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# Cloning of a cDNA Encoding Cellobiose Dehydrogenase, a Hemoflavoenzyme from *Phanerochaete chrysosporium*

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**Cellobiose dehydrogenase (CDH) is an extracellular hemoflavoenzyme produced by cellulose-degrading cultures of the wood-degrading basidiomycete *Phanerochaete chrysosporium*. CDH contains one flavin adenine dinucleotide (FAD) and one heme *b* per molecule, and it oxidizes cellobiose to cellobionolactone. In this report, a 2.4-kb cDNA encoding CDH was isolated by screening an expression library of *P. chrysosporium* OGC101 with a CDH-specific polyclonal antibody. The cDNA encodes a 755-amino-acid protein with a predicted mass of 80,115 Da. Sequence analysis suggests that the heme domain is located at the N terminus and that the falvin domain is located at the C terminus. The flavin domain shows a β1-αA-β2 motif for FAD binding and has high sequence similarity to several FAD-dependent enzymes. Little sequence similarity to hemoflavoenzymes is found. CDH binds to cellulose similarly to cellulases. However, little sequence similarity is observed with the conserved cellulose-binding sequences of cellulases. This suggests that CDH might possess a specific sequence for cellulose binding which is different from that of cellulases. Northern (RNA) blot analysis of total RNA from cellulose-, glucose-, and cellobiose-grown *P. chrysosporium* indicated that CDH mRNA is produced only in cellulose-grown cells. This suggests that CDH expression is regulated at the transcriptional level by either cellulose or one of its degradation products. Southern blot analysis suggests the presence of only a single gene for CDH in *P. chrysosporium* OGC101.**

Cellulolytic fungi such as *Phanerochaete chrysosporium*, *Sporotrichum thermophile*, and *Coniophora puteana* produce extracellular cellobiose dehydrogenases (CDHs). CDH is a novel hemoflavoenzyme containing one heme *b* and one flavin adenine dinucleotide (FAD) per molecule (4, 8, 14, 42). CDH oxidizes the reducing ends of cellobiose, cellooligosaccharides, and even cellulose in the presence of electron acceptors such as cytochrome *c*, quinones, and Fe(III) and Mn(III) complexes (4, 8, 26, 28, 40). Steady-state kinetic studies suggest that cellobiose is the preferred substrate and that cytochrome *c* is the preferred electron acceptor (8). In the absence of suitable electron acceptors, oxygen functions as an electron acceptor and is reduced to $H_2O_2$ (8). The heme and flavin cofactors of CDH are bound to separate domains (25, 26). Extracellular protease(s) from cellulose-degrading cultures of *P. chrysosporium* and papain hydrolyze CDH into a flavopeptide and a heme peptide (25, 26). The flavopeptide is catalytically active and oxidizes cellobiose in the presence of all electron acceptors for CDH except cytochrome *c* (25, 26). Cellulose-degrading cultures of *P. chrysosporium*, in addition to CDH, produce another cellobiose-oxidizing flavoenzyme, cellobiose:quinone oxidoreductase (6, 49). However, recent proteolysis experiments suggest that cellobiose:quinone oxidoreductase is possibly the flavopeptide formed from extracellular proteolytic degradation of CDH (25, 26). The heme iron of CDH is ferric and hexacoordinate (4, 8). Cox et al. have suggested that CDH has a histidine and a methionine as the fifth and sixth coordinates to the heme iron (19).

CDH appears to be part of the cellulolytic system of *P. chrysosporium*, because CDH is produced only when cellulose is provided as the carbon source (6) and its substrate cellobiose is formed from exocellobiohydrolase hydrolysis of cellulose. Bao and Renganathan (7) demonstrated that CDH enhances microcrystalline cellulose hydrolysis by *Trichoderma viride* cellulase. Cellobiose is an inhibitor of cellulase; CDH could be reducing this inhibition by oxidizing cellobiose to cellobionolactone (7). CDH has also been suggested to play a role in lignin degradation by *P. chrysosporium* (2). Depolymerization of lignin by lignin and manganese peroxidases generates reactive phenoxy radicals which tend to condense with themselves and with the lignin substrate. Reduction of such phenoxy radicals by CDH and cellobiose:quinone oxidoreductase has been proposed to prevent these polymerization reactions and thus increase the rate of depolymerization (2).

Among the cellulolytic enzymes produced by *P. chrysosporium*, only the cellobiohydrolase gene has been cloned and sequenced (17, 18, 43). Cellobiohydrolase appears to be encoded by a family of genes (17, 18, 43). Although endoglucanase has been purified from *P. chrysosporium*, a specific gene for that enzyme has not been isolated and sequenced (43). Sims et al. have suggested that a cellobiohydrolase I-like protein might be exhibiting endoglucanase activity in *P. chrysosporium* (43). The *P. chrysosporium* cellulolytic system is unique in that all of its enzymes—cellobiohydrolase, endoglucanase, β-glucosidase, and CDH—apparently can bind to cellulose (30, 37). We recently demonstrated that the extracellular β-glucosidase from *P. chrysosporium* binds to cellulose and that it might be organized into two domains: a cellulose-binding domain and a catalytic domain (30). Herein, we report the molecular cloning and characterization of a cDNA encoding CDH from *P. chrysosporium*. Recently, Raices et al. (36) reported a cDNA sequence for a CDH from *P. chrysosporium* K3. Although the two sequences are similar, differences in the cDNA and the deduced amino acid sequences have been observed.

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## MATERIALS AND METHODS

**Organisms.** *P. chrysosporium* OGC101 (a derivative of BKM-F-1767) was obtained from Michael H. Gold of the Oregon Graduate Institute (1). *Escherichia coli* XL1-Blue, XL1-Blue MRF′, and SOLR were obtained from Stratagene, La Jolla, Calif.

**Enzymes and nucleotides.** λZAP-cDNA synthesis and Picoblue immunoscreening kits and Gigapack II Gold packaging extract were purchased from Stratagene. [α-Thio-$^{35}$S]ATP was obtained from DuPont NEN Research Products, Boston, Mass. Oligonucleotides were prepared by Oligos Etc., Wilsonville, Oreg. The plasmid isolation kit was obtained from Qiagen, Inc., Chatsworth, Calif. Papain was purchased from Boehringer Mannheim, Inc.

**CDH polyclonal antibody.** A polyclonal antibody against CDH was raised in rabbits at the Pocono Rabbit Farm and Laboratory (Canadensis, Pa.). The CDH antiserum was used in immunoscreening without further purification. A 2,000-fold-diluted antiserum was able to detect 1 ng of CDH.

**N-terminus protein sequence analyses.** The N-terminus sequences of CDH and its heme and flavin domains were analyzed. CDH was purified from cellulose-degrading cultures of *P. chrysosporium* as described previously (6, 8). The flavin and heme domains were prepared by incubation of homogeneous CDH (3 mg) with papain (75 μg) in 0.1 M phosphate buffer (pH 7 [1 ml]) containing EDTA (2 mM) and dithiothreitol (2 mM) for 3 h at room temperature as described previously (26). The reaction products were purified on a Sephacryl S-200 column (58 by 2.8 cm) equilibrated with 50 mM phosphate (pH 6). CDH elution was monitored by cytochrome *c* assay (8); elution of the flavin and heme domains was monitored by their $A_{420}$ and $A_{450}$, respectively. Heme and flavin domains were further purified by fast protein liquid chromatography (FPLC) with a Mono-Q column (Pharmacia Fine Chemicals, Piscataway, N.J.). FPLC separations were performed in 10 mM Tris-HCl (pH 8), and proteins were eluted with a 1 M NaCl gradient. Fractions containing heme and flavin domains were separated and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The N-terminus sequence was analyzed by the Edman degradation method. These analyses were performed at the Center for Gene Research and Biotechnology, Oregon State University, Corvallis, Oreg.

**cDNA library construction and screening.** *P. chrysosporium* was grown in a succinate medium containing 1% cotton linters as the carbon source (6). On the 11th day, cells were harvested by filtration and homogenized with a Polytron homogenizer in sodium citrate buffer (25 mM [pH 7]) containing guanidinium isothiocyanate (50%) and lauryl sarcosine (0.5%). The total RNA was isolated from the cell extract by CsCl centrifugation (16). Poly(A) RNA was separated from the total RNA with an oligo(dT) cellulose column (3). A cDNA library was prepared from the poly(A) RNA by the method of Gubler and Hoffman (23), with a commercial λZAPII cDNA synthesis kit (Stratagene). The lambda library was packaged (Gigapack II Gold packaging extract [Stratagene]) and plated on *E. coli* XL1-Blue MRF′. The plaques were screened with anti-CDH antibody and a secondary antibody labeled with alkaline phosphatase.

**cDNA sequencing.** The pBluescript SK(−) plasmid containing a putative CDH cDNA insert was rescued by in vivo excision with a helper phage. The plasmid was purified with a commercial plasmid isolation kit (Qiagen, Inc.) (10). The cDNA was sequenced by the dideoxy method with the primer walking strategy (41, 44). All DNA sequencing was performed with a TAQuence version 2.0 sequencing kit (U.S. Biochemicals, Cleveland, Ohio). Initial sequencing was performed with vector primers flanking the cDNA. The internal sequences were obtained with cDNA-specific 17-mer oligonucleotides. Chain extension products were labeled with [α-$^{35}$S]ATP. 7-Deaza-dGTP was substituted for dGTP to avoid compression artifacts in G+C-rich regions of cDNA. Sequence analysis was performed with DNASTAR. PCR was used to determine the 5′-end sequence of CDH cDNA. The cDNA library was amplified with the vector-flanking T3 primer as the 5′ primer and a 3′ primer (ACAGGGTCGGTGATACCAGTG) designed from a CDH cDNA sequence (32). The PCR conditions used were 35 cycles of 92°C for 1 min, 55°C for 1 min, and 72°C for 5 min, with 2.5 U of *Taq* polymerase (Promega, Madison, Wis.). The PCR product was subcloned into the PGEM-T vector (Promega) and sequenced as described previously (32).

**Northern (RNA) blot analysis.** Total RNA was isolated from 11-day-old mycelia of *P. chrysosporium* cultured with 1% cotton linters, cellobiose, or glucose as the carbon source. RNA was electrophoresed in 1.5% agarose gel containing 2.2 M formaldehyde, transferred to Magnagraph nylon membranes (Microseparations, Inc.), and probed with CDH cDNA at 42°C as described previously (13).

**Southern blot analysis.** DNA from *P. chrysosporium* OGC101 and K3 was restriction digested and electrophoresed with a 0.7% agarose gel. The DNA was transferred to Magnagraph nylon membranes and hybridized to a $^{32}$P-labeled CDH cDNA fragment (nucleotides 192 to 591) (12). For this purpose, a PCR product of CDH cDNA from nucleotide 192 to nucleotide 591 was prepared, and this DNA fragment was used as a template for random-primed synthesis of a $^{32}$P-labeled probe.

**Nucleotide sequence accession number.** The *P. chrysosporium* OGC101 CDH cDNA sequence data reported here have been deposited in GenBank under accession number U46081.

## RESULTS AND DISCUSSION

CDH is a novel hemoflavoenzyme containing one FAD and one heme *b* per molecule (4, 8). Other enzymes that could be characterized as hemoflavoenzymes include flavocytochrome $b_2$ or lactate dehydrogenase from *Saccharomyces cerevisiae* (51), flavocytochrome *c* or fumarate reductase from *Shewanella putrefaciens* (34), spermidine dehydrogenase from *Serratia marcescens* (45), mandelate dehydrogenase from *Rhodotorula graminis* (52), rubredoxin oxidase from *Desulfovibrio gigas* (15), fatty acid monooxygenase from *Bacillus megaterium* (33), and the nitric oxide synthetases from murine macrophages, rat cerebellum, and bovine aortic endothelial cells (31). Except for CDH, all of the other hemoflavoenzymes are intracellular (15, 31, 33, 34, 45, 51, 52). Among these enzymes, flavocytochrome $b_2$, fumarate reductase, fatty acid monooxygenase, and nitric oxide synthetase have been cloned and sequenced (11, 24, 31, 34, 39).

**CDH cDNA sequence.** Twenty-six positive clones of CDH were isolated by immunoscreening of the cDNA library. A clone with the largest insert (2.5 kb) was sequenced and analyzed (Fig. 1). The sequence at the 5′ end was obtained by PCR amplification of the library with a 3′-specific primer and a T3 primer. Sequence analysis revealed an open reading frame consisting of 2,319 bp encoding 773 amino acids. The open reading frame reported by Raices et al. (36) has 2,310 bp encoding 770 amino acid residues. The open reading frame reported here is flanked by 17 bp in the 5′-noncoding region and 88 bp in the 3′-noncoding region, excluding the poly(A) tail. The G+C contents of the coding region and 5′- and 3′-noncoding regions are 59.2, 58.8, and 38.7%, respectively. The G+C contents of the coding and the 5′-noncoding regions are similar to that of *P. chrysosporium* genomic DNA (59%); however, the G+C content of the 3′-noncoding region is lower than that of genomic DNA (35). The order of preference for the third codon in a codon family is C>>G>T>>A. Highly expressed or constitutive genes of filamentous fungi prefer codons ending in C and avoid codons ending in A (5); similar codon bias has been observed for lignin and manganese peroxidases produced by *P. chrysosporium* (20, 38). CDH constitutes approximately 1% of the extracellular proteins found in cellulose-degrading cultures of *P. chrysosporium* (6).

**Authenticity of the CDH cDNA clone.** To verify that this cDNA codes for CDH, the protein sequence of CDH was compared with the cDNA sequence. The N terminus of CDH is blocked; consequently an N-terminus sequence of CDH protein could not be obtained for comparison with the cDNA sequence. The holoenzyme was hydrolyzed to two peptide fragments, one containing the heme and the other containing the flavin cofactor (26); the individual peptides were purified, and their N termini were sequenced. The heme domain did not provide any sequence, suggesting that its N terminus is blocked; this finding also suggested that this domain is located at the N terminus of the holoenzyme. A sequence of 28 amino acids at the N terminus of the flavin domain was obtained. This sequence, TGPXVXAXPYDYIIVGAGPGGIIAADRL, matched residues 203 to 235 of the cDNA translation product except at locations 211 (T), 213 (S), and 215 (T) (Fig. 1). Since the unidentified residues are hydroxyamino acids, they might be glycosylated and thus could have escaped detection. Expression cloning with a CDH antibody and the match between the cDNA and protein sequences suggest that this cDNA encodes CDH.

**CDH structure.** CDH is a secreted enzyme and, therefore, is likely to possess a signal peptide (SP) sequence. Since the N-terminus amino acid of mature CDH was not determined,

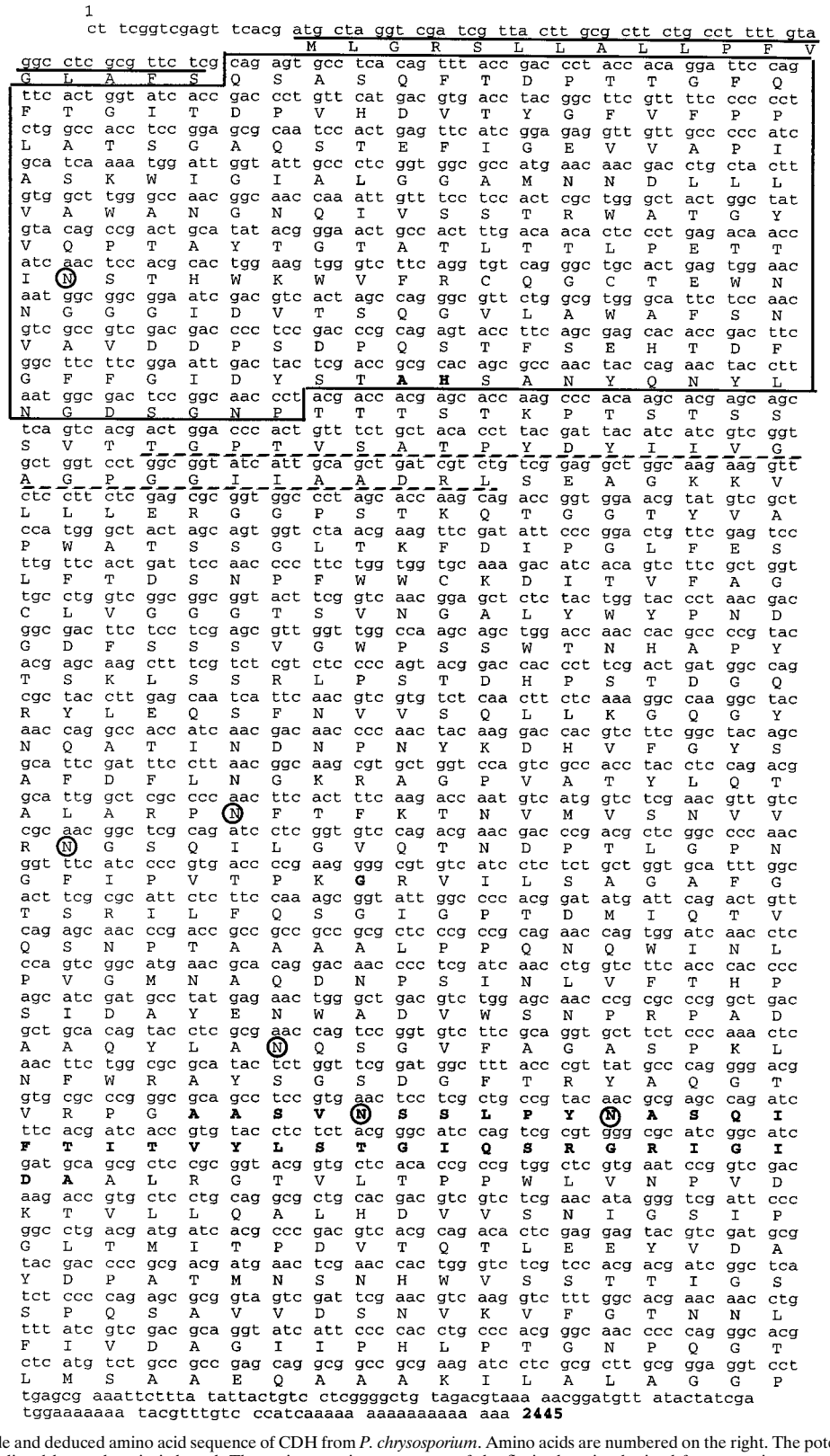

FIG. 1. Nucleotide and deduced amino acid sequence of CDH from *P. chrysosporium*. Amino acids are numbered on the right. The potential signal peptide sequence is overlined. The predicted heme domain is boxed. The amino-terminus sequence of the flavin domain obtained from protein sequencing is indicated by a dotted underline. Potential N-glycosylation sites are circled. Amino acid sequence which does not correspond to that of Raices et al. (36) is indicated in boldface type.

the SP cleavage site and the size of the signal peptide could not be deduced. However, the empirical approach of von Heijne (47, 48) for SP identification suggests that the first 18 amino acids of the cDNA translation product constitute the SP sequence, and the most probable cleavage site is located between Ser and Gln. The latter is presumably the N-terminus amino acid and is numbered 1 in Fig. 1. This is in agreement with the findings of Raices et al. (36). Thus, the mature protein of CDH appears to consist of 755 amino acids and has an apparent molecular weight of 80,115. According to Raices et al. (36), the mature protein of CDH consists of 752 amino acids and has an apparent molecular weight of 80,313. The CDH molecular weight as determined by SDS-PAGE is 90,000 (4, 8). CDH is a glycoprotein (8), and the difference in molecular weight could be attributable to the carbohydrate portion. The cDNA sequence revealed six potential N glycosylation sites conforming to the general rule Asn-X-Thr/Ser in which X is not a proline (9, 27). In addition, numerous O glycosylation sites are possible.

In the yeast flavocytochrome $b_2$, the heme domain is at the N terminus and the flavin domain is at the C terminus. The two domains are joined by a linker region (24, 51). A similar organization is found in CDH. Amino acids 1 to 192 could form the heme domain; residues 193 to 207, which are enriched with hydroxyamino acids, could form the linker region; and residues 208 to 755 could form the flavin domain. A methionine and a histidine have been suggested as the fifth and sixth coordinates of the heme iron (19). Identification of four histidines and at least one methionine in the heme domain sequence further supports this suggestion.

**Comparison with FAD-dependent enzymes.** FAD-dependent enzymes possess a conserved β1-αA-β2 motif for binding the ADP substructure of FAD (50). This motif is usually located at the N terminus of the protein. In this motif, comprising about 30 amino acids, there are three conserved glycine residues with the sequence Gly-X-Gly-X-X-Gly, where X is any residue (50). In addition, there are six hydrophobic residues, which form a hydrophobic core between the helix and the β-strand, and one conserved Asp (50). In CDH, this FAD-binding fingerprint is located at the N terminus of the flavin domain between residues 218 and 246. The flavin domain of CDH exhibited sequence homology at the N terminus with other FAD-dependent enzymes, such as glucose oxidase from *Aspergillus niger* (21), methanol oxidase from *Hansenula polymorpha* (29), and alcohol dehydrogenase from *Pseudomonas oleovorans* (46) (Fig. 2). The C terminus of CDH also exhibits extensive sequence similarity with these enzymes. The level of sequence similarity between CDH and these FAD-dependent enzymes is approximately 50%.

**Comparison with hemoflavoenzymes.** Hemoflavoenzymes are organized into two domains—an N-terminal heme-binding domain and a C-terminal flavin-binding domain (31, 33, 34, 51, 52). In the case of flavocytochrome $b_2$ and mandelate dehydrogenase, the flavin mononucleotide-binding domain oxidizes the organic substrate by two electrons and subsequently transfers these electrons to the heme domain one electron at a time (51, 52). The heme domain in turn transfers the electrons to ferricytochrome *c*. The heme domain of the dehydrogenase class is a hexacoordinated cytochrome $b_5$ or cytochrome *c* (34, 51, 52), whereas the corresponding domain for monooxygenases such as fatty acid monooxygenase and nitric oxide synthetases is a cytochrome P-450 (31, 33). Although many biochemical properties of CDH are very similar to those of flavocytochrome $b_2$ (4, 8), it exhibits very little sequence homology with flavocytochrome $b_2$.

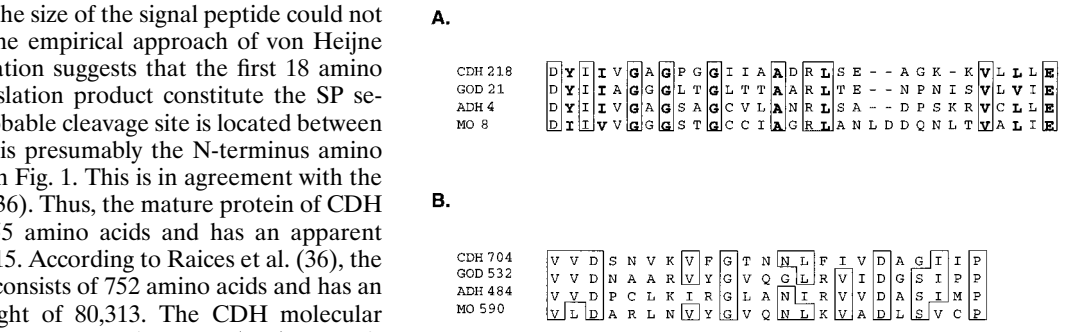


FIG. 2. Comparison of the CDH flavin domain with FAD-dependent enzymes. (A) The amino-terminus region of CDH (amino acids 218 to 246) is compared with that of glucose oxidase (GOD) (amino acids 21 to 50) from *Aspergillus niger* (21), alcohol dehydrogenase (ADH) (amino acids 4 to 33) from *Pseudomonas oleovorans* (46), and methanol oxidase (MO) (amino acids 8 to 39) from *Hansenula anomala* (29). (B) The carboxy-terminus region of CDH (amino acids 704 to 726) is compared with those of GOD (amino acids 532 to 555), ADH (amino acids 484 to 506), and MO (amino acids 590 to 612). N-terminus residues involved in FAD binding are indicated in boldface type. Identical amino acids are enclosed in solid boxes.

**Cellulose binding by CDH.** Earlier we demonstrated that CDH binds to microcrystalline cellulose and enhances the hydrolysis of this cellulose by *Trichoderma* cellulases (7, 37). Recent experiments suggest that CDH can also bind to cotton linters and filter paper and can also enhance the hydrolysis of these celluloses by *Trichoderma* cellulases (44a). The flavin domain of CDH binds to crystalline cellulose, whereas the heme domain does not bind to cellulose, suggesting that the

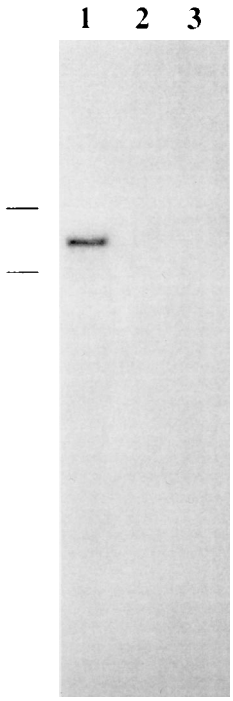


FIG. 3. Northern analysis of *P. chrysosporium* RNA. Total RNA was isolated from 11-day-old mycelia obtained from 1% cellulose (lane 1), glucose (lane 2), or cellobiose (lane 3). The RNA was fractionated by electrophoresis in 1% agarose containing 2.2 M formaldehyde and transferred to a Magnagraph nylon membrane (13). The blot was probed with $^{32}$P-labeled CDH cDNA. Bars to the left indicate the positions of 18S and 28S rRNA.

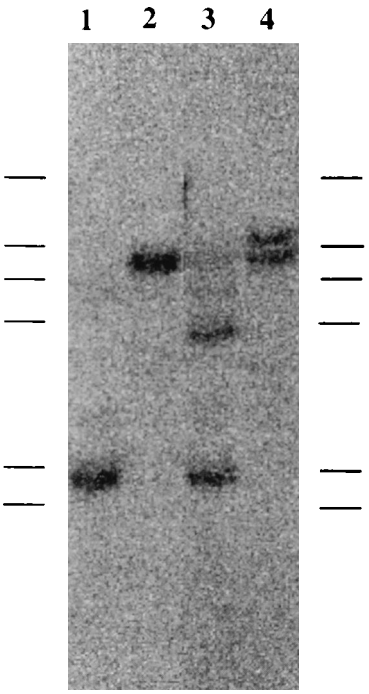


FIG. 4. Southern analysis of genomic DNA from *P. chrysosporium* OGC101 and K3. Genomic DNA, isolated by standard procedures, was digested with the restriction enzymes *Hin*dIII (lanes 1 and 3) and *Sac*I (lanes 2 and 4). The DNA was electrophoresed in 0.7% agarose and transferred to a Magnagraph nylon membrane. The blot was probed with a $^{32}$P-labeled fragment (nucleotides 192 to 591) of CDH cDNA. Bars indicate the positions of molecular size standards (from top to bottom) 23, 9.4, 6.6, 4.4, 2.3, and 2.0 kb.

cellulose-binding characteristic of CDH resides in the flavin domain (25, 26).

Cellulose-binding bacterial and fungal cellulases are organized into two domains, a cellulose-binding domain (CBD) and a catalytic domain (22). These two domains are usually connected by a linker region consisting of proline and threonine repeats. In fungal cellulases such as those of *Trichoderma reesei* and *P. chrysosporium*, the CBD consists of a conserved sequence of 33 amino acids (22). In bacterial cellulase, the CBD is larger and consists of approximately 100 amino acids (22). Proteolytic hydrolysis experiments have not indicated a separate CBD in CDH (25, 26). Also, comparison of the CDH amino acid sequence with that of fungal and bacterial cellulases did not reveal any obvious sequence similarity. Thus, the amino acid sequence which enables CDH to bind to cellulose remains unknown.

*P. chrysosporium* produces CDH abundantly only when cellulose is provided as the sole carbon source (6). To obtain further evidence that CDH expression is regulated by cellulose, total RNA was isolated from 11-day-old cellulose, cellobiose, or glucose cultures and analyzed by Northern blotting (Fig. 3). A band corresponding to 2.5 kb was observed only with the RNA isolated from cellulose-grown cells. Also, the size of this RNA was very similar to the size of the cDNA insert. These preliminary findings suggest that either cellulose or one of its degradation products is controlling the expression of CDH at the transcriptional level.

A Southern blot of DNA from *P. chrysosporium* OGC101 and K3 is shown in Fig. 4. Strain OGC101 was used in this study, and strain K3 was used by Raices et al. (36). DNA was digested with *Hin*dIII and *Sac*I. Southern blot analysis of

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1830 CCG GGC GCA GCC TCC GTG AAC TCC TCG CTG CCG TAC AAC GCG AGC
 587 P   G   A   A   S   V   N   S   S   L   P   Y   N   A   S
1807 CCG GGC --A GCC TCC GTG AAC TCC TCG CTG CCG TAC AAC GCG AGC
 585 P   G     S   L   R   E   L   L   A   A   V   Q   R   E   P

     CAG ATC TTC ACG ATC ACC GTG TAC CTC TCT ACG GGC ATC CAG TCG
     Q   I   F   T   I   T   V   Y   L   S   T   G   I   Q   S
     CAG ATC TTC ACG ATC ACC GTG TAC CTC TCT ACG GGC ATC CAG TCG
       D   L   H   D   H   R   V   P   L   Y   G   H   P   V   A

     CGT GGG CGC ATC GGC ATC GAT GCA GCG CTC 1949
     R   G   R   I   G   I   D   A   A   L   626
     CGT GGG CGC ATC GGC ATC GAT -CA GCG CTC 1923
       W   A   H   R   H   R   S     A   L   623
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FIG. 5. Comparison of nucleotide and deduced amino acid sequences from nucleotides 1830 to 1949 of this study (top) and 1807 to 1923 of the study of Raices et al. (36) (bottom). Nucleotides that were absent in the sequence from Raices et al. (36) are indicated in boldface type.

OGC101 DNA showed that only one restriction fragment (2.1 kb for *Hin*dIII, 8.0 kb for *Sac*I) from each digest hybridized to the probe, which suggests that CDH is probably encoded by a single gene in this strain (Fig. 4). However, two fragments (2.1 and 4.0 kb for *Hin*dIII, 8.0 and 9.4 kb for *Sac*I) from strain K3 hybridized to the probe, suggesting the presence of a potentially distinct allele for CDH in strain K3 (Fig. 4).

The findings of this study with regard to the heme, flavin, and CBD structures of CDH are similar to those of Raices et al. (36). The two cDNA sequences are 99.6% similar; however, variations in the amino acid sequences deduced from the corresponding cDNA sequences have been observed. In the heme domain at residues 176 and 177, this study predicts an Ala and a His, and the corresponding cDNA sequences are GCG and CAC. In the previous report (36), a single Asp-176 with the cDNA sequence GAC was predicted in the place of Ala-176 and His-177. In the flavin domain at position 460, this study predicts a Gly, which is absent in the other sequence. Protein sequence similarity was not observed between residues 587 and 621 of Raices et al. (36) and 589 and 624 of this study; however, the nucleotide sequence in that region showed 97% similarity (Fig. 5). Two base pairs, G-1836 and C-1837, found at the 5′ end of this cDNA sequence are absent in the other sequence, possibly resulting in a reading frame that has been shifted by 2 bp. The reading frame-shifted sequence apparently returns to the original reading frame by the loss of G-1941 of this cDNA sequence (Fig. 5). The net result is that the earlier sequence lacks 3 bp or one amino acid in this region. A portion of the sequence in question in the flavin domain is partially conserved in FAD-dependent enzymes (Fig. 6). Only the sequence reported here indicates a similar conserved sequence (Fig. 6). The amino acid composition calculated from the cDNA sequence of this study correlates with the amino acid composition of the CDH protein reported by Raices et al. (36). A large deviation is found in the numbers of His and Arg residues. The earlier study reports 15 His and 21 Arg residues, whereas protein amino acid analysis indicates 11 His and 17 Arg resi-

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CDH 615   Q S R G R I G I D
ADH 390   K S R G R I G L K
CHD 392   P S R G H V R I K
GDH 457   R S R G F I G L R
GOD 431   F T R G Y V H I L
CDH 612   P V A W A H R H R
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FIG. 6. Comparison of dissimilar protein sequences of CDH with those of FAD-dependent enzymes. The CDH sequence at the top is from this study. The CDH sequence at the bottom was reported by Raices et al. (36). ADH, alcohol dehydrogenase from *P. oleovorans* (46); CHD, choline dehydrogenase from *E. coli* (36); GDH, glucose dehydrogenase from *Drosophila melanogaster* (36); GOD, glucose oxidase from *A. niger* (21). Identical amino acids are enclosed in boxes.

dues (36), and this sequence predicts 11 His and 18 Arg residues.

It is possible that different alleles were sequenced in the two studies and that the observed variations therefore reflect the allelic sequence differences. This possibility is supported by Southern analysis (Fig. 4), which suggests that whereas the CDH from strain OGC101 is encoded by one gene, the CDH from strain K3 might be encoded by different alleles. Differences could also be due to sequencing error(s) in one or both studies, in which case further studies will be necessary to identify the correct sequence. A genomic sequence of CDH from strain OGC101 obtained by this laboratory (29a) matched the CDH cDNA sequence reported here.

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