CHROM. 5987

A rapid chromatographic method for the separation of nucleotide sulphates

In the course of studies involving the nucleotide sulphates, 3′-phosphoadenylyl sulphate (PAPS) and adenylyl sulphate (APS), a number of methods have been reported for the separation of PAPS, APS and inorganic sulphate. These methods have involved chromatography on Whatman No. 1 paper[1] or electrophoresis on Whatman No. 1[2], Whatman No. 3[1] or Beckman S + S No. 2043A paper[3]. The paper chromatography method is time-consuming and, for the analysis of large numbers of samples, paper electrophoresis is not a convenient method. The present communication describes a rapid chromatographic method of separating PAPS, APS and inorganic sulphate that readily allows large numbers of samples to be assayed at the same time.

Experimental

Preparation of materials. Inorganic [^{35}S]sulphate (code SJS-1) was obtained from the Radiochemical Centre, Amersham, Great Britain. Authentic [^{35}S]PAPS was prepared by the method described by BAILEY-WOOD *et al.*[2]. Authentic [^{35}S]APS was prepared by the method of CHERNIAK AND DAVIDSON[4].

Paper chromatography. Samples (5 μl) of aqueous solutions (0.01–0.3 mM) of the materials were spotted onto Whatman paper Chromedia DE-81 and chromatograms were developed (descending) with 0.1 M phosphate buffer pH 6.0. The developed chromatograms were dried and cut into strips. Radioactive spots were located and quantitated by scanning with a Tracerlab 4π Radiochromatogram Scanner.

Results and discussion

Chromatographic separations carried out under the above conditions were highly reproducible. The hR_F values obtained were: [^{35}S]PAPS, 9; [^{35}S]APS, 27; inorganic [^{35}S]sulphate, 80. Identical hR_F values were obtained when the compounds were chromatographed singly or as a mixture. The separation method was subsequently applied to mixtures of [^{35}S]PAPS, [^{35}S]APS and inorganic [^{35}S]sulphate in solutions from enzyme assays. These solutions contained, in addition to protein, Tris–HCl or sodium acetate–acetic acid buffer in a final concentration of 0.1 M and at pH values throughout their respective buffering ranges. After the removal of protein by boiling and centrifuging, samples (5 μl) of supernatant were chromatographed. It was found that the presence of the buffer in the samples affected neither the migration nor the resolution of the radioactive components. However, when sodium chloride was also present in these samples in concentrations exceeding 0.2 M, the resolution of [^{35}S]PAPS and [^{35}S]APS was impaired and accurate quantitation of these components by scanning was difficult.

In addition, it is interesting to note that samples (5 μl) of aqueous solutions containing authentic inorganic [^{35}S]sulphate in the low concentrations tested ($<$ 0.3 mM) chromatographed as a single spot. But when these solutions also contained 5 mM $CoCl_2$, the inorganic [^{35}S]sulphate chromatographed as a double spot (hR_F, 80 and 90). Chromatography of mixtures of [^{35}S]PAPS, [^{35}S]APS and inorganic [^{35}S]-sulphate containing 5 mM $CoCl_2$ also resulted in the same double spot of inorganic

[^{35}S]sulphate without affecting the migration or resolution of the [^{35}S]PAPS and [^{35}S]APS.

In the interest of maximum economy of time and materials, it has been found that a distance of 20 cm from the point of application of the sample to the solvent front produces sufficient resolution of [^{35}S]PAPS and [^{35}S]APS for accurate quantitation by scanning. Using this method, a chromatographic separation of approximately 70 samples can be effected simultaneously on four sheets (46 × 28.5 cm) of DE-81 paper in a single Shandon Model 500 Chromatank within 90 min. This illustrates the excellent resolving power of ion-exchange paper and underlines the advantage of using this type of paper when the handling of large numbers of samples makes time an important factor.

The authors are grateful to the Science Research Council for financial support.

Department of Biochemistry,
University College,
P.O. Box 78, Cardiff CF1 1XL (Great Britain)

W. H. B. DENNER
A. M. STOKES

1 S. SUZUKI AND J. L. STROMINGER, *J. Biol. Chem.*, 235 (1960) 257.
2 R. BAILEY-WOOD, K. S. DODGSON AND F. A. ROSE, *Biochim. Biophys. Acta*, 220 (1970) 284.
3 J. AUSTIN, D. ARMSTRONG, D. STUMPF, T. LUTTENEGGER AND M. DRAGOO, *Biochim. Biophys. Acta*, 192 (1969) 106.
4 R. CHERNIAK AND E. A. DAVIDSON, *J. Biol. Chem.*, 239 (1964) 2986.

Received February 21st, 1972

J. Chromatogr., 69 (1972) 426–427