	92.6		
On Al ₂ O ₃ after contact for 4 h																
Total	1.4	—	0.8	0.5	—	17.5	0.6	2.4	28.9	47.9	—	—	22.6	77.4		
1	0.5	—	1.1	0.6	—	30.7	0.8	4.8	24.6	36.9	—	—	37.7	62.3		
2	2.3	—	0.5	0.4	—	4.3	0.4	—	33.2	58.9	—	—	7.5	92.5		
On Al ₂ O ₃ * after contact for 4 h																
Total	1.2	—	0.7	0.8	—	17.3	0.4	2.9	29.1	47.6	—	—	22.9	77.1		
1	0.9	—	0.8	1.0	—	30.5	0.5	5.8	24.8	35.7	—	—	39.0	61.0		
2	1.5	—	0.6	0.6	—	4.1	0.3	—	33.4	59.5	—	—	6.8	93.2		
On Al ₂ O ₃ after contact for 24 h																
Total	2.6	1.6	1.6	1.2	—	15.9	2.4	3.4	25.4	45.9	—	—	26.3	73.7		
1	0.8	1.2	1.4	1.0	—	28.0	3.2	6.8	22.2	35.4	—	—	39.2	60.8		
2	4.4	2.0	1.8	1.4	—	3.8	1.6	—	28.6	56.4	—	—	13.4	86.6		
On Al ₂ O ₃ * after contact for 24 h																
Total	3.1	—	3.6	3.1	—	18.1	2.9	5.1	24.5	39.6	—	—	33.0	67.0		
1	0.2	—	5.7	5.1	—	31.6	4.0	10.2	20.0	23.2	—	—	52.8	47.2		
2	6.0	—	1.5	1.1	—	4.6	1.8	—	29.0	56.0	—	—	13.2	86.8		
Lyso-PC	3.4	2.7	3.8	4.3	—	22.9	4.6	8.8	22.2	27.3	—	—	45.9	54.1		
On Al ₂ O ₃ after contact for 48 h																
Total	1.7	2.0	1.4	2.3	2.6	14.9	4.2	6.9	22.4	35.1	4.6	1.9	38.3	61.7		
1	3.4	4.0	2.8	3.7	4.3	23.6	7.2	13.8	18.9	11.2	7.0	0.1	62.7	37.3		
2	—	—	—	0.9	0.9	6.2	1.2	—	25.9	59.0	2.2	3.7	13.9	86.1		
Lyso-PC	3.3	0.5	0.7	1.9	1.1	17.7	2.0	4.7	24.6	40.4	1.0	2.0	33.0	67.0		
On Al ₂ O ₃ * after contact for 48 h																
Total	—	0.3	—	0.6	0.6	15.2	1.6	5.2	26.2	47.8	—	—	2.5	24.4	75.6	
1	—	0.6	—	0.6	0.8	26.1	1.6	10.4	24.6	31.9	—	—	3.4	41.9	58.1	
2	—	—	—	0.6	0.4	4.3	1.6	—	27.8	63.7	—	—	1.6	6.9	93.1	
Lyso-PC	0.3	0.3	0.9	0.8	—	15.8	1.4	3.9	27.8	48.1	—	—	0.4	22.7	77.3	

The yield of PC on silica gel amounted to practically 100% in all cases; on Al₂O₃ to 92.2% and on Al₂O₃* to 93.2% with contact for 4 hours, 86.4% (PC) and 6.9% of lyso-PC, and 86.9% (PC) and 6.4% of lyso-PC at 24 h; 17.5% (PC) and 27.5% lyso-PC and 46.3% and 23.7% lyso-PC at 48 h, respectively. On Al₂O₃ the yield of PC fell from 92.2% to 86.4% to 17.5% and on Al₂O₃* from 93.2% to 83.9% to 46.3% for 4, 24, 48 h, respectively. The amount of lyso-PC increased from 6.9 and 6.4% at 24 h to 27.5 and 23.7% at 48 h for Al₂O₃ and Al₂O₃*, respectively. The lowest yield and most pronounced deacylation of the PC was observed on Al₂O₃ and Al₂O₃* with contact for 48 hours. In actual fact, in this case the deacylation of the PC took place more slowly on the Al₂O₃* and the yield of PC was three times higher than on Al₂O₃.

From the results of the experimental work we have drawn the following conclusions:

On silica gel and in TLC the yield is practically 100% and no degradation of the PC is observed;

Al₂O₃* has proved to be a milder adsorbent than Al₂O₃, but in both cases on prolonged contact the yield of PC decreased sharply and its deacylation increased.

TABLE 2. Compositions and Position Distributions of the Fatty Acid Radicals in Phosphatidylcholines Taking Their Elution from the Column into Account

Phosphatidylcholine	Acid													ΣS	ΣU
	10:0	11:0	12:0	14:0	15:0	16:0	16:1	18:0	18:1	18:2	X	e-18:0			
On Al ₂ O ₃ after contact for 4 h (yield 92.2%)															
Total	1,2	—	0,7	0,4	—	16,1	0,5	2,2	26,6	44,1	—	—	20,8	71,4	
1	0,4	—	1,0	0,5	—	28,3	0,7	4,4	22,6	34,0	—	—	34,7	57,5	
2	2,2	—	0,4	0,3	—	3,9	0,3	—	30,6	54,2	—	—	6,9	85,3	
On Al ₂ O ₃ * after contact for 4 h (yield 93.2%)															
Total	1,4	—	0,6	0,7	—	16,1	0,3	2,7	27,1	44,3	—	—	21,3	71,9	
1	0,8	—	0,7	0,9	—	28,4	0,4	5,4	23,1	33,3	—	—	36,3	55,8	
2	1,4	—	0,5	0,5	—	3,8	0,2	—	31,1	55,3	—	—	6,3	86,9	
On Al ₂ O ₃ after contact for 24 hours (yield 86.4%)															
Total	2,2	1,4	1,4	1,0	—	13,7	2,1	2,9	21,9	39,6	—	—	22,3	63,7	
1	0,6	1,7	1,2	0,8	—	24,2	2,9	5,8	19,1	30,5	—	—	33,8	52,6	
2	3,8	0,7	1,6	1,2	—	3,2	1,3	—	24,7	48,7	—	—	11,6	74,8	
On Al ₂ O ₃ * after contact for 24 hours (yield 86.9%)															
Total	2,7	—	3,1	2,7	—	15,7	2,5	4,4	21,3	34,4	—	—	28,6	58,8	
1	0,2	—	4,9	4,4	—	27,4	3,4	8,8	17,4	20,1	—	—	45,8	41,5	
2	5,2	—	1,3	1,0	—	4,0	1,6	—	25,2	48,7	—	—	11,4	77,1	
On Al ₂ O ₃ after contact for 48 hours (yield 17.5%)															
Total	0,3	0,3	0,2	0,4	0,5	2,6	0,7	1,2	3,9	6,1	0,8	0,3	6,7	10,8	
1	0,6	0,6	0,4	0,6	0,8	4,1	1,2	2,4	3,3	1,9	1,2	—	11,0	6,5	
2	—	—	—	0,2	0,2	1,1	0,2	—	4,5	10,3	0,4	0,6	2,4	15,1	
On Al ₂ O ₃ * after contact for 48 hours (yield 46.3%)															
Total	—	0,1	—	0,2	0,2	7,0	0,7	2,4	12,1	22,1	—	1,1	11,3	35,0	
1	—	0,2	—	0,2	0,3	12,1	0,7	4,8	11,4	14,7	—	1,5	19,4	26,9	
2	—	—	—	0,2	0,1	1,9	0,7	—	12,8	29,5	—	0,7	3,2	43,1	

The next stage of our investigations was the study of the structural changes taking place in the PC molecule on contact with the adsorbents mentioned.

We calculated the amount of fatty acids of the PCs, taking their elution from the column into account (Table 2). As follows from the figures given, changes take place mainly as the result of a decrease in the amount of the main fatty acids.

On Al₂O₃ and Al₂O₃* the losses of fatty acids for 4, 24, and 48 h were as follows: in the case of the 16:0 acid, 1.3 → 4.3% and 1.7 → 14.8% and 10.4%; for the 18:1 acid, 1.2 and 0.7% → 5.9% and 6.5% → 23.9% and 15.7%; for the 18:2 acid, 4.7% and 4.5% → 9.2% and 14.4% → 42.7% and 26.7%, respectively. In parallel with this, the quantitative yield of the main molecular species, 16:0-18:1, 16:0-18:2, 18:1-18:2, 18:1-18:1, 18:2-18:1, and 18:2-18:2, decreased, while the complexity of molecular composition increased on Al₂O₃ and Al₂O₃* with contact for 48 hours from 64 to 96 and 72 species, respectively.

Thus, with respect to the compositions and position distributions of the fatty acids in the PCs the following conclusions can be drawn:

The amount of unsaturated fatty acids present in both the first and second positions of the PCs decreases on prolonged contact with Al₂O₃ and Al₂O₃*;

The number of main molecular species decreases but the complexity of molecular composition in the PCs increases.

After 48 h contact with Al₂O₃, epoxystearic acid (e-18:0) was identified, this apparently having been formed as the result of the oxidation of oleic acid.

In the case of prolonged contact with Al₂O₃ and Al₂O₃*, a nonspecific migration of the acyl radicals in the PC molecule takes place.

Thus, for the separation, isolation, and structural studies of phospholipids, KSK silica gel treated as described in the Experimental part has proved to be most suitable.

EXPERIMENTAL

Granules of KSK silica gel were ground in a ball mill to a size of less than 250 μ . They were covered with dilute nitric acid (1:1) and were stored in it for a month, and were then washed with distilled water on a filter to neutrality. The silica gel was dried in the air and washed with chloroform-methanol (1:1), and was then dried in the air again and in an oven at 600°C for 8 h.

The Al_2O_3 was treated with ethyl acetate by the method of Meakins and Swindells [5].

For TLC, 5% of gypsum was added. For column chromatography, 2 \times 50 cm steel columns filled with 10 g of adsorbent were used, and before the charging of the PCs (80 mg) they were first washed with 100 ml of chloroform and with 100 ml chloroform-methanol (1:1). The PCs were eluted from the column with chloroform (100 ml), chloroform-methanol (1:1) (200 ml), methanol (100 ml), and chloroform-methanol-25% NH_4OH (10:30:5) (150 ml). Elution was monitored with aid of TLC in the chloroform-methanol-25% NH_4OH (70:30:5) system.

Enzymatic hydrolysis was performed with the aid of phospholipase A_2 (snake venom) at pH 9.5, and alkaline hydrolysis and the treatment of the hydrolysis products as described previously [7].

The methyl esters of the fatty acids were analyzed on a Chrom-41 chromatograph. The solid phase was Celite 545, 60-80 mesh, impregnated with 17% of poly(ethylene succinate) and 19% of poly(diethyleneglycol succinate), the temperature of the thermostat being 198-200°C, that of the detector 230°C, and that of the evaporator 230°C, with a column 2.5 m long.

SUMMARY

No structural changes take place in a phosphatidylcholine on silica gel.

In columns with the adsorbents Al_2O_3 and Al_2O_3^* on contact for 4, 24, and 48 h there is a marked fall in yield, and at the same time the amount of deacylated phosphatidylcholine increases. Furthermore, migration of the acyl radicals in the phosphatidylcholine molecule is observed.

We consider that alumina is unsuitable for structural investigations of phospholipids.

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