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Fractionation of a natural mixture of alkenyl acyl and diacyl ethanolamine phosphatides by argentation adsorption thin-layer chromatography*

Ethanolamine phosphatides were implicated in the intrinsic[1–4], and extrinsic[5] prothrombin activation of blood coagulation processes and their activity increased with increase in degree of unsaturation[2,5]. Although plasmalogens were shown to be ineffective by indirect means[1,6], the evidence was not very convincing and, surprisingly enough, the platelets[4] and brain tissues[7] which are the sources of ethanolamine phosphatides for intrinsic[1–4] and extrinsic[8] prothrombin activation, respectively, contain appreciable amounts of plasmalogens. This raises a question about whether the plasmalogens or the less unsaturated ethanolamine phosphatides have an inhibitory effect on the procoagulant activity of the highly unsaturated ethanolamine phosphatides. The question can be answered only when one has available different molecular species of alkenyl acyl and diacyl ethanolamine phosphatides which vary in their degree of unsaturation.

Although various molecular species of diacyl ethanolamine phosphatides may be obtained by synthetic means[9], the available methods are tedious and time-consuming, while methods available for the synthesis of ethanolamine plasmalogens are not promising[10]. An alternative is the argentation adsorption chromatographic technique which has been used so successfully in the fractionation of choline phosphatides[11–17]. Recent investigations[14] have indicated that plasmalogens from beef heart choline phosphatides may be concentrated by this technique and suggested the possibility of isolating native plasmalogens without destroying the accompanying diacyl analogs, in contrast to other methods[18–21].

The present communication describes the fractionation of a natural mixture of alkenyl acyl and diacyl ethanolamine phosphatides by argentation adsorption chromatography and discusses the possible use of the fractionated molecular species in the blood coagulation studies.

Experimental

The preparation of phosphatides from beef heart, isolation of a mixture of alkenyl acyl and diacyl ethanolamine phosphatides from the total beef heart phosphatides, the preparation of Silica Gel G plates (plain and impregnated with silver nitrate), fractionation of the ethanolamine phosphatides on silver nitrate impregnated Silica Gel G plates using chloroform–methanol–ammonia (70:30:4.5) as the developing solvent, methanolysis of original and fractionated samples, and subsequent gas–liquid chromatographic (GLC) analysis of the methyl esters and dimethyl acetals, were carried out as described previously[14].

Results

The argentation fractionation of a mixture of alkenyl acyl and diacyl ethanolamine phosphatides obtained from beef heart is shown in Fig. 1. Five main fractions were clearly visible. However, fractions III and IV overlapped to a considerable extent

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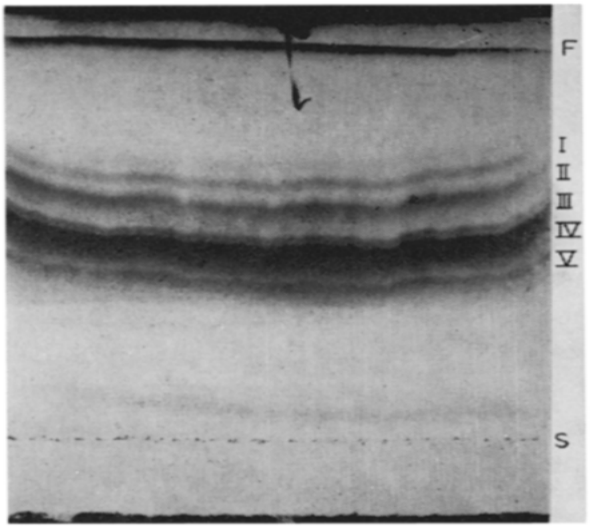

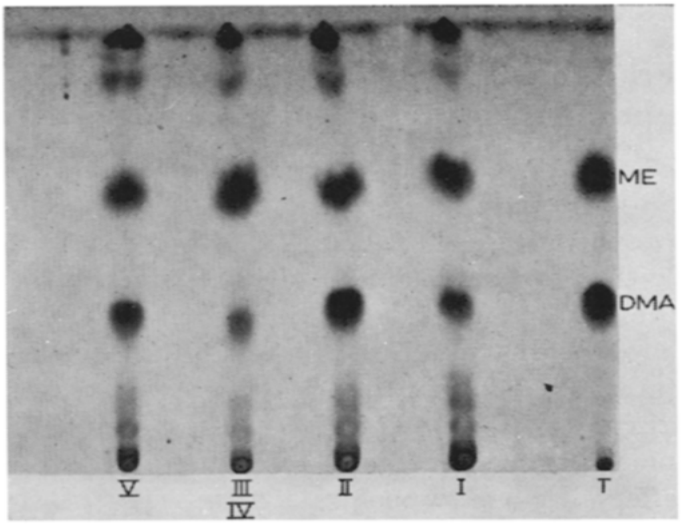

Fig. 1. Fractionation of a mixture of alkenyl acyl and diacyl ethanolamine phosphatides from beef heart. Adsorbent: Silica Gel G impregnated with silver nitrate. Activation: 2 h at 100°. Solvent: chloroform–methanol–water (70:30:4.5). Spray reagent: 50% aqueous sulfuric acid (plate charred for 10 min at 160°). S = Starting line; F = solvent front.

Fig. 2. Methanolysis products of original mixture of alkenyl acyl and diacyl ethanolamine phosphatides, and different fractions thereof obtained by argentation adsorption TLC of the original mixture. T = Methanolysis products of original mixture of alkenyl acyl and diacyl ethanolamine phosphatides; I = methanolysis product of fraction I; II = methanolysis product of fraction II; III, IV = methanolysis product of fractions III and IV; V = methanolysis product of fraction V; ME = methyl esters; DMA = dimethyl acetals.

TABLE I

FATTY ACID AND ALDEHYDE COMPOSITION OF BEEF HEART ALKENYL ACYL AND DIACYL ETHANOLAMINE PHOSPHATIDES AND THEIR MOLECULAR SPECIES OBTAINED BY ARGENTATION ADSORPTION THIN-LAYER CHROMATOGRAPHY

Carbon number of fatty chain	*Percent of total fatty acids*					*Percent of total fatty aldehydes*				
	Original phosphatide mixture	*Fractions*				*Original phosphatide mixture*	*Fractions*			
		I	*II*	*III and IV*	*V*		*I*	*II*	*III and IV*	*V*
14:0	—	—	—	—	—	0.5	Tr.	—	—	1.1
15:0	0.3	Tr.	Tr.	—	—	Tr.	Tr.	—	—	—
16:0	1.9	16.0	1.4	0.8	—	35.4	37.8	33.2	45.9	31.3
16:1	0.5	Tr.	Tr.	—	3.6	2.9	2.8	2.8	1.8	3.2
17:Br } 17:0 }	—	—	—	—	—	6.7	5.3	7.6	5.6	11.1
18:0	34.3	25.9	3.6	40.9	4.6	50.1	54.0	55.6	37.8	49.8
18:1	4.8	15.6	1.5	—	4.2	4.4	—	0.8	8.9	3.5
18:2	16.3	42.5	86.6	Tr.	Tr.					
18:3	0.6	—	Tr.	Tr.	Tr.					
20:3	3.7	—	6.9	6.5	Tr.					
20:4	35.9	—	—	51.8	78.4					
20:5	1.7	—	—	—	9.2					

and therefore were collected as a single fraction. Two additional fractions, one just above fraction I, and another near the starting line, were scarcely visible and their small quantities precluded further investigation.

The separation of fatty acid methyl esters (ME) and dimethyl acetals (DMA) obtained by methanolysis of the original mixture of alkenyl acyl and diacyl ethanolamine phosphatides and fractions thereof is shown in Fig. 2. Visual observation followed by densitometry (based on the relative intensities of ME and DMA) indicated high plasmalogen content in fractions II and V.

The GLC analysis of ME and DMA obtained from the original ethanolamine phosphatide mixture, and fractions thereof, is given in Table I.

Discussion

Although contaminations because of overlapping are not unusual in such separations, Table I and Fig. 2 aid in the interpretation of this fractionation. It is clear from Table I that fractions I and II both contain high amounts of linoleic acid compared to other unsaturated constituents, but nevertheless may be separated. The explanation for this apparent anomaly lies in the high plasmalogen content of fraction II which, due to its vinyl group, makes available one more double bond for silver ion-complex formation[22]. This explanation finds further support in the analyses recorded in Table II, which show appreciably higher amounts of linoleic acid in the alkenyl acyl

TABLE II*

FATTY ACID DISTRIBUTION IN THE ORIGINAL MIXTURE OF ALKENYL ACYL AND DIACYL ETHANOLAMINE PHOSPHATIDES

Carbon number of fatty acids	*Percent fatty acids of total fatty chains in alkenyl acyl ethanolamine phosphatides*	*Percent fatty acids of total fatty acids in diacyl ethanolamine phosphatides*
C16:0	0.2	1.9
C18:0	0.7	49.1
C18:1	0.9	5.0
C18:2	30.6	9.2
C20:3	2.1	—
C20:4	15.5	34.8
Aldehydes	Rest (50%)	

* The analysis reported in this table is on another sample of alkenyl acyl and diacyl ethanolamine phosphatides isolated from another beef heart and analyzed by a two-dimensional reaction thin-layer chromatography technique[24].

ethanolamine phosphatides than in the diacyl ethanolamine phosphatides. Similar explanations seem justified in explaining the separation of combined fractions III and IV, and fraction V, both of which possess larger proportions of arachidonic acid.

ROUSER AND SCHLOREDT[2] and NEMERSON[5] in their studies of blood coagulation used ethanolamine phosphatides from different sources in order to vary unsaturation and plasmalogen content. The technique described herein employs ethanolamine phosphatides from the same source but fractionated to give varied unsaturation and

plasmalogen content. One may easily obtain plasmalogen-free ethanolamine phosphatides from any of the fractions referred to earlier by simple acid hydrolysis, followed by thin-layer separation (reaction thin-layer chromatography)[23,24]. Studies involving the role of molecular species of diacyl and alkenyl acyl ethanolamine phosphatides in blood coagulation will be reported elsewhere.

A combination of the above fractionation technique with reversed phase chromatography (as suggested by ARVIDSON[13]) may further aid in subfractionation.

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