Paper chromatographic separation of 2-phosphoglyceric acid and 3-phosphoglyceric acid

Studies by this author of the purification and enzymic interconversion of 2-phosphoryl-D-glyceric acid (2-PGA) and 3-phosphoryl-D-glyceric acid (3-PGA) were facilitated by a paper chromatographic method for separation and identification of these two compounds. The general procedures described in the literature for separation of phosphate esters were found unsatisfactory for this purpose. The separation of 2-PGA and 3-PGA was accomplished by the use of paper impregnated with molybdate salts. Other techniques for the separation are those conventionally used for chromatography of sugar phosphates.

Procedure

1. Pretreatment of the paper.

Strips of Whatman #52 paper* of a convenient size were passed once through an aqueous solution of 0.5% (w/v) $Na_2MoO_4 \cdot 2H_2O$**. The wet sheets were hung in an air current to dry. One-half inch strips were trimmed from the top and bottom edges before the paper was used***.

2. Formation of the chromatogram.

Spots of 0.1–1.0 μM of the phosphate ester were applied. The rate of spot migration, at least for the phosphoglyceric acids, was not affected by the pH of the solution or by the presence of moderate amounts of salts. The chromatogram was developed with solvent flow in descent for a period of 20 hours at room temperature§. The solvent was 1 part 88–90% formic acid, 29 parts water and 70 parts 95% ethanol.

3. Development of spots.

The procedure of AXELROD AND BANDURSKI[1] was employed with slight modification. The dried chromatogram was sprayed with the acid molybdate spray[1] and heated in an oven at 100° C for 5 minutes§§. The paper was briefly steamed to restore flexibility and was then exposed to an ultraviolet lamp of the capacity described by AXELROD AND BANDURSKI[1]. If the paper was placed 10 cm from this light, all phosphate esters listed below gave blue spots within 10 minutes.

4. Location of spots.

The distance from the center of each spot to the origin is reported relative to the corresponding distance of the 2-PGA spot.

Compound	R_{2PGA}	*Compound*	R_{2PGA}
2-PGA	1.00	Fructose-1,6-PO_4	0.28
3-PGA	0.4	Glucose-1-PO_4	0.56
Inorganic PO_4	2.1	Glucose-6-PO_4	0.67
α- and β-glycerol-PO_4	0.95	Glyceraldehyde-3-PO_4	0.95

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[1] R. S. BANDURSKI AND B. AXELROD, *J. Biol. Chem.*, 193 (1951) 405.

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* Whatman #1 paper may be used although spots were not as well formed as with #52.

** A solution of 0.4% $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (w/v) may be substituted for 0.5% $Na_2MoO_4 \cdot 2H_2O$.

*** The direction of drainage of the paper must influence the ultimate degree of impregnation of the paper. However, the writer has found no difference in the developed chromatogram whether one end or the other of the paper was taken as the origin for the spot.

§ Solvent will flow off the bottom of the paper before the 20 hour period is over; therefore, the bottom edge should be serrated to maintain uniform solvent flow.

§§ A shorter time of heating than 5 minutes led to a heavy blue background when the chromatogram was exposed to the u.v. lamp. A period greatly in excess of 5 minutes led to extensive destruction of the paper.