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Note

Identification of *myo*-inositol monophosphates in mycelia of *Pholiota nameko*

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Inositol monophosphates from the mycelia of *Pholiota nameko* were analyzed by gas chromatography. Mycelia were found to contain inositol-1-phosphate and inositol-2-phosphate isomers in a proportion of 7 : 1, the amounts being 50 and 7.2 pmol/mg dry weight, respectively. We assume that inositol-2-phosphate is a hydrolysis product of the phytase reaction with phosphatase. It was also found that the amount of inositol monophosphates in the mycelia was affected by the concentration of inorganic phosphate in the medium.

Key Words——acid phosphatase; gas chromatography; *myo*-inositol monophosphate; *Pholiota nameko*; phytase.

Inositol is regarded as an essential element in many organisms. In animal nutrition, inositol plays a vitamin-like function and its deficiency causes incomplete development, fatty liver, and alopecia. Inositol is contained abundantly in vegetables and fruits, and it is also present in mushrooms. Hayakawa et al. (1991) reported that inositol was the major free sugar alcohol in young pileus of the basidiomycete *Agrocybe cylindracea* (Fr.) Maire. Yoshida et al. (1982, 1984) detected inositol in 13 edible mushrooms. However, the mechanism of inositol metabolism in mushrooms is not known. In the absence of an extracellular supply of inositol, animal and plant cells acquire inositol by hydrolysis of both D and L enantiomers of *myo*-inositol-1-phosphate (Ins1P) with *myo*-inositol monophosphatase (EC 3.1.3.25). L-Ins1P is synthesized from glucose-6-phosphate, while D-Ins1P is derived from phosphatidylinositol (Karen et al., 1987; Loewus, 1990). *myo*-Inositol monophosphate (InsMP), the precursor of inositol, has been identified in rat brain (Hirvonen et al., 1988), lilly pollen (Manthey and Dickinson, 1978), and *Neurospora crassa* Shear et Dodge (Hanson, 1991). However, we know of no reported study of InsMP in mushrooms. In this study, we examined the presence of InsMP in the mycelia of *Pholiota nameko* (T. Ito) S. Ito et Imai in Imai, a widely consumed mushroom in Japan.

Strain N114 of *P. nameko* obtained from Tohoku Shiitake Ltd. (Sendai, Japan) was used in the present study. The mycelia were inoculated into 30 ml of liquid medium in a 200-ml Erlenmeyer flask. The composition of the medium was as follows: glucose, 20.0 g; vitamin assay casamino acids (Difco), 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KH_2PO_4, 0.5 g; KCl, 0.27 g; $CaCl_2$, 0.1 g; $FeSO_4 \cdot 7H_2O$, 10 mg; thiamin·HCl, 10 mg; $ZnSO_4 \cdot 7H_2O$, 3 mg; $MnSO_4 \cdot 5H_2O$, 3 mg; $CuSO_4 \cdot 5H_2O$, 1 mg; $(NH_4)_6Mo_7O_{24} \cdot 7H_2O$, 1 mg; in 1 L of deionized water. pH was adjusted to 6.5 before autoclaving. The culture was grown at 25°C in darkness and harvested after 20 d. The procedure for extraction of InsMP was based on that described by Hanson (1991). The mycelia were extracted twice with 20 ml of ethanol : H_2O: diethyl ether : pyridine (15 : 15 : 5 : 1, v/v) at 60°C for 15 min with occasional shaking. The extracts were combined and concentrated in a rotary evaporator. The concentrated solution was adjusted to pH 7 with conc. NH_4OH, then loaded onto an AG 1-X8 column ($HCOO^-$ type; 1 × 13 cm, Bio-Rad). The column was first washed with deionized water to remove non-adsorbed materials. The adsorbed compounds were eluted in a stepwise manner with 50 ml of 0.1, 0.2, and 1.0 M ammonium formate. The fractions were lyophilized separately and an aliquot of each dried material was allowed to react with 100 μl of a trimethylsilylating reagent, TMS-PZ (Tokyo Kasei Kogyo). The derivatized materials were analyzed on a gas chromatograph (Hitachi, model 163) equipped with a flame-ionization detector and a 1% silicone OV-17 packed column (2 m × 3 mm internal diam). Injector and column temperatures were maintained at 220 and 180°C, respectively. The flow rate of the nitrogen carrier gas was 40 ml/min.

Figure 1 shows gas chromatograms of the TMS derivatives of fractions eluted with 0.1 and 0.2 M ammonium formate. Both fractions showed two peaks with retention times of 6.17 and 8.23 min. Standards of D,L-Ins1P and *myo*-inositol-2-phosphate (Ins2P) were detected at retention times of 6.17 and 8.21 min, respectively, under the same conditions, and the two peaks from the fractions were co-eluted with standards when the fractions and standards were cochromatographed (data not

shown). These results suggest that the mycelia of *P. nameko* contain InsMP in a mixture of two isomers, Ins1P (the major isomer) and Ins2P (the minor one). The Ins1P/Ins2P ratio was calculated from the peak areas to be approximately 7/1. In the fractions eluted with water and 1.0 M ammonium formate, no peak corresponding to InsMP was observed on the chromatograms. The finding that InsMP was eluted from anion exchange resin with ammonium formate ranging in concentration from 0.1 to 0.2 M agreed with the results of other workers (Christensen, 1990; Downes and Michell, 1981; Hanson, 1991).

Ins1P has been shown in many organisms to be synthesized from glucose-6-phosphate or phosphoinositides (Allison et al., 1976; Cosgrove, 1980; Hanson, 1991; Karen et al., 1987; Loewus, 1990; Pina et al., 1972). However, Ins2P has been detected only in plants and microorganisms as the final product of inositol hexaphosphate hydrolysis by phytase (Cosgrove, 1970, 1980; Hayakawa et al., 1990; Maiti et al., 1974). Therefore, phytase activity was examined in the mycelia of *P. nameko*. After homogenizing the mycelia in 10 mM acetate buffer (pH 5.5) by Polytron PT10-35 (Kinematica), the homogenate was centrifuged at 10,000 × *g* for 30 min at 4°C and phytase activity in the supernatant was measured as described by Hayakawa et al. (1989). As shown in Table 1, phytase activity was detected at the same level as acid phosphatase activity in the mycelia. Although the presence of inositol polyphosphates, the substrates of phytase reaction, has not been revealed in the mycelia of *P. nameko*, the Ins2P detected might be a hydrolysis product of inositol polyphosphate by the enzyme with phytase activity.

The amount of Ins1P in the mycelia of *P. nameko* was 50 pmol/mg dry weight of mycelia (Table 1), being one-third to one-seventh of the levels reported in rat brain (0.170 to 0.339 nmol/mg dry weight; Allison et al., 1976; Hirvonen, 1991) and *N. crassa* (0.216 nmol/mg dry weight; Pina et al., 1972). It has been reported that Ins1P level is affected by concentrations of lithium (Allison et al., 1976; Hanson, 1991; Hirvonen, 1991) and inorganic phosphate (Pi) (Pina et al., 1972) in the medium. Therefore, the effect of concentration of Pi in the medium on the amount of InsMP produced in the mycelia was examined. The mycelia of *P. nameko* were grown in a Pi-depleted medium omitting KH_2PO_4 from the above Pi-supplied liquid medium and the fraction containing InsMP was prepared by the procedure described above. In the Pi-depleted culture, the amounts of phosphorus from vitamin assay casamino acids and from inoculum were determined to be 55 and 1 mg/L medium as KH_2PO_4, respectively. In this case, neither Ins1P nor Ins2P were detected in the above fraction by gas chromatography (Table 1). When acid phosphatase activity was examined in the crude enzymes from the mycelia grown in Pi-supplied and -depleted media, the mycelia in Pi-depleted culture had 14-fold higher activity than those in Pi-supplied culture (Table 1). It has been reported that under Pi-depleted condition many plants and microorganisms increase phosphatase activity in the cells to achive a quick metabolic turnover of phosphate (Elliott et al., 1986; Lefebvre et al., 1990; Tadano and Sakai, 1991; Toh-e and Ishikawa, 1971). In animals and plants, Ins1P is hydrolyzed to *myo*-inositol and Pi by a specific enzyme, *myo*-inositol-1-phosphatase (EC 3.1.3.25) (Gee et al., 1988; Loewus and Loewus, 1982). However, no such specific enzyme has yet been found in fungi to our knowledge, and acid phosphatases induced by Pi-deficiency in *P. nameko* have a high *myo*-inositol monophosphatase activity (data not shown). This sug-

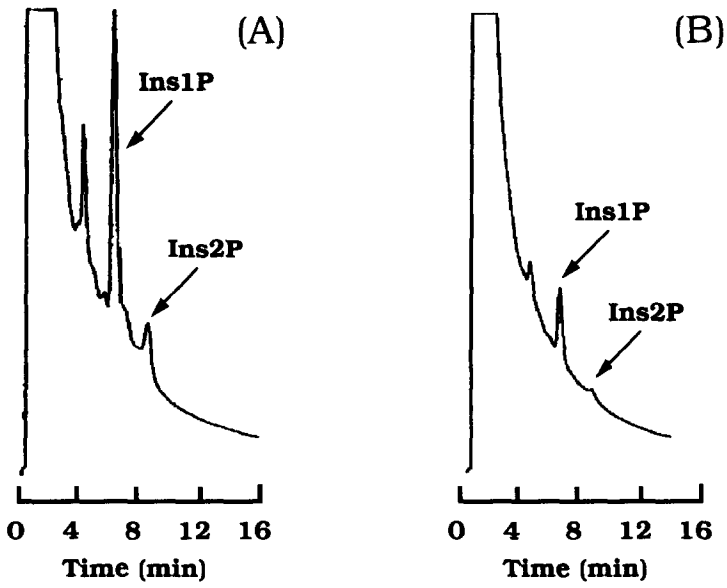

Fig. 1. Gas chromatogram of inositol monophosphate extracted from the mycelia of *Pholiota nameko*.
The effluents from an AG 1X8 column eluted with 0.1 M (A) and 0.2 M (B) ammonium formate were lyophilized, and each dry material was trimethylsilylated and analyzed by gas chromatography.

Table 1. Comparison of InsMPs and enzyme activities in *Pholiota nameko* grown in Pi-supplied and -depleted media.

	InsMP (pmol/mg dry weight)		Enzyme activity[a] (U/mg protein)	
	Ins1P	Ins2P	Phytase	Acid phosphatase
Pi-supplied culture	50	7.2	0.50	0.55
Pi-depleted culture	N.D.[b]	N.D.	0.51	7.93

a) Phytase and acid phosphatase activities were assayed as described by Hayakawa et al. (1989) using inositol hexaphosphate and *p*-nitrophenylphosphate, respectively, as substrates. One unit of the enzyme activity was defined as the amount of enzyme releasing 1 μmol of inorganic phosphate per min.
b) N.D.: not detected.

gests that the absence of InsMPs in the mycelia grown in Pi-depleted medium is due to degradation of InsMPs by the induced acid phosphatases.

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