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Note

Paper ionophoresis of pancreatic islet phosphate esters in a magnesium-containing buffer

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Paper ionophoresis has been widely used for separating the phosphate esters liberated as partial degradation products of phospholipids[1] and attempts have also been made to examine the phosphate esters which occur as normal constituents of biological tissues[2–4]. Recently we have used a single-dimensional, high-voltage, paper-ionophoretic separation in volatile buffer at pH 4.8 to examine the ^{32}P-labeling of the β cells of pancreatic islets[5,6]. However in our hands and others, the value of the technique has been undermined by inconsistent running of components, in particular the nucleotides which change their position relative to other compounds leading to difficulties in identification and overlapping of spots. Prior acid washing of the paper used, although considered necessary for the removal of interfering substances[3], led to poorer resolutions than could be obtained by the better runs on untreated commercially available paper. We now attribute this inconsistency to variability in the divalent metal ion content of the paper. Completely reproducible separations can be obtained by using washed papers and incorporating $MgCl_2$ into the electrophoretic buffer. As developed, the method provides a useful rapid method for examining the ^{32}P-labeling of the main phosphate ester components of a tissue and considerably smaller samples need to be used than are necessary by techniques such as ion-exchange chromatography.

METHODS

The ^{32}P-labeled pancreatic islets, frozen in liquid nitrogen, were deproteinized and extracted with ice-cold 3% perchloric acid solution containing phosphate ester markers by a procedure already described[5]. The perchloric acid was then eliminated from the extract as insoluble $KClO_4$ by neutralization at 0° with 15% KOH using phenol red as internal indicator, followed by centrigufation.

A suitable aliquot of the supernatant was applied to Whatman No. 1 paper (46 × 57 in.), the narrow application strip being dried with a stream of air at room temperature. The paper was wetted by spraying, with buffer containing 5 ml pyridine,

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5 ml acetic acid and 90 ml glass distilled water containing 0 to 2 m*M* $MgCl_2$. The paper was subjected to a potential difference of 4000 V so that anodic mobility occurred along the full length of the paper. The paper was removed when the visible phenol red spot had run 11–12 cm (about 70 min). After drying at 50°, reference and carrier phosphate esters were located with acid molybdate spray[1] and ^{32}P-labeled components were located by radioautography.

RESULTS AND DISCUSSION

If Whatman No. 1 papers were used unwashed then the system suffered from slow mobilities, trailing of certain phosphate esters, and lower resolution by the phosphate detection spray. A thorough preliminary soaking and washing of the paper with 2 *M* acetic acid, followed by elution with distilled water to neutrality before drying undoubtedly eliminated some of these problems, with the changed texture of the paper causing more rapid mobility. Undoubtedly the washing also inhibits the slow hydrolysis of certain labile phosphate esters under the slightly acid conditions of the ionophoresis buffer possibly due to the catalytic action of certain contaminating heavy metal ions[7].

Fig. 1 shows the result of including graded quantities of $MgCl_2$ into the wetting buffer used for ionophoresis. Increasing $MgCl_2$ effected a significant retardation of

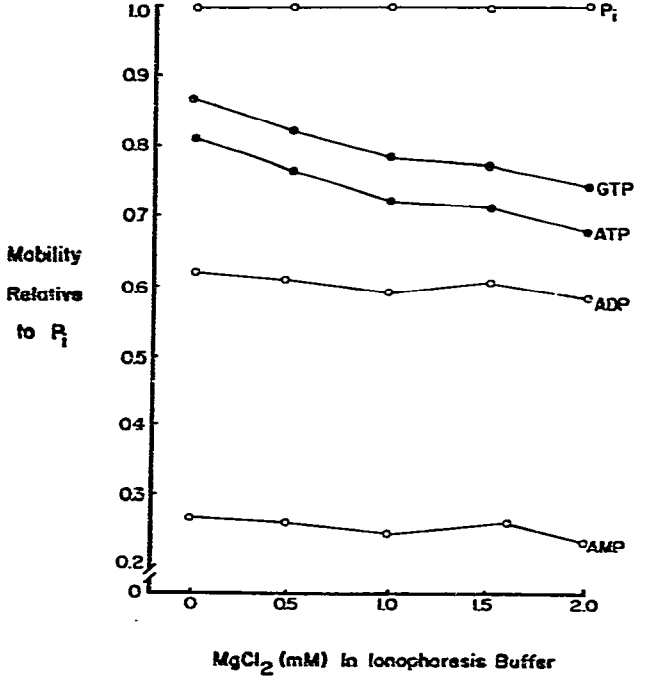

Fig. 1. The effect of increasing concentrations of $MgCl_2$ in the paper wetting buffer on the mobilities of organophosphates relative to inorganic orthophosphate (P_i) during high voltage paper ionophoresis.

the mobilities of the nucleotide triphosphates ATP and GTP relative to inorganic phosphate (P_i) while retarding ADP and AMP only minimally (Fig. 1). $MgCl_2$ added to the initial tissue extract before application to the paper was ineffective. Undoubtedly the retarding action is due to the Mg ions reversibly and continuously interacting with the nucleotide triphosphates during the ionophoresis and retarding the movement of the anions during any time when they exist as Mg complexes rather than the fully charged ATP anion. The complexing of Mg by ATP is a well documented phenomenon[8] while ADP and AMP interact far less readily with this divalent cation. Moreover, the potentiality for modifying the ionophoretic mobilities of nucleotides by the addition of metal ions has been recognized previously[9] although it has not been applied in a standardized fashion to offset the variable content of divalent cations in chromatography paper.

The present simple procedure produces an acceptable and consistent rapid method of separating the phosphate esters which occur in the highest quantities in

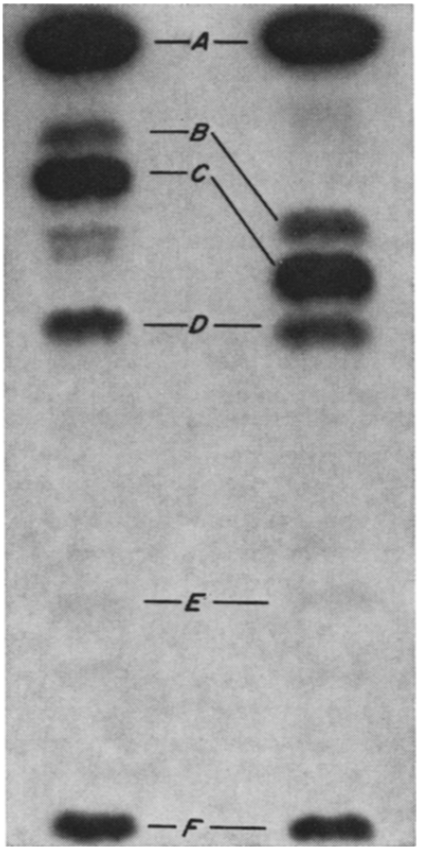

Fig. 2. Radioautographs of ionophoretograms of a perchloric acid extract of ^{32}P-labeled rat pancreatic islets: Paper ionophoresis shown on the left had been performed without the inclusion of $MgCl_2$ in the paper wetting buffer; 2 m*M* $MgCl_2$ had been added to the wetting buffer for the ionophoresis shown on the right. The letters denote the following labeled components in the extract: A, P_i; B, GTP; C, ATP; D, ADP; E, AMP; F, phosphorlycholine and phosphorylethanolamine (at origin).

mammalian tissues. We now routinely incorporate 2 m*M* $MgCl_2$ into the wetting buffer to achieve a predictable and highly reproducible shift of ATP and GTP relative to other organophosphates in extracts of ^{32}P-labeled pancreatic islets (Fig. 2). Of course, it is to be expected that normal tissues contain multiple very minor and trace water-soluble P components other than those identified in Fig. 2 (*e.g.*, hexose, phosphates); however we have documented that the components cited in Fig. 2 account for more than 80% of total water-soluble radioactivity in ^{32}P-labeled pancreatic islets[5]. In other tissues, it would be necessary to assess by paper chromatography or thin-layer chromatography[10] of the phosphorus containing spots separated by ionophoresis, that a similar conclusion was valid.

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