A procedure for detecting phosphonolipids on thin-layer chromatograms

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Summary A simple modification of a phospholipid-specific spray for thin-layer chromatograms allows it to be used as a specific detection reagent for phosphonolipids and as a general lipid detection reagent.—**Stillway, L. W., and S. J. Harmon.** A procedure for detecting phosphonolipids on thin-layer chromatograms. *J. Lipid Res.* 1980. **21:** 1141–1143.

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The phospholipid spray reported by Dittmer and Lester (1) has been used extensively to detect specifically phospholipids on thin-layer chromatograms. This reagent contains molybdenum trioxide and metallic molybdenum in dilute H_2SO_4. The relatively high concentration of sulfuric acid (8.3N) allows the use of "phospholipid specific spray" to be extended to the detection of other lipids with minor modifications and without loss of its specificity for phospholipids. We have used this spray as a general lipid detection reagent for a number of years by heating plates after

Abbreviations: 1-AEP, 1-aminoethylphosphonate; 2-AEP, 2-aminoethylphosphonate.

spraying. It was noted that polar lipid samples from certain marine organisms contained phospholipid components which could easily be distinguished from the conventional phospholipids because they retained the characteristic blue color. It became clear that these components were phosphonolipids and that this reagent could be used specifically to detect phosphonolipids. This report describes the use of this reagent as a specific detection reagent for phosphonolipids and as a general thin-layer chromatography detection reagent for non-phospholipids.

METHODS AND RESULTS

Total lipids were extracted from clam tissues (*Mercenaria mercenaria*) and rat liver with chloroform–methanol (2). Cerebrosides and sulfatides were purchased from Applied Science Laboratories, Inc. (State College, PA). 1-Aminoethylphosphonate (1-AEP) and 2-aminoethylphosphonate (2-AEP) were purchased from Analabs, Inc. (North Haven, CT). The spray reagent was prepared as described by Dittmer and Lester (1):

"Solution I. To 1 liter of 25N H_2SO_4 40.11 g of MoO_3 is added and the mixture is boiled gently until the MoO_3 is dissolved.

Solution II. To 500 ml of Solution I 1.78 g of powdered molybdenum is added and the mixture is boiled gently for 15 min. The solution is cooled and decanted from any residue that may be present.

Molybdenum Spray. Equal volumes of Solutions I and II are mixed and the combined solution is mixed with two volumes of water. The final solution is greenish yellow in color. If too little water is used it will be blue; if too much, yellow. The spray is stable for months."

Samples of total lipid (100–300 μg) were spotted on thin-layer chromatography plates coated with Supelcosil 42A (Supelco, Inc., Bellefonte, PA). Plates were developed two-dimensionally in chloroform–methanol–water 65:35:4 and chloroform–acetone–methanol–acetic acid–water 5:2:1:1:0.5 as described by Rouser (3). The solvent was allowed to evaporate, and the plates were uniformly sprayed with phospholipid spray. As expected, phospholipids appeared blue on a white background. The plates were heated at 100°C for 15 min. The background of the plate became a dark blue color, and the lipid spots became a dark brown color. Upon cooling for 15–30 min the blue background became colorless. In the clam lipid sample, one of the phospholipid spots appeared a brilliant blue color, but all the others remained dark brown. The blue color remained stable and was still visible after several months, but the other spots gradually faded after several days.

The spot remaining blue after heating proved to be ceramide aminoethylphosphonate, a phosphonolipid known to occur in clams (4), oysters (5), and other marine organisms. According to the method of Aalbers and Bieber (6) it contained 94.6% phosphonate phosphorus. Its relative chromatographic position to other phospholipids was similar to that reported in the literature (5), and it was resistant to mild alkaline hydrolysis (7).

To confirm the specificity for phosphonolipid, other plates were spotted and developed in the same way, but were sprayed with 50% H_2SO_4 and charred at 120°C. These spots were scraped off, and phosphonate phosphorus was determined by the method of Aalbers and Bieber (6). To determine sensitivity, graded series of lipids were spotted on TLC plates and the total phosphorus was determined in the phosphonolipid spots. The lower limit of detection of phosphonolipids was 0.03 μmoles. None of the lipids from rat liver retained blue color after heating.

To test the specificity for non-lipid phosphonate, 1-AEP and 2-AEP were spotted on a TLC plate, sprayed with the reagent and heated. These compounds also yielded a blue color which was stable to heating, but the intensity of the color was much weaker than that displayed by phosphonolipid and the lower limit of sensitivity was 0.5 μmoles. Cerebrosides, sulfatides and cholesterol displayed the same characteristic color reactions as encountered by spraying with sulfuric acid and heating (7), but the color reactions were greatly diminished.

DISCUSSION

As shown, use of phospholipid spray as reported by Dittmer and Lester (1) can be extended to detect phosphonolipids specifically and to detect other lipids generally by a simple procedural modification. To date, a method has not been reported which will directly detect phosphonolipids, and it has been necessary to identify phosphonolipids by indirect methods such as the chemical determination of phosphonate phosphorus (6) or the release of 2-AEP (ciliatine) (5). If used according to the original procedure (1), the phosphonolipids react equally as well as other phospholipids, but our modified procedure for utilizing this spray reagent considerably simplifies the detection of phosphonolipids on thin-layer chromatograms and makes it possible to screen a large number of lipid samples for phosphonolipids in virtually one step.

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