Preliminary

A Possible Intramolecular Electron Transfer Pathway of Glyoxylate Dehydrogenase in a Brown-rot Fungus *Tyromyces palustris*[*1]

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Introduction

Oxalic acid has been receiving much attention in relation to its important multiple roles in our ecosystem, including wood decay by wood-rotting fungi[1]. Thus, the elucidation of the enzymatic mechanism for the oxalate formation in wood destroying fungi is very important.

We successfully purified a novel flavohemoprotein glyoxylate dehydrogenase from the mycelial homogenate of the brown-rot fungus *Tyromyces palustris*[2]. This enzyme which has been found to catalyze dehydrogenation of glyoxylate to produce oxalate in the presence of cytochrome *c*, is distinguished from previously reported ones[3–6]. The present paper describes a possible intramolecular electron-transferring pathway of the enzyme during the dehydrogenation of glyoxylate. A role of the enzyme is also discussed in relation to the production of oxalic acid by the fungus.

Materials and Methods

Glyoxylate dehydrogenase was purified from the crude extracts of *T. palustris* cultivated at 33°C as previously reported[2]. FMN fragment and heme fragment were prepared by digestion of the enzyme with papain[7] followed by gel chromatography. The glyoxylate: cytochrome *c* oxidoreductase activity was determined spectrophotometrically for each of two fragments. The qualitative and quantitative analysis for the organic acids produced in the culture was done by GC-MS.

Results and Discussion

Oxalic acid was identified by GC-MS as a major organic acid produced in the culture of *T. palustris* (2.16 g/l at maximal concentration), but neither lactate nor pyruvate was detected. Amounts of oxalate accumulated in the culture increased, as the glyoxylate dehydrogenase activity increased until day 6 of the cultivation. However, after day 6 the oxalic acid accumulation further increased and then the enzyme activity decreased (Fig. 1). Since the parallel relationship was observed between the enzyme activity and production of oxalate, the results strongly suggest that the glyoxylate dehydrogenase participated in producing oxalate in the former part of the cultivation of *T. palustris*. Although lactate was found to be much a better substrate for the enzyme *in vitro*, the enzyme may not serve for oxidation of lactate to produce pyruvate. In fact, neither lactate nor pyruvate was found in a detectable amount.

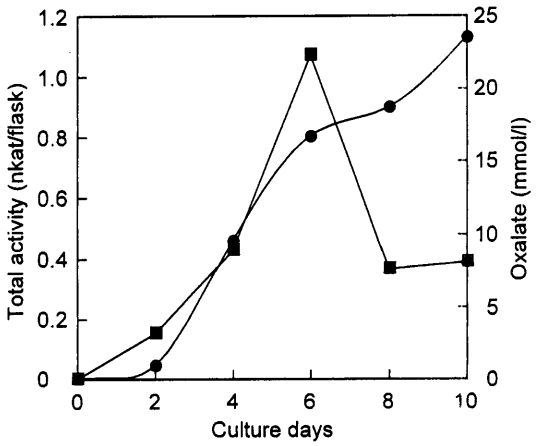

Fig. 1. Changes in the production of oxalate and the total glyoxylate dehydrogenase activity during the cultivation of *Tyromyces palustris*. Circles, oxalate production; squares, total enzyme activity.

The FMN and heme fragments (Mr=ca 12,000 and Mr=ca 17,000, respectively) were separated and purified by gel chromatography after the limited proteolysis of the enzyme with papain. The absorption spectrum of the FMN fragment rapidly changed to the reduced form after the addition of glyoxylate. The reduction of the FMN fragment was confirmed because the obtained spectrum was almost identical with that of dithionite-reduced FMN. However, the absorption spectrum of the heme fragment did not change to the reduced form after the addition of glyoxylate, but showed slowly the reduced form after the addition of both the FMN fragment and glyoxylate (Fig. 2). This finding clearly indicate that electrons from the substrate were not directly transfered to the heme

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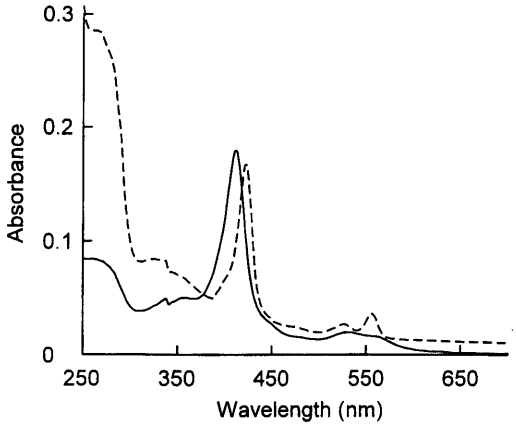

Fig. 2. UV and visible spectra of the heme fragment measured in the presence of glyoxylate and FMN fragment. ——, Without supplement; ·····, after the addition of glyoxylate and FMN fragment.

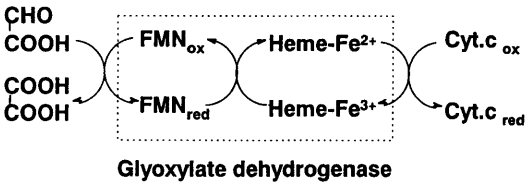

Fig. 3. A proposed scheme of electron-transferring function of the flavohemoprotein glyoxylate dehydrogenase during the dehydrogenation of glyoxylate to form oxalate in the presence of cytochrome *c*.

fragment. Therefore, the results suggest that the electron was transferred from glyoxylate to the FMN fragment first, then to the heme fragment, and finally to cytochrome *c* as shown in Fig. 3. The proposed intramolecular electron transfer pathway was similar to those reported for FMN-containing L-lactate dehydrogenase from yeast[8–11] and FAD-containing cellobiose dehydrogenase from wood-rotting fungi[12,13]. However, further research is needed to reveal the electron transfer pathway in detail.

References

1) M. Shimada *et al.*: *J. Biotechnol.*, **53**, 103–113 (1997).
2) T. Tokimatsu *et al.*: *FEBS Letters*, **437**, 117–121 (1998).
3) J.R. Quayle and G.A. Taylor: *Biochem. J.*, **78**, 611–615 (1961).
4) A.J. Balmforth and A. Thompson: *Biochem. J.*, **218**, 113–118 (1984).
5) T. Kasai *et al.*: *J. Gen. Appl. Microbiol.*, **9**, 49–58 (1963).
6) Y. Akamatsu and M. Shimada: *Phytochemistry*, **37**, 649–653 (1994).
7) G. Henriksson *et al.*: *Eur. J. Biochem.*, **196**, 101–106 (1991).
8) C.A. Appleby and R.K. Morton: *Nature*, **173**, 749–752 (1954).
9) C.A. Appleby and R.K. Morton: *Biochem. J.*, **75**, 258–269 (1960).
10) R.K. Morton and J.M. Sturtevant: *J. Biol. Chem.*, **239**, 1614–1624 (1964).
11) H. Suzuki and Y. Ogura: *J. Biochem.*, 7, 277–289 (1970).
12) G.D. Jones and M.T. Wilson: *Biochem. J.*, **256**, 713–718 (1988).
13) M.S. Rogers *et al.*: *Biochem. J.*, **298**, 329–334 (1994).