Preliminary

An Enzymatic Study on Isocitrate Metabolism in the Ectomycorrhizal Fungus *Laccaria amethystea*[*1]

Masayuki ITAYA[*2], Takefumi HATTORI[*2],
Akira OHTA[*3] and Mikio SHIMADA[*2]

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Introduction

Ectomycorrhizal fungi are supplied with carbohydrates as a sole carbon source from their host plants[1]. The metabolism of carbohydrates in these fungi associated with woody plants has been of widespread interest, but their organic acid metabolism has not been fully investigated.

This study focussed on isocitrate metabolism in the ectomycorrhizal fungi because isocitrate is the key intermediate at a diverging point of the glyoxylate cycle and TCA cycle. Although the regulatory mechanisms for the isocitrate flux to the glyoxylate cycle by isocitrate lyase (ICL) and TCA cycle by isocitrate dehydrogenase (ICDH) have been studied for *Escherichia coli*[2], no enzymatic experiment has been reported on the comparison of the activity of ICDH with that of ICL in ectomycorrhizal fungi.

Here we report that ICDH activity was always greater than that of ICL in the mycelial extracts from the ectomycorrhizal fungus *Laccaria amethystea* grown in the pure culture and the symbiotic culture associated with *Pinus densiflora* seedlings. The results are discussed in relation to a possible isocitrate flux in the organic acid metabolism in the fungus.

Materials and Methods

Fungal culture

L. amethystea was grown stationary in the 100 ml of glucose (1%) and yeast extract (0.1%) medium at 21°C under the dark condition.

Symbiotic cultivation by the sandwich method

The symbiotic culture of *P. densiflora* seedlings and *L. amethystea* was conducted by the modified methods of Chilvers *et al.*[3].

Enzyme assay

The crude enzyme solution was prepared by extraction of the mycelia with 0.1 M potassium phosphate buffer (pH 7.0) which contains 0.5 mM phenylmethylsulfonyl fluoride. The activities of NAD-ICDH (EC1.1.1.41), NADP-ICDH (EC1.1.1.42) and ICL (EC4.1.3.1) were determined spectrophotometrically[4,5].

Results and discussion

Table 1 shows the growth of *L. amethystea* and the specific activities of NADP-ICDH and ICL in the cell-free extracts from the mycelia grown in the free-living culture. The fungus did not grow better in the sucrose media than in the glucose and the fructose media. However, the activities of NADP-ICDH were always greater than that of ICL in all cases. This finding is contrasted with the recent report on wood rotting fungi, in which the activity of ICL is greater than that of ICDH in vegetative mycelia of *Fomitopsis palustris*[6]. Thus, in relation to the isocitrate metabolism, the induction of ICL by ethanol was investigated for *L. amethystea*. Because it has been known that the glyoxylate cycle enzymes appear when microorganisms were grown on the non-sugar substrate such as ethanol and acetate[7]. However, the mycelia of *L. amethystea* could not sufficiently grow in the ethanol media and ICL activity was not induced by the addition of ethanol (data not shown). Thus, it has been found that *L. amethystea* can little utilize ethanol as a carbon source. Furthermore, the results suggest that the enzymic system for the isocitrate metabolism in this fungus is probably different from that of

Table 1. The growth and the activities of ICDH and ICL of *L. amethystea* in the free-living culture with different carbon source.

Culture days	Carbon source	Growth (mg D.W)	Specific activity (nkat/mg protein)	
			ICDH	ICL
21	Glucose	128.5	1.528	0.123
21	Fructose	153.8	1.548	0.218
21	Sucrose	53	0.754	0.047

The values presented are averages of two independent replicates.

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[*2] Laboratory of Biochemical Control, Wood Research Institute, Kyoto University, Uji, Kyoto 611-0011, Japan.
[*3] Shiga Forest Research Center, 978-95 Kitasakura, Yasu-cho, Yasu-gun, Shiga 520-2321, Japan.

other microorganisms[7], including the wood rotting fungus *F. palustris*[6].

During 5 weeks for symbiotic cultivation, ectomycorrhizal synthesis was microscopically observed on the roots of *P. densiflora* associated with *L. amethystea*. In contrast to the poor growth of fungus on the sucrose media in the free-living cultivation, the mycelia could grow well near the roots of *P. densiflora* when sucrose was used as a carbon source. The NADP-ICDH activity was detected but ICL was not in the extracts from mycelia grown near the roots. However, the details of the results will be reported elsewhere.

These results suggest that NADP-ICDH probably play a more important role than ICL in the isocitrate metabolism of *L. amethystea*. ICDH is one of the key enzymes in TCA cycle and regulates the flux of carbon to nitrogen metabolism through synthesis of 2-oxoglutarate, which is necessary for glutamate biosynthesis[1]. Therefore further research is needed to elucidate the metabolic pathway for 2-oxoglutarate whether it is metabolized in TCA cycle or through γ-aminobutyrate (GABA) shunt, which is reported for other basidiomycetes[8].

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