

CHROM. 6109

Separation of carbamyl phosphate and phosphate by paper chromatography

In this report we present a simple method for the separation of carbamyl phosphate and phosphate by paper chromatography (PC). An effective solvent system developed in this laboratory is methanol-isopropyl alcohol-formic acid-water (9:6:3:2). The application of this method to the studies of the ornithine transcarbamylase catalyzed isotopic exchange between carbamyl phosphate and phosphate will be presented elsewhere.

A number of methods which have been used successfully for similar separations were tried and found unsatisfactory for the separation of carbamyl phosphate and phosphate. These included: methanol-isopropyl alcohol-25% ammonia-water (45:30:15:10) used for the separation of creatine phosphate and phosphate on paper¹; 0.6 M ammonium formate, pH 3.1, used for the separation of nucleotides on DEAE-cellulose thin-layer sheets²; 0.5 M lithium chloride, used for the separation of acetyl phosphate and phosphate on DEAE-cellulose thin-layer sheets³. An attempt to separate the compounds with 0.5 M, pH 5.5 acetate buffer on DEAE-cellulose thin-layer sheets was also unsuccessful.

Experimental procedure

Whatman 3MM paper in 20 × 25 cm sheets was used. 10 μ l of sample were spotted with a 10- μ l pipette, 1.5 cm from the edge of the paper. After drying, the

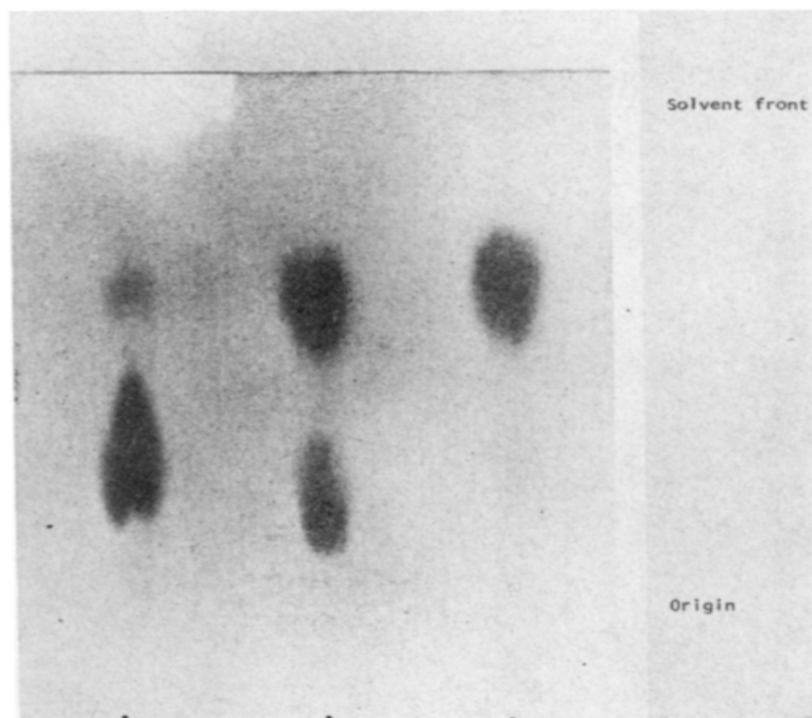


Fig. 1. PC separation of carbamyl phosphate and phosphate. 10 μ l of sample were spotted on Whatman 3MM paper and developed at 4° in methanol-isopropyl alcohol-formic acid-water (9:6:3:2). Left-hand spot, 10⁻³ M carbamyl phosphate; right-hand spot, 10⁻³ M phosphate; center spot, 10⁻³ M carbamyl phosphate and 10⁻³ M phosphate. Approximate R_F values in this system are: carbamyl phosphate, 0.41; phosphate, 0.67.

paper was developed by the ascending technique in a rectangular developing tank (30 × 10 × 30 cm, Brinkmann) at 4° in order to prevent the decomposition of carbamyl phosphate. The solvent system used was methanol-isopropyl alcohol-formic acid-water (9:6:3:2). The development required approximately 3 h. The paper was sprayed with the molybdate reagent of ROSENBERG⁴, allowed to dry and then sprayed with reducing reagent⁴. After drying, blue spots were produced on a white background which turned blue slowly in 24 h. An example of the separation of carbamyl phosphate and phosphate is shown in Fig. 1.

This work was supported by The Robert A. Welch Foundation, Grant Q-317. The authors are grateful to Dr. S. H. BISHOP for his suggestion of ROSENBERG's⁴ detection method for phosphates.

*Department of Biochemistry,
Baylor College of Medicine,
Houston, Texas 77025 (U.S.A.)*

YAN-FENG SHEN
ALBERT HIMOE

- 1 E. GERLACH AND J. JANKE, *Biochem. Z.*, 330 (1958) 565.
- 2 J. F. MORRISON AND W. W. CLELAND, *J. Biol. Chem.*, 241 (1966) 673.
- 3 R. F. ANTHONY AND L. B. SPECTOR, *J. Biol. Chem.*, 245 (1970) 6739.
- 4 H. ROSENBERG, *J. Chromatogr.*, 2 (1959) 487.

Received March 14th, 1972

J. Chromatogr., 70 (1972) 210-211