notes on methodology

Thin-layer chromatography of the phosphoinositides

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SUMMARY Thin-layer chromatography for the separation of mono-, di-, and triphosphoinositides (0.3–3 μg total phosphorus) is described.

KEY WORDS phosphoinositides · thin-layer chromatography · calcium sequestration · oxalate

The presence in brain of a phospholipid fraction containing inositol was reported by Folch in 1942 (1). This fraction was later shown to comprise three lipids differing in their phosphorus content—the mono-, di-, and triphosphoinositides (2–4)—the chemical structures of which were subsequently established (5–7). Up to now these compounds have been identified and estimated quantitatively by chromatography on formaldehyde-treated paper (8) and on silicic acid-impregnated paper (9).

In an attempt to apply to the estimation of phosphoinositides the rapid and convenient procedure of thin-layer chromatography, it was found that when Silica Gel G was used, triphosphoinositides (TPI) did not migrate with several of the usual solvent mixtures. Since the calcium salts of phosphoinositides in general, and of TPI in particular, are insoluble in water, we wondered whether the lack of mobility of TPI on Silica Gel G might be due to the calcium sulfate binder. We found that on Silica Gel H to which potassium oxalate had been added for the purpose of sequestering any small amounts of calcium that might be present, TPI do migrate.

Glass plates were coated with a 0.25 mm layer prepared from a slurry of 30 g of Silica Gel H (E. Merck A. G., Darmstadt, Germany) in 80 ml of a 1% aqueous solution of potassium oxalate (Merck). The plates were dried at room temperature and activated at 110°C for 30 min. The samples (generously supplied by Dr. K. Hayashi) consisted of mono-, di-, and triphosphoinositides obtained from ox brain, isolated according to Hendrickson and Ballou (10) and identified by the procedure of Dawson and Dittmer (5). The purity of the samples was checked by chromatography on formaldehyde-treated paper (8). The phosphoinositides were applied to the plates, in amounts ranging from 0.3 to 3.0 μg of total phosphorus, in water or in chloroform–methanol–water 75:25:2. The plates were developed upward with *n*-propanol–4 N NH_4OH 2:1 or with chloroform–methanol–4 N NH_4OH 9:7:2 (Fig. 1). Spots were revealed either by exposure to iodine vapor followed by spraying with 1% starch solution, or by spraying with Kaggi–Micher reagent (glacial acetic acid–concd sulfuric acid–*p*-anisaldehyde 100:1:0.4) and heating in an oven at 120°C for 15 min. Table 1 gives the average R_f values observed for the three phosphoinositides in the two solvents used.

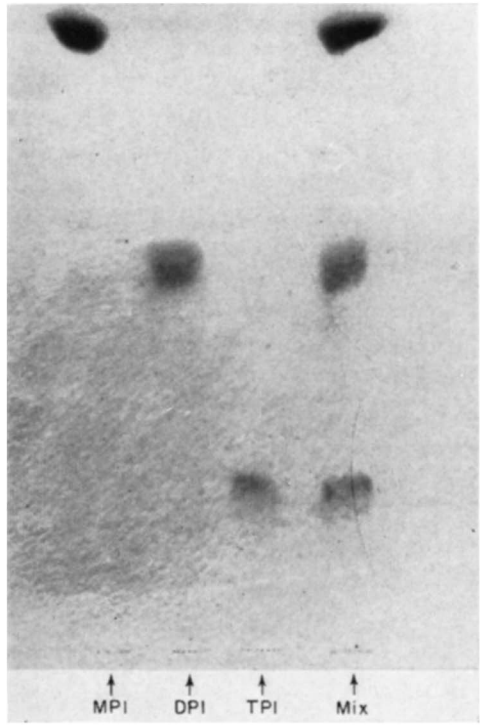

Fig. 1. Thin-layer chromatography of phosphoinositides on Silica Gel H. MPI, monophosphoinositide (1 μg of P); DPI, diphosphoinositide (0.4 μg of P); TPI, triphosphoinositide (0.3 μg of P); Mix, MPI + DPI + TPI. Solvent: chloroform–methanol–4 N NH_4OH 9:7:2.

TABLE 1 R_f Values for Mono-, Di, and Triphosphoinositides

	Chloroform–Methanol–4 N NH_4OH 9:7:2	*n*-Propanol–4 N NH_4OH 2:1
Monophosphoinositide	0.78	0.55
Diphosphoinositide	0.36	0.43
Triphosphoinositide	0.14	0.29

Silica Gel H plates prepared in potassium oxalate solution.

Abbreviation: TPI, triphosphoinositides.

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The presence of potassium oxalate appears to be an absolute requirement when the chloroform–methanol–ammonia solvent is used; it seems to be less critical with propanol–ammonia.

TPI were examined in the form of the sodium, potassium, lithium, ammonium, calcium, and magnesium salts. Mono- and diphosphoinositides were assayed as ammonium and calcium salts. The same relative mobilities were observed for the different salts of each of the three phosphoinositides. The R_f values did not change with variations in the amount of sample applied within the range indicated.

With both developing solvents, the lipids other than phosphoinositides that are present in the lower phase of Folch brain extract (11) show R_f values above 0.6. The chromatographic procedure described can therefore be used for the qualitative detection of di- and triphosphoinositides in lipid mixtures. It also provides the basis for the development of a procedure for their quantitative estimation (work in progress).

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