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Phosphate levels and expression of phosphoribosylpyrophosphate synthetase isozymes in suspension-cultured *Arabidopsis thaliana* cells

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ABSTRACT

In plants, as well as the generally distributed phosphate-dependent phosphoribosylpyrophosphate (PRPP) synthetase (class I), phosphate-independent PRPP synthetase (class II) is also present. To investigate the effect of the inorganic phosphate (Pi) level on the two classes of PRPP synthetase, we first estimated the cellular phosphate level using ^{33}Pi in suspension-cultured *Arabidopsis thaliana* cells. ^{33}Pi in the culture medium was taken up by the cells, and the concentration of Pi in cells increased up to 5.5 μmol/g fresh weight within 24 h once the cells were transferred to the fresh medium; its concentration then fell because of the conversion of Pi to organic compounds. *In vitro* activity of PRPP synthetase increased after inoculation and maintained a high activity until the early exponential growth stage. The transcript levels of *PRS1* and *PRS2* encoding class I and *PRS3* encoding class II enzymes increased rapidly after the cells were transferred to the fresh medium, then remained almost constant during the early exponential growth phase. In contrast, constitutive expression of *PRS4* encoding cytosolic class II enzyme was observed during culture. During long-term Pi-starvation the transcript levels of *PRS1* and *PRS2* were reduced, but *PRS3* and *PRS4* were expressed continually during the Pi-starvation. Pi-dependent PRPP synthetase activity was simultaneously reduced, but Pi-independent activity did not change. Expression of *PRS1* and *PRS2* and the activity of Pi-dependent enzyme grew to normal rates by 24 h after supply of Pi.

1. Introduction

PRPP synthetase (EC 2.7.6.1, ATP:D-ribose-5-phosphate diphosphotransferase) catalyses the formation of PRPP from ribose-5-phosphate (Khorana et al., 1958). PRPP is utilised by several phosphoribosyltransferases that are involved in the *de novo* and salvage synthesis of the purine, pyrimidine and pyridine nucleotides, and synthesis of histidine and tryptophan. PRPP synthesis is therefore related to the synthesis of secondary metabolites derived from these compounds, such as caffeine and nicotine in plants (Ashihara and Crozier, 1999; Katoh and Hashimoto, 2004).

Distinct from bacterial and mammalian PRPP synthetases (Becker, 2001; Becker et al., 1979; Jensen, 1983) for which Pi is necessary for their activity, Pi-independent PRPP synthetase has been found in plants; its activity was somewhat inhibited by high concentrations of Pi (Ashihara and Komamine, 1974; Ashihara, 1977, 1990; Ukaji and Ashihara, 1987; Gallois et al., 1997).

Krath and Hove-Jensen (1999) reported four genes encoding spinach PRPP synthetase (*PRS*); *PRS1* and *PRS2* are the genes for Pi-dependent (class I) PRPP synthetases, and *PRS3* and *PRS4* are genes for Pi-independent (class II) enzymes. All four genes have since been found in *Arabidopsis thaliana* (Krath et al., 1999). Table 1 shows genes coding isoforms of PRPP synthetases and their subcellular localisation as suggested by Krath and Hove-Jensen (1999). Genes of a class II enzyme have also been isolated from sugarcane (Sculaccio et al., 2008).

We have previously studied fluctuations in PRPP content of cultured plant cells and the role of phosphate in PRPP synthesis (Hirose and Ashihara, 1983; Ukaji and Ashihara, 1987). The gene information regarding *PRS* led us to make a further investigation of the physiological role of PRPP synthetase in plant cells. To study the detailed relation between cellular Pi status and PRPP synthetase isoforms, we used suspension-cultured *A. thaliana* cells. The cellular level of Pi was estimated from the uptake and metabolism of [^{33}P]phosphate supplied to the culture medium. We determined changes in activity of PRPP synthetase and the expression of the four *PRS* genes during culture.

2. Results and discussion

2.1. Growth and chlorophyll content

Following inoculation of suspension-cultured starved cells into fresh medium, the cells begin to grow using constituents of the

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Table 1
Isoforms of PRPP synthetase in *Arabidopsis thaliana*.

Gene name	Locus	Class	Pi-requirement	Localization[a]
PRS1	At2g44530	I	Yes	Chloroplasts
PRS2	At1g32380	I	Yes	Chloroplasts
PRS3	At1g10700	II	No	Mitochondria
PRS4	At2g42910	II	No	Cytosol

[a] Possible localization suggested by Krath and Hove-Jensen (1999).

medium. Fig. 1A shows the growth of a suspension culture of *A. thaliana* together with the concentration of chlorophyll during culture. When 10-day-old cells were transferred into the fresh medium, their fresh weight began to increase after a 2-day lag. Exponential growth began at day 3 and continued to day 7. After day 7, growth reached the stationary phase. This changing pattern of growth is essentially the same as that observed in *Catharanthus roseus* cultures (Kanamori et al., 1979). The chlorophyll concentration remained almost constant during culture, although a slight decrease was observed in the early, lag phase of cell growth (Fig. 1A). To prepare long-term Pi-deficient cells, 10-day-old cells were transferred to Pi-free fresh medium and were cultured for 7 days. During Pi-starvation, growth of the cells was greatly retarded (the net increases in fresh weight during 7 days in the Pi-free and Pi-fed medium were 280 ± 30 mg/flask and $1460 \text{ mg} \pm 100$ mg/flask, respectively), and the chlorophyll concentration was reduced by 17%. Growth started immediately after addition of Pi, and the fresh weight increased by 150 ± 20 mg/flask in the first 24 h. Similar fluctuations were observed in *C. roseus* cultures (Yin et al., 2007).

2.2. Uptake and metabolism of [^{33}P]Pi

We estimated the uptake of Pi by *A. thaliana* cells and its conversion of organic phosphates using [^{33}P]Pi; the results are shown in Fig. 1B. The fresh culture medium contained 570 μM Pi (14.2 μmol/25 ml). Nearly 85% of Pi in the culture medium was taken up by the cells during the first 4 days, and most Pi was metabolised to organic phosphates including nucleotides, sugar phosphates, nucleic acids and phospholipids, as demonstrated by Ashihara and Tokoro (1985). The cellular Pi concentration increased during the first day (5.5 μmol/g fresh weight), then decreased gradually. At 10 days the values were less than 0.7 μmol/g fresh weight. When the 10-day-old cells were cultured in the Pi-deficient culture medium for 7 days, no ^{33}Pi was detected in the cells, although we found small amounts of radioactivity in organic compounds (data not shown).

Intracellular compartmentalization of Pi in *A. thaliana* has not been examined, but by using ^{31}P-NMR, Rebeille et al. (1983) found that the average Pi concentration in suspension-cultured *Acer psudoplatanus* cells was 2 mM, which consist of *ca.* 6 mM of cytoplasmic Pi and *ca.* 1.5 mM vacuolar Pi. During Pi-starvation, Pi in the cytoplasm was maintained at the expense of the vacuolar Pi. In our *A. thaliana* cells, if we assume that Pi is uniformly distributed in the cells, the highest cellular concentration was 5.5 mM (day 1), and the values decreased gradually during culture to 0.54 mM at day 10. If we suppose that subcellular distribution of Pi in *A. thaliana* cells is similar to *Acer psudoplatanus*, the Pi concentration in cytoplasm would be *ca.* 6 mM until day 5, and then decrease. In the long-term phosphate deficient cells, Pi appears to be absent from all compartments of the cells.

2.3. Changes in levels of PRPP synthetase activity and PRS transcripts during growth

The activities of PRPP synthetase were measured in the presence and absence of 20 mM Pi in the reaction medium.

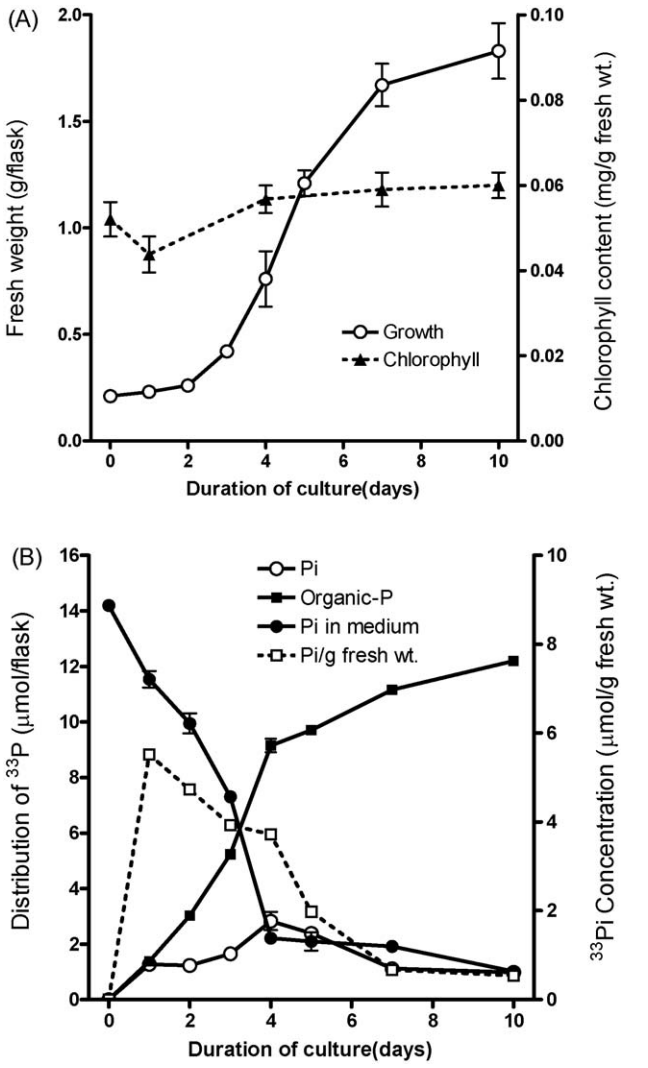

Fig. 1. Changes in fresh weight and chlorophyll concentration (A) and in distribution of ^{33}P-phosphate (B) during growth of suspension-cultured *A. thaliana* cells. Fresh weight (g) and distribution of ^{33}P in culture medium, in cellular Pi and in organic phosphates (μmol) are shown per culture (as solid lines); the concentration of chlorophyll (mg) and Pi (μmol) was expressed per g fresh weight (shown as dotted lines). Mean values and S.D. ($n = 3$) are shown.

Activity increased after transferring the cells to fresh medium, and decreased in the later stage of the culture (Fig. 2). Pi (20 mM) always stimulated PRPP synthetase activity. Consequently, Pi-dependent class I enzyme is present in *A. thaliana* cells. This differs from the results obtained from *C. roseus* cells, in which no Pi-dependent PRPP synthetase was detected (Ukaji and Ashihara, 1987). This discrepancy may be due to the difference in plant species studied, but also the culture conditions; *C. roseus* cells were cultured in the dark. Since the Pi-dependent enzyme appears to be located in chloroplasts (Krath and Hove-Jensen, 1999), its activity might be greatly reduced in *C. roseus* grown in the dark. Seven days after the 10-day-old cells were transferred to the Pi-free medium, *in vitro* PRPP synthetase activity measured with 20 mM Pi had fallen from 16.9 ± 0.8 to 15.0 ± 0.7, but Pi-independent activity had increased slightly, from 9.6 ± 0.8 to 12.0 ± 0.3. Since Pi in the cells fell greatly in the Pi-starved cells, Pi-dependent PRPP synthetase may be not fully functional in plants.

According to the summary in Table 1, *PRS1* and *PRS2* are genes for plastidic class I enzyme, whereas *PRS3* and *PRS4* code for mitochondrial and cytosolic class II enzymes, respectively (Krath