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Note

Separation of [^{3}H]inositol monophosphates and [^{3}H]inositol on silica gel glass-fiber sheets

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Interest in inositol 1,2-cyclic phosphate and inositol 1-phosphate as metabolic products of phosphatidylinositol breakdown in animal tissues[1,2] has encouraged studies of the water-soluble products of phosphatidylinositol breakdown under various conditions. Previous studies have used [^{32}P]phosphatidylinositol as a radioactive substrate[1]. The use of [^{3}H]phosphatidylinositol, labeled with [2-^{3}H]inositol, is more versatile since the production of free [^{3}H]inositol can also be measured. The methods in general use for the chromatographic separation of inositol monophosphates from tissue extracts[1] are not suitable for such studies. The paper chromatographic systems of Pizer and Ballou[3] do not separate free inositol from inositol 1,2-cyclic phosphate; and in the paper electrophoresis method of Dawson *et al.*[1] inositol stays near the baseline. During acetylcholine-responsive breakdown of phosphatidylinositol labeled with [2-^{3}H]inositol[4,5], we found a liberation of much free [^{3}H]inositol[6]. To examine other possible metabolic products it was necessary to develop chromatographic systems which would give good resolution of [^{3}H]inositol 1,2-cyclic phosphate, [^{3}H]inositol 1-phosphate, [^{3}H]inositol 2-phosphate, glyceryl-phosphoryl-[^{3}H]inositol and [^{3}H]inositol, in the presence of high amounts of radioactivity in the latter compound. The present work describes the rapid separation of these compounds on silica gel glass-fiber sheets.

MATERIALS AND METHODS

Reagents

ITLC Type-SA and ITLC Type-SG glass-fiber sheets were obtained from Gelman, Ann Arbor, Mich., U.S.A. [2-^{3}H]Inositol was obtained from New England Nuclear, Boston, Mass., U.S.A.; it was recrystallized from ethanol with carrier myoinositol to a specific activity of 18.5 mCi/mmole. Inositol 1,2-cyclic phosphate, synthesized from inositol 2-phosphate by the method of Pizer and Ballou[3], was a kind gift from Dr. Frank Eisenberg, Jr., National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Md., U.S.A. Inositol 1-phosphate and inositol 2-phosphate were from the California Corporation for Biochemical Research, Los Angeles, Calif., U.S.A. Glyceryl-phosphoryl-inositol was from Supelco, Bellefonte, Pa., U.S.A. All solvents were analytical reagent grade.

Procedures

The separations were carried out on silica gel glass-fiber sheets. Two systems were used. System A uses ITLC Type-SA sheets with ethanol–13.5 *N* NH_4OH (3:2) as developing solvent. System B uses ITLC Type-SG sheets with propan-2-ol–6 *N* NH_4OH (14:5) as developing solvent. All sheets were 20 cm × 20 cm; they were activated by heating at 110° for 30 min immediately before use. The chromatograms were developed by ascending chromatography for 15 cm on racks in sealed glass tanks which were lined with solvent-soaked filter paper. System A took approximately 2 h to develop; System B took approximately 90 min to develop. For both systems, carrier inositol monophosphates and inositol were always present in the tissue extracts which were being assayed for ^3H radioactivity. The chromatograms were air-dried and the spots were located by spraying with freshly prepared 0.1% ferric chloride in ethanol, followed, after air-drying, with 1% sulfosalicilic acid in ethanol[7]. Phosphate-containing compounds show as white, inositol shows as purple, on a pink background. This visualization procedure preserves the ^3H of the compounds. To assay the radioactivity, the spots were cut up, placed in scintillation vials with 0.5 ml of water, and counted in 10 ml of Bray's fluid in a Packard scintillation photospectrometer.

RESULTS AND DISCUSSION

In the presence of very high ^3H radioactivity in [^3H]inositol, we were unable to obtain complete resolution of [^3H]inositol and the [^3H]inositol monophosphates in a single system, so that two systems were used. System A was used for the separation of [^3H]inositol 1,2-cyclic phosphate from [^3H]inositol, and System B was used for the separation of [^3H]inositol 1-phosphate from [^3H]inositol.

System A gives a good separation of inositol 1,2-cyclic phosphate from [^3H]inositol and from inositol 1-phosphate, inositol 2-phosphate, and glyceryl-phosphoryl-inositol (Fig. 1A). System B gives a good separation of [^3H]inositol from inositol 1-phosphate (Fig. 1B). Although there appears to be reasonable separation of all the compounds in System B, under conditions in which there was high radioactivity in [^3H]inositol and relatively low ^3H radioactivity in inositol 1,2-cyclic phosphate, this system was not suitable for the separation of inositol 1,2-cyclic phosphate, because the [^3H]inositol could not be relied on not to have some forward overlap into the inositol 1,2-cyclic phosphate area. This is illustrated by a comparison of the strip counted areas and the staining areas of [^3H]inositol in Fig. 1B.

We have found these systems useful for the rapid separation of possible water-soluble inositol-containing products of [^3H]phosphatidylinositol breakdown in tissues and tissue extracts, using carrier compounds for identification. For assay of ^3H-labeled compounds in tissues, the tissues were homogenized from the frozen state in an aqueous solution which contained 0.017% each of inositol, inositol 1,2-cyclic phosphate, inositol 1-phosphate, inositol 2-phosphate, and glyceryl-phosphoryl-inositol, added as carriers for the radioactivity. Two volumes of methanol followed by four volumes of chloroform were added, essentially as described by Dawson *et al.*[1]. Aliquots of the upper, aqueous methanol phase were chromatographed. The bulk of the material chromatographed is composed of the carrier compounds. The assay consists in measurement of the ^3H radioactivity associated with each compound.

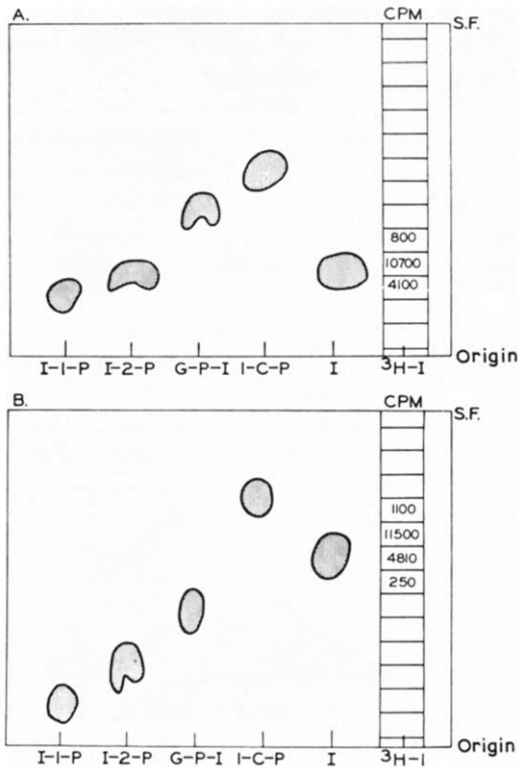

Fig. 1. Separation of inositol monophosphates and [^{3}H]inositol on silica gel glass-fiber sheets. A, ITLC Type-SA with ethanol–13.5 *N* NH_4OH (3:2), ascending. B, ITLC- Type-SG with propan-2-ol–6 *N* NH_4OH (14:5), ascending. All compounds were applied in 10-μg amounts. I-1-P = Inositol 1-phosphate; I-2-P = inositol 2-phosphate; G-P-I = glyceryl-phosphoryl-inositol; I-C-P = inositol 1,2-cyclic phosphate; I = inositol; ^{3}H-I = [2-^{3}H]inositol, recrystallized with carrier myoinositol from ethanol. The columns above ^{3}H-I show serial strip counting of the radioactivity; blank strips had radioactivity which counted at less than twice the background of 20 cpm. S.F. = Solvent front.

The separations are satisfactory with [^{3}H]inositol-labeled compounds because of the specificity of labeling with [^{3}H]inositol. It should be emphasized that the systems have not been used for separations of compounds derived from [^{32}P]phosphatidylinositol by enzyme activity in tissue extracts.

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