Biotechnology Letters Vol 9 No 5 361-364 (1987)
Received as revised April 14

THIOUREA EFFECT ON THE LIGNIN BIODEGRADATION.

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SUMMARY.

Lignin degradation by peroxidase or an extracellular crude ligninase in the presence of thiourea is enhanced. Veratryl alcohol, exhibits the same behaviour in both cases. Thiourea is proposed as an additive in biopulping

INTRODUCTION

Recently the catalytic efficiency of anthraquinone: thiourea mixtures as additives in alkaline pulping was investigated. A 3:1 anthraquinone/ thiourea ratio was as effective as plain anthraquinone. This additive reduces the chemical cost of alkaline pulping (Werthermann,1983), and the mechanism of its effect has been discussed (Mortimer and Fleming, 1983).

Paper producers have been long concerned with the high cost and environmental impact of current methods of separating lignin from cellulose, and it is not surprising that they have taken an active interest in new methods of biopulping, that offer the possibility of being cleaner and more efficient than conventional methods (Trewhitt and Jones,1986).

An important step was the isolation and characterization of lignin peroxidase (Kirk et al.,1986; Renganathan et al.,1986;Umezawa and Higuchi,1985).

Thiourea inhibited lignin degradation by Phanerochaete chrysosporium (Glenn and Gold 1983), but on contrary no inhibition was observed on Streptomyces viridosporum, suggesting that a different mechanism is occuring. In view of the importance of thiourea in chemical pulping we have decided to study the effect of this compound on a new isolated ascomycete from a xylophagous insect (Campos et al, 1986; Durán et al, 1987) which has cellulolytic and lignolytic capacity (Durán et al, 1986a) in order to improve the lignin biodegradation.

For this purpose we investigated a ligninase model (horseradish peroxidase /H2O2/O2 system) (Durán et al. 1984; Durán and Mansilla, 1984) and a ligninase from extracellular culture medium of Chrysonilia sitophila TFB 27441.

MATERIALS AND METHODS

Chemicals. Dioxane-lignin was obtained from rice hull as described before (Durán and Mansilla, 1984). Horseradish peroxidase (Type VI), H2O2, thiourea were obtained from SIGMA. Veratryl alcohol was synthetized from vanillin and purified by vacuum distillation (Buck, 1943).

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Microorganism. C. sitophila (TFB-27441 strain) was isolated from a previous strain (Campos et al. 1986; Durán et al. 1986a).

Methods.

(a)Peroxidase/lignin or veratryl alcohol/H2O2/O2 system: Experiments with peroxidase/substrate/H2O2/O2 system were carried out using the method of Young and Steelink (1973) as modified by Durán et al (1986b). The kinetics of substrate degradation was followed by absorption spectroscopy (Zeiss DMR-10) at 280 nm or by the fluorescence technique (Hitachi-Perkin-Elmer Mod. MPF-44B) (λ exc. 360 nm) at em. 460 nm.

(b)Ligninase(crude extract)/lignin or veratryl alcohol/ H2O2/O2 system:
Experiments with ligninase (crude extract) were carried out following the method of Kirk et al.(1986).

(c)Ligninase activity: Enzyme activity was measured by UV spectroscopy of veratraldehyde (ε (310 nm) = 9300 $M^{-1} cm^{-1}$) formed by the oxidation of veratryl alcohol. The assay misture contained 275 µl of supernatant culture , 0.1 sodium tartrate buffer pH 2.5, 2 mM veratryl alcohol and 0.4 mM H2O2 in a total volume of 0.5 ml (Kirk et al. 1986).

(d)Culture conditions: Culture of C. sitophila (TFB-27441 strain) were grown in non agitated conditions in a 125 ml Erlenmeyer flask at 27ºC. with a culture medium at pH 5.6 (Czapeck modified medium)(Durán et al., 1987). The extracellular culture medium (supernatant) was separated by vaccum filtration at room temperature under aseptic conditions and then stored (frozen). This strain produced a total ligninase (veratryl alcohol oxididizing) activity of 67.3 ± 12.5 U/l (Durán et al. 1987).

RESULTS AND DISCUSSION

Fig.1A shows the kinetics of lignin degradation, measured by UV aabsorption, in the presence and absence of thiourea . Fig.1B shows the same type of kinetics by the fluorescence technique , following the appearance of the chromophoric group which emits at 410 nm. This chromophore was previously correlated with lignin degradation (Durán and Mansilla, 1984). Apparently the higher rate in the presence of thiourea is related to a previous reaction of thiourea with lignin to form an intermediate which is a better substrate for the peroxidase than lignin itself. This is in agreement with the the existence of an isothiouronium derivative of lignin (Mikawa and Sato, 1958). Thiourea is not a specific substrate for peroxidase/H2O2 oxidation (Ugarova et al., 1980).

Fig.2, shows the crude extract of ligninase from C. sitophila acting on lignin in the presence and absence of thiourea, followed by fluorescence (disappearance of the fluorescence maximum at 400 nm (λ exc. 360 nm)). As with the peroxidase, a faster rate was observed in the presence of thiourea. Fig.2 shows similar results as observed by absorption at 280 nm (see TABLE I). Thus thiourea could be an important additive in the biopulping process (Jurasek and Paice, 1986).

To understand the the mechanism of thiourea acting on lignin in the presence of ligninase, we have studied a lignin model, veratryl alcohol, on the basis of the

existence of the isothiouronium derivative of this alcohol (Mikawa and Sato, 1958). Fig.3A shows the kinetics of veratryl alcohol degradation by peroxidase in the presence and absence of thiourea. The same effect with lignin was observed. The inserted Fig.3B shows the molar ratio (thiourea)/(veratryl alcohol) indicating a maximum reactivity at 1:1 molar ratio. This probably is a good indication that an intermediate such as the isothiouronium derivative was formed.

From these data it is evident that thiourea catalyzes the lignin degradation in both peroxidase and ligninase systems in the same manner. TABLE I summarizes the ratio of the rates in the presence and absence of thiourea.

TABLE I. RATIO OF RATE CONSTANT IN THE OXIDATION OF LIGNIN AND VERATRYL ALCOHOL BY PEROXIDASE AND LIGNINASE IN THE PRESENCE AND ABSENCE OF THIOUREA.

	LIGNIN (a)	VERATRYL ALCOHOL (b)
PEROXIDASE	10.0	2.0
LIGNINASE	1.8 (1.9)(c)	2.5

a) At 285 nm; b) At 310 nm; c) At λ exc. 360 nm, λ em. 410 nm.

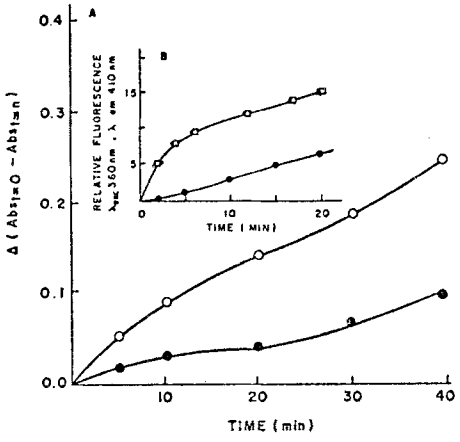

FIG.1. (A). Lignin degradation (disappearance of absorption at 285 nm) in 0.1 M phosphate buffer pH 5.5 by HRP(4 μM)/ lignin (0.1 mg/ml, dioxane:H2O (1:1)/H2O2 (9 mM/O2 system: (- ● -) in the absence of thiourea; (- O -) in the presence of 25 mM thiourea.
(B). Lignin degradation (appearance of fluorescence band at 410 nm (exc.360 nm); otherwise as (A).

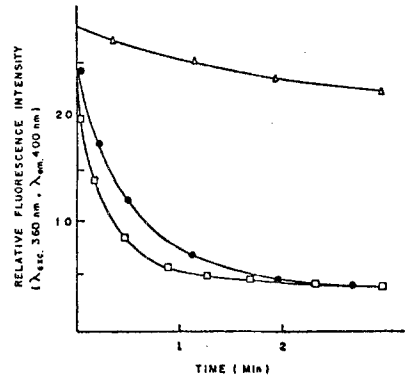

Fig.2 Lignin degradation (dissappearance of fluorescence band at em. 400 nm (λ exc. 360 nm)), in 0.1 M tartrate buffer pH 2.5 , by the extracellular culture medium of ligninase (0.1 ml)/ lignin (0.2 mg/ml, dioxane:H2O (1:1)/H2O2 (0.67 mM)/O2 system (final volume: 3 ml): (- △ -) Control, lignin and H2O2; (- ● -) complete system in the absence of thiourea; (- □ -) complete system in the presence of 25 mM of thiourea.

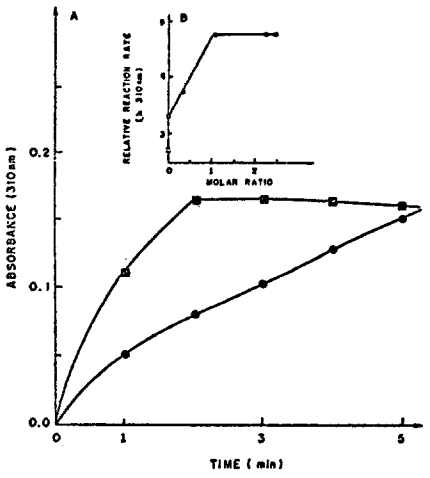

FIG.3. (A) Kinetics of veratryl alcohol degradation (appearance of veratraldehyde absorption at 310 nm) in 0.1 M phosphate buffer pH 5.5 by HRP(4 μM)/ veratryl alcohol (4.1 mM)/H2O2 (9 mM)/O2 system: (- ● -) in the absence of thiourea; (- ■ -) in the presence of 50 mM thiourea. (B) Plot of relative rate versus molar ratio for the system described in Fig.3A at different concentrations of thiourea.

In order to evaluate the effectiveness of thiourea biopulping of pinus wood is actually in progress.

ACKNOWLEDGMENT. This work was supported by FAPESP, CNPq, FINEP, PADCT, OAS Program (Chile-Brazil), CT/INTRA(BID) (Brazil-Chile), PNUD(UNESCO) Program and Direccion de Invest. Pont. Univers. Catol.Chile (DIUC).

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