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A possible role of unique TCA cycles in wood-rotting basidiomycetes

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Abstract

We have reported that the copper tolerant brown-rot fungus, *Fomitopsis palustris*, acquires metabolic energy by use of the constitutively-occurring Kornberg's glyoxylate cycle coordinating with oxalate biosynthesis and glucose oxidation (Erman Munir et al. *Proc. Natl. Acad. Sci. USA*, (2001) 98, 11126-11130). Furthermore, this fungus does not have the normal TCA cycle, lacking 2-oxoglutarate dehydrogenase which is a key enzyme of the TCA cycle of most living things. This paper reports that most wood decay fungi tested lack 2-oxoglutarate dehydrogenase (ODH) and that much greater activities of glutamate dehydrogenase compensating the absence of ODH were detected from both white- and brown-rot fungi.

Keywords: Wood-rotting fungi, oxalic acid, TCA cycle, glyoxylate cycle, copper-tolerant brown-rot fungi, *Fomitopsis palustris*

Introduction

Metabolism of oxalic acid, which widely occurs in a wide variety of higher plants, cultures of molds, and even in human urine as an end product, has long been investigated from a medicinal viewpoint (1). Oxalic acid has three chemical natures such as proton and electron sources, and a strong metal chelater, despite its simple chemical formula of (COOH)₂. Due to its unique multiple chemical natures, it has been receiving much attention from various ecological aspects as follows: a) bioremediation of a wide variety of organic pollutants (2) with lignin biodegradation systems (3–7), b) inactivation of copper-containing wood preservatives by wood-rotting fungi (8, 9), c) detoxication of aluminum toxicity in Al-resistant buckwheat (10), d) crop damages caused by oxalic acid-producing phytopathogens (11, 12), e) a biofertilizer effect of ectomycorrhizal fungi (13, 14), and f) an electron source for nitrogen-fixation in symbiotic

rhizobia in a legume plant (15). In addition, it is known as a general physiological trait that most of brown-rot basidiomycetes, including *Fomitopsis palustris*, accumulate oxalic acid at greater concentrations in culture fluid, whereas white-rot ones do not because they metabolize and/or decompose it by various mechanisms (16–19). Nevertheless, the white-rots were observed to accumulate Ca-oxalate during wood decay processes (20).

Previously, we have reported the occurrence of the two oxalate-producing enzymes, glyoxylate (GLOX) oxidase and oxaloacetase (OXA), in wood-rotting fungus *F. palustris* (21, 22). We have purified and characterized a novel flavohemoprotein glyoxylate dehydrogenase (GLOXDH) which catalyzes dehydrogenation of glyoxylate to oxalate in the presence of cytochrome c (23). Quite recently, we have also found that the glyoxylate-producing enzyme, isocitrate lyase (ICL), and the glyoxylate-utilizing enzyme, malate synthase (MS), which are both the GLOX cycle key enzymes, commonly occur in white-rot and brown-rot basidiomycetes, although they were grown on the glucose medium (24). The oxalate biosynthesis in this fungus was characterized as the oxalate fermentation, through which biochemical energy is generated for growth of the fungus. Thus, it was found that the oxalate biosynthesis is linked with the TCA and GLOX cycles (24). We have reported the unprecedented metabolic system which explains an important physiological role of the oxalate biosynthesis in a wood-rotting basidiomycete *F. palustris* (25).

In this context, we were motivated to investigate the reason why GLOX cycle constitutively occurs in the wood-rotting fungi, although they are grown on the glucose medium. Here we report that most of the wood-rotting fungi tested lack 2-oxoglutarate dehydrogenase (ODH), a key enzyme of the TCA cycle, whose dysfunction is in turn compensated largely by glutamate pathways and the GLOX cycle linked with the oxalate biosynthesis.

Materials and Methods

Chemicals

All chemical and biochemical reagents were of reagent grades. NADP, DL-isocitric acid, and 2-oxoglutaric acid were obtained from Nacalai Tesque (Kyoto, Japan). Protein assay kit was from BIO-RAD Laboratories (Hercules, CA, USA).

Organism and Growth Conditions

Wood-rotting fungi listed in Table 1, including *Coriolus versicolor*, and *Fomitopsis palustris* (formerly called *Tyromyces palustris*) that are Japanese Industrial Test fungi for wood-preserved efficacy tests, were cultivated in the modified Kirk's medium (200 ml) containing 2% (w/v) glucose and 24 mM ammonium tartrate as the carbon and the nitrogen source, respectively (26).

Preparation of Cell-Free Extracts and Enzyme Assays

Cell-free extracts were prepared from the fungal mycelia in the same way as previously reported (24). All the enzyme activities in the fungal extracts were determined spectrophotometrically using a double beam spectrophotometer (Hitachi model U-3000) equipped with a temperature controller and external recorder. ICL activity was assayed by measurement of the increase in absorbance at 324 nm, due to the formation of phenylhydrazone derivative of glyoxylate produced from isocitrate (27). 2-Oxoglutarate dehydrogenase (ODH), and isocitrate dehydrogenase (IDH) activities were determined by measuring the increase in absorbance at 340 nm due to the reduction of NAD or NADP based on the reported methods (28). Glutamate dehydrogenase was assayed according to the method of Moore and Ewaze (29).

The enzyme activities were expressed as the specific activity (mmole min⁻¹ mg⁻¹ protein. One unit of enzyme activity is defined as the amount of enzyme which catalyzes the formation of 1 mmole product per minute or the consumption of 1 mmole substrate per minute under the conditions described.

Results and Discussion

Figure 1 indicates that ICL is a central and pivotal enzyme in the metabolic cycles (A, B, C, and D) producing oxalate from glucose in the copper-tolerant fungus *Fomitopsis palustris*. In fact, inhibition of the key enzyme by the ICL inhibitor of itaconate caused a decrease in oxalate biosynthesis and the fungal growth (24). Thus, ICL will be a target enzyme of most of the copper-tolerant brown-rot fungi. However, it has not yet been examined whether the ICL enzyme generally occurs as a major constitutive enzyme in preference to IDH in TCA cycle (A) among many white- and brown-rot fungi. The preference of either of the two key enzymes determines the route to the TCA or to GLOX cycle, because these two enzymes are located at the branch-point leading to either of the cycles.

The results (Table 1) shows that only 3 fungi (*Ganoderma applanatum*, *Lentinus edodes*, and *Pycnoporus cinnbarinus*) out of 14 white-rot fungi tested and 3 fungi (*F. palustris*, *L. sulphureus*, and *Poria cocos*) out of 7 brown-rot fungi exhibit greater ICL activities than IDH activities, which indicates that as a rule ICL occurs at higher ratio in a group of brown rotters, whereas IDH is major in a group of the white-rotters tested in this experiment.

Now, Fig. 1 shows that there is another interesting branch-point at 2-oxoglutarate which is produced from isocitrate by IDH; oxoglutarate is converted to glutamate (GLT) or to succinyl-CoA, catalyzed by glutamate dehydrogenase (GDH) or ODH, respectively. The results (Table 1) indicate that all the fungi tested except for *L. sulphureus* exhibit markedly greater activities of GDH, whereas no activity of ODH was found except for *Panus rudis*, *Phanerochaete chrysosporium* and *Lentinus lepideus* that are even negligible. Markedly lower activity of ODH but greater activities of GDH in both white- and brown-rot fungi clearly indicate that GLT metabolite plays an important intermediary role in bypassing the metabolite in the TCA cycle. "Absence" of ODH were also reported for *Coprinus cinereus* (29), *Agaricus bisporus* (30), and *Flammulina velutipes* (31) by other workers.

Thus, it is safely stated that neither GLOX cycle nor the normal TCA cycle is major but the dysfunctional or short-cut TCA cycle is commonly major in the wood-rotting fungi, because lacking or (repression of the enzyme _expression of the responsible DNA) is compensated with the greater activity of GDH, which may bypath GLT to succinate via the GABA route by decarboxylation of GLT to form gamma-aminobutyric acid (GABA) as Moore and Ewaze reported (29). Quite, recently we have found that the glutamate pathway plays an important metabolic role during the fruit body formation of the copper-tolerant fungus *F. palustris*, because the GLOX cycle and the oxalate biosynthesis disappear during this period (32).

In conclusion, it has been established that unique TCA cycle is operative with GLT pathway and the GLOX cycle in wood decay fungi, because ODH is commonly lacking in both white- and brown-rot fungi. It is likely that the oxalate producing brown rotters have the preference of the GLOX cycle to the TCA cycle during vegetative growth.

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Fig.1. Novel metabolic cycles linked with oxalic acid biosynthesis in the copper-tolerant fungus *F. palustris*.

Table 1. Activity of isocitrate utilizing enzymes and oxoglutarate utilizing enzymes detected in wood-rotting fungi grown on glucose rich-medium

Fungi	Isocitrate-utilizing		Oxoglutarate-utilizing	
	ICL ¹	IDH	ODH	GDH
White-rotters				
<i>Bjerkandera adusta</i> ²	0.018	0.238	nd ³	1.487
<i>Ceriporiopsis subvermispora</i>	0.009	0.067	nd	0.328
<i>Coriolus versicolor</i>	0.024	0.069	nd	0.642
<i>Flamullina velutipes</i>	0.029	0.131	nd	1.053
<i>Ganoderma applanatum</i>	0.390	0.220	nd	1.340
<i>Ganoderma lucidum</i>	0.025	0.159	nd	1.443
<i>Lentinus edodes</i>	0.138	0.084	nd	0.124
<i>Panus rudis</i>	0.022	0.104	0.015	0.834
<i>Phanerochaete chrysosporium</i>	0.093	0.134	0.021	0.459
<i>Pleurotus cystidiosus</i>	0.006	0.116	nd	0.510
<i>Pleurotus eringii</i>	0.004	0.179	nd	0.352
<i>Pleurotus ostreatus</i>	0.017	0.312	nd	0.885
<i>Pycnoporus cinnabarinus</i>	0.087	0.062	nd	0.609
<i>Schizophyllum commune</i>	0.075	0.110	nd	0.506
Brown-rotters				
<i>Coniophora puteana</i>	0.064	0.250	nd	0.023
<i>Daedalea dickinsi</i>	0.103	0.156	nd	0.830
<i>Fomitopsis palustris</i>	0.170	0.032	nd	0.586
<i>Gloeophyllum trabeum</i>	0.012	0.132	nd	0.247
<i>Laetiporus sulphureus</i>	0.608	0.052	nd	nd
<i>Lentinus lepideus</i>	0.004	0.034	0.016	0.364
<i>Poria cocos</i>	0.494	0.120	nd	0.286

¹Enzyme activity is expressed as the specific activity ($\mu\text{mol min}^{-1} \text{mg}^{-1}$ protein).

²Culture time: 1 to 2 weeks.

³nd, no activity was detected.