2. Clark, A. A., Hodge, R. L., Molony, M. and Pilkington, T. R. E., *Lancet*, 1967, i, 1135.
3. Rizza, R., Haymond, M., Cryer, P. and Gerich, J., *Am. J. Physiol.*, 1979, **237**, E356.
4. Weinkove, C., in *Variey's Practical Clinical Biochemistry* (ed. Gowonlock, A., McMurray, J. R. and Mclaughlin, D. M.), Heinemann Medical Books London, 1987, pp. 877–893.
5. Ganong, W. F., in *Review of Medical Physiology*, Maruzen 12th Asian edn, Lang Medical Publications, California, 1985, pp. 293–317.
6. David, S. and Bernadette, D., *Annu. Rev. Physiol.*, 1986, **48**, 335.
7. Spicer, L. M., *Life Sci.*, 1986, **39**, 1701.
8. Moudgal, R. P. and Razdan, M. N., *Nature*, 1981, **293**, 738.
9. Moudgal, R. P. and Razdan, M. N., *Zbl. Vet. Med.*, 1985, **A32**, 573.
10. Moudgal, R. P., Razdan, M. N., Kajal, S. and Singal, S. P., *Indian J. Exp. Biol.*, 1985, **23**, 343.
11. Davis, P. B. and Silski, C., *Clin. Sci.*, 1987, **73**, 507.
12. Kolta, A., Diop, L. and Reader, T. A., *Life Sci.*, 1987, **41**, 281.
13. Mannelli, M., Pubilli, C., Fabbri, G., Masante, R., Defeo, M. L., Tranchi, F. and Giusti, G., *J. Clin. Endocrinol. Metabol.*, 1988, **66**, 626.
14. Hughes, J. M., Beck, T. R., Rose, C. E., Jr and Carey, R. M., *J. Clin. Endocrinol. Metabol.*, 1988, **66**, 518.
15. Lee, M. R., *Clin. Sci.*, 1982, **62**, 439.
16. Edens, F. W. and Siegel, H., *Poult. Sci.*, 1973, **52**, 2024.
17. Freeman, B. M., *World's Poult. Sci. J.*, 1976, **32**, 249.
18. Freeman, B. M., *World's Poult. Sci. J.*, 1985, **41**, 45.
19. McBride, G. and Foenander, F., *Nature*, 1962, **194**, 102.
20. Glatz, P. C. and Frensham, A. B., *Br. Poult. Sci.*, 1987, **28**, 119.
21. Moudgal, R. P. and Razdan, M. N., *Br. Poult. Sci.*, 1983, **24**, 173.

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Presence of phosphoribosylpyrophosphate synthetase in chloroplasts from fronds of *Bryopsis*

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The subcellular localization of phosphoribosylpyrophosphate (PRPP) synthetase activity in the fronds of a green alga, *Bryopsis* sp. was examined. The subcellular components of *Bryopsis* cells were squeezed out from the algar fronds without any requirement for homogenization, and intact organelles were easily obtained. Most of the activity of PRPP synthetase was distributed in the cytosol and chloroplasts.

5-PHOSPHORIBOSYL-1-PYROPHOSPHATE (PRPP) is a donor of phosphoribosyl groups for the synthesis of nucleotides. PRPP synthetase [ATP: D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1] from higher plants has been characterized[1,2]. Preliminary studies on the subcellular distribution of PRPP synthetase in spinach indicated that the enzyme is located predominantly in the cytosol[1]. However, our recent studies on purine metabolism in intact chloroplasts purified from spinach leaves suggest that a salvage pathway for adenine operates in the chloroplasts (H. Ashihara, unpublished observation). Salvage of adenine seems to be performed by adenine phosphoribosyltransferase which is present in the chloroplasts[3]. PRPP, required for the reaction catalysed by the enzyme, may be transported only with difficulty from the cytosol to the interior of the chloroplasts. It can, therefore, be presumed that machinery for the synthesis of PRPP is present in chloroplasts. In the present study, the localization of PRPP synthetase in cellular extracts of algal fronds of *Bryopsis* sp. was examined. This material was chosen because of the ease of preparation of intact organelles[4].

Bryopsis sp., collected at Futtu in Chiba Prefecture, Japan, was used as plant material. This green alga appears to be an intraspecific variant of *Bryopsis plumosa* (Hudson) C. Agardh from its morphological characteristics[4]. The cellular organelles and cytosol from fronds of *Bryopsis* sp. were extracted by the methods of Misonou *et al.*[4] with a slight modification. The algal fronds (ca. 40 g fresh wt) were cut with scissors and the components of cells were squeezed into 40 ml of ice-cold extraction medium (50 mM Tris-HCl buffer, pH 8.5, 0.5 M sorbitol, 10 mM sodium EDTA) through two layers of nylon cloth (pores, 100 μm in diameter). The suspension was filtered through a layer of Miracloth (Calbiochem, La Jolla, CA, USA), and the filtrate was centrifuged at 1500 g for 5 min. The supernatant fraction was further centrifuged at 10,000 g for 20 min. Chloroplasts were pelleted by the first centrifugation, and mitochondria and nuclei by the second one. The pellets were washed once with washing medium (25 mM Tris-HCl buffer, pH 7.6, 0.25 M sorbitol, 5 mM sodium EDTA). In order to check the intactness of the chloroplasts, the chloroplast fraction was examined by gradient centrifugation in different concentrations of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden)[3,5]. A single band of chloroplasts was always observed. Purity of chloroplasts was checked by light microscopy. The preparation was not contaminated by nuclei or mitochondria.

For the preparation of the enzyme, pellets of particulate fractions were dissolved in 50 mM HEPES–NaOH buffer (pH 7.6), and homogenized with a Polytron homogenizer, Type PT 10–35 (Kinematica, Littau, Switzerland), operated at maximum speed for 15 sec at 2°C. In the case of separation of stroma from chloroplast membranes, the homogenate was further centrifuged at 10,000 g for 20 min at 2°C.

The activity of PRPP synthetase was determined as described earlier[1]. The reaction mixture contained the following components in a final volume of 2.0 ml. Twenty-five mM HEPES–NaOH buffer (pH 7.6), 75 μM ribose 5-phosphate, 300 μM ATP, 7.5 mM $MgCl_2$, 50 μM [7–^{14}C]orotate (specific activity, 37 kBq

Table 1. Subcellular distribution of PRPP synthetase in fronds of *Bryopsis* sp.

Fraction	Activity (A) nmol/h/g fr wt (%)	Protein (B) (mg/g fr wt)	Specific activity (A/B)	Chlorophyll content (μg/g fr wt)
Chloroplasts	8.5 ± 1.1 (45.2)	0.61 ± 0.10	13.9	3.9 ± 0.6
Mitochondria	0.2 ± 0.0 (0.5)	0.03 ± 0.01	6.7	< 0.1
Cytosol	10.2 ± 2.7 (54.3)	0.50 ± 0.01	20.4	< 0.1

Activity (A) is expressed as nmol of PRPP synthesized per h per g fresh wt. Protein content (B) is expressed as mg per g fresh wt of samples. Specific activity (A/B) is expressed as nmol of PRPP synthesized per h per mg protein. The mitochondrial fraction contained nuclei. The distribution of the enzymes in the three fractions is expressed as percentages of the total activity (parentheses). The averaged values and s.ds were obtained from triplicate samples.

μmol^{-1}; Amersham International Ltd, Amersham, UK), an excess (16.7 mU) of orotate phosphoribosyltransferase and orotidine-5′-monophosphate decarboxylase (Sigma Chemical Company, St. Louis, USA) and the preparation of the enzyme. ATP was omitted from the reaction mixture for blank controls. The reaction was initiated by addition of the preparation of enzymes, and the mixture was incubated at 30°C for 60 min. The proportionality of activity to the amount of enzyme was checked for every assay by plotting the initial velocity against at least three different amounts of the preparation of enzyme. Levels of protein and chlorophyll were determined by the methods of Bradford[6] and Arnon[7], respectively.

The difficulty in subcellular fractionation of plants has been overcome by the use of a green alga, *Bryopsis*. The components of its cells are easily squeezed out from the algal fronds without any requirement for homogenization. Furthermore, a method for the fractionation of the subcellular components was recently established[4]. The chloroplasts of *Bryopsis* have large volumes ($10 \times 7\ \mu m^2$ in average cross-section), so that they precipitate more rapidly than nuclei.

Table 1 shows the subcellular distribution of PRPP synthetase in fronds of *Bryopsis*. The yield of recovery of chloroplasts based on the content of chlorophyll was greater than 95%. More than half of the total activity of PRPP synthetase was recovered in the cytosol, and most of the rest was found in the chloroplasts. Less than 1% of the total activity was found in the mitochondrial and nuclear fractions. The specific activity of the enzyme in the cytosol was 1.5 times higher than that in the chloroplasts. These data suggest that PRPP synthetase is located both in the cytosol and in the chloroplasts. A similar situation has been observed for the enzymes involved in glycolysis, such as phosphofructokinase[8], and those of the oxidative pentose phosphate pathway such as glucose-6-phosphate and 6-phosphogluconate dehydrogenases[9].

Table 2. Distribution of PRPP synthetase in chloroplasts from *Bryopsis* sp.

Fraction	Activity (nmol/h/mg)	Recovery (%)
Chloroplast homogenate	31.6 ± 4.5	100
Stroma (10,000 *g*, 15 min, sup.)	29.8 ± 6.0	94
Pellet (10,000 *g*, 15 min, ppt.)	< 0.1	0

Activity is expressed as nmol of PRPP synthesized per h per mg chlorophyll of the initial homogenate. Averaged values and s.ds were obtained from triplicate samples.

In order to examine the localization of PRPP synthetase within the chloroplasts, disrupted chloroplasts, were fractionated by centrifugation at 10,000 *g* for 20 min. The activity of PRPP synthetase was recovered exclusively in the supernatant fraction (Table 2). Thus, it seems probable that PRPP synthetase is a stromal enzyme.

PRPP synthetases from mammals have an absolute requirement for Pi for activity, and their activity is enhanced dramatically by addition of Pi[10]. However, PRPP synthetase from several plant materials does not require Pi for its activity and the activity may even be somewhat inhibited by Pi[1,2,11,12]. When HEPES–NaOH buffer in the reaction medium was replaced by 25 mM sodium phosphate buffer, 48% of the activity of PRPP synthetase from *Bryopsis* chloroplasts disappeared (data not shown). Thus, PRPP synthetases from plants seem to differ in terms of their requirements from those of mammals[10].

The present study clearly demonstrated that *Bryopsis* PRPP synthetase is present in the chloroplasts as well as in the cytosol. PRPP is an essential substrate for several biosynthetic pathways which include those involved in the synthesis of purine and pyrimidine nucleotides, nicotinamide nucleotides, histidine and tryptophan. Although studies on the intracellular localization of enzymes involved in nucleotide metabolism in photosynthetic plant cells are fragmentary, it has been reported that several enzymes involved in the biosynthesis of the pyrimidine nucleotides *de novo*[13,14] and in purine salvage pathways[3] are located in chloroplasts. Thus, it is quite plausible that at least some aspects of nucleotide metabolism in plant cells take place within chloroplasts. In chloroplasts, a substrate for PRPP synthetase, ribose 5-phosphate, may be supplied directly from the photosynthetic reductive pentose phosphate pathway.

1. Ashihara, H., *Z. Pflanzenphysiol.*, 1977, **83**, 379.
2. Ashihara, H., *Z. Pflanzenphysiol.*, 1977, **85**, 383.
3. Ashihara, H. and Ukaji, T., *Int. J. Biochem.*, 1985, **17**, 1275.
4. Misonou, T., Ishihara, J., Pak, J. Y. and Nitta, T., *Phycologia*, 1989, **28**, 40.
5. Walker, D. A., *The Use of the Oxygen Electrode and Fluorescent Probes in Simple Measurements of Photosynthesis*, Oxographics Ltd, Sheffield, 1988.
6. Bradford, M. M., *Anal. Biochem.*, 1976, **72**, 248.
7. Arnon, D. I., *Plant Physiol.*, 1949, **24**, 1.
8. Kelley, G. J. and Latzko, E., *Nature*, 1975, **256**, 429.
9. Schnarrenberger, C., Oeser, A. and Tolbert, N. E., *Arch. Biochem. Biophys.*, 1973, **154**, 438.
10. Becker, M. A., Raivio, K. O. and Seegmiller, J. E., *Adv. Enzymol.*, 1979, **49**, 281.
11. Ashihara, H. and Komamine, A., *Plant Sci. Lett.*, 1974, **2**, 119.
12. Ukaji, T. and Ashihara, H., *Int. J. Biochem.*, 1987, **19**, 1127.
13. Deremus, H. D. and Jagendorf, A. T., *Plant Physiol.*, 1985, **79**, 856.
14. Shibata, H., Ochiai, H., Sawa, Y. and Miyoshi, S., *Plant Physiol.*, 1986, **80**, 126.

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