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Note

Simple and rapid purification of acidic lipids from rat brains

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Although the primary role of lipids is to provide a molecular "cement" for the construction of biomembranes and other cellular organelles, some dynamic function would also be expected. For example, Loh and Law[1] suggested that one of the dynamic functions of lipids in receptor mechanisms is to act directly as binding sites themselves and the most likely candidates for this role are the acidic lipids, as the large numbers of neurotransmitters and drug molecules are cationic in the physiological environment. In fact, several investigators[2–4] have reported the implication of sulphatides (CS), phosphatidylserine (PS) and phosphatidylinositol (PI) in the proteolipid nature of opioid and serotonin receptors. These aspects have indicated the need for highly purified acidic lipids in order to be able to examine the neurophysiological function of these acidic lipids in detail.

For the purification of acidic lipids from central nervous tissue, a column chromatographic separation has already been reported[5]. However, to our knowledge, this method is unsuitable with respect to purity, yield and convenience. In this paper, a simple and rapid purification procedure is described, by means of which CS, PS and PI can be separated from rat brains with reasonable purity and yield.

EXPERIMENTAL

DEAE-cellulose was obtained from Brown (Berlin, NH, U.S.A.) and silicic acid from (analytical-reagent grade) Mallinckrodt (St. Louis, MO, U.S.A.). Chloroform and methanol were redistilled before use. Whole rat brains (male Wistar, 150–200 g), except for the cerebellum and olfactory lobe, were extracted with chloroform–methanol (2:1) and then treated to give the so-called Folch-washed extracts[6]. The purities of the isolated lipids were checked by two-dimensional thin-layer chromatography (TLC) on Kieselgel 60 (E. Merck, Darmstadt, G.F.R.) with chloroform–methanol–ammonia solution (14:6:1) followed by chloroform–acetone–methanol–acetic acid–water (10:4:2:2:1). The determination of phospholipids (*i.e.*, PS and PI) and CS was performed by the methods of Chen *et al.*[7] and Kean[8], respectively.

RESULTS AND DISCUSSION

As a first step toward the purification of three acidic lipids from whole rat

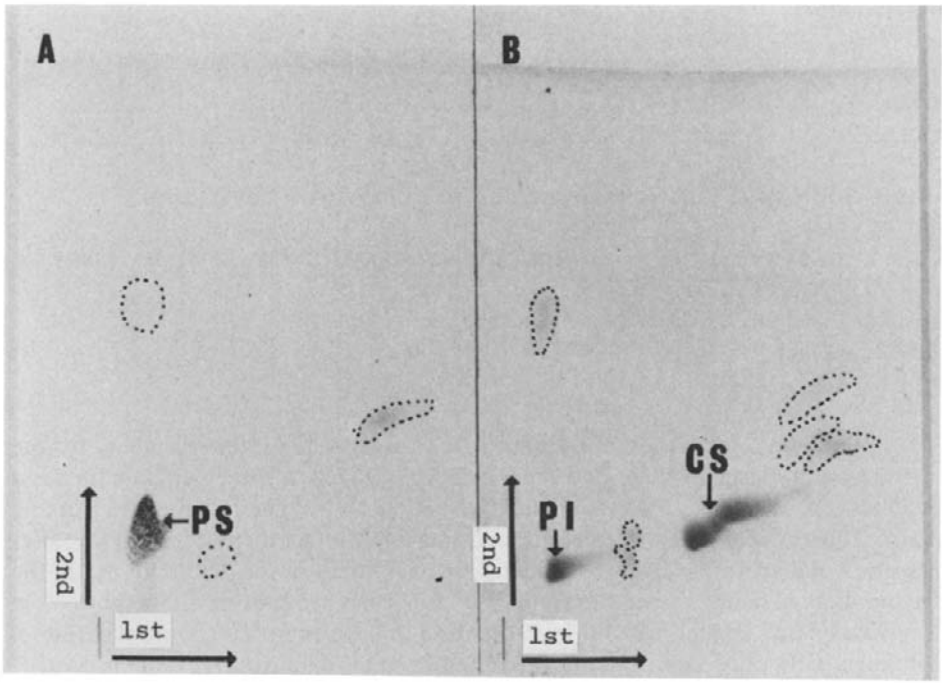

Fig. 1. Thin-layer chromatograms of the F-I and F-II fractions separated by DEAE-cellulose column chromatography. The plates were developed in a two-dimensional solvent system as described and compounds were detected with 50% sulphuric acid. (A) Acetic acid fraction (F-I); (B) chloroform–methanol–ammonia-ammonium acetate fraction (F-II).

brains, silicic acid and DEAE-cellulose (acetate form) column chromatography was used according to the methods of Norton and Autilio[9] and Rouser *et al.*[5], respectively. Briefly, the Folch-washed extracts (250 mg as total lipids) were loaded on to a silicic acid column (30 × 1.3 cm I.D.) and discontinuous elution was carried out with chloroform–methanol of increasing polarity (98:2, 96:4, 90:10, 80:20, 60:40 and 50:50). The fractions eluted with 80:20 and 60:40 chloroform–methanol were pooled and these mixtures (100 mg as total lipids) were subsequently chromatographed on DEAE-cellulose column (25 × 2 cm I.D.). Stepwise elution was carried out with the following solvents: chloroform–methanol (7:3), methanol, acetic acid (F-I fraction), methanol and then chloroform–methanol–ammonia solution (2:1:0.06) containing 0.05 *M* ammonium acetate (F-II fraction). Detection of three acidic lipids was performed by two-dimensional TLC as described above. As shown in Fig. 1, the chromatograms clearly revealed PS and CS + PI in the F-I and F-II fractions, respectively. However, both fractions also included several other lipids. These observations indicate the need for further purification.

The F-I fraction (8 mg as total lipids) was passed through a silicic acid column (30 × 1.3 cm I.D.). Elution was carried out with chloroform–acetone–methanol–acetic acid–water (10:4:2:2:1). PS was appeared as the third peak and the elution profile of each lipid in this system was in good agreement with the results of TLC (see Figs. 1 and 2). The F-II fraction, containing mainly CS and PI (18 mg as total lipids)

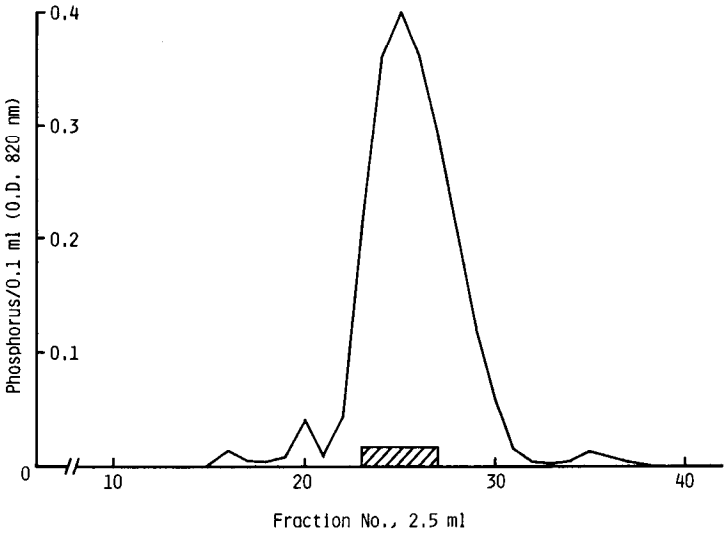

Fig. 2. Separation of phosphatidylserine from the F-I fraction by silicic acid column chromatography. The acetic acid fraction (F-I) separated by DEAE-cellulose column chromatography was loaded on to a silicic acid column and elution was carried out with chloroform–acetone–methanol–acetic acid–water (10:4:2:2:1). The elution pattern of lipid phosphorus is presented. The hatched area represents the separated fraction as PS.

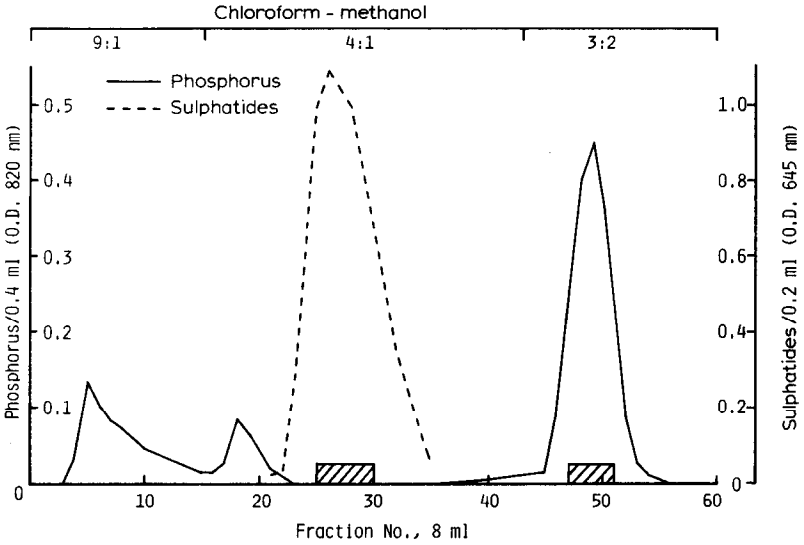

Fig. 3. Separation of sulphatides and phosphatidylinositol from the F-II fraction by silicic acid column chromatography. The chloroform–methanol–ammonia–ammonium acetate fraction (F-II) separated by DEAE-cellulose column chromatography was loaded on to a silicic acid column. Stepwise elution was carried out with the following solvents: 120 ml of chloroform–methanol (9:1), 240 ml of chloroform–methanol (4:1) and 120 ml of chloroform–methanol (3:2). The elution profiles of lipid phosphorus and CS (as the coloured complex formed with CS and azure A) are indicated. The hatched areas represent the separated fraction as CS and PI.

was loaded on to a silicic acid column (30 × 1.3 cm I.D.) and stepwise elution was carried out with chloroform–methanol (9:1, 4:1 and then 3:2). As shown in Fig. 3, CS was eluted with chloroform–methanol (4:1), whereas PI appeared in the chloroform–methanol (3:2) fraction. On the other hand, most of the contaminants appeared in both the chloroform–methanol (9:1) and (4:1) solvent fronts.

In addition, we have already found that the absorption maximum of the coloured complex formed with CS and azure A is 46 absorbance units (AU)/μmole, whereas those of ethanolamine phosphatides and choline phosphatides are 0.051 and 0.018 AU/μmole, respectively. Thus, we can recommend the application of an azure A colorimetric analysis for the determination of the concentration of CS in this system.

The isolated acidic lipids were identified by their R_F values, using 50% sulphuric acid and 0.1% ninhydrin as the spray reagent.

Finally, the yields of CS, PS and PI were 0.9, 2.5 and 0.3 mg/g of tissue, respectively, and these three acidic lipids were more than 96% pure.

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