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Note

Separation of diphosphoinositide and triphosphoinositide on oxalate-impregnated silica gel columns

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Diphosphoinositide and triphosphoinositide have been prepared from the Folch "diphosphoinositide fraction" of brain[1] by column chromatography using DEAE-cellulose[2]. A second method of separating these phospholipids has now been developed using oxalate-impregnated silica gel. It is based on the thin-layer chromatography procedure of Gonzalez-Sastre and Folch-Pi[3]. Silica gel (100 g, 100–200 mesh) was slurried with 230 ml 1% (w/w) aqueous potassium oxalate and dried overnight at 110°. The next day the oxalate-impregnated silica gel was allowed to cool in a desiccator and quickly ground to a powder using a mortar and pestle. A slurry was then prepared with chloroform–methanol (7:3) and made into a column 30 cm × 2.5 cm. Folch "diphosphoinositide fraction" was applied to the column in a small volume of the same solvent with the addition of a little chloroform as necessary to give a clear solution. The loading ratio was 1 mg lipid phosphorus per gram of silica gel. Gradient elution followed, using 2 l chloroform–methanol–water (9:7:2) in the

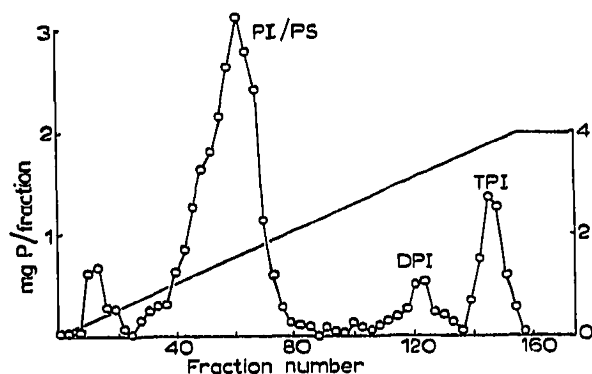

Fig. 1. Separation of phosphoinositides by column chromatography on silica gel. The sloping line indicates the gradient elution, figures on the right-hand ordinate representing the molarity of NH_4^+ in the aqueous portion of the solvent (see text).

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mixing vessel and 2 l chloroform–methanol–4 *M* ammonium hydroxide (9:7:2) in the reservoir. Fractions of 25 ml were collected and monitored by thin-layer chromatography[3], by paper chromatography[4] and also by phosphorus estimation[5]. Phosphatidylinositol (PI) was eluted first, followed by diphosphoinositide (DPI) and then triphosphoinositide (TPI) with little overlap (Fig. 1). A further 500 ml chloroform–methanol–4 *M* ammonium hydroxide was passed through the column to remove any remaining triphosphoinositide. Suitable fractions were then combined, neutralised to pH 7.0 with glacial acetic acid and the solvents removed by rotary evaporation *in vacuo* at 40°. The resulting aqueous dispersion of lipid was then dialysed against disodium EDTA (0.05 *M*) overnight and then against distilled water for two days with several changes. The phospholipids were lyophilised for storage. The fractions containing phosphatidylinositol, which were contaminated with phosphatidylserine (PS) and phosphatidylethanolamine, were further purified by alumina and silicic acid chromatography[6,7].

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