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Note

Determination of plasmalogen contents of phospholipid classes by reaction micro-thin-layer chromatography

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Plasmalogens are widespread in nature and appear to be essential in some biological processes[1–3]. Different methods for their analysis have been described[4,5] and new ones have recently been suggested[6]. Reaction thin-layer chromatography (TLC) is the most convenient procedure for the determination of plasmalogen contents of separated lipids[7–9]. We have combined reaction TLC principles with our micro-TLC technique of lipid analysis[10,11] and obtained a simple and sensitive method for plasmalogen quantitation in phospholipid classes.

EXPERIMENTAL

Chloroform, methanol, acetone, acetic acid, hydrochloric acid, sulphuric acid and 28% ammonia solution were commercial products and were used without additional purification.

Starfishes *(Distolasterias nipon)* were collected in Posiet Bay, Sea of Japan, in the winter of 1974–75. Brain tissue was obtained from adult albino rats. Lipids were extracted by the method of Bligh and Dyer[12] as modified by Christie[13]. Phosphatidylethanolamine (PE) was isolated from the lipid extract of starfishes by column chromatography on silica gel L, 100–160 mesh (Chemapol, Prague, Czechoslovakia), with chloroform–methanol mixtures. Butylated hydroxytoluene was added to the extract and elution mixtures.

Micro-TLC plates were prepared as described earlier[10].

In order to establish the optimal concentration of hydrochloric acid in methanol, about 10 μg of PE were spotted in an angle of a micro-TLC plate and developed in the first direction with chloroform–methanol–28% ammonia (65:35:5). The chromatogram was dried in a cold current of air for 5 min, and a 1-cm wide zone from the start to the front was sprayed with hydrochloric acid in methanol at concentrations from 0.5 to 6.0 N. The chromatogram was dried again in a current of air and developed in the second direction with chloroform–acetone–methanol–acetic acid–water (100:40:20:20:10). The spots were detected with 10% sulphuric acid in methanol after heating at 180°. Phosphorus in PE and lyso-PE spots was quantitated by our previous method[11].

Non-polar products of PE hydrolysis were separated on micro-TLC plates by the modified method of Mahadevan *et al.*[14]. After hydrolysis of PE, the plate was

developed in the second direction with *o*-xylene and in the third direction with light petroleum (b.p. 80–90°)–diethyl ether (100:5).

In order to determine plasmalogen contents of separate phospholipid classes, a portion of lipid extract containing 1.5–2.0 μg of phosphorus was spotted in an angle of a micro-TLC plate. The chromatogram was treated as described above for PE. Hydrolysis of plasmalogen was carried out with 2 *N* hydrochloric acid in methanol.

RESULTS AND DISCUSSION

The essential stage in the analysis of plasmalogens by reaction TLC is their splitting on the thin-layer chromatogram. Owens[7], who was the first worker to use this method, used an aqueous solution of mercury(II) chloride, while Brockhuyse[15] used a solution of mercury(II) chloride in 0.1 *M* acetic acid. Horrocks[8] treated the plates with hydrochloric acid vapour and Viswanathan *et al.*[16] used 12% hydrochloric acid in methanol.

For our micro-TLC, we used hydrochloric acid in methanol as the most simple and convenient reagent. In order to establish the optimal acid concentration, we used PE from starfishes as a model substance as starfishes are rich in plasmalogen[17]. The results are given in Table I, from which it is apparent that hydrochloric acid in concentrations from 1.5 to 6 *N* splits plasmalogens completely. We decided to use 2 *N* hydrochloric acid in methanol.

TABLE I

PERCENTAGE OF STARFISH PE PLASMALOGEN HYDROLYSIS WITH DIFFERENT HYDROCHLORIC ACID CONCENTRATIONS

HCl concentration (N)	*Hydrolysis (%)*
0.5	86.4
1.0	97.3
1.5	99.7
2.0	100.0
4.0	100.0
6.0	100.0

As the analysis of starfish PE gave a content of more than 80% of plasmalogens, we decided to check whether or not it is a result of partial splitting of ester bonds, and examined the non-polar products of PE hydrolysis. Dimethylacetals and free aldehydes were identified, but not methyl esters or free fatty acids.

We then tested the reaction micro-TLC method on the well studied rat brain, and found that its PE and phosphatidylcholine (PC) contained 55.6 and 4.9% of plasmalogens, respectively. These values compare with literature values[18–24] of 50–66 and 3.5–4.0%, respectively.

Analysis of the phospholipid composition of starfishes (*D. nipon*) gave the following results (as the percentage of phosphorus in separate phospholipid classes based on total lipid phosphorus): PE (diester and ester–ether forms) 5.8%; PE plasmalogen 30.6%; PC (diester and ester–ether forms) 42.0%; PC plasmalogen 6.2%; lyso-PE 1.2%; lyso-PC 3.6%; phosphatidylserine 9.3%; and phosphatidylinositol 1.6%. Hence, starfish PE contains 84.1% and PC 12.8% of plasmalogen.

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