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Note

Simple separation of adenosine-5′-phosphosulphate, adenosine-5′-phosphoramidate and cyclic adenosine-5′-monophosphate nucleotides involved in adenosine-5′-phosphosulphate degradation

FRIEDRICH KRAUSS and AHLERT SCHMIDT*
Botanisches Institut, Universität München, Menzinger Str. 67, D-8000 Munich 19 (F.R.G.)
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Adenosine-5′-phosphosulphate (APS), a very important intermediate of assimilatory sulphate reduction of higher plants, algae and a part of blue green algae[1], is degraded by several APS sulphohydrolases, as observed with *Anabaeba*[2] and *Chlamydomonas*[3], which forms sulphate and adenosine-5′-monophosphate (5′-AMP). In *Chlorella*, a specific sulphohydrolase, APS cyclase, was described by Tsang and Schiff[4], which should form adenosine-3′,5′-monophosphate (cyclic AMP; cAMP). Recent work has shown that the nucleotide formed is, in fact, adenosine-5′-phosphoramidate (APA) and not cAMP[5]. This confusion is caused by the similar behaviour of the compounds concerned in the methods used[5] in thin-layer chromatography (TLC), paper electrophoresis and degradation by phosphodiesterase (PDE)[6]. Further, in the presence of ammonia APA will be formed chemically by cleavage of APS at high temperature[6].

One of the several APS sulphohydrolases observed in *Chlamydomonas reinhardtii*[3] actually forms cAMP, which was demonstrated by the same behaviour, on labelling with [^{14}C]APS, in TLC, electrophoresis, beef heart phosphodiesterase treatment, Bio-Rad column chromatography and protein binding assay[3]. The TLC method used in that work[3] was modified by us to achieve a better separation of APS, APA and cAMP, necessary for product analysis of APS cyclase and the synthesis of APS.

EXPERIMENTAL

Thin-layer chromatography

For four glass plates (20 × 20 cm), 40 g of silica gel GF_{254} (Merck, Darmstadt, F.R.G.), and either 1 g of barium acetate or 0.5 g calcium chloride were mixed with 80 ml of water in a Braun homogenizer. After drying and activation for 30 min at 110°C, the nucleotides, listed in Table I, were separated with the solvent system 2-propanol–25% ammonia solution–ethanol (6:3:1) for 4 h and detected by UV quenching.

Chemicals

APS was prepared according to Cooper and Trüper[7]. APA was purchased

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TABLE I

THIN-LAYER CHROMATOGRAPHY OF THE NUCLEOTIDES

Separation of the nucleotides on silica gel mixtures with salts as described under Experimental.

Nucleotide	R_F *value*	
	Silica gel–barium acetate (40:1)	*Silica gel–calcium chloride (40:0.5)*
Adenine	0.77	0.95
Adenosine	0.68	0.94
5′-AMP	0.02	0.02
5′-ATP	0.02	0.02
APS	0.03	0.03
APA	0.25	0.26
cAMP	0.44	0.45
5′-GMP	0.02	0.03
cGMP*	0.20	0.17

* Guanosine-3′,5′-monophosphate.

from Sigma (St. Louis, MO, U.S.A.). All other nucleotides were obtained from Boehringer (Mannheim, F.R.G.) and other chemicals from Merck.

RESULTS AND DISCUSSION

The TLC behaviour of nucleotides on the silica gel mixture described under Experimental is demonstrated in Table I.

Nucleotides with two negative charges, such as APS, 5′-AMP, adenosine-5′-triphosphate (5′-ATP) or guanosine-5′-monophosphate (5′-GMP), cannot migrate, because they are precipitated by the divalent cationic salts of barium and calcium. Less concentrated salt–silica gel mixtures (*e.g.*, 0.1:40, w/w) or other divalent cations such as magnesium, manganese or zinc(II) are not recommended because of their poorer separation properties (data not shown). Because the R_F values of APA and cAMP are very different, we are able to analyse the reaction products of APS sulphohydrolases, which form 5′-AMP, APA or cAMP and sulphate. This is very important in the purification of APS cyclase, because APA, formed by APS adenyltransferase, masks cAMP in the methods used previously[4,5].

The nature of APA was confirmed by IR and NMR spectroscopy[5]. Using our procedure, it is possible to examine the synthesis of APS by APS reductase of *Thiobacillus*[7] for contamination with cAMP, formed by an unknown side-reaction of the enzyme[3], and APA, produced by heating APS in the presence of ammonium salts for a short time[6]. Both nucleotides will be separated quantitatively from APS, which can be desalted by Dowex or Trisacryl GF05 chromatography[8]. Hence we have overcome the difficulties in the analysis of APS cyclase.

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