PRODUCTION, PURIFICATION, AND CHARACTERIZATION OF ALCOHOL OXIDASE OF A LIGNIN — DEGRADING BASIDIOMYCETE, *PHANEROCHAETE CHRYSOSPORIUM*

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Alchol oxidase was found in the mycelial extract of a lignin — degrading basidiomycete, *Phanerochaete chrysosporium*.

The high enzyme activity was detected in the mycelia grown under the condition of nutrient nitrogen limitation （low — N） and high oxigen atmosphere （high — O_2） , which coincided with the culture condition that this fungus shows the high ligninolytic activity. The enzyme productivity was markedly repressed when *P. chrysosporium* was grown in the medium containing a high amount of glucose. The enzyme was purified to homogeneity and characterized. The enzyme has a molecular weight of about 440,000 and consists of six subnits identical in molecular weight （74,000） . The enzyme shows a typical absorption spectrum of a flavoprotein, and contains 6 mol of FAD per mol of enzyme. The isoelectric point is pH 5.3. The enzyme catalyzes the oxidation of methanol to yield an equimolar amount of formal dehyde and hydrogen peroxide with the equimolar amount of oxygen consumption. In addition to methanol, which is the most preferred substrate, some short — chain normal primary aliphatic alcohols can serve as substrates of the enzyme. The enzyme activity is significantly inhibited by sulfhydryl reagents.

Keywords : alcohol oxidase ; *Phanerochaete chrysosporium* ; lignin — degrading basidiomycete ; lignin — degradation ; methanol ; hydrogen peroxide.

Introduction

Phanerochaete chrysosporium is a well — known lignin — degrading basidiomycete and has been studied extensively about its ligninolytic system. It has been reported that methanol is formed during the degradation of lignin [1] and lignin — related aromatic substances [2] by *P. chrysosporium*. The formation of methanol is due to the demethoxylation of methoxyl groups of these compounds probably catalyzed by peroxidase, and may be concerned with the ligninolytic system of this fungus [1,2].

Alcohol oxidase is considered to be one of enzymes concerning the metabolism of methanol thus formed. Alcohol oxidase was first found in several basidiomycetes [3-5] other than *P. chrysosporium*, and subsequently has been detected in a number of methanol — utilizing yeasts [6-9]. In the latter organisms, the enzyme plays a key role in the metabolism of C_1 compounds as the enzyme catalyzing the first step of the sequential methanol metabolism. Alcohol oxidase has been detected also in a brown rot basidiomycete, *Poria contigua* [10]. The existence of alcohol oxidase in a mycelial extract of *Sporotrichum pulverulentum*, an anamorph of *P. chrysosporium*, was suggested in the report of Ander and Eriksson [1], however little is known in details.

In this paper, we describe about the production, purification, and characterization of alcohol oxidase of *P. chrysosporium*.

Materials and Methods

Microorganism and culture conditions. Cultures of *P. chrysosporium* ME446 was maintaind on slants

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as described previously [11]. *P. chrysosporium* was grown in media containing 0.5 % glucose unless otherwise stated, either 1.2 mM (low－N) or 12 mM (high－N) ammonium tartrate, 20 mM sodium 2, 2－dimethylsuccinate buffer (pH 4.5), 0.02 % yeast extract, and salts as described by kirk *et al*. [12]. A suspension of conidia was inoculated into the media (25 ml in a 500－ml Erlenmeyer flask), and the stationary cultures were carried out at 37 ℃. *P. chrysosporium* was grown under air (low－O_2) or high oxygen atmosphere (high－O_2) in which the cultures were purged with 100 % oxigen at 48－h intervals after growth under air for 3 days.

Preparation of mycerial extracts. All operations were performed at 0－4 ℃. The mycelia harvested by centrifugation were washed twice with 0.1 M potassium phosphate buffer (pH7.5). The washed mycelia were disrupted in a mortar chilled on ice by grinding with appropriate amounts of sea sands and 0.1 M potassium phosphate buffer (pH7.5), and then suspended in the same buffer. The supernatant solution obtain by centrifugation was dialyzed against 100 volumes of 10 mM potassium phosphate buffer (pH7.5). The dialyzed solution was used as a mycelial extract.

Enzyme assay. 〔Method A〕 ： The enzyme activity was assayed by determining the formation of hydrogen peroxide according to the method of Tani *et al*. [13] with a slight modification. The standard assay system consisted of 50 μ mol of potassium phosphate buffer (pH7.5), 25 μ mol of methanol, 0.8 μ mol of 2,2'－azino－bis－(3－ethylbenzthiazoline－6－sulphonate) (ABTS), 10 units of horseradish peroxidase,and alcohol oxidase solution in a final volume of 1.0 ml. The reaction was initiated by the addition of methanol and the reaction velocity was measured at 30 ℃ by following the increase in absorbance at 420 nm. 〔Method B〕 ： The enzyme activity was also assayed by determining the formation of formaldehyde. After incubation of the reaction mixture mentioned above without ABTS and horseradish peroxidase at 30 ℃ for 5 min,the reaction was terminated by the addition of 0.1 ml of 2 N HCl. The enzyme solution was replaced by 10 mM potassium phosphate buffer (pH 7.5) in a blank. The supernatant solution obtained by brief centrifugation was used for the determination of formed formaldehyde by the method of Nash [14]. Unless otherwise stated, routinely Method A was employed. One unit of enzyme is defined as the amount that catalyzes the formation of 1 μ mol of hydrogen peroxide per min.

Protein determination. The protein concentration was determined by the method of Bradford [15] with bovine serum albumin as a standard.

Determination fo molecular weight. The molecular weight of the native enzyme was determined by TSK－gel G 3000SW$_{XL}$ (0.75 × 30 cm, Tosoh) gel permeation chromatography with a high performance liquid chromatograph according to the method of Fukano *et al*. [16]. A calibration curve was made with standard proteins ： bovine thyroglobulin (molecular weight, 660,000), bovine liver glutamate dehydrogenase (350,000), yeast glutamate dehydrogenase (290,000), porcine heart lactate dehydrogenase (140,000), bovine serum albumin (68,000), and egg albumin (43,000). The enzyme and standard proteins were eluted with 10 mM potassium phosphate buffer (pH 7.0) containing 0.2 M NaCl at a flow rate of 0.2 ml per min. The molecular weight of the subunits was estimated by sodium dodecyl sulfate (SDS)－polyacrylamide gel electrophoresis according to the method of Weber and Osborn [17]. The enzyme was dialyzed against 10 mM sodium phosphate buffer (pH 7.0) and then denatured by treatment with 1 % SDS solution containing 1 % 2－mercaptoethanol at 100 ℃ for 5 min.The standard proteins, rabbit muscle phosphorylase b (94,000), bovine serum albumin (68,000), bovine liver cutalase (60,000), egg albumin (43,000), and bovine pancreas α－chymotrypsinogen A (25,000), were treated in the same manner.

Determination of Isoelectric point. The isoelectric point of the enzyme was estimated by isoelectric

forcusing on a flat — bed polyacrylamide gel （Ampholine PAG plate, pH range 3.5 to 9.5, Pharmacia） with a pI — marker kit （Oriental Yeast） as a standard.

Spectrophotometry. Spectrophotometric measurements were made with a Hitachi Model U — 3200 recording spectrophotometer with a 1.0 — cm light path.

Materials. Horseradish peroxidase was purchased from Wako Pure Chemical Ind. Ltd. The other chemicals were of analytical grade.

Results

Effect of culture conditions on the enzyme production by *P. chrysosporium*

The time course of alcohol oxidase activity in mycelial extracts of *P. chrysosporium* was examined under four different culture conditions （low — N ; high — O_2, low — N ; low — O_2, High — N ; high — O_2, and high — N ; low — O_2） . *P. chrysosporium* was grown in media containing 0.5 % glucose, and the mycelial extracts were prepared from the mycelia harvested at the times indicated. As shown in Fig. 1A, the
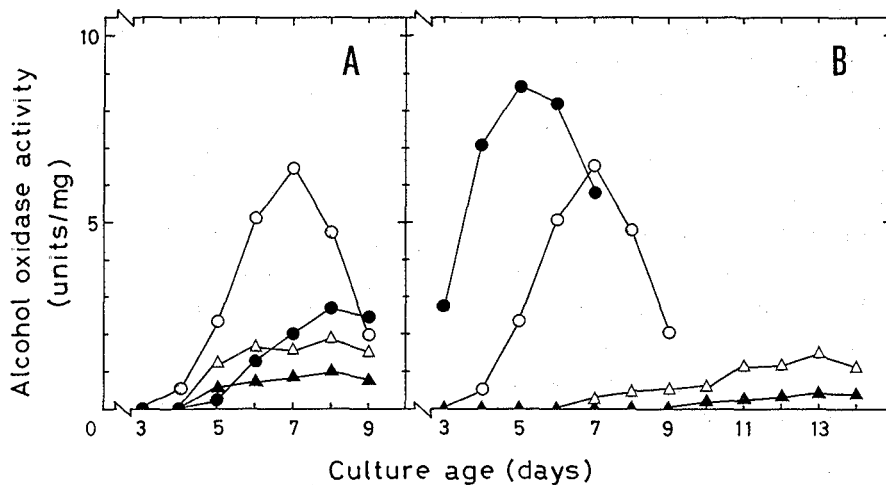

Fig. 1 Effect of culture conditions on the production of alcohol oxidase by *P. chrysosporium* . Each point represents the mean of five replicate cultures. （A） ： Cultures were carried out in madia containing 0.5 % glucose under the conditions of low — N ; high — O_2 （—○—） , low — N ; low — O_2 （—●—） , high — N ; high — O_2 （—△—） , and high — N ; low — O_2 （—▲—） . （B） ： Cultures were carried out in madia containing 0.25 % （—●—） , 0.5 % （—○—） , 1.0 % （—△—） , and 2.0 % （—▲） glucose under the condition of low — N ; high — O_2.

enzyme productivity was significantly affected by both the concentrations of nutrient nitrogen in the medium and oxygen in the gas phase of culture as in the case of the ligninolytic activity of this fungus [12]. Thus,the highest activity of the enzyme was found in the mycelia grown under the condition of nutrient nitrogen limitation （low — N） and high oxygen atmosphere （high — O_2） . This culture condition coincided with that appropriate for the ligninolytic activity [12], indicating that alcohol oxidase plays a certain role in the ligninolytic system of *P.chrysosporium* as well as the methanol formation [1,2]. Alcohol oxidase productivity was also affected by the glucose concentration in the medium. As shown in Fig.1B, the enzyme

productivity was repressed markedly when *P. chrysosporium* was grown in the medium containing high concentration （more than 1.0 %）of glucose. The appearance of the enzyme activity in the mycelia showed the tendency to delay as glucose concentration in the medium increased, and the highest activity detected became lower also as the glucose concentration increased. This indicates the catabolite repression of alcohol oxidase production.The enzyme was not induced when methanol was added into the media, while its production was repressed （data not shown）.

Purification of the enzyme

Steps 1 to 3 were carried out at $0 - 4$ ℃, and Step 4 at room temperature.

Step 1 ： A mycelial extract was prepared from the mycelia （about 12 g, wet weight） grown for 7 days under the condition of low $-$ N ; high $-$ O$_2$.

Step 2 ： The mycelial extract was brought to 60 % saturation with ammonium sulfate. The precipitate collected by centrifugation was re $-$ dissolved in an appropriate volume of 10 mM potassium phosphate buffer （pH 7.5）. The enzyme solution was dialyzed twice against 100 volumes of the same buffer.

Step 3 ： The dialyzed enzyme solution was applied to a DEAE $-$ TOYOPEARL 650 M （Tosoh） column （1.8 × 6.0 cm） equilibrated with the dialysis buffer. After the column was washed thoroughly with the same buffer to remove the unadsorbed protein, the enzyme was eluted with the same buffer containing 50 mM NaCl. The active fractions were pooled, dialyzed against 10mM potassium phosphate buffer （pH 7.5）, and concentrated to about 0.3 ml with a Centricon $-$ 10 microconcentrator （Amiocon）.

Step 4 ： A portion （0.1 ml） of the enzyme solution was subjected to the gel permeation chromatography with a high performance liquid chromatograph equipped with a TSK $-$ gel G 3000SW$_{XL}$ （0.75 × 30 cm）. The enzyme was eluted with 10 mM potassium phosphate buffer （pH 7.5） at a flow rante of 0.2 ml per min. The remainder of the enzyme solution was also subjected to the gel permeation chromatography. The active fractions were pooled and concetrated as mentioned above.

Approximately 46 $-$ fold purification was achieved with an over $-$ all yield of 54 %. A summary of the enzyme purification procedure is presented in Table 1.

Table 1. Purification of alcohol oxidase from *P. chrysosporium* .

Step	Total Protein (mg)	Total activity (units)	Specific activity (units／mg)	Yield (%)
1. Mycelial extract[a]	85.1	519	6.1	100
2. Ammonium sulfate fractionation （0 $-$ 60 %）	69.1	470	6.8	91
3. DEAE $-$ TOYOPEARL 650 M	11.2	374	33.4	72
4. TSK $-$ gel G 3000SW$_{XL}$	1.0	280	280.4	54

[a] A mycelial extract was prepared from the mycelia （about 12 g, wet weight） of *P. chrysosporium* grown for 7 days under the condition of low $-$ N ; high $-$ O$_2$.

Purity, molecular weight, and subunit structure

The purified enzyme was shown to be homogenous upon disc gel electrophoresis performed in 5.0 % polyacrylamide gel by the method of Davis [18].

The molecular weight of the enzyme was determined to be about 440,000 by the gel filtration method. The

subunit structure of the enzyme was examined by polyacrylamide disc gel electrophoresis in the presence of 0.1 % SDS. There was a single band of stained protein. The molecular weight of the subunits was estimated to be about $74,000$ on the basis of its mobility relative to those of standardproteins, indicating that the enzyme is composed of six subunits identical in molecular weight.

Stability of the enzyme

The purified enzyme in 10 mM potassium phosphate buffer （pH 7.5） could be stored at -20 ℃ for at least several weeks with little loss of activity when the protein concentration was higher than 0.6 mg per ml. When heated for 10 min in 0.1 M potassium phosphate buffer （pH 7.5）, the enzyme was stable up to 35 ℃. Upon incubation at 35 ℃ for 10 min. the enzyme was stable in the pH range of 7.0 to 9.5. The stability decreased markedly below pH 6.0.

Absorption spectrum

The enzyme showed a typical absorption spectrum of a flavoprotein having maxima at 278, 388, and 460 nm and a shoulder around 490 nm as shown in Fig.2. No appreciable spectral shift occurred on varying the pH （6.0 to 9.0） .
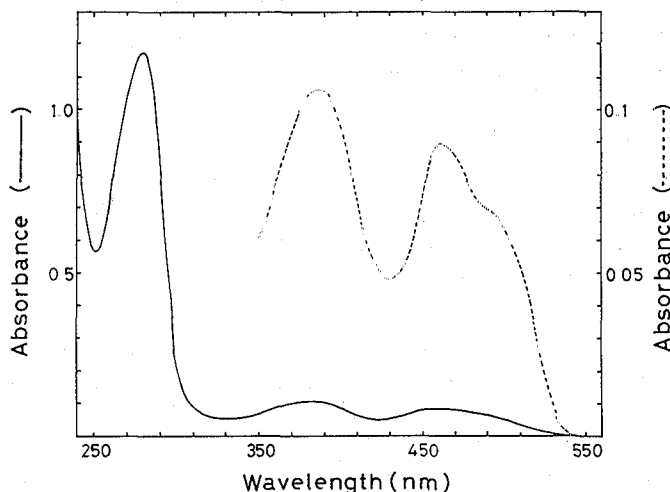

Fig. 2 Absorption spectrum of alcohol oxidase in 10 mM potassium phosphate buffer （pH 7.5） . The enzyme concentration was 0.75 mg per ml.

Prosthetic group

The absorption spectrum indicated that flavin nucleotide is a prosthetic group of the enzyme. The flavin moiety in the enzyme was indentified as follows. The enzyme （0.75 mg）, dissolved in 0.2 ml of water, was kept in boiling water for 5 min and then centrifuged to remove denatured protein. The yellow supernatant solution was examined by thin layer chromatoglaphy on silica gel plates （Silica gel 60 F $_{254}$, Merck） with two solvent systems of *tert* — pentanol — formic acid — water （$3:1:1$, v／v／v） and 1 — butanol — acetone — acetic acid — water （$5:2:1:3$, v／v／v／v） . A single yellow spot （Rf $=0.08$, 0.30, respectively） which corresponded to the authentic flavin adenine dinucleotide （FAD） was observed, and the Rf values of flavin mononucleotide and riboflavin were 0.19, 0.42, and 0.34, 0.68, respectively, under these conditions. Thus, the flavin moiety was identified as

FAD. To measure the FAD content, 0.1 ml of 25 % trichloroacetic acid was added to 0.6 ml of the enzyme solution containing 1.65 mg of protein （3.75 nmol）, and then the mixture was centrifuged. After the supernatant was neutralized with 1 M K_2HPO_4, the volume was adjusted to 1.0 ml with water, and the absorbance at 450 nm was measured. The amount of FAD was estimated to be 21.7 nmol on the basis of the molar absorption coefficient of FAD （ε = 11,300）, indicating that 6 mol of FAD were bound per mol of the enzyme protein.

Isoelectric point

The isoelectric point of the enzyme was estimated to be pH 5.3 upon isoelectric focusing on polyacrylamide gel.

Effect of pH and temperature on the enzyme activity

The enzyme activity was assayed by Method B described in ” Materials and Methods ” on varying the pH or temperature. The enzyme showed the maximum activity in the broad pH range of 7.0 to 9.0 when examined in the presence of 0.1 M potassium phosphate and sodium borate buffers.The reaction velocity declined markedly below pH 6.0.When the enzyme activity was assayed at various temperatures, the maximum activity was found at 35 ℃. The reaction velocity increased almost linearly when the temperature was raised in the range of 20 to 35 ℃, and declined rapidly over 40 ℃.

Stoichiometry of the reaction

The ratio of the amount of oxygen consumed during the enzyme reaction to those of formalehyde and hydrogen peroxide formed was approximately 1 : 1 : 1, as shown in Table 2. Therefore, it is evident that the enzyme reaction proceeds in the following equation :

$$CH_3OH + O_2 \longrightarrow HCHO + H_2O_2$$

Table 2. Stoichiometry of methanol oxidation catalyzed by alcohol oxidase. The reaction was carried out in the vessel of an oxygen electrode （Sensonix）, and the oxygen consumption was followed. The reaction mixture contained 150 μ mol of potassium phosphate buffer （pH 7.5）, 75 μ mol of methanol, and 0.35 μ g of alcohol oxidase in a final volume of 3.0 ml. After incubation at 30 ℃ for 5 min, the reaction was terminated by the addition of 0.1 ml of 5 N HCl. Formaldehyde formed ws determined by the method of Nash[14]. Hydrogen peroxide formed was measured by the ABTS — peroxidase system[13].

Oxygen consumed （μ mol）	Formaldehyde formed （μ mol）	Hydrogen peroxide formed （μ mol）
0.37	0.41	0.39

Substrate specificity

The ability of the enzyme to catalyze the oxidation of various alcohols was investigated, and the initial velocities for the substrates are shown in Table 3. In addition to methanol, which is the most preferred substrate, some short — chain normal primary aliphatic alcohols were oxidized to a lesser extent. The reactivity decreased as the chain length of the alcohols increased. The substituted ethanols, especially 2 — halogenated ethanols, were also oxidized less efficiently than ethanol. The enzyme showed little or no activity toward the secondary, tertiary, branched chain, and aromatic alcohols, under the assay conditions used in this

Table 3. Substrate specificity of alcohol oxidase.

Substrate	Relative activity (%)	Substrate	Relative activity (%)
Methanol[a]	100	2 — Chloroethanol[a]	38
Ethanol[a]	87	Methyl cellosolve[a]	4
1 — Propanol[a]	52	Ethylen glycol[a]	2
1 — Butanol[a]	20	Propylen glycol[a]	0
1 — Pentanol[a]	12	Glycerol[a]	0
2 — Propanol[a]	3	Acetaldehyde[a]	0
2 — Butanol[a]	0	Benzyl alcohol[b]	0
2 — Pentanol[a]	0	Anise alcohol[b]	0
Isobutanol[a]	2	2 — Methoxybenzyl alcohol[b]	0
Isopentanol[a]	1	3 — Methoxybenzyl alcohol[b]	0
tert — Butanol[a]	0	Vanilly alcohol[b]	0
tert — Pentanol[a]	0	Veratryl alcohol[b]	0
2 — Bromoethanol[a]	23		

[a] The oxidation of each aliphatic alcohol and acetaldehyde was determined bby the ABTS — peroxidase system (Method A) as described in " Materials and Methods " with the exceptions that methanol was replaced by each substrate at the concentration of 50 mM, and that 50 units of horseradish peroxidase was contained.

[b] The oxidation of each aromatic alcohol (final concentration, 1 mM) was assayed spectrophotometrically in the reaction mixture without ABTS and horseradish peroxidase. The formation of each corresponding aldehyde was determined by following the initial velocity of the increase in absorbance at λ_{max} of the aldehyde.

study. The apparent Michaelis constants were determined to be as follows : 1.6 mM for methanol, 6.7 mM for ethanol, and 43.5 mM for 1 — propanol.

Effect of various compounds on the enzyme activity

Various compounds were investigated for their effects on the enzyme activity (Table 4). Almost metal cations tested and ethylenediamine tetraacetic acid (EDTA) did not appreciably influence the enzyme activity except that Cu^{2+} and Hg^{2+} showed the strong inhibitory effect on the enyme activity at the concetration of 1mM. The enzyme activity was inhibited completely by such sulfhydryl reagents as *p* — chloromercuribenzoate (PCMB) and $HgCl_2$ at the concentration of 1 mM, indicating that the sulfhydryl group (s) of the enzyme is essential for the catalytic action of the enzyme.Potassium cyanide and sodium

Table 4. Effect of various compounds on a alcohol oxidase activity.

Compound	Relative activity[a] (%)	Compound	Relative activity[a] (%)
None	100	$ZnSO_4$	98
KCl	100	EDTA[b]	95
LiCl	99	*o* — Phenanthroline	100
NaCl	100	PCMB[c]	0
$BaCl_2$	100	Potassium cyanide	28
$CaCl_2$	101	Sodium azide	24
$CuSO_4$	3	Thiourea	88
$HgCl_2$	0	Dithiothreitol	96
$MgSO_4$	100	2 — Mercaptoethanol	101
$MnCl_2$	100		

[a] The enzyme activity was measured by Method B as described in " Mateials and Methods " with the standard reaction mixture in the presence of each compound at the concentration of 1 mM.

[b] ethylendiamine tetraacetic acid.

[c] p — chloromercuribenzoate.

azide were also inhibitory.

Discussion

Previous findings on methanol formation resulted from the demethoxylation of lignin [1] and lignin — related aromatic substrances [2] during the degradation of them by *P.chrysosporium*, prompted us to investigate about the enzyme of this fungus concerning the metabolism of methanol such as alcohol oxidase. Alcohol oxidase has been detected in a number of basidiomycetes [3-5], methanol — utilizing yeasts [6-9], and a brown rot basidiomycete [10]. Although the existence of alcohol oxidase in a mycelial extract of *S. pulverulentum* [1], an anamorph of *P. chrysosporium*, very little is known about *P. chrysosporium*.

In the present paper, we have shown that alcohol oxidase is produced in the mycelia of *P. chrysosporium*. The culture conditions that affect the entire ligninolytic activity of this fungus, *i.e.* oxidation of lignin to CO_2 [1,2], exhibited a parallel effect on alcohol oxidase productivity. Thus, the highest activity of the enzyme was detected in the mycelia grown under the condition of nutrient nitrogen limitation (low — N) and high oxygen atmosphere (high — O_2), which is also appropriate for the ligninolytic activity. This indicates that alcohol oxidase plays a certain role in the ligninolytic system of *P. chrysosporium*. The enzyme productivity was also affected markedly by the glucose concentration in a medium, and it was remarkably repressed by high amount of glucose in a medium.This result is in good agreement with the fact reported previously for several white — rot basidiomycetes including *S. pulverulentum* that glucose showed the strong repressive effect on the metabolism of methoxyl groups of the lignin — related aromatic substances such as vanillic acid and syringic acid to CO_2 [19,20]. Ander *et al.* [19] also reported that the metabolism of methanol to CO_2 by *S. pulverulentum* is slower in the high glucose concentration medium than in the low glucose concentration medium, whereas the metabolism of formaldehyde is not influenced by defferent glucose levels in the medium. These results are also compatible with our result obtaind in this study that the appearance of alcohol oxidase activity in the mycelia was delayed in the medium containing higher amount of glucose.

Then, we have purified alcohol oxidase to homogeneity from a mycelial extract of *P. chrysosporium* and somewhat charactetrized it. The stoichiometric study has shown that methanol is oxidized by the enzyme with oxygen as a hydrogen acceptor to yield an equimolar amount of formaldehyde and hydrogen peroxide. The enzyme is a flavoprotein containing FAD as a prosthetic group as usual oxidases. The physicochemical and enzymatic properties of alcohol oxidase from *P. chrysosporium* showed a common feature in many aspects with those of alcohol oxidases from a basidiomycete belonging to the family of polyporaceae [4], methanol — utilizing yeasts [6-9], and a brown rot basidiomycete *Poria contigua* [10] thus far reported.

It has been demonstrated that under the ligninolytic culture condition, *P. chrysosporium* produces extracellular hydrogen peroxide which plays an essential role in the ligninolytic system [21]. The importance of extracellular hydrogen peroxide in the ligninolytic system has been revealed by the findings that *P. chrysosporium* produces extracellular lignin — degrading peroxidases (named lignin peroxidase [22,23], and manganese — dependent peroxidase [24,25]) which catalyze the oxidation and partial depolymerization of lignin and the degradation of lignin substructual model compounds only in the presence of hydrogen peroxide.Ander and Eriksson [1] reported that the cultures of *P. chrysosporium* under the ligninolytic condition without added lignin or lignin — related aromatic substances in the medium were also found to contain methanol, which probably emanated from the demethoxylation of veratryl alcohol synthesized *de novo* from glucose by the fungus. Thus, it seems that alcohol oxidase may participate in the ligninolytic

system as one of donors of hydrogen peroxide. In the case of methanol — utilizing yeasts, alcohol oxidases were reported to be localized in the microbodies of the peroxisome type [26]. Therefore, alcohol oxidase of *P. chrysosporium* also may be localized in the periplasmic microbodies which was postulated to be the site of extracellular hydrogen peroxide production of *P. chrysosporium* [27]. Alcohol oxidase of *P. contigua* was also reported to be localized in the microbodies [28]. Several other schemes have been proposed for the production of extracellular hydrogen peroxide in the ligninolytic cultures of *P. chrysosporium* : three intracellular enzymes, *i. e.* fatty acyl — coenzyme A oxidase [29], glucose — 1 — oxidase [30], and glucose — 2 — oxidase [31], and two extracellular enzymes, *i.e.* manganese — dependent peroxidase [24,25,32], and glyoxal oxidase [33].

It may be also possible that alcohol oxidase plays a role of the first step in the energy — producing system from methanol, in which NAD — dependent formaldehyde dehydrogenase may be coupled with the energy reproduction system, although at this time we are unable to show the strict evidence for the existence of formaldehyde dehydrogenase in *P. chrysosporium*. We are currently investigating to show the direct evidence for the participation of alcohol oxidase to the ligninolytic system of *P. chrysosporium*.

Acknowledgements

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リグニン分解性担子菌，*Phanerochaete chrysosporium* による
アルコール酸化酵素の生産，およびその精製，諸性質

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　リグニン分解担子菌，*Phanerochaete chrysosporium* はその菌糸体中にアルコール酸化酵素を生産した。本酵素は，低窒素源濃度の培地を用い，培養気相を高酸素濃度とする培養条件において高生産された。この培養条件は本菌が良好なリグニン分解活性を示すものと完全に一致したことなどから，本酵素のリグニン分解作用への関与が推測された。また，本酵素の生産はカタボライトリプレッションを受けることが示唆された。本酵素をポリアクリルアミドゲル電気泳動的に均一状態にまで精製し，精製酵素を用いて，本酵素の蛋白化学的，酵素化学的諸性質について種々検討した。