

日本農芸化学会関西支部
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Purification and Characterization of Malate Synthase from the Oxalic Acid-Producing Basidiomycete *Fomitopsis palustris*

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Extracellular production of oxalic acid by brown-rot basidiomycetes, including *Fomitopsis palustris*, has been recognized as their unique physiological trait. We have recently discovered that the oxalate biosynthesis is prerequisite for respiratory metabolism, which involves a novel metabolic coupling of the TCA and glyoxylate cycles when they are grown on glucose.¹⁾ Furthermore, we have demonstrated the coordination of malate synthase with malate dehydrogenase in generating NADH by oxidation of malate to oxaloacetate as an oxalate precursor.

Here we report the first example of purification and characterization of malate synthase from any basidiomycetes. Malate synthase (EC 4.1.3.2), one of the two key enzymes of the glyoxylate cycle, catalyzing the C-C bond formation of glyoxylate and acetyl-CoA to yield malate, was purified to a homogenous protein from *F. palustris* grown on glucose. The purified enzyme, with a molecular mass of 520 kDa, was found to apparently consist of eight identical 65-kDa subunits, and to have high affinity for both glyoxylate (K_m of 45 μM) and acetyl-CoA (2.2 μM). No C-S bond cleavage of acetyl-CoA was observed in the presence of pyruvate, oxaloacetate, 2-oxoglutarate, or glyoxal. The potent inhibition of the enzyme activity by *p*-chloromercuribenzoate suggests that the enzyme has a sulfhydryl group at the active center. The enzyme activity was competitively inhibited by oxalate (K_i , 8.5 μM) and glycolate (K_i , 17 μM), and uncompetitively by coenzyme A (K_i , 100 μM).

Importantly, because oxalic acid (78 mM) accumulated in the culture fluid of the fungus and the acid was also found to be a potential inhibitor of the glyoxylate cycle enzymes, it is hypothesized that *F. palustris* has an elaborate system to transport oxalate from the intracellular sites to the extracellular sites.

1) E. Munir, J.J. Yoon, T. Tokimatsu, T. Hattori, and M. Shimada. *Proc. Natl. Acad. Sci. USA* **98**, 11126 (2001).