

Assessing the function of CDH and CDH-like hemoproteins in the degradation of lignocellulosic biomass in the fungus *Myceliophthora thermophila* C1

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Summary

We have analyzed the genome of *Myceliophthora thermophila* C1 for cellobiose dehydrogenases (CDHs) and found three such encoding genes. One CDH was found to be a class IIa enzyme containing a C-terminal type 1 cellulose binding module (CBM). A second CDH belonged to class IIb, lacking such a CBM, and a third CDH was identified that, surprisingly, lacked the N-terminal heme domain. Interestingly, in addition to these three CDHs, a number of genes was found encoding small extracellular enzymes that are homologous to the CDH-heme domain. Moreover, the genome of C1 contains a large number of GH61s encoding genes. We propose a short electron-transfer chain where the electron equivalents produced by the three CDHs are transferred to the GH61s aided by the small hemoproteins, thus enhancing the degradation of cellulosic materials.

Introduction

Dyadic develops the filamentous fungus *M. thermophila* C1, an ascomycete which was previously classified as *Chrysosporium lucknowense*, as a proprietary protein expression platform for the efficient production of enzyme mixtures for the complete degradation of lignocellulosic biomass. The genome of C1 wild-type strain VKM F-3500-D was initially sequenced in 2005 by Sanger-sequencing and recently re-sequenced by a paired-end approach using the 454 pyro-sequencing technology. Both datasets were assembled into a new C1 draft genome. The genome size was approximately 38.5 Mbp of which 37.2 Mbp were assigned to the 10 largest scaffolds. The data revealed an impressive number of (secreted) carbohydrate active enzymes, including 26 proteins belonging to family GH61. Members of this family enhance the degradation of cellulosic materials and are proposed to be redox-active hydrolytic enzymes (oxidohydrolases), that require a reducing environment for optimal activity¹. A possible source of reducing equivalents in C1 is CDH. Here we present a systematic analysis of the C1 genome for CDH and CDH-like genes and discuss their involvement in the degradation of lignocellulosic material.

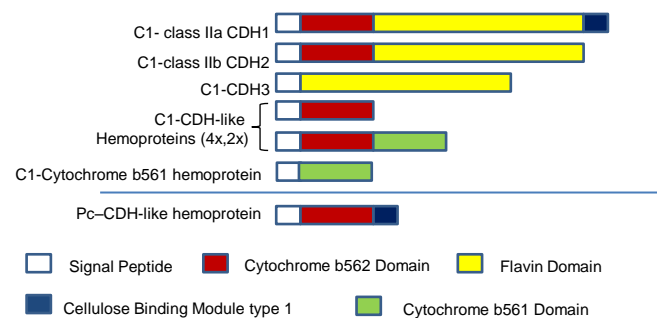


Fig. 1. Domain organization of CDH and CDH-like hemoproteins identified in the C1 genome. The *Phanerochaete chrysosporium* cytochrome b562 protein is also shown for comparison.

Cellobiose dehydrogenases

CDHs are extracellular flavocytochromes that catalyze the oxidation of cellobiosaccharides such as cellobiose and have previously been linked to the degradation of lignocellulosic materials in basidiomycetes as well as ascomycetes. CDHs have been divided into three classes. Class I represents only basidiomycetous CDHs and class II comprises exclusively ascomycetous CDHs. Class II enzymes are subdivided into class IIa enzymes containing a type 1 CBM and class IIb enzymes that lack such a domain. C1 contains both a IIa and a IIb CDH (Fig. 1) and these two CDHs were isolated from a C1 culture (Fig. 2). In addition to these Class II CDHs a third gene was identified that, surprisingly, lacked the N-terminal heme domain. Work is currently in progress to isolate and characterize the remaining CDH.

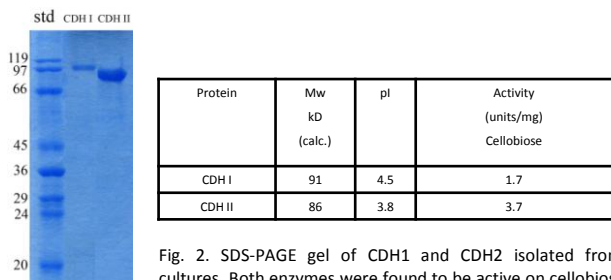


Fig. 2. SDS-PAGE gel of CDH1 and CDH2 isolated from C1 cultures. Both enzymes were found to be active on cellobiose.

CDH-like hemoproteins

The CDH-like hemoproteins are small secreted proteins containing a cytochrome b562-type heme. One such hemoprotein from *Phanerochaete chrysosporium* was shown to be redox active². Interestingly, this protein contained a C-terminal CBM suggesting a function in the degradation of cellulosic materials (Fig. 1). The genome of *M. thermophila* C1 contains six of these hemoproteins that are all predicted to be secreted. Two of these proteins contain a second heme domain that is similar to cytochrome b561 proteins.

Cytochrome b561

Cytochrome b561 proteins (cd08760) might act as a ferric-chelate reductase, catalyzing the reduction of Fe³⁺ to Fe²⁺. We propose that these proteins in C1 may be involved in the transfer of electrons supplied by CDHs or other secreted oxidoreductases. In addition to the two CDH-like hemoproteins that have this cytochrome b561 domain, the genome of C1 contains one additional small hemoprotein predicted to be a secreted single domain protein (Fig. 1).

Gene organization

The possible link between CDHs, the small hemoproteins and the GH61 oxidohydrolases is also suggested by the genetic organization. In the C1 genome we found two examples where these enzymes are clustered (Fig. 3). A GH61 was found to be clustered to the type IIb CDH and a small hemoprotein in addition to three hypothetical proteins. The non-heme containing CDH3 was found to be clustered with two GH61s.

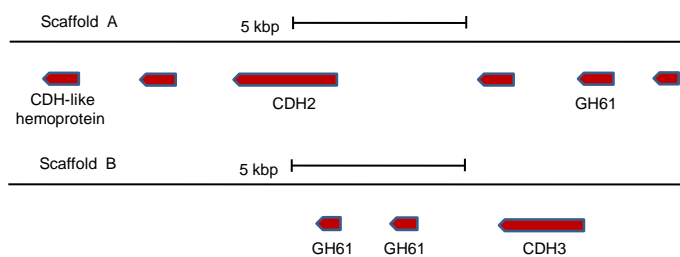


Fig. 3. Gene organization of CDHs, hemoproteins and GH61s found in the genome of C1

Proposed Mechanism

We propose that the different proteins described here closely interact in the (partial) degradation of cellulosic material, by formation of a short electron transfer chain (Fig. 4). The GH61 proteins use the reducing equivalents to catalyze the oxidation of the C1 carbon of crystalline cellulose and the subsequent hydrolysis. At this stage it is unclear whether the GH61s themselves are reduced since these enzymes also function with non redox-active metal such as calcium. We propose that the GH61 may form a Fenton-like radical species in the presence of hydrogen peroxide. A peroxide species may be formed when non-redox metals such as calcium are bound to the GH61.

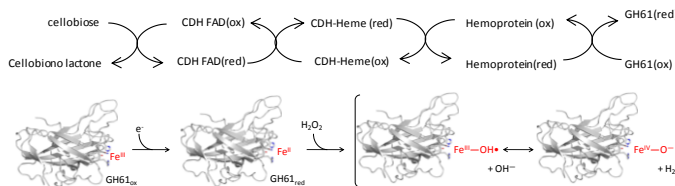


Fig. 4. Proposed mechanism of CDH, hemoproteins and GH61s in the degradation of cellulosic and other structural polysaccharide materials.

References

¹Vaae-Kolstad et al. (2010) Science 330 ²Yoshida et al. (2005) AEM 71