Homology between cellobiase oxidase from \textit{Phanerochaete chrysosporium} and other proteins

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The white-rot fungus \textit{Phanerochaete chrysosporium} releases two enzymes that oxidize cellobiase and higher cellodextrins when grown with cellulose as carbon source [1]. Cellobiase oxidase (EC 1.1.3.25) has haem and flavin prosthetic groups and M, by SDS-PAGE of 90 kDa [1]. It has recently been cleaved by papain into fragments containing the flavin and haem, with molecular weights of approximately 35 and 35 kDa respectively [2]. Cellobiase quinone oxidoreductase (EC 1.1.5.1; abbreviated CBQase) has flavin as sole prosthetic group and M, by SDS-PAGE of 60 kDa [3].

We have studied the relationship between these enzymes using immunological blot and partial proteolytic digestion analyses [4]. Partial digestion with Staphylococcal V8 protease or with cyanogen bromide yielded fragments which when separated on SDS-PAGE gave many identical bands. Polyclonal antibodies were raised in rabbits against both purified proteins. Both antibodies were found to cross-react with both proteins on western blots. These findings indicate a high degree of homology between cellobiase oxidase and CBQase.

A haem protein has been partially purified from the extracellular medium which ran on LDS-PAGE with M, 31 kDa. This haem protein cross-reacted with anti-cellobiase oxidase antibody, but not with antibody to CBQase. Sulphite bleached the flavin of cellobiase oxidase, but did not react with the 31 kDa haem protein, suggesting an absence of flavin. It therefore seems likely that CBQase and the 31 kDa haem protein are formed from cellobiase oxidase by proteolytic cleavage.

We have obtained information on the primary structure of cellobiase oxidase. Amino acid sequence analysis was carried out by pulsed-liquid degradations in an Applied Biosystems 477A protein sequencer. The first samples tested were the intact native proteins: cellobiase oxidase had a blocked N-terminal and the N-terminal sequence of CBQase was

\[ S \ G \ N \ P \ (K) \ (P). \]

No further sequence could be obtained since the proline(s) were cleaved with low efficiency.

In addition, a 4 kDa fragment obtained from a 7 hour CNBr digest of cellobiase oxidase has been sequenced. CNBr derived peptides were separated by electrophoresis in a 16.5% polyacrylamide-tricine-SDS gel with a 10% spacer gel and 4% stacking gel [5], then electroblotted onto a sheet of ProBlott polyvinylidene difluoride membrane. Protein was detected by Coomassie blue staining and then bands of interest were excised for sequencing. The sequence of the 4 kDa fragment was:

\[ N \ S \ N \ H \ W \ V \ S \ S \ T \ T \ I \ G \ (S). \]

This sequence was used to search the Swiss protein database at the European Molecular Biology Laboratory, Heidelberg using the Genetics Computer Group Sequence Analysis Software Package [6]. This fragment scored 45% identity and 64% similarity in an 11 amino acid overlap with endoglucanase I from the related fungus \textit{Trichoderma reesei} [7].

2 SNHWSSTTTIG 12 CNBr fragment

409 STTRSSSTSS 419 Endoglucanase I

This region of endoglucanase I is part of the linker joining the catalytic domain to the cellulose-binding domain [8]. Linkers in cellulases are thought to form extended, flexible hinges between domains [8]. Typically, they are rich in hydroxyamino acids and may also be relatively rich in hydrophobic amino acids [8].

The substrates oxidized by cellobiase oxidase include microcrystalline cellulose [S.M. Kremer & P.M. Wood, to be published]. Henriksson et al. [2] have shown that cellobiase oxidase binds to cellulose to a similar extent as cellobiohydrolase I, and that cleavage by papain gives a flavin domain with similar affinity for cellulose, and a non-cellulose binding haem domain. Renganathan et al. [9] have proposed that cellobiase oxidase and CBQase both contain a cellulose-binding domain. We have found that cleavage of CBQase with CNBr appears to generate the same 4 kDa fragment. Therefore, it is likely that this fragment forms part of a linker joining the catalytic flavin domain to the cellulose-binding domain of cellobiase oxidase.

Cellobiase oxidase was cleaved with hydroxylamine according to ref. [10] and two fragments of M, approximately 70 and 25 kDa were generated. CBQase however was not cleaved. The 70 kDa fragment cross-reacted with both anti-cellobiase oxidase and anti-CBQase antibodies on western blots, whereas the 25 kDa fragment only reacted with anti-cellobiase oxidase antibody. Hydroxylamine cleavage under these conditions is diagnostic for Asn-Gly, implying that cellobiase oxidase contains a single Asn-Gly bond located within the haem domain.

The information we have allows us to speculate that cellobiase oxidase contains at least three domains: an N-terminal haem domain which is N-terminally blocked and contains a single Asn-Gly bond; a central flavin domain which is joined via a flexible linker to the third domain; and a C-terminal cellulose-binding domain.

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References