A SIMPLE PREPARATION OF PHOSPHO-ADENYLYL SULPHATE LABELLED WITH ^{35}SULPHUR

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1. Introduction

It has been well established that PAPS[+] is the sulphate donor in the formation of sulphate esters of a wide range of biological compounds such as phenols, steroids, cerebrosides and acidic glycoaminoglycans.

Methods of synthesising PAPS labelled with ^{35}S have employed enzymic preparations of mammalian liver [1–3] or mouse mastocytoma [4] to synthesise PAPS from ATP and sulphate ions. These methods are convenient if only small quantities of PAPS are required but are impracticable for the synthesis of milligram quantities of [^{35}S]PAPS. Chemical syntheses of PAPS so far reported [5–7] have not been suitable for the preparation of [^{35}S]PAPS due to difficulty in obtaining suitably labelled starting materials. The method reported allows [^{35}S]PAPS to be easily prepared from readily available starting materials.

2. Materials and methods

Adenosine 3′, 5′-diphosphate dilithium salt (Boehringer Corporation, London), dicyclohexylcarbodiimide (B.D.H. Ltd.) and 98% [^{35}S]sulphuric acid (Radiochemical Centre, Amersham) were used without purification. Charcoal (Norit A, Sigma) was refined according to the method of Chemiak and Davidson [7]. Sartorius SF dense membrane was purchased from A.V. Howe Ltd., London. Paper chromatography was carried out on Whatman 3 MM paper (W. and R. Balston Ltd., Maidstone). Solvents were of B.D.H. Analar grade used without further purification.

Adenosine 3′, 5′-diphosphate dilithium salt (50 mg), dicyclohexylcarbodiimide (250 mg), [^{35}S]sulphuric acid (20 μl) and 60% aqueous pyridine (80 ul) were shaken for 5 hr at room temperature in a stoppered vessel. Dicyclohexylcarbodiimide (250 mg) in pyridine (150 μl) was then added and the mixture shaken for a further 5 hr at room temperature.

The reaction was terminated by the addition of ice-cold water (10 ml). The solution was made up to 100 ml and the pH adjusted to 1 by the addition of 1 N hydrochloric acid, and the aqueous phase was extracted several times with diethyl ether to remove pyridine, unreacted dicyclohexylcarbodiimide, and dicyclohexylurea. Purified charcoal (10 g) was added to the aqueous solution and the mixture was shaken gently for 30 min. The solution was filtered and [^{35}S]PAPS was eluted from the charcoal cake by washing with ethanol: 0.1% ammonium hydroxide (1:1), or 20% aqueous pyridine. Colloidal charcoal was removed from the filtrate by passage through Sartorius SF dense membrane [8]. The filtrate was concentrated under reduced pressure at room temperature to a volume of about 500 μl.

This solution was chromatographed on Whatman 3 MM paper by descending paper chromatography. The

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[+]*Abreviations:* PAPS, 3′-phosphoadenylyl sulphate; PAP, adenosine 3′, 5′-diphosphate.

solvent system was isobutyric acid: 0.3 N ammonium hydroxide, 5.3 [9]. The chromatograph was allowed to develop for 16 hr. [^{35}S]PAPS was located by scanning a strip of the chromatogram on a Panax Thin Layer Scanner RTLA-IA fitted with a paper chromatogram attachment. Inspection under ultraviolet Light (260 nm) showed [^{35}S]PAPS and PAP to be well separated (with R_f values of 0.16 and 0.33 respectively).

The paper chromatogram was eluted with water. The solution pH was adjusted to 7.5 with 1 N NaOH, and [^{35}S]PAPS was stored at −15°C in 1 ml fractions each containing approximately 1 μmole [^{35}S]PAPS.

The adenine content of the [^{35}S]PAPS solution was estimated by measuring the ultraviolet absorbance at 260 nm, using the extinction coefficient of 14 500 [7, 10]. [^{35}S]PAPS was determined enzymically by the method of Spencer [11] which is specific for PAPS.

3. Results and discussion

Yields of about 25% were normally obtained. The low yield could be attributed to poor elution from charcoal. Recovery experiments on this step indicated that only 35–40% of [^{35}S]PAPS was recovered. However, the omission of this step gave paper chromatograms with distorted bands and poor separation of [^{35}S]PAPS and PAP. The ultraviolet extinction quotients of [^{35}S]PAPS which had not been treated with charcoal indicated the presence of other ultraviolet absorbing materials (see table 1).

Total nucleotides were determined by measuring the ultraviolet absorbance at 260 nm. Absorbance at this wavelength is due to the adenine moiety. The adenine concentration was calculated from the absorbance and the extinction coefficient of 14 500 [7, 10].

Table 1
Extinction quotient [^{35}S]PAPS.

Quotient	[^{35}S]PAPS without charcoal treatment	[^{35}S]PAPS with charcoal treatment	ATP
E_{250nm}/E_{260nm}	0.86	0.79	0.80
E_{280nm}/E_{260nm}	0.79	0.19	0.15
E_{290nm}/E_{260nm}	0.62	0.02	0.01

The compound prepared was assayed by the method of Spencer [11] which measured the enzyme transfer of sulphate from PAPS to a receptor molecule, in this case *p*-nitrophenol, using rat liver as a source of phenol sulphotransferase. If less than 50% of the *p*-nitrophenol is converted to *p*-nitrophenyl sulphate, there is little interference from the reverse reaction and 98% of the sulphate group is transferred.

Comparison of results by the two assay methods of [^{35}S]PAPS, freshly prepared, and rechromatographed after several weeks storage showed that [^{35}S]-PAPS can be estimated accurately by either the ultraviolet- or sulphotransferase-assay.

[^{35}S]PAPS is normally used in the study of sulphotransferases. The activity of at least some such enzymes are strongly inhibited by PAP [12] and it is therefore important to remove all traces of this material from the final product. Under the conditions of storage, (1 ml samples, −15°C), [^{35}S]PAPS was stable for at least four weeks, but, when necessary, the compound could be purified by paper chromatography, as described, to separate the required labelled compound from the decomposition product which appeared to be PAP since it gave the same R_f value on paper chromatography as the authentic compound. Good agreement was obtained between [^{35}S]PAPS content and adenine content. [^{35}S]PAPS and PAP were well separated by paper chromatography.

This method would appear to be suitable for the preparation of PAPfree [^{35}S]PAPS in milligram quantities.

References

[1] Adams, J.B. (1960) Biochem. J. 76, 520–533.
[2] Robbins, P.W. (1963) in: Methods in Enzymology (Colowick, S.P. and Kaplan, N.O., eds), Vol. 6, p. 770–775, Academic Press, New York.
[3] Banerjee, R.K. and Roy, A.B. (1966) Mol. Pharmacol. 2, 56–66.
[4] Balasubramanian, A.S., Spolter, L., Rice, L.I., Sharon, J.B. and Marx, W. (1967) Anal. Biochem. 21, 22–33.
[5] Baddiley, J., Buchanan, J.G., Letters, K. and Sanderson, A.R. (1959) J. Chem. Soc. 1731–1734.
[6] Baddiley, J. and Sanderson, A.R. (1963) Biochem. Prep. 10, 3–5.
[7] Cherniak, R. and Davidson, E.A. (1964) J. Biol. Chem. 239, 2986–2990.
[8] Bailey-Wood, R., Dogson, K.S. and Rose, F.A. (1970) Biochim. Biophys. Acta 220, 284–299.

[9] Suzuki, S. and Strominger, J.L. (1960) J. Biol. Chem. 235, 257–266.
[10] Robbins, P.W. and Lipmann, F. (1957) J. Biol. Chem. 229, 837–851.
[11] Spencer, B. (1960) Biochem. J. 77, 294–304.
[12] Roy, A.B. and Trudinger, P.A. (1970) in: The Biochemistry of Inorganic Compounds of Sulphur, p. 107, Cambridge University Press, Cambridge.