Inositol hexakisphosphate synthesis in plant suspension-cultured cells and measurement of inositol phosphate isomers with anion chromatography

Tetsuro Mimura$^{1,2}$, Naoto Mitsuhashi$^{1,2}$, Miwa Ohnishi$^{1,2}$, Sung-Kee Chung$^3$, Hitoshi Yagisawa$^4$

$^1$Department of Biology, Faculty of Science, Kobe University, Rokkodai 1-1, Nada-ku, Kobe, 657-8501, Japan
$^2$Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency (JST), Chuou-ku, Tokyo, 113-0027, Japan
$^3$Department of Chemistry, Pohang University of Science and Technology, San 31 Hyoja Dong, Pohang 790-784, Korea
$^4$Department of Life Science, Graduated School of Science, University of Hyogo, Harima Science Garden City, Hyogo 678-1297, Japan

Summary. We have established a new system for studying for phytic acid (InsP$_6$) synthesis in suspension-cultured cells of Catharanthus. InsP$_6$ and other intermediates of myo-inositol phosphate metabolism were measured using a newly developed ion chromatography method. The detection limit for InsP$_6$ was less than 50 nM, which was sufficient level to analyze Ins phosphates in living cells. Synthesis of myo-inositol phosphates was induced by incubation in high phosphate medium. InsP$_6$ was mainly accumulated in vacuoles and was enhanced when cells were grown in high concentration of phosphate with the cations of K$^+$, Ca$^{2+}$ or Zn$^{2+}$. However there was a strong tendency for InsP$_6$ to accumulate in the vacuole in the presence of Ca$^{2+}$ and in non-vacuolar compartments when supplied with Zn$^{2+}$, possibly due to precipitation of InsP$_6$ with Zn$^{2+}$ in the cytosol. Expression of some enzymes involving inositol phosphate metabolism are investigated.

Results and discussion. Myo-Inositol (Ins) phosphates play crucial roles in both animal and plant cells. In plants, a large amount of InsP$_6$ is synthesized and accumulated in seeds as a phosphorus reservoir instead of inorganic phosphates. InsP$_6$ also has various physiological roles other than storage of phosphorus, such as mRNA export and chromatin remodeling. Although there are many reports dealing with InsP6 synthesis in plants, we report the development of a more convenient experimental system for the in vivo investigation of the dynamics of synthesis and compartmentation of InsP$_6$ using suspension cultured cells of Catharanthus.

Induction InsP$_6$ of accumulation in suspension-cultured cells. When grown in Murashige-Skoog (MS) with 1.25 mM phosphate, the cells depleted phosphate in the medium and were effectively starved after 7 d (Low-phosphate cells) and contained negligible amounts of InsP$_6$ (Figure 1A). If the medium was supplemented with 7.5 mM phosphate at day 3 and day 5, cells then accumulated high concentrations of both phosphate and InsP$_6$ (High-phosphate cells). In addition to InsP$_6$, various other isomers of InsP$_4$ and InsP$_5$ were detected in High-phosphate cells (Fig. 1B), but InsP$_1$, InsP$_2$ and InsP$_3$ were either absent or below the detection limit. InsP$_6$ and other intermediates of Ins phosphate metabolism were measured using a newly developed ion chromatography method.

Cellular localization of Ins phosphates. The sub-cellular localization of Ins phosphates in the cultured cells was investigated by comparing the profiles of Ins phosphates in protoplasts and in vacuoles isolated from the protoplasts. High-phosphate cells were found to contain more than half of the InsP$_6$ in their vacuoles, while vacuoles from Low-phosphate
cells accumulated very low level of InsP₆. Low levels of InsP₄s and InsP₅s were found in protoplasts of High-phosphate cells, but were absent from the vacuole.

**Effect of cations on accumulation of InsP₆ in vacuoles.** In mature dry seeds, InsP₆ is usually bound to Ca²⁺ and Mg²⁺, forming phytin globoids. Thus, accumulation of InsP₆ might be closely related to storage of cations. The effects of cations on accumulation of InsP₆ were investigated by growing Catharanthus cells in High-Pi medium with K⁺, Ca²⁺, Mg²⁺, Zn²⁺ or Mn²⁺ for 7 days. The amount of InsP₆ in cells incubated with Pi plus K⁺, Ca²⁺ or Zn²⁺ increased markedly compared to that in cells supplied only with phosphate, but decreased slightly in cells incubated with Mg²⁺ or Mn²⁺.

The location of InsP₆ in *Catharanthus* cells was investigated by isolating protoplasts and vacuoles. In High-phosphate + Ca²⁺, InsP₆ was mainly accumulated in vacuoles, in contrast, in High-phosphate + Zn²⁺ cells, InsP₆ was predominantly accumulated in non-vacuolar compartments. Furthermore, in some inhibitor experiments, vesicle transport system from ER to vacuole may be concerned with InsP₆ synthesis. These results suggest that InsP₆ is synthesized in cytosol and then incorporated into vacuoles directly or indirectly.

**Analysis of enzymy expression in InsP₆ synthesis.** The level of MIPS, Ins(1,4,5)P₃ 6-/3-kinase (Ipk2) and Ins(1,3,4)P₃ 5-/6-kinase in Catharanthus was investigated with specific antibodies against recombinant Arabidopsis proteins. The level of InsP₆ does not appear to relate to the levels of any above enzymes.

**References**