Enzyme and Microbial Technology 43 (2008) 199–204

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

journal homepage: www.elsevier.com/locate/emt

Diastereomer selectivity in the degradation of a lignin model compound of the arylglycerol β-aryl ether type by white-rot fungi

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ARTICLE INFO

Article history:
Received 6 November 2007
Received in revised form 18 February 2008
Accepted 1 March 2008

Keywords:
Lignin biodegradation
Lignin model compound
Diastereomers
White-rot fungi

ABSTRACT

Mixtures of about equal amounts of the diastereomers of arylglycerol β-aryl ether 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) were added to cultures of *Trametes versicolor*, *Phanerochaete chrysosporium*, and *Pycnoporus cinnabarinus*. Samples taken from the fungal cultures were analyzed with respect to the fraction of **1** degraded, the *erythro*:*threo* ratio in the remaining **1**, and the product profile, using HPLC and UV-diode array detector. The *P. cinnabarinus* cultures exhibited laccase activity and partially decolorized Remazol Brilliant Blue R (RBBR), but they did not degrade **1** under the conditions studied. Cultures of *T. versicolor* and *P. chrysosporium* preferentially degraded the *threo* isomer of **1**. This is consistent with the fact that the product profiles showed larger amounts of *threo*- than *erythro*-veratrylglycerol. The results can be discussed in relation to the diastereomer selectivity of various oxidants implicated in lignin degradation by white-rot fungi. Preferential degradation of the *threo* isomer of arylglycerol β-aryl ethers is not in agreement with the action of Fenton's reagent, since this reagent does not exhibit any stereopreference.

1. Introduction

Biodegradation of wood by fungi is an important process in nature and is also of interest in applied contexts. The mode in which various enzymic and non-enzymic oxidants generated by wood-rotting fungi may co-operate in the decay of wood polymers is not completely elucidated. White-rot fungi secrete enzymes, such as peroxidases and laccases, with different capacities to oxidize simple lignin model compounds such as methoxylated benzyl alcohols [1]. *In vitro* studies of the oxidative degradation of lignin model compounds increase the possibility of identifying the oxidants that white-rot fungi produce to effect biodegradation of lignin and contribute to the understanding of the properties of different oxidants [2,3].

Lignin is one of the major structural components in vascular plants [4,5]. It consists of phenylpropane units connected to each other by a series of characteristic linkages, of which the β-O-4 ether linkage is the most common. The fraction of arylglycerol β-aryl ether bonds is ~35% and ~45% in softwood and hardwood lignins, respectively. The arylglycerol β-aryl ether substructures that arise from β-O-4 coupling exist in two diastereomeric forms, referred to as *erythro* and *threo* isomers. The isomer ratio has been found to correlate with the syringyl:guaiacyl ratio [6]. The *erythro* isomer is predominant in hardwood lignins, while softwood lignins contain about equal amounts of the *erythro* and *threo* forms.

In vitro experiments in which the arylglycerol β-aryl ethers 2-(2,6-dimethoxyphenoxy)-1-(3,4-dimethoxyphenyl)-1,3-propanediol and 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) were oxidized by lignin peroxidase showed that the *threo* isomers are preferentially degraded [3,7]. Experiments in which **1** was oxidized using laccase-mediator systems revealed that the stereopreference was dependent on the nature of the mediator [3]. Fenton's reagent, which has been said to be involved in the degradation of wood polymers by both brown-rot and white-rot fungi (reviewed by Hammel et al. [8]), did not exhibit any stereopreference [3].

This investigation addresses stereopreference in the degradation of arylglycerol β-aryl ethers by white-rot fungi. The fungi studied were *Trametes* (*Coriolus*, *Polyporus*) *versicolor*, *Phanerochaete chrysosporium*, and *Pycnoporus cinnabarinus*. Of these, *T. versicolor* is known to produce lignin peroxidase, manganese peroxidase, and laccase [9–12], and *P. chrysosporium* is known to produce lignin peroxidase and manganese peroxidase (reviewed in [13]). *P. chrysosporium* has also been reported to produce laccase [14], but recent investigations have shown that the genome of *P. chrysosporium* does not encode any conventional laccase [15,16]. *P. cinnabarinus* is known to produce laccase, and this enzyme is believed to play an important role for the ability of the fungus to

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doi:10.1016/j.enzmictec.2008.03.002

degrade lignin [17]. Mixtures of diastereomers of **1** (Fig. 1) were added to fungal cultures under ligninolytic conditions, as indicated by the conversion of Remazol Brilliant Blue R (RBBR). The effects of the fungi on the degradation of the *erythro* and *threo* isomers were investigated.

2. Materials and methods

2.1. Chemicals

Veratryl alcohol (**2**) and veratraldehyde (**3**) (Fig. 1) are commercially available. The *erythro* (**1e**) and *threo* (**1t**) forms of 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol (**1**) (Fig. 1) were synthesized as described by Ibrahim and Lundquist [18] and Li et al. [19]. 1-(3,4-Dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-1-propanone (**4**) (Fig. 1) was synthesized according to Adler et al. [20]. The synthesis and assignment of the diastereomers of veratrylglycerol [1-(3,4-dimethoxyphenyl)-1,2,3-propanetriol] (**5**) (Fig. 1) is described by Adler and Gustafsson [21]. Reagent grade chemicals were used unless otherwise stated.

2.2. Organisms and culture conditions

T. versicolor (PRL 572), *P. chrysosporium* (BKM-F-1767), and *P. cinnabarinus* (FP-100490-Sp) were stored on malt agar slants at 4 °C as described in [22].

2.3. Fungal pre-cultures

Pieces of mycelia were transferred from malt agar slants to growth at room temperature (~22 °C) in stationary liquid cultures (50 ml medium in 250 ml Erlenmeyer flasks). The pre-culture medium used for *T. versicolor* [23] contained 20 g/l glucose. *P. chrysosporium* was grown in a nitrogen-limited medium [22]. *P. cinnabarinus* was grown in a malt medium [22]. Veratryl alcohol (**2**) was not included in any of the media. After growth, the fungi formed a mycelial mat covering the surface of the growth medium. The flasks contained glass beads at the bottom to make it possible to disintegrate the mycelium.

2.4. Treatment of *1* with fungal cultures

Stationary liquid cultures of *T. versicolor*, *P. chrysosporium*, and *P. cinnabarinus* were grown at ~22 °C in different series with a minimum of ten cultures in each series. Three of them contained Remazol Brilliant Blue R. Five of them were used in trials with a mixture of **1e** and **1t**. One culture was used for cell-free oxidation of **1e** and **1t**. Finally, one culture was a control to which no RBBR or **1e**/**1t** mixture was added.

Prior to inoculation, the mycelial mats of the pre-cultures were disintegrated by vigorous shaking with the glass beads. The cultures (20 ml growth medium in 100 ml Erlenmeyer flasks) were inoculated with 0.4 ml of the suspension of disintegrated mycelium prepared from the pre-cultures. *T. versicolor* and *P. cinnabarinus* were grown in a carbon-limited medium [23], which contained glucose (2 g/l) and $CuSO_4$ (concentration, 0.1 mM). *P. chrysosporium* was grown in the nitrogen-limited medium described in [22], which contained $CuSO_4$ (concentration, 0.1 mM). Veratryl alcohol (**2**) was not added to the media.

The cultures were allowed to grow until ligninolytic conditions had been established as indicated by visual inspection of the cultures to which RBBR had been added. The initial concentration of RBBR in the growth medium ranged from 0.1 to 1 mM. When a change in the color of the RBBR-containing cultures was observed, a mixture of **1e** and **1t** was added to some of the cultures without RBBR. *T. versicolor* cultures were grown for 8 days before the addition of **1e** and **1t**. Cultures of *P. chrysosporium* and *P. cinnabarinus* were grown for 14 days before **1e** and **1t** were added.

The mixture of **1e** and **1t** was added by first withdrawing 3 ml of the medium of each culture to which the lignin model compound was to be added. A mixture of about equal amounts of **1e** and **1t** was then dissolved in the withdrawn culture medium. A sample (200 μl) was taken for analysis and the medium containing dissolved **1e** and **1t** was then added to the culture.

From cultures of *T. versicolor*, samples of 200 μl were withdrawn daily during 4 days after the addition of the **1e**/**1t** mixture. From cultures of *P. chrysosporium* and *P. cinnabarinus*, samples of 200 μl were withdrawn every second day over a period of 22 days after the addition of the **1e**/**1t** mixture.

In each experimental series, samples taken from a control culture were also analyzed in order to determine the concentration of secondary metabolites, such as veratryl alcohol (**2**).

2.5. Cell-free oxidation experiments

Several experimental series were made with each one of the three fungi. In each experimental series, culture medium (5 ml) was withdrawn from one of the cultures for cell-free oxidation of the **1e**/**1t** mixture. The culture fluid was filtered through a 0.2 μm filter to remove fungal cells and 3.4 mg of a mixture of about equal amounts of **1e** and **1t** was then added. This culture fluid with dissolved **1e** and **1t** was then transferred to an Erlenmeyer flask (20 ml), which was kept at ~22 °C. Samples (200 μl) for analysis were taken according to the same schedule as for the corresponding fungal cultures.

2.6. In vitro experiments with laccase and 3-hydroxyanthranilic acid

Since it was found that *P. cinnabarinus* cultures did not degrade the diastereomers, trials were also carried out *in vitro* with mixtures of **1e** and **1t** as the substrate in reactions with *T. versicolor* laccase and 3-hydroxyanthranilic acid (HAA), a natural

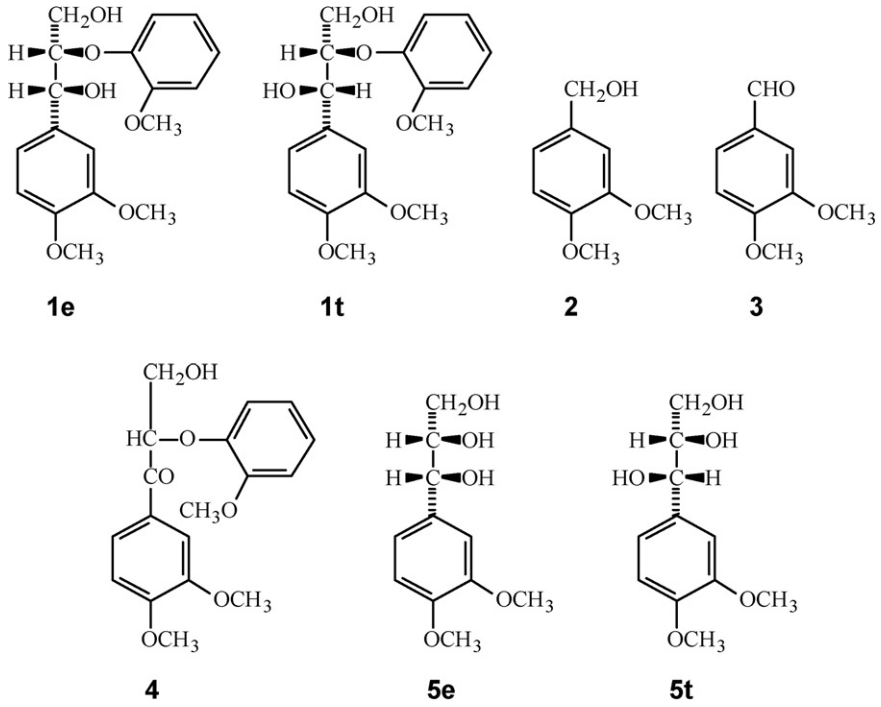

Fig. 1. The *erythro* (**1e**) and *threo* (**1t**) isomers of the lignin model compound 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol, veratryl alcohol (**2**), veratraldehyde (**3**),1-(3,4-dimethoxyphenyl)-3-hydroxy-2-(2-methoxyphenoxy)-1-propanone (**4**), and the *erythro* (**5e**) and *threo* (**5t**) isomers of veratrylglycerol.