Journal of Chromatography, 176 (1979) 478–479

CHROM. 11,919

Note

Analysis of 3′-phosphoadenylylsulphate and related compounds by paired-ion high-performance liquid chromatography

E. J. M. PENNINGS and G. M. J. VAN KEMPEN
Department of Biochemistry, Psychiatric Institute Endegeest, 2342 AJ Oegstgeest (The Netherlands)
(Received April 12th, 1979)

3′-Phosphoadenylylsulphate (PAPS, active sulphate) is considered as the universal sulphate donor for enzymic sulphation processes in nature. For the *in vitro* studies of the rat-brain phenol sulphotransferase (EC 2.8.2.1) reaction we prepared PAPS enzymically from freeze-dried rat liver and purified it by the method of Tsang *et al.*[1], with some minor modifications as described in a recent paper[2].

Adenosine 3′,5′-bisphosphate (PAP) may be formed by hydrolysis of PAPS during storage. As PAP is a strong inhibitor of sulphotransferases, preparations of PAPS to be used must be free of any contaminating PAP. Therefore, a routine analysis of PAPS is needed, which is capable of monitoring the hydrolysis products of PAPS. The technique should also provide a method for monitoring ATP, as this compound is eluted close to PAPS during the purification of PAPS on DEAE-Sephadex A-25 (ref. 2). Thin-layer chromatography (TLC) and electrophoresis have been used to assay the amount of PAP in preparations of PAPS[1,2]. However, with the advent of high-performance liquid chromatography (HPLC) an excellent technique is at hand, which is rapid and reliable. As a routine analysis technique we have selected reversed-phase paired-ion HPLC on a LiChrosorb 5 RP-18 column, with tetrabutylammonium perchlorate as the pairing agent.

EXPERIMENTAL

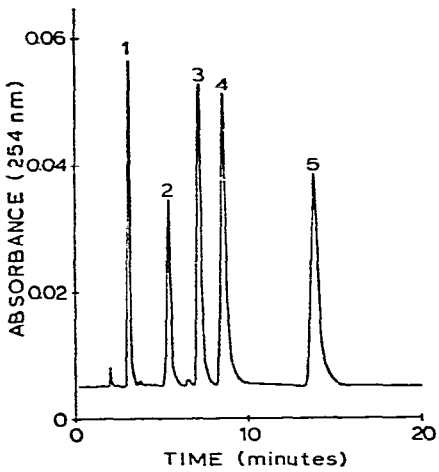
All analyses were carried out on a Waters Assoc. (Milford, Mass., U.S.A.) liquid chromatograph consisting of a M-6000A pump, a Model U6K injector and a Model 440 absorbance detector, capable of monitoring 254 nm. The solvent system consisted of 3 m*M* tetrabutylammonium perchlorate (Fluka, Buchs, Switzerland) and 30 m*M* KH_2PO_4 in methanol–water (1:3, v/v), pH 7.0. It was prepared by dissolving the pairing agent in methanol and KH_2PO_4 in water. These solutions were filtered through 0.45-μm Millipore (Molsheim, France) filters before mixing. The resulting solution was adjusted to pH 7.0 with 2 *M* KOH. The analyses were carried out at room temperature on a 15 cm × 4.6 mm I.D. stainless-steel column packed with LiChrosorb 5 RP-18 (Chrompack, Middelburg, The Netherlands). The flow-rate was 1.3 ml/min.

PAPS was prepared and purified as described earlier[2]. Adenosine 5′-monophosphate (AMP), adenosine 5′-sulphatophosphate (APS), PAP and ATP were ob-

tained from Boehringer (Mannheim, G.F.R.). All other chemicals were of analytical grade.

RESULTS AND DISCUSSION

Fig. 1 shows the optimal separation of AMP, APS, PAP, ATP and PAPS, respectively. The retention times for these compounds are 3.1, 5.4, 7.2, 8.5 and 13.5 min, respectively. AMP is co-chromatographed as it appeared to be present as a contaminant in the batch of APS. Assuming these adenosine nucleotides to have approximately equal molar absorption coefficients at 260 nm[3,4] and equal detector responses, the amounts of contaminating APS, PAP and ATP have been determined in the preparation of purified PAPS. The chromatogram is shown in Fig. 2. PAPS contains a maximum of 0.5% of APS, PAP and ATP, together. A purified preparation of PAPS is stable for at least 1 month when stored dry at −20°.



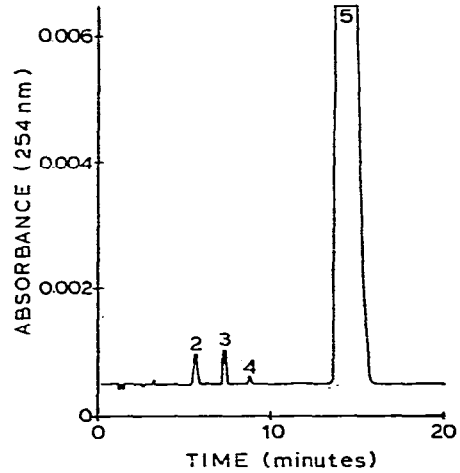


Fig. 1. Optimal separation of a test mixture of AMP (1), APS (2), PAP (3), ATP (4) and PAPS (5). Column: 150 × 4.6 mm I.D., LiChrosorb 5 RP-18. Eluent: 3 m*M* tetrabutylammonium perchlorate, 30 m*M* KH_2PO_4 in methanol-water (1:3, v/v), pH 7.0. Flow-rate: 1.3 ml/min. Detection: UV, 254 nm. Temperature: ambient.

Fig. 2. Analysis of a purified preparation of PAPS. Same conditions as in Fig. 1.

It should be mentioned that ADP and APS have identical retention times under the HPLC conditions used, but, if needed, they can be separated by increasing the concentration of tetrabutylammonium perchlorate in the solvent system. In fact, the amount of pairing agent and the amount of methanol in the solvent system are elegant tools for adapting separation factors and retention times of the compounds studied.

REFERENCES

1 M. L. Tsang, J. Lemieux, J. A. Schiff and T. B. Bojarski, *Anal. Biochem.*, 74 (1976) 623.
2 E. J. M. Pennings, R. Vrielink and G. M. J. van Kempen, *Biochem. J.*, 173 (1978) 299.
3 R. H. de Meio, in D. M. Greenberg (Editor), *Metabolic Pathways, Vol. VII, Metabolism of Sulfur Compounds*, Academic Press, New York, San Francisco, London, 3rd ed,. 1975, Ch. 8, p. 291.
4 In M. Windholz, S. Budavari, L. Y. Stroumtsos and M. N. Fertig (Editors), *The Merck Index*, Merck & Co., Rahway, N.J., 9th ed., 1976, p. 21.