Preliminary

The Degradation of β-*O*-4 Type Lignin Carbohydrate Complex (LCC) Model Compounds by Lignin Peroxidase*[1]

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Introduction

In our laboratry, a number of investigations have been reported on the enzymatic degradations of lignin and its model compounds[1-3]. Recently, Tokimatsu *et al.*[4] reported the first example of lignin peroxidase (LiP)-catalyzed oxidations of the four diastereomers. These results suggested that the sugar moiety etherified at the benzyl position could reduce the reactivity of the β-*O*-4 type LCC model compounds in the LiP system, although much still remain to be done before the final conclusion is drawn. In the present investigation, experiments were further carried out to evaluate the effect of the sugar moiety in the reactivity more precisely.

Experiments

LiP preparation

LiP from *P. chrysosporium* was partially purified as previously reported[5]. LiP thus obtained was further purified by use of the TSKgel G3000SW$_{XL}$ and Protein-Pak^{TM} DEAE-8HR.

Oxidation of substrates by LiP

The reaction mixture (total, 1 ml) contained substrates (β-*O*-4 type LCC model compounds, LCC$_{e_1}$, LCC$_{e_2}$, LCC$_{t_1}$, and LCC$_{t_2}$[6], or β-*O*-4 dimers, β-*O*-4e and β-*O*-4t (Fig. 1), 1 mM), LiP (0.5 U), and H_2O_2 (25 mM) in 0.1 M sodium tartrate buffer (pH 3.0).

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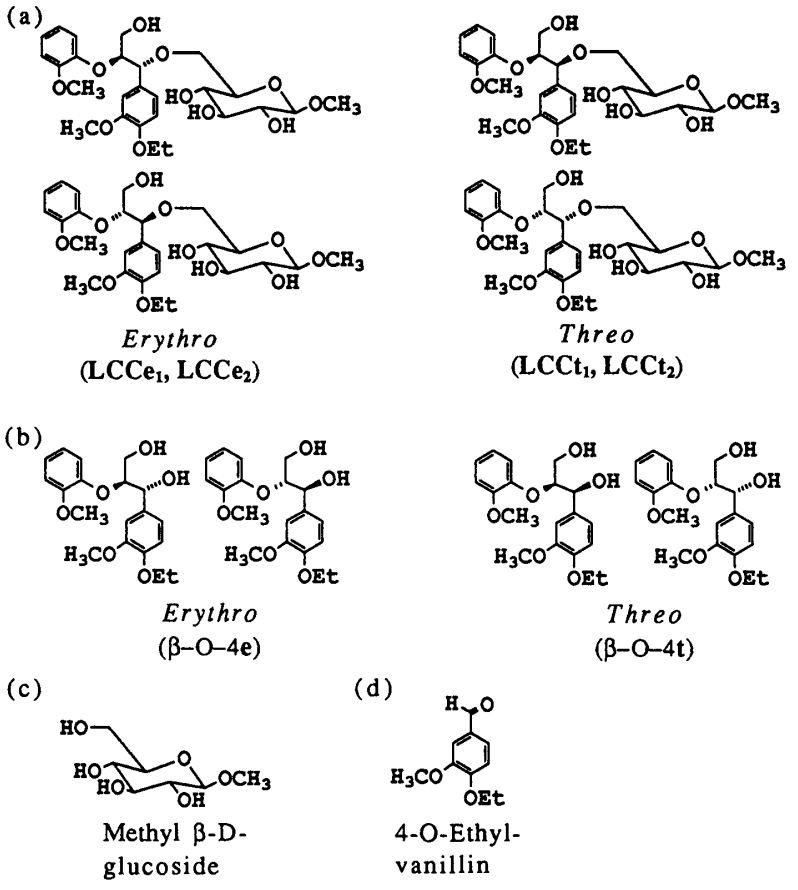

Fig. 1. Structures of Compounds. a) *β-O*-4 type LCC model compounds, b) *β-O*-4 dimers, c) methyl *β*-D-glucoside (**3**), d) 4-*O*-ethylvanillin (**4**), Et=CH_2CH_3.

The reaction mixture terminated by the addition of *N*, *N*-dimethylformamide (DMF) was freeze-dried and extracted with methanol. The methanol extracts were subjected to product identification by GC-MS (acetylation: acetic anhydride/piridine=1/1) and to product quantification by HPLC.

Results and Disccusion

Methyl *β*-D-glucoside (Fig. 1) as the oxidation product from the *β-O*-4 type LCC model compounds was identified for the first time. The amounts of the C_α-C_β cleavage product, 4-*O*-ethylvanillin (Fig. 1), from the *β-O*-4 type LCC model compounds was about half those from the corresponding *β-O*-4 dimers.

The quantitative analysis of the *β-O*-4 type LCC model compounds in the crude LiP system indicated that no significant differences were observed between the amounts of each isomer oxidized under the condition. In addition, no significat differences were observed between the amounts of the *β-O*-4 type LCC model compounds oxidized and those of the *β-*

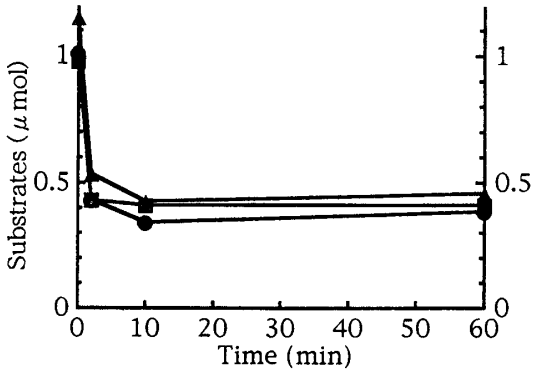

Fig. 2. Oxidation of *β-O*-4 type LCC model compounds and *β-O*-4 dimers by crude LiP. ▲, LCC_{t_2}; ●, LCC_{c_1}; ■, *β-O*-4t.

O-4 dimers oxidized (Fig. 2). Thus it was indicated that the reactivity of the *β-O*-4 type LCC model compounds was close to that of the *β-O*-4 dimers.

In conclusion, the present study indicated that the reactivity of the *β-O*-4 type LCC model compounds was almost equal to that of the *β-O*-4 dimers ; the sugar moiety etherified at the benzyl position of the *β-O*-4 type LCC model compounds does not seem to deter the reactivity of the *β-O*-4 moiety by LiP.

References

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5) T. Hattori, M. Shimada and T. Higuchi: *Mokuzai Gakkaishi*, **35**, 933–937 (1989).
6) The absolute configuration of each compound has not yet been determined, the *erythro* isomer having higher Rf value on TLC chromatogram than the other *erythro* isomer in referred to as LCC_{c_1}, and LCC_{c_2} refers to the *erythro* isomer having the lower Rf value. Similarly, the *threo* isomers with the higher and lower Rf values are referred to as LCC_{t_1} and LCC_{t_2}, respectively.