紅藻スサビノリにおける PAPS の酵素的産生

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3′-Phosphoadenosine 5′-Phosphosulfate Synthesis by Cell-Free Enzyme System of Marine Alga *Porphyra yezoensis*

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3′-Phosphoadenosine 5′-phosphosulfate (PAPS) synthesis from sulfate and ATP by the cell-free enzyme system of marine alga *Porphyra yezoensis* was demonstrated. PAPS formed in the reaction mixture was isolated by DEAE-Toyo Pearl column chromatography, and identified by the behavior on TLC, HPLC and UV spectra.

PAPS synthesizing activity was inhibited by molybdate and selenate. PAPS was synthesized also from ATP and APS as substrates. These results suggest the presence of the PAPS synthesizing system involving ATP-sulfurylase and APS-kinase in the alga.

It is generally postulated that 3′-phosphoadenosine 5′-phosphosulfate (PAPS)*2 is the donor of the sulfate in the biosynthesis of marine algal sulfated polysaccharide as in that of biological sulfate esters in other organisms.[1,2] However, very little has been published on the PAPS synthesis in marine algae, apart from the works of Goldberg and Delbruck,[3] and Moller and Evans[4] which deal very briefly with [^{35}S]-PAPS synthesis from inorganic [^{35}S]-SO_4^{2-}.

The active sulfate, PAPS, is produced from sulfate and ATP *via* adenosine 5′-phosphosulfate (APS) by the successive sulfate activating reactions involving ATP-sulfurylase (EC 2.7.7.4), APS-kinase (EC 2.7.1.25) and inorganic pyrophosphatase (EC 3.6.1.1) which catalyze reactions (1), (2) and (3), respectively.

$$SO_4^{2-} + ATP \rightleftarrows APS + PPi \quad (1)$$

$$APS + ATP \rightleftarrows PAPS + ADP \quad (2)$$

$$PPi \rightleftarrows 2Pi \quad (3)$$

The occurrence of ATP-sulfurylase and the APS synthesizing activity was already investigated in the marine red alga *Porphyra yezoensis*,[5,6] which contains considerable quantities of sulfated galactan "porphyran".[7] The purpose of this paper is to demonstrate the PAPS synthesis by the *Porphyra* enzyme system, and to provide evidence of the presence of APS-kinase.

Materials and Methods

Chemicals

APS, PAPS, 3′-phosphoadenosine 5′-phosphate (PAP) and 2′-phosphoadenosine 5′-phosphate (2′,5′-ADP) were purchased from Sigma Chemicals. APS was also synthesized by the method of Cherniak and Davidson,[8] and separated using DEAE-Sephadex A25 column. The other nucleotides were from Boehringer Mannheim, G.m.b.H. All other reagents were of the highest purity available.

Preparation of Cell-Free Enzyme System

Porphyra yezoensis, which was cultivated in the Ofunato Bay, Iwate, Japan, and harvested during the winter in 1986–1987, was used in the study. The laver fronds were stored at −20°C until use.

The fronds were ground in a chilled mortar with acid-washed sea sand and ice-cold 0.1 M Tris-HCl buffer (pH 8.0) containing 2 mM EDTA and 2 mM 2-mercaptoethanol. The homogenate was centrifuged for 30 min at 10000 rpm and 4°C. The superantant was fractionated with solid ammonium sulfate. The precipitate formed at 40–75% saturated ammonium sulfate was recovered by centrifugation, dissolved in a minimal volume of 30 mM Tris-HCl buffer (pH 8.0), and dialyzed satisfactorily against the same buffer at 4°C. The dialysate was then centrifuged, and the supernat-

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*2 Abbreviations used: APS, adenosine 5′-phosphosulfate; PAPS, 3′-phospho adenosine 5′-phosphosulfate; PAP, 3′-phospho adenosine 5′-phosphate; PPi, pyrophosphate; Pi, orthophosphate; HPLC, high performance liquid chromatography; AUFS, absorption unit, full scale; TLC, thin layer chromatography.

Table 1. Retention times of compounds

Compound	Retention Time (min)	
	System I	System II
Adenosine	2.60	—*
5′-AMP	8.50	2.53
3′-AMP	12.50	—*
5′-ADP	16.70	2.93
2′,5′-ADP	18.62	—*
3′,5′-ADP (PAP)	19.80	—*
5′-ATP	22.20	3.80
APS	32.00	15.10
PAPS	36.50	22.50

* -: not determined.

ant was used as the cell-free enzyme system.

Determination of PAPS Synthesizing Activity

All enzyme reactions were performed at 25°C for 1 h under N_2, and terminated by immersing the reaction mixture in boiling water for 2 min. Nucleotides were recovered from the heat-stopped mixture by passing through a collodion bag (SM 13200, Sartorius G.m.b.H.). The nucleotides thus obtained were identified and determined by separating on a HITACHI GEL 3013-N packed column (4×150 mm) with a linear ionic strength gradient elution as described in the previous paper,[6] except the gradient was generated from 20 m*l* of each buffer (System I), or with an isocratic elution by 0.05 M K_2HPO_4/0.05 M KH_2PO_4/0.3 M NH_4Cl in 6% CH_3CN (System II). The eluate was monitored at 260 nm, and peak areas and retention times were calculated with a Shimadzu C-R1B. Retention times of the adenosine nucleotides are presented in Table 1.

Results and Discussion

In the primary experiment, PAPS synthesis from inorganic sulfate and ATP was determined using 10 m*l* of the reaction mixture containing 0.1 M Tris-HCl buffer (pH 8.0), 10 mM ATP (neutralized), 5 mM $MgCl_2$, 10 mM Na_2SO_4 and 2.0 m*l* of the cell-free system (4.0 mg protein/m*l*,[9] prepared from the laver havested in January 1987). As shown in Fig. 1-A, accumulation of the compound that had the same retention time with authentic PAPS on HPLC chromatogram was observed. In order to confirm the identity, the PAPS like compound was isolated by DEAE-Toyo Pearl 650 M column (1×20 cm) chromatography.[6] Elution by a linear gradient of $(NH_4)HCO_3$ (0–0.4 M, 400 m*l*) gave three main peaks (Fig. 2). The PAPS like compound was

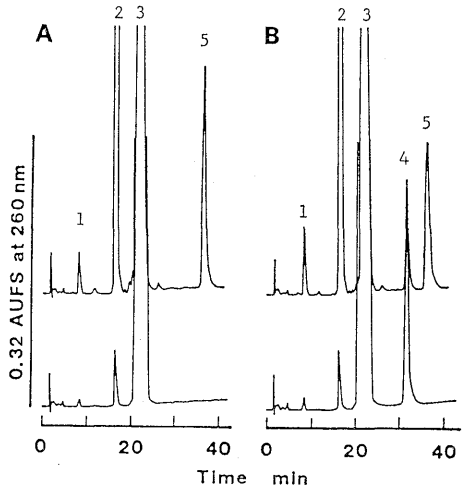

Fig. 1. Chromatographic examination of the products of the enzymatic sulfate activation. 25 μl of nucleotide solution prepared from the reaction mixture was chromatographed on a HITACHI 3013-N column (4×150 mm) with System I. A, nucleotides recovered from a reaction mixture using sulfate and ATP as substrates; B, from a reaction mixture using APS and ATP. The lower panels show the nucleotides from blank tubes using heat treated enzymes (2 min in boiling water). The details of the enzyme reactions are in the text and Table 2. Peak identification: 1, AMP; 2, ADP; 3, ATP; 4, APS; 5, PAPS-like compound.

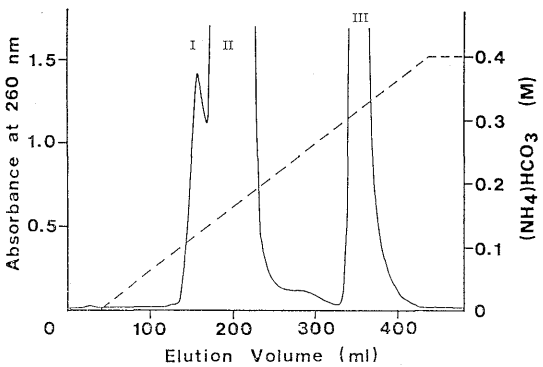

Fig. 2. DEAE-Toyo Pearl column (1×20 cm, formate form) chromatography of the nucleotides recovered from the reaction mixture in which the cell-free enzyme system was incubated with sulfate and ATP. The gradient profile of ammonium bicarbonate is shown by the broken line.

recovered from Peak III, and was almost a single component on HPLC (Fig. 3). The isolated PAPS like compound was characterized by the following criteria. Behavior on the TLC[6] was indistinguishable from authentic PAPS. The UV

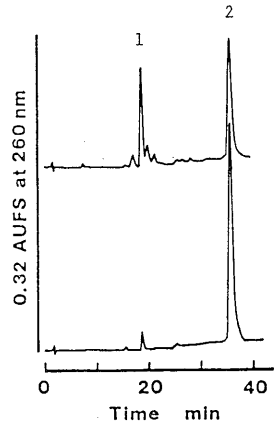

Fig. 3. Chromatographic comparison of the authentic PAPS (upper) and the compound recovered from Peak III of DEAE-Toyo Pearl column chromatography (lower). Each compound was chromatographed on HPLC with System I. 1, PAP (main degradative contaminant of PAPS); 2, PAPS.

Table 2. PAPS synthesizing activity in several reaction mixtures

Reaction mixture*1	PAPS formed (μmol/ml/h)*2
10 mM ATP+10 mM Na_2SO_4	0.52
10 mM ATP	0.08
10 mM ATP+10 mM Na_2SO_4 +2.5 mM Na_2MoO_4	not detected
10 mM ATP+10 mM Na_2SO_4 +2.5 mM Na_2SeO_4	0.22
10 mM ATP+10 mM Na_2SO_4 +5 mM DTT*3	0.34
10 mM ATP+10 mM Na_2SO_4 +5 mM GSH*4	0.31
10 mM ATP+0.5 mM APS	0.35
0.5 mM APS	not detected

*1 Reaction mixture contained in a final volume of 1.0 ml; 0.1 M Tris-HCl buffer (pH 8.0), 5.0 mM $MgCl_2$, 200 μl of the enzyme system (prepared from the laver harvested in January 1987), and the presented compound (final concentration).
*2 PAPS was determined using HPLC with System II.
*3 Dithiothreitol.
*4 Glutathione (reduced).

absorption spectrum at pH 7.0 was typical of the adenine compound (λ_{max}=258.0 nm, λ_{min}=228.0 nm).[10] The molar ratio of adenosine to phosphate[11] to sulfate[12] was 1:1.98:0.96. The compound was very acid-labile and easily hydrolyzed (0.1 N HCl at room temperature for 1 h) to PAP. Nuclease P1 (Seikagaku Kogyo Co.) which showed 3′-phosphohydrolase activity degraded the compound to APS, and 5′-nucleotidase (Sigma Co.) degraded it to 3′-AMP. Consequently, the isolated compound was identified as PAPS (Fig. 4).

It is known that all the group VI anions other

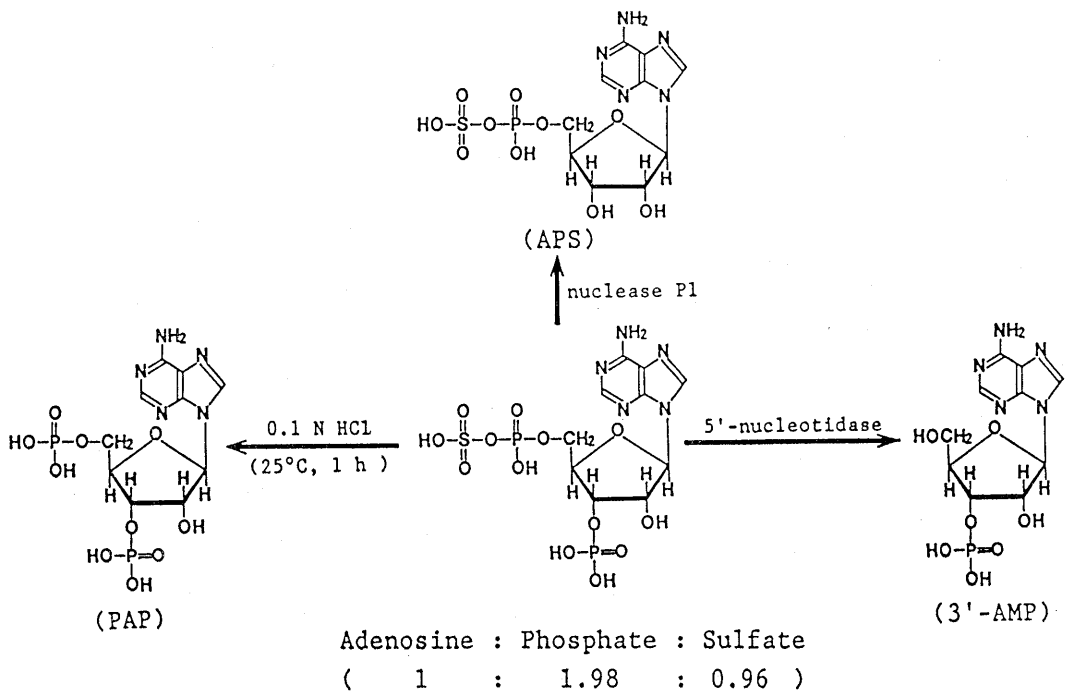

Fig. 4. Scheme for the identification of the product of the enzymatic sulfate activation.

than sulfate, such as molybdate and selenate, react as the substrate of ATP-sulfurylase without formation of stable adenyl anion like APS.[6,13] Thus these are competitive inhibitors of sulfate in the ATP-sulfurylase reaction. The addition of molybdate or selenate inhibited the PAPS synthesis (Table 2). This result gives evidence that PAPS is synthesized *via* APS.

PAPS synthesis was also observed when the *Porphyra* system was incubated with APS and ATP (Fig. 1-B, Table 2). These results suggest the presence of APS-kinase which catalyzes the conversion of APS into PAPS. However, *Porphyra* APS-kinase activity in the cell-free system could not be measured by the spectrophotometric method of Burnell and Whatley.[14] It appears that this method does not suited for the study using the crude enzyme, as pointed out by Renosto *et al.*[15]

Several attempts were performed to determine the optimal conditions for the PAPS synthesizing reaction. However, it was difficult to establish the conditions. For example, although PAPS synthesis was observed over a broad pH range (6.0–9.5), the optimal pH was varied with other factors, such as the concentration of sulfate, the concentration and species of divalent cations (Mn^{2+} and Co^{2+} were substitutive for Mg^{2+}), and the presence or absence of external inorganic PPase (from yeast, Boehringer Mannheim). Furthermore, the optimal pH differed between the enzyme systems prepared from the laver harvested in the early and the latter part of the season.

It is noteworthy that the addition of the thiol inhibited the PAPS synthesis (Table 2). It is expected that the inhibition is a result of the APS consumption by APS-sulfotransferase (APS: thiolsulfotransferase), since a significant increase of AMP was also observed. The presence of APS-sulfotransferase, which catalyzes the first reaction in the assimilatory sulfate reduction formed AMP in the presence of APS and thiol, had been demonstrated in several algae and plants by Schmidt.[16]

The presented result indicated the presence of the PAPS synthesizing system involving ATP-sulfurylase and APS-kinase. However, further information on the property of the PAPS synthesizing system could not be obtained. The uncertainty of the PAPS synthesizing system seems to result from the presence of interfering enzymes in the cell-free system, *e.g.*, ATP-ase by which the concentration of ATP is significantly fallen, and enzymes which catalyze the degradation of APS and PAPS as summarized by De Meio.[2] It is also expected as the cause of the uncertainty that the sulfate metabolism in algae seems to utilize not only PAPS but also APS as a sulfur donor, differing from the metabolism in animals and bacteria which requires PAPS.[17] Therefore, the isolation and characterization of APS-kinase is necessary for the evaluation of sulfate activation in the alga.

References

1) J. A. Schiff and R. C. Hodson: *Ann. Rev. Plant Physiol.*, **24**, 381–414 (1973).
2) R. H. De Meio: in "Metabolic Pathways" (ed. by D. M. Greenberg), Vol. 7, Academic Press, New York, 1975, pp. 287–358.
3) I. H. Goldberg and A. Delbruck: *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **18**, 235 (1959).
4) M. E. Moller and L. V. Evans: *Phytochemistry*, **15**, 1623–1626 (1976).
5) N. Kanno, M. Sato, and Y. Sato: *Nippon Suisan Gakkaishi*, **53**, 1047–1050 (1987).
6) N. Kanno, M. Sato, and Y. Sato: *Nippon Suisan Gakkaishi*, **53**, 1867–1870 (1987).
7) E. Percival and R. H. McDowell: Chemistry and Enzymology of Marine Algal Polysaccharides, Academic Press, London, 1967, pp. 127–175.
8) R. Cherniak and E. A. Davidson: *J. Biol. Chem.*, **239**, 2986–2990 (1964).
9) O. H. Lowly, N. J. Rosebrough, A. L. Farr, and J. Randall: *J. Biol. Chem.*, **193**, 265;275 (1951).
10) J. Baddiley, J. G. Buchanan, R. Letters, and A. R. Sanderson: *J. Chem. Soc.*, 1731–1734 ((1959).
11) B. L. Horecker, T. S. Ma, and E. Haas: *J. Biol. Chem.*, **136**, 775–776 (1940).
12) H. N. S. Schafter: *Anal. Chem.*, **39**, 1719–1726 (1967).
13) L. G. Wilson and R. S. Bundurski: *J. Biol. Chem.*, **233**, 975–981 (1958).
14) J.N. Burnell and F.R. Whatley: *Anal. Biochem.*, **68**, 281–288 (1975).
15) F. Renosto, P. A. Seubert, and I. H. Segel: *J. Biol. Chem.*, **259**, 2113–2123 (1984).
16) A. Schmidt: *Planta*, **124**, 267–275 (1975).
17) L. M. Siegel: in "Metabolic Pathways" (ed. by D. M. Greenberg), Vol. 7, Academic Press, New York, 1975, pp. 217–285.