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Research Article

Cellobiose dehydrogenase: An essential enzyme for lignocellulose degradation in nature – A review

Cellobiosedehydrogenase: Ein essentielles Enzym für den Lignozelluloseabbau in der Natur – Eine Übersicht

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Summary

The flavin and heme cofactor containing enzyme cellobiose dehydrogenase (CDH) is ubiquitously distributed in wood-degrading fungi. Current research provides compelling evidence that CDH is an activator for cellulolytic monooxygenases, which enhance the accessibility of crystalline cellulose surfaces for hydrolases. Such oxidative cellulose degradation contributes to the overall cellulolytic capabilities of wood decaying fungi to a large extent, and holds great potential to improve the efficiency of commercial enzyme mixtures for biomass processing and biofuel production. This review summarizes current literature with regard to the distribution, structure and physiological role of CDH in the light of recent findings.

Keywords: Cellobiose dehydrogenase, cellulose degradation, lytic polysaccharide monooxygenase, wood-rotting fungi, biofuels

Zusammenfassung

Das Flavin- und Häm-Kofaktor-hältige Enzym Cellobiosedehydrogenase (CDH) ist ubiquitär in holzabbauenden Pilzen verbreitet. Aktuelle Forschungergebnisse zeigen eindeutig, dass CDH ein Aktivator für Zellulose spaltende Monooxygenasen ist, welche die Zugänglichkeit von kristalliner Zellulose für Hydrolasen erhöhen. Dieser oxidative Zelluloseabbau trägt wesentlich zur holzzersetzenden Fähigkeit von Pilzen bei und birgt ein großes Potenzial, die Effizienz kommerzieller Enzymmischungen zur Biomasseverzuckerung zu verbessern und eine effizientere Produktion von Biokraftstoffen aus diesen nachwachsenden Rohstoffen zu ermöglichen. Dieser Übersichtsartikel fasst die aktuelle Literatur in Bezug auf die Verteilung, die Struktur und die physiologische Rolle von CDH im Lichte der jüngsten Erkenntnisse zusammen.

Schlagworte: Cellobiosedehydrogenase, Zelluloseabbau, lytische Polysaccharidmonooxygenase, holzabbauende Pilze, Biokraftstoffe

1. Introduction

The foreseeable shortage of fossil fuels will eventually require major structural changes in the global economy. At the current extraction rate of Earth's fossil reservoirs, the coal, oil and gas reserves are expected to run dry in less than a century (Henrich et al., 2015). In addition, with the world population increasing, its collective consumption and its desire to increase affluence will put further strain on society and economy (Hoekstra and Wiedmann, 2014). Together, these developments will create a tremendous challenge for the humanity, which can only be tackled with novel industrial bioprocesses that employ renewable resources in order to sustain the global need for hydrocarbons. The development and market introduction of *green* materials as feedstock for the production of goods and energy has to be managed in time before a shortage provokes economic, social and political conflicts. Plant biomass is expected to play a critical role in the future energy mix, and could be potentially converted into a broad spectrum of goods and products (Esposito and Antonietti, 2015). However, the deconstruction into its monomeric building blocks remains a technological challenge due to the inherent recalcitrance of woody tissues. The method of choice to achieve a controlled degradation is by nature's set of highly specialized biocatalysts (Cragg et al., 2015). Understanding the complex functions and interactions of these enzymes, which evolved over several hundred million years in specialized organisms, is the *conditio sine qua non* for their application.

2. Microbial degradation of plant cell walls

Plant biomass in the form of living or dead vegetation represents a major carbon reservoir on Earth that accounts for approximately 30% (or 500 billion tons) of the total terrestrial carbon (Scharlemann et al., 2014). The balance between carbon storage and CO_2 release into the atmosphere is greatly influenced by microbial plant degraders, such as wood-rotting fungi. Decomposer fungi degrade and assimilate the most recalcitrant organic polymers in plant cell walls across all terrestrial habitats. This is mainly attributed to their highly adaptive lifestyles, reflected by a large phylogenetic and phenotypic diversity (Cragg et al., 2015). Fungi use specific sets of enzymes which they release into the environment during their growth to degrade plant cell walls into digestible sugars. Degradation is an energy-rewarding, but complex process. The plant biomass mainly consists of three structural polymers: cellulose, hemicelluloses and lignin, which are woven into a compact, recalcitrant matrix called lignocellulose. Cellulose is the main structural polymer in plant cell walls, and hence one of the largest carbohydrate reservoirs on earth. Depending on the plant species, the cellulose content varies between 35 and 50% (Willför et al., 2005). Cellulose is composed of long chains formed by β-(1,4)-interlinked D-glucoses, which are arranged in large three-dimensional networks stabilized by extensive inter-chain hydrogen bonding and van der Waals stacking interactions (Jordan et al., 2012). The partially crystalline character of cellulose is a major barrier for its biological degradation; its overall crystallinity can vary from 40 to 50% (Klemm et al., 2005). Cellulose is frequently associated with heterogeneous β-(1,4)-linked polysaccharides collectively called hemicelluloses. The composition and abundance of hemicelluloses varies within plant species and tissue types, but usually represents approximately 20% of the total plant biomass. Major polysaccharides in hemicellulose are xyloglucans, xylans, mannans and glucomannans (Scheller and Ulvskov, 2010). Both cellulose and hemicellulose are embedded in lignin, a dense aromatic polymer formed by the monolignols *p*-coumaryl, coniferyl and sinapyl alcohol. About two thirds to three quarters of all intermolecular bonds in lignin are ether bonds, which greatly contribute to the overall physical stability of the cell wall. Like hemicelluloses, lignins show a plant-specific and tissue-specific composition (Vanholme et al., 2010). In order to overcome the physical and chemical stability of lignocellulose, the wood-degrading fungi typically employ large sets of oxidative and hydrolytic enzymes with different specificities. Lignin degradation is mainly accomplished by manganese-dependent peroxidases, lignin peroxidases, versatile peroxidases and laccases (Pollegioni et al., 2015). Peroxidases *per se* are too large to penetrate the dense lignocellulosic matrix; rather, they are thought to generate small molecular radical species which catalyze the oxidation of lignin. Laccases oxidize phenolic lignin structures, but can also employ diffusible mediators which act as electron shuttles to perform oxidation reactions at non-accessible sites (Cragg et al., 2015). Fungal cellulose degradation is accomplished by a consortium of carbohydrate-active enzymes with complementary catalytic activities. Internally chain-cleaving endoglucanases introduce chain breaks in amorphous cellulose, providing access points for processive chain-end cleaving cellobiohydrolases (Ganner et al., 2012; Payne et al., 2015). The main product of cellobiohy-

drolases is the disaccharide cellobiose, which is cleaved into glucose units by the enzyme β-glucosidase. The carbohydrate-active enzymes (CAZy) database (http://www.cazy.org/) provides a comprehensive compilation of polysaccharide-degrading or -modifying enzymes, and describes families of structurally related "CAZymes" (Henrissat, 1991). The recent discovery of oxidative enzymatic processes that augment cellulose degradation prompted the introduction of a new CAZy family, termed as "Auxiliary Activity (AA)" (Levasseur et al., 2013). Especially the cellulose-active lytic polysaccharide monooxygenases (LPMO; CAZy: AA9) attracted ample attention due to their ability to directly oxidize crystalline substrate surfaces, which greatly enhances the overall degradability of cellulose (Horn et al., 2012). This review aims to highlight the role of oxidative enzymes involved in the deconstruction of plant matter. The discovery of biocatalysts targeting the highly recalcitrant cellulose surfaces not only introduced a new enzymatic paradigm, but also provides new opportunities for the refinement of commercial enzyme mixtures used for biomass processing and biofuel production.

3. Identification and general properties of cellobiose dehydrogenase (CDH)

Cellobiose dehydrogenase (CDH; EC1.1.99.18; CAZy: AA3.1) was first described in 1974 as a component of the exo-enzyme system of the wood-degrading fungus *Phanerochaete chrysosporium* (Westermark and Eriksson, 1974). Studies of the isolated enzyme revealed fast oxidation of the soluble cellulose breakdown products (cello-oligosaccharides) generated during wood decay. It was initially suggested that the concomitant reduction of various lignin-derived quinoid compounds is required to fulfill a catalytic cycle (Westermark and Eriksson, 1975; Ayers et al., 1978). The comparatively slow interaction with molecular oxygen later established the catalytic function of CDH as those of a dehydrogenase with some residual oxidase activity (Bao et al., 1993). Soon after its discovery, it became obvious that CDH is a ubiquitously distributed enzyme in the wood-decaying fungi. Consequently, CDH received ample attention in literature because of its intriguing two-domain architecture, which comprises a small electron-transferring cytochrome moiety (CYT) fused to a sugar oxidizing flavodehydrogenase (DH) domain. But despite great efforts, CDH remained in search of a function for almost four decades. A panoply of possible physiological roles was suggested by several research groups, including the reduction of aromatic radicals generated by laccases and peroxidases to prevent their repolymerization (Ander et al., 1990). Several authors also speculated that CDH may relieve cellulase inhibition via oxidation of cellobiose to cellobionic acid (Ayers et al., 1978; Igarashi et al., 1998). Cellobionic acid efficiently chelates manganese, and could thereby also support manganese peroxidases (Roy et al., 1994). Nevertheless, no single hypothesis provided a satisfactory explanation for the function of the enigmatic CYT domain. For a detailed, critical reflection on these hypotheses, see Henriksson et al. (2000) and Cameron and Aust (2001). The most widely supported hypothesis regarding CDH's true activity suggested a participation in a Fenton chemistry to create highly reactive hydroxyl radicals via the reduction of ferric compounds (Kremer and Wood, 1992a; 1992c; Mason et al., 2003). Hydroxyl radicals were previously shown to degrade cellulose and, to some extent, also lignin model compounds (Henriksson et al., 1995), and are thought to be the main lignocellulose-degradation mechanism of the brown-rot fungi (Arantes et al., 2012). To date, a series of recent publications rather support the role of CDH as a catalytic activator for lytic polysaccharide monooxygenase (LPMO). The fast interaction of CDH with LPMO makes the participation of CDH in a Fenton-type chemistry kinetically unfavorable (Tan et al., 2015), and many CDHs show little activity at low pH values (~3) (Harreither et al., 2011), which are required for efficient reduction of ferric iron complexes (Baldrian and Valášková, 2008). LPMOs cleave the most recalcitrant crystalline parts of cellulose and provide attack points for cellulases. Thus, the CDH/LPMO system greatly enhances the degradation of cellulose in an iron-independent manner, as is documented in several recent publications (Langston et al., 2011; Phillips et al., 2011b). As a consequence, CDH was recently reclassified in the CAZy database as a member of the Auxiliary Activity (AA) family AA3 (Levasseur et al., 2013).

4. Diversity and classification of cellobiose dehydrogenases

4.1 Characterization of CDH from wood-degrading fungi

To date, the literature reports the characterization of about 30 CDHs from their native fungal producers. The formation of CDH *in vitro* requires the presence of insoluble cellulose or soluble cello-oligosaccharides, which act as an

inducer (Hori et al., 2012). CDH is secreted into the extracellular space and typically represents about 0.5-2.5% of the total secreted protein on a mass basis (Zámocký et al., 2006). Most of the characterized CDHs were isolated from basidiomycetous white-rot fungi, which are ubiquitous wood degraders in boreal forests. They secrete a battery of oxidative and hydrolytic enzymes allowing them to completely mineralize complex lignocellulosic substrates. Notable examples of CDH-producing strains are the extensively studied model organisms *P. chrysosporium* and *Trametes versicolor*, which are also the first reported CDH-producers (Westermark and Eriksson, 1974). A recent, unexpected finding was the formation of CDH by the selective delignifier *Ceriporiopsis subvermispora* (Harreither et al., 2009) when grown on cellulose as the sole carbon source. A secretome analysis of the fungus grown on more complex lignocellulosic carbon sources showed that CDH is secreted in the late stages of growth, and most likely supports the weak cellulose-degrading machinery of the fungus after the depletion of lignin (Hori et al., 2014). In contrast to white-rot fungi, the cellulose-specific brown-rots contain a drastically reduced set of lignocellulose degrading enzymes. To date, CDH secretion by these fungi has only been shown for *Coniophora puteana* (Schmidhalter and Canevascini, 1993). Most brown-rot fungi lack *cdh*-encoding genes, which supports the view that they rely on other, non-enzymatic mechanisms for wood decay (Floudas et al., 2012). CDH has also been isolated from the secretomes of some plant-pathogenic species, suggesting a role in the host-pathogen interaction. The phytopathogenic fungus *Sclerotium rolfsii*, which has a host-spectrum of approx. 600 plants, is recognized as a potent producer of CDH, whose secretion has been studied and optimized (Sachslehner et al., 1997; Ludwig and Haltrich, 2003). Moreover, a proteome analysis showed the presence of CDH in the "early" secretome of the grape-infecting fungus *Botrytis cinerea*, which was collected 16 hrs post-infection. The enzymes isolated at this stage of growth presumably play an important role in establishing a successful infection (Espino et al., 2010). Another recent example is the ascomycete *Mycosphaerella fijiensis*, which secreted CDH during *in vitro* cultivation on banana leaves (Escobar-Tovaret et al., 2015). In contrast, a mass spectrometric analysis did not detect CDH in the secretomes of the ascomycetes *Aspergillus nidulans* and *Aspergillus niger* during plant infection (Couturier et al., 2012), although these organisms contain putative *cdh* sequences in their genomes. The precise role of CDH in phytopathology therefore remains unclear, but may be connected to the initial penetration of plant cell walls. In recent years a growing number of CDHs from ascomycete fungi has been reported. In contrast to mesophilic basidiomycetes, ascomycetes commonly degrade lignocellulose in more extreme habitats at elevated temperatures, higher moisture contents or at alkaline pH (Worrall et al., 1997). Ascomycetes preferentially metabolize cellulose and hemicellulose, while lignin remains generally unaffected, or is only slightly modified (Worrall et al., 1997). A comprehensive screening of 40 ascomycete fungi showed significant CDH activity in 13 strains using cellulose-based media and shaking flask cultures (Harreither et al., 2011). The cellulose concentration in the cultivation medium had the highest impact on CDH activity, while the availability of the nitrogen source (peptone) seemed less important in most cases. The genome of the ascomycete model organism *Neurospora crassa* contains two active CDHs (IIA and IIB), both of which were previously expressed and characterized (Zhang et al., 2011; Sygmund et al., 2012). A proteome analysis showed a high prevalence of CDH IIA in the secretome (2.4% mass abundance) when cellulose was the sole carbon source (Phillips et al., 2011a). The corresponding *cdh* gene was significantly (at least three-fold) upregulated when the fungus was grown on complex substrates such as barley, corn, rice, soybean and wheat (Wang et al., 2015). Thus, *cdh* belongs to a core-set of genes in *N. crassa* which are induced under lignocellulolytic conditions. In contrast, growth on xylan and pectin did not enhance transcription of *cdh* (Wang et al., 2015). Among the known ascomycetous CDHs, several show elevated (neutral or alkaline) pH optima and a high thermostability. Examples are CDHs isolated from the thermophilic fungi *Corynascus thermophilus* (Harreither et al., 2011; 2012b) and *Chaetomium* sp. INBI 2-26(–) (Karapetyan et al., 2006), which have maximal catalytic activities at a neutral pH around 7. This is considerably higher than the pH optima of "classical" basidiomycete CDHs, which usually exhibit highest catalytic activities in an acidic milieu (Table 1).

4.2 Classification of CDHs

In recent years large-scale genome sequencing projects, such as the 1000 Fungal Genomes Project (Hibbett and Taylor, 2013; Hori et al., 2013), uncovered a plethora of putative *cdh* sequences which show a high overlap with *lpmo* and cellulase-encoding genes (Beeson et al., 2015).

CDH sequences are abundant in both Ascomycota and Basidiomycota, with sequence identities as low as 35%. A generally high sequence homology of the CYT domains in all *cdh* sequences, including the rare His/Met iron coordination (see Chapter 5.1 for details), suggests a common ancestor that probably existed before the separation of Dikarya into the phyla Ascomycota and Basidiomycota (Zámocký et al., 2004) about 600 mio. years ago (Floudas et al., 2012). While basidiomycete genomes contain single copies of *cdh* genes, up to three copies are found in ascomycetes. A general classification of these sequences based on maximum likelihood phylogenetic reconstruction has been proposed (Zámocký et al., 2006; 2008). As shown in Figure 1, CDHs form three well-supported phylogenetic branches: Class-I CDHs from basidiomycetes and Class-II or Class-III CDHs from ascomycetes. Class-II CDHs can be further divided into Class-IIA harboring a CBM1, with the remainder of sequences forming Class-IIB. The existence of Class-III CDHs was suggested previously (Zámocký et al., 2008), but to the best of our knowledge, none of these sequences has been functionally expressed or isolated so far.

4.3 Substrate specificity

CDHs show a high preference for soluble, β-(1,4)-interlinked saccharides, but scarcely oxidize monosaccharides. Highest catalytic efficiencies are observed for β-D-cellobiose, the dimeric subunit of cellulose, and higher soluble cello-oligosaccharides. Lactose, an epimer of cellobiose, displays comparable catalytic constants. The catalytic capabilities of many CDHs partially correlate to their phylogenetic classification, as is exemplified in Table 1. Generally, Class-I CDHs exhibit higher turnover numbers (k_{cat}, s-1) for the native substrate cellobiose, while higher affinities (lower K_M values) are reported for Class-II CDHs. The substrate spectrum of Class-II CDHs appears to be more relaxed, and also extends to the (starch-derived) β-(1,4)-linked maltose which is oxidized at a reasonable rate. In addition, the discrimination against monosaccharides in this class seems to be less pronounced compared to basidiomycetous CDHs. Notable examples are *Thielavia terrestris* and *C. thermophilus* CDHs, both of which have 4 – 70-fold lower K_M values for glucose relative to other characterized CDHs. Members from both classes

Table 1 (Tabelle 1). Kinetic properties of CDHs from various sources (Kinetische Eigenschaften von CDHs unterschiedlicher Herkunft). All values were determined with dichloroindophenol (DCIP) as terminal electron acceptor, with the exception of the *N.* crassa CDHs IIA and IIB, for which benzoquinone was used.

Source of CDH	Cellobiose		Xylobiose		Maltose		Glucose		pH	References
	k_{cat}	K_M	k_{cat}	K_M	k_{cat}	K_M	k_{cat}	K_M		
Class I										
Phanerochaete chrysosporium	16	0.11	0	0	1.1	240	2.6	1600	4.5	Henriksson et al., 1998
Trametes villosa	24	0.21	17	7.7	2.1	350	1.9	1300	4.5	Ludwig et al., 2003
Sclerotium rolfsii	27	0.12	27	5.4	0.8	240	1.5	1250	3.5	Baminger et al., 2001
Trametes hirsuta	11	0.04	0	0	2.8#	*n.d.*	1.0#	*n.d.*	5.0	Nakagame et al., 2006
Ceriporiopsis subvermispora	25	0.14	*n.d.*		1.3	300	1.0	3300	4.5	Harreither et al., 2009
Schizophyllum commune	13	0.03	*n.d.*		0	0	0	0	4.5	Fang et al., 1998
Cerrena unicolor	19	0.29	*n.d.*		*n.d.*		0	0	4.5	Sulej et al., 2015
Coprinopsis cinerea	97	0.16	*n.d.*		*n.d*		34.4	354	5.0	Turbe-Doan et al., 2013
Class II										
Humicola insolens	14	0.05	3.0	7.1	1.7	7.6	0	0	7.0	Schou et al., 1998
Neurospora crassa IIA§	46	0.09	45	3.6	0.4	17	55	3700	6.0	Sygmund et al., 2012
Neurospora crassa IIB§	11	0.02	5.8	1.3	3.5	3.4	8.1	550	6.0	Sygmund et al., 2012
Thielavia terrestris	1.2	0.04	*n.d.*		0.2	2.5	1	54	6.0	Langston et al., 2012
Myriococcum thermophilum§	13	0.01	*n.d.*		1.2	11	14	250	6.0	Zámocký et al., 2008
Corynascus thermophilus	13	0.08	*n.d.*		*n.d.*		13	93	7.5	Harreither et al., 2012
Podospora anserina	8.6	0.13	*n.d.*		*n.d.*		1.6	329	6.0	Turbe-Doan et al., 2013

k_{cat}, (s^{-1}); K_M, (mM); n.d., not determined; § recombinantly produced enzyme; # estimated from rel. activity (cellobiose=100%)

(I and II) are reported to oxidize hemicellulose-derived sugars, such as xylobiose and higher xylooligosaccharides, and also conversion of galactosyl-mannose was reported (Henriksson et al., 1998). *P. chrysosporium* CDH also oxidized the β-(1,4)-linked disaccharide 2α-mannobiose (Bao et al., 1993), which was not observed for *N. crassa* CDH IIA and IIB. However, the latter enzymes readily oxidized mannopentaose (Sygmund et al., 2012). Several studies also showed the potential of CDH to oxidize the reducing end groups of insoluble cellulose fibers. Some authors reported catalytic activity of *P. chrysosporium* CDH in the presence of cellulose (Morpeth, 1985; Kremer and Wood, 1992b). Pricelius et al. (2010) observed that incubation of pure *M. thermophilum* CDH with cot-

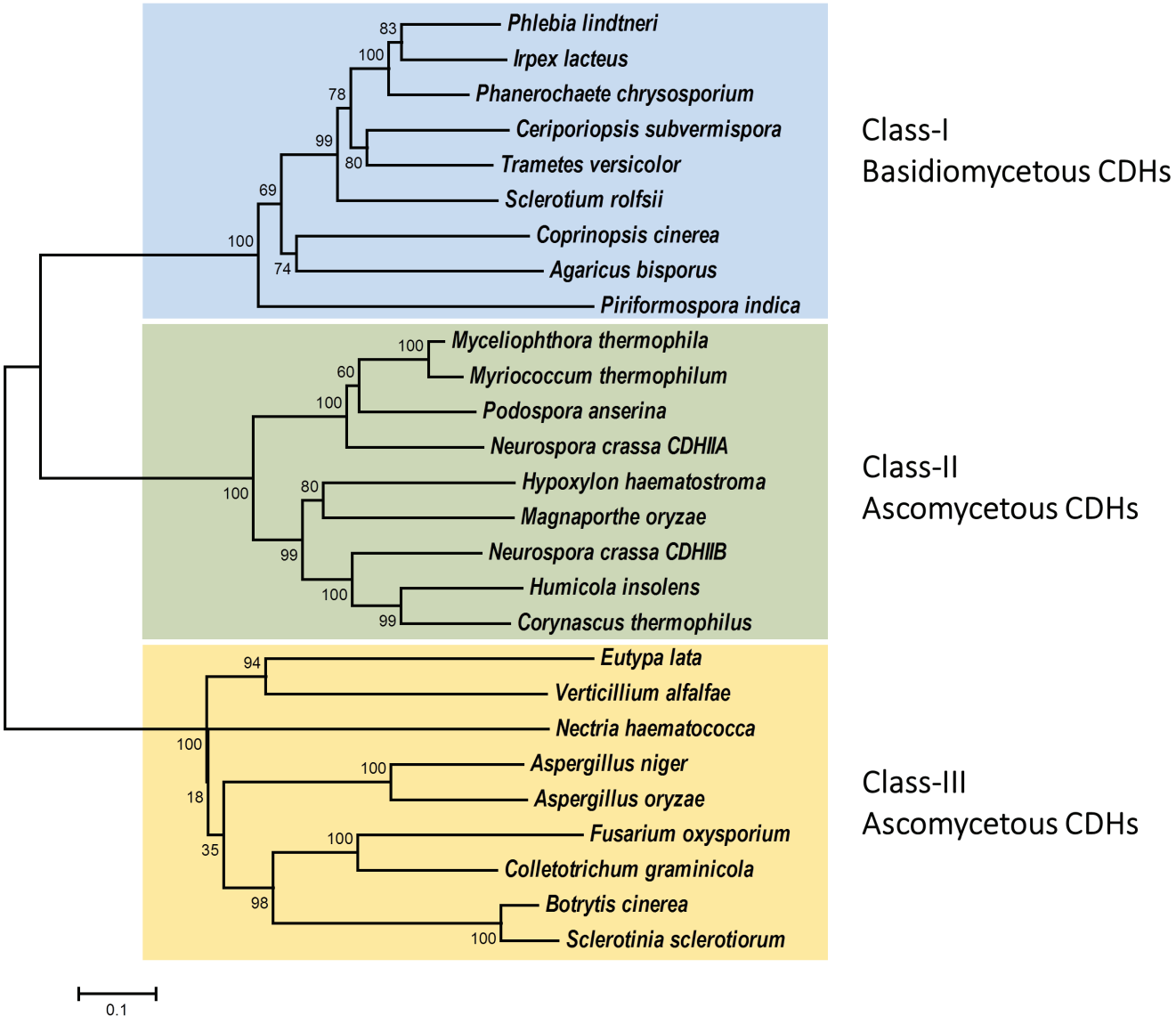

Figure 1 (Abbildung 1). Unrooted phylogenetic tree comprising 27 cellobiose dehydrogenases (Ungewurzelter phylogenetischer Stammbaum von 27 Cellobiosedehydrogenasen). CDH sequences were derived from the NCBI database (http://www.ncbi.nlm.nih.gov/protein). Signal sequences were identified using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP) and removed before further analysis. Evolutionary relationships were inferred with the minimum-evolution method (Rzhetsky and Nei, 1992) using the MEGA 7 software package (http://www.megasoftware.net). The tree was generated by the neighbor-joining method and the bootstrap consensus tree was inferred from 1000 replicates.

ton or microcrystalline cellulose led to the formation of minor amounts of hydrogen peroxide in the absence of soluble CDH substrates.

5. Structure of CDH

A common feature of all the CDH sequences is their complex bipartite structure, which comprises a C-terminal cytochrome-binding fragment (CYT) and a larger, catalytic flavodehydrogenase (DH) domain encoded within a single polypeptide chain. Both domains are connected by a linear, papain-sensitive linker peptide, which typically comprises 15–33 amino acids. The supposed mobility of the domains relative to each other has long been considered a key factor for CDH's function, but the supposed flexibility of the domains hampered initial crystallographic studies. The first attempts to investigate the three-dimensional structure of CDH using small-angle X-ray scattering (SAXS) indicated a "cigar-shaped" conformation in solution, but details regarding its dynamic behavior remained unclear (Lehner et al., 1996).

5.1 Structure of CYT

Later, crystal structures of separated CYT and DH domains were reported. In 2000, the X-ray crystal structure of the isolated CYT domain of *P. chrysosporium* CDH was resolved at 1.9 Å (pdb: 1D7C) and revealed a scaffold similar to the antibody fold of the heavy-chain Fab fragment (Hallberg et al., 2000). The 190 amino-acid long CYT has a β-sandwich topology forming a compact globular structure with a diameter of approx. 35 Å. The hydrophobic heme *b*-binding pocket is located at the surface of CYT. Notably, the propionate-A arm of the heme cofactor is partly exposed to the surrounding solvent. The heme iron is hexa-coordinated by Met and His as axial ligands (Cox et al., 1992). The replacement of either of these ligands in *P. chrysosporium* CDH by alanine resulted in a weaker binding of the heme and destabilized the CYT fragment, which was rapidly degraded in the cultivation medium due to proteolytic cleavage (Rotsaert et al., 2001). Besides CDH, only few examples of heme proteins with Met/His ligation exist in literature. Among these are the ubiquitous electron transfer protein cytochrome *c* (Kleingardner and Bren, 2015), which is part of the mitochondrial electron transport chain, and cytochrome b_{562} found in the periplasm of *Escherichia coli* (Mathews et al., 1979). In addition, a secreted cytochrome b_{562} domain featuring a cellulose-binding CBM1 has also been identified in the supernatant of *P. chrysosporium* (Yoshida et al., 2005). While its function remains to be clarified, a high homology to CDH`s CYT fragment, including a conserved pair of His/Met heme ligands, suggests a role in extracellular electron transfer. The cytochrome moiety of the pyrroloquinoline quinone (PQQ)-dependent pyranose dehydrogenase isolated from the fungus *Coprinopsis cinerea* (Matsumura et al., 2014) also shows a well-conserved His/Met pair analogous to the CYT domain in CDH (Takeda et al., 2015).

5.2 Structure of DH

The crystal structure of the *P. chrysosporium* DH domain (1.6 Å resolution, pdb: 1KDG) was reported in 2002. DH is a member of the large, structurally related family of glucose-methanol-choline (GMC) sugar oxidoreductases. The structure shows a global resemblance with the *p*-hydroxybenzoate hydroxylase (PHBH)-fold, which is common for flavin-containing enzymes (Hallberg et al., 2002). Two structurally distinct subdomains are observed: a flavin-binding domain (F-domain) and a substrate-binding domain (S-domain). The F-domain features a typical, flavin-binding βαβ-motif, the Rossman-fold, which is a common structural motif in FAD or NAD-binding proteins. The FAD moiety is non-covalently bound to the enzyme and shows a butterfly-like bending with a torsion angle of 22° in *P. chrysosporium* DH. It is well buried in the interior of the enzyme, approximately 12 Å below the protein surface. However, the active site can be accommodated via a substrate channel located at the subdomain interface.

5.3 Full-length structures of CDH

The recently reported full-length crystal structures of two CDHs from the ascomycetes *M. thermophilum* and *N. crassa* allowed for a more detailed insight into the dynamic interactions between CYT and DH (Tan et al., 2015). Two structures of *N. crassa* CDH IIA showed an "open" conformation in which DH and CYT were spatially separated, whereas a structure of *M. thermophilum* CDH showed a "closed" conformation (Figure 2A). Analysis by SAXS also suggested a number of possible intermediate conformers that exist in solution. The crystal structure

of *M. thermophilum* CDH was determined at a resolution of 3.2 Å and revealed a tight association of DH and CYT. CYT was oriented with the heme *b* facing the active site of DH, and its propionate-A chain protruding into the substrate binding pocket. The observed close distance of 9 Å between the heme *b* and FAD cofactors (edge-to-edge) in this conformation would theoretically allow direct electron transfer between the redox centers. Interestingly, the substrate channel leading to the active site was not blocked by CYT, and substrates as well as products could spatially accommodate the active site in closed conformation. The site-directed mutagenesis of amino acids along the interface of DH and CYT provided further evidence for the relevance of the "closed" state of CDH. Mutation of Ser298, Met309 and Arg698 on the surface of DH barely affected the substrate oxidation, but selectively weakened the interaction between the FAD and heme *b* cofactors as was shown by a kinetic analysis. These amino acids also formed the docking platform for CYT in the "closed" structure. Furthermore, two "open" conformations of *N. crassa* CDH IIA were resolved at a resolution of 2.9 Å. In both the structures, the domains showed different relative orientations, as well as different conformations of the linker peptides. The maximal edge-to-edge distance between FAD and heme *b* in these conformers was approx. 60 Å, which indicates a high mobility of the two subdomains. In both structures the heme *b* propionate-A was fully solvent-exposed. These observations were corroborated by the SAXS analysis of

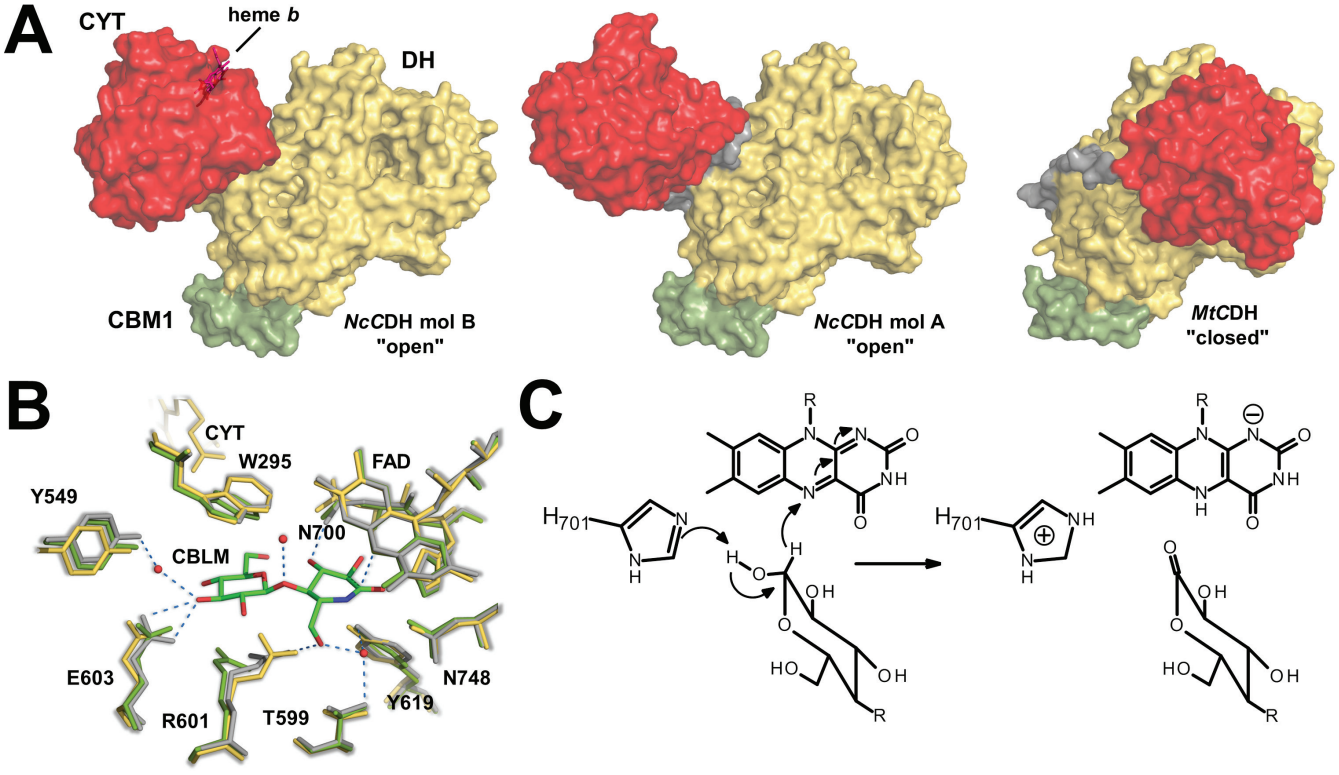

Figure 2 (Abbildung 2). Structures and kinetic mechanism of cellobiose dehydrogenase (Strukturen und kinetischer Mechanismus von Cellobiosedehydrogenase). (A) Crystal structures of full-length CDHs from *N. crassa* (pdb: 4QI7) and *M. thermophilum* (pdb: 4QI6). Shown are the relative orientations of the cytochrome (CYT) domains (red), the dehydrogenase (DH) domains (yellow) and the linker peptides (grey) in the "open" state of two *N. crassa* CDHs ("mol A" and "mol B") in comparison to the "closed" state of *M. thermophilum* CDH. Note the different orientations of the CYT domains in the structures of *N. crassa*. Family 1 carbohydrate binding modules (CBM1s) are shown in green. (B) Active-site architecture of *M. thermophilum* CDH (yellow) in comparison to the CBLM-bound DH (grey; pdb: 4QI5) and ligand-free DH (green; pdb: 4QI4). Figure modified from Tan et al. (2015). The propionate-A chain of CYT is approx. 9 Å away from the FAD. (C) Proposed catalytic hydride-transfer mechanism of CDH. Indicated is the suggested catalytic histidine. Bound substrates are eventually converted to a lactone. Numbering of amino acids is for *M. thermophilum* CDH. Figure adapted from Wongnate and Chaiyen (2013).

full-length CDH using the ensemble-optimization method (EOM). In solution, intermediate conformers were detected which included the closed and open state, and the addition of the inhibitor cellobionolactam (CBLM) restricted the number of observed conformers. While the "closed" conformation of CDH favors interaction between the redox centers, the open conformation may allow for interaction of CYT with external electron acceptors, such as LPMO.

5.4 Catalytic mechanism

Co-crystallization with the competitive inhibitor CBLM, which resembles a possible transition state, revealed the active site architecture and identified key amino acid residues for substrate binding and catalysis (Hallberg et al., 2003; Tan et al., 2015). A series of ligand interactions with conserved amino acid residues was observed, which formed a dense hydrogen bonding network with CBLM (Figure 2B). Generally, the active site is composed of two subsites, a catalytic subsite C and a substrate-binding subsite B. CBLM was positioned in the active site with the C1 carbon (corresponding to the reducing end in cellobiose) facing the N5 atom of the isoalloxazine ring at a distance of approximately 2.9 Å. This orientation would allow oxidative attack of cellobiose by a catalytic histidine (His701 in *M. thermophilum* CDH), which is the only basic residue in the vicinity of C1. The binding pose of CBLM strongly supports a general hydride transfer mechanism, in which the catalytic His as the general base initially abstracts a proton from the C1 hydroxyl group. The transfer of the anomeric (C1) hydrogen to the N5 atom of the FAD cofactor then results in a 2-electron reduced FAD (hydroquinone form), while cellobiose is oxidized to cellobionolactone. The role of His as a catalytic base was confirmed by mutation of the structurally conserved His689 in *P. chrysosporium* CDH, which resulted in kinetically impaired enzymes (Rotsaert et al., 2003). Later, it was also shown that mutations of His701 in *M. thermophilum* had the same detrimental effect (Sygmund et al., 2013). The rational engineering of amino acid residues in the substrate binding subsite B also allowed changing the substrate specificity of the enzyme. In *P. chrysosporium* CDH, Glu279 specifically interacts with the 2nd hydroxyl group of the non-reducing end sugar moiety (Hallberg et al., 2003). Replacement of this residue by Gln had little effect on cellobiose turnover, but completely abolished the activity towards lactose (Desriani et al., 2010). Using the CDH of *M. thermophilum*, Sygmund et al. (2013) demonstrated that replacement of Asn700 (Asn688 in *P. chrysosporium*) by Ser enhanced the re-oxidation of $FADH_2$ by artificial electron acceptors and oxygen. This amino acid is located in-between subsite B and subsite C, and steric changes at this position may influence the accessibility of the FAD cofactor for diffusible substrate or acceptor molecules (Tan et al., 2015).

6. Electron transfer in CDH

Being an extracellular flavocytochrome, CDH bears the hallmarks of an electron transfer protein. However, the role of CYT in the complex reaction mechanism of the enzyme remained unexplained for many years. For instance, removal of CYT by papain cleavage resulted in a perfectly active DH domain, which alone was found to oxidize cellobiose (Henriksson et al., 1991). Later, rapid kinetic techniques revealed the importance of CYT for the interaction with external electron acceptors. These experiments showed that mixing of CDH with cellobiose resulted in rapid reduction of the FAD cofactor, followed by a slower interdomain electron transfer to the heme *b* moiety (Jones and Wilson, 1988; Samejima et al., 1992). Redox titration experiments showed that a fully reduced CDH carries three electrons, and that the flavin and heme cofactors react as two- and one-electron acceptors, respectively (Igarashi et al., 2002).

One-electron transfer from the fully (two-electron reduced) flavin cofactor to the heme *b* should theoretically result in the formation of a flavin-semiquinone. Direct spectroscopic observation of such a radical intermediate has not been reported so far, which might be due to its weak absorbance or the possible overlap with the strong absorbance of the heme cofactor. Igarashi et al. (2002) reported the detection of a semiquinone intermediate by electron paramagnetic resonance (EPR) spectroscopy, but also noted that in-depth analyses remain to be performed to support this finding. The idea of a sequential electron transfer chain from FAD to CYT (Figure 3) was further strengthened by the observation that the oxidative potential of heme *b* is always higher than that of flavin. The redox potential denotes the tendency of a chemical species to acquire electrons; thus, in CDH, electron transfer is expected to proceed from the reduced FAD ($FADH_2$) to the oxidized heme *b* cofactor.

The measured redox potentials of the FAD cofactor in the DH domain of *P. chrysosporium* CDH ranged from 106

mV at pH 3.0 to -132 mV at pH 7.0. The heme *b* redox potential of the same enzyme at pH 3.0 and 7.0 was found at 190 and 130 mV, respectively (Igarashi et al., 1999). The reported heme redox potentials of ascomycetous (Class-II) CDHs are similar and range from 90 mV (*C. thermophilus* CDH, pH 7.4 (Tasca et al., 2011)) to 158 mV (*N. crassa* CDH IIB, pH 7.5 (Sygmund et al., 2012)). The role of CYT as a dedicated electron mediator was finally established by the observation of a fast and specific one-electron transfer from the reduced CYT-domain to the artificial electron acceptor cytochrome *c* (Rogers et al., 1992; Samejima et al., 1992; Henriksson et al., 1993; Rogers et al., 1994). The bimolecular reduction rate for cytochrome *c* turnover by CYT is very high (6.0×10^6 $M^{-1}s^{-1}$ (Igarashi et al., 2005)), and is not the rate limiting step in the transfer reaction. Therefore, the reduction of cytochrome *c* is commonly used in spectroscopic assays as a measure for the much slower IET. While the redox-gradient formed by CDH´s cofactors thermodynamically allows electron transfer to occur, it does not fully explain the kinetic behavior of IET in *P. chrysosporium* CDH. Several groups reported a sharp decline of the electron transfer rate between DH and CYT at pH values higher than 5.0, and a complete inhibition at pH 6.0 (Wilson and Liu, 1994; Igarashi et al., 2002). In contrast, the dehydrogenase function of the enzyme remained unperturbed even at alkaline pH. A similar trend is observed for other basidiomycetous (Class-I) CDHs, all of which show acidic pH optima for IET. Such low pH values are usually found during lignocellulose degradation *in vivo*. A possible mechanistic explanation for the pH dependency of IET was provided by Stoica et al. (2006), which speculated that electrostatic repulsion between DH and CYT at high pH values could prevent effective inter-domain electron transfer. Indeed, the experimentally determined isoelectric points of the *P. chrysosporium* DH and CYT domains were found at pH 5.45 and 3.42, respectively, while the pI of the holoenzyme was at pH 4.18 (Henriksson et al., 1991). Thus, it appears plausible that the individual domains are predominantly negatively charged at high pH (above 6), leading to a charge-induced repulsion that prevents the closed conformation of DH and CYT, and thus, effective electron transfer.

In contrast to basidiomycete CDHs, CDHs from ascomycetes seem to be more diverse in their kinetic behavior. For a number of Class-II CDHs, less acidic or even alkaline IET pH-optima were observed when using cytochrome *c* activity as proxy for IET (Harreither et al., 2011; 2012a). This is particularly remarkable, since the reported isoelectric points for most of these CDHs do not differ from those of basidiomycetous CDHs (Harreither et al., 2011). For example, CDH from the thermophilic ascomycete *C. thermophilus* has an alkaline pH optimum (pH 8.0) for cytochrome *c*, but the isoelectric point of the enzyme was found at pH 3.8 (Harreither et al., 2011). It was specu-

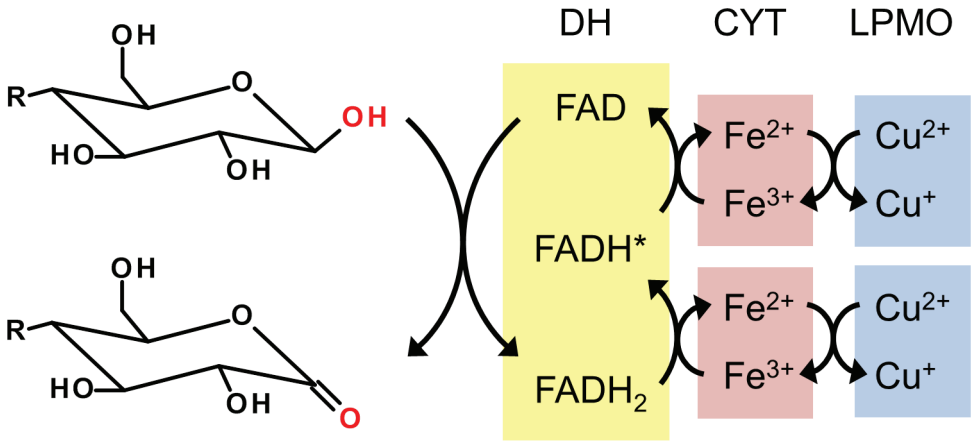

Figure 3 (Abbildung 3). Electron-chain model for electron transfer in CDH (Das Elektronenketten-Modell für den Elektronentransfer in CDH). Following substrate oxidation, electrons are transferred sequentially from the reduced flavin to the heme cofactor and eventually to a suitable electron acceptor such as LPMO. FADH, flavin semiquinone. Figure adapted from Henriksson et al. (2000).

lated that the presence of transient patches of oppositely charged or non-charged amino acid residues along the interaction surface of DH and CYT could allow IET even at high pH (Kracher et al., 2015).This idea is also corroborated by the pronounced domain mobility observed in the crystal and solution structures of *M. thermophilum* and *N. crassa* CDHs, which suggests that the domains form a short-lived, transient complex involving relatively weak forces (Tan et al., 2015).

The collapse of IET at high pH can be – at least partly – reversed in the presence of millimolar amounts of dications (Schulz et al., 2012; Kracher et al., 2015). In the case of *M. thermophilum* CDH, hydrogen exchange experiments provided a structural explanation for this phenomenon by showing that divalent cations primarily bridge or neutralize negative charges at the interface of CYT and DH (Kadek et al., 2015). This induces the formation of the "closed" state of CDH also at alkaline pH, as was confirmed by docking and fast kinetic studies (Kracher et al., 2015). A number of CDHs from basidiomycetes and ascomycetes responded differently to the presence of dications, which most likely reflects the diverse pH optima of these enzymes (Kracher et al., 2015). The conclusion that can be drawn from these reports is that domain interaction in CDH is mainly an electrostatics-driven process.

7. Interaction of CDH with lytic polysaccharide monooxygenase

7.1 General characteristics

Oxidative enzymes greatly contribute to the overall degradation capacity of lignocellulolytic fungi (Horn et al., 2012). A distinct reaction catalyzed by the so-called lytic polysaccharide monooxygenase (LPMO, CAZy: AA9) introduces chain breaks into otherwise recalcitrant crystalline cellulose surfaces, which creates isolated glucan chains susceptible for attack by cellulases (Eibinger et al., 2014). Consequently, LPMOs came to be known as "cellulase booster", and have since been employed in commercial enzyme cocktails to increase the degradation of lignocellulosic biomass.

LPMOs activate molecular oxygen, which is inserted at the C1 or C4 atom adjacent to a glycosidic bond to create an

Table 2 (Tabelle 2). Literature overview of *in vitro* assays demonstrating the synergy of LPMO and CDH (Literaturübersicht über *in vitro* Analysen, die den Synergismus zwischen LPMO und CDH verdeutlichen).

CDH		LPMO		Substrate	Cellulase	Reference
Organism	**Name**	**Organism**	**Name**			
H. insolens	*Hi*CDH	*T. aurantiacus*	*Ta*GH61A	PASC, MC	+	Langston et al., 2011
T. terrestris	*Tt*CDH	*T. terrestris*	*Tt*GH61E	PASC, MC	+	Langston et al., 2011
M. thermophila	*Mt*CDH-2	*N. crassa*	NCU01050	PASC	-	Phillips et al., 2011b
M. thermophila	*Mt*CDH-2	*N. crassa*	NCU07898	PASC	-	Phillips et al., 2011b
M. thermophila	*Mt*CDH-2	*N. crassa*	NCU08760	PASC	-	Phillips et al., 2011b
T. terrestris	*Tt*CDH	*T. aurantiacus*	*Ta*GH61A	PASC	+	Langston et al., 2012
N. crassa	CDH IIA	*N. crassa*	NCU01867	MC	-	Kittl et al., 2012
N. crassa	CDH IIA	*N. crassa*	*Nc*LPMO9C	MC	-	Kittl et al., 2012
N. crassa	CDH IIA	*N. crassa*	*Nc*LPMO9F	MC	-	Kittl et al., 2012
N. crassa	CDH IIA	*N. crassa*	NCU08760	MC	-	Kittl et al., 2012
P. cinnabarinus	*Pc*CDH	*P. anserina*	*Pa*LPMO9A	PASC	-	Bey et al., 2013
P. cinnabarinus	*Pc*CDH	*P. anserina*	*Pa*LPMO9B	PASC	-	Bey et al., 2013
M. thermophilum	*Mt*CDH	*N. crassa*	*Nc*LPMO9C	Cellopentaose	-	Isaksen et al., 2014
M. thermophila	*Mt*CDH-2	*N. crassa*	NCU08746	Amylopectin	-	Vu et al., 2014
P. anserina	*Pa*CDHB	*P. anserina*	*Pa*LPMO9A	PASC	-	Bennati-Granier et al., 2015
P. anserina	*Pa*CDHB	*P. anserina*	*Pa*LPMO9E	PASC	-	Bennati-Granier et al., 2015
P. anserina	*Pa*CDHB	*P. anserina*	*Pa*LPMO9H	PASC	-	Bennati-Granier et al., 2015

PASC, phosphoric acid swollen cellulose; MC, microcrystalline cellulose

instable intermediate that eventually leads to strand breaks (Phillips et al., 2011b). LPMO employs a mononuclear type-II copper atom in the active site, which is located on a surface-exposed flat patch oriented towards the cellulose surface during catalysis (Figure 4A).The precise reaction mechanism by which LPMOs cleave cellulose and the type of the intermediate oxygen species are presently not known. Importantly, the activity of LPMO depends on the reductive activation by an external electron donor, which could be an artificial small-molecule reductant such as ascorbic acid (Quinlan et al., 2011) or potentially an electron transferring partner enzyme. Since its discovery, the importance of CDH for the degradation of cellulose was noted, and an increasing number of transcriptome and proteome studies report the co-expression of CDH and LPMO during lignocellulose degradation (see, e.g., Phillips et al., 2011a; Hori et al., 2014; Couturier et al., 2015). Furthermore, putative *cdh* and *lpmo* genes show a high overlap in fungal genomes, although some fungi contain a smaller number of solitary *lpmo* genes (Rytioja et al., 2014). A notable example is the industrially exploited fungus *Trichoderma reesei*, which does not contain putative CDH-encoding genes but actively secretes LPMOs under cellulolytic conditions (Adav et al., 2013). Lignin-derived compounds have recently been identified as additional electron sources for LPMO (Dimarogona et al., 2012; Westereng et al., 2015). Such soluble lignin breakdown products are released during fungal lignocellulose degradation, and could potentially augment or substitute CDH activity. The exact physiological role of these alternative reducing agents still remains elusive, but is currently of high scientific interest.

Before the discovery of LPMO activity, Bao and Renganathan realized in 1992 that the addition of purified CDH to crude extracts of *T. reesei* enhanced the depolymerization of crystalline cellulose by up to 49% (Bao and Renganathan, 1992). The same behavior was observed for *T. viride* (Bao and Renganathan, 1992), which contains putative LPMOs in its genome, but lacks CDH-encoding genes. Likewise, a CDH-deficient strain of *T. versicolor* showed a severely impaired cellulose catabolism (Dumonceaux et al., 2001; Canam et al., 2011). In 2011, Langston et al. (2011) were the first to conclusively link the electron transfer properties of CDH to the enigmatic activity of LPMO. In a landmark paper, they reported that a combination of LPMO from *T. terrestris* (*Tt*GH61E) and CDH from the same organism increased the performance of various cellulases approximately two-fold; an effect that was seen for crystalline and amorphous (phosphoric acid swollen) cellulose substrates (Langston et al., 2011). However, the specific mechanism underlying the synergy of the CDH/LPMO system remained unclear. These results were soon after corroborated by a study which showed that deletion of a single *cdh* gene in the wood-degrading fungus *N. crassa* reduced its cellulose-degrading abilities by approximately two-fold (Phillips et al., 2011a). The cellulolytic capabilities of the deletion strain could be fully recovered by supplementation with exogenous *Myceliophthora thermophila* CDH. The same study also showed that purified LPMOs from *N. crassa* in combination with full-length CDH directly oxidized cellulose (Phillips et al., 2011b). Thereafter, a number of reports showed that CDH instigates LPMO, leading to the formation of soluble oxidized cello-oligosaccharides which are not observed when adding only one of the enzymes (Table 2).

Interestingly, many studies have shown that single CDHs can activate multiple LPMOs. This observation is in line with transcriptome or secretome studies; for example, the secretome of the ascomycete *N. crassa* contains four LPMOs along with one CDH when cultivated in the presence of cellulose (Phillips et al., 2011a). The interaction across species borders was also observed in many cases. The multitude of *lpmo* sequences found in many fungal genomes (e.g., 30 in *C. cinerea;* Harris et al., 2011) might be explained by a functional and catalytic diversity of LPMOs. For example, LPMO9C from *N. crassa* was shown to cleave hemicellulosic polymers as well, such as xyloglucan (Agger et al., 2014), and LPMO9A from *M. thermophila* catalyzed the oxidative cleavage of xylan (Frommhagen et al., 2015). A new family of starch-degrading LPMOs (CAZy: AA11) found in *N. crassa* was recently introduced (Vu et al., 2014). Along these lines, it has to be noted that the substrate spectrum of CDHs largely coincides with the polysaccharide-degrading capabilities of these fungal LPMOs. This provides the possibility for a functional regulation mechanism, in which the differential expression of LPMOs with diverse catalytic capabilities may allow a fungus to specifically adapt to different growth conditions or habitats (Figure 5). The catalytic promiscuity of some CDHs, for example, towards starch-derived malto-oligosaccharides or hemicellulosic sugars, may allow activation of LPMOs during the degradation of these polysaccharides.

7.2 Electron transfer between CDH and LPMO

The detailed mechanism of electron transfer between CDH and LPMO is currently a subject of great scientific

interest. The work of Phillips et al. (2011b) indicated for the first time that the cellulolytic machinery of *N. crassa* requires full-length CDH and oxygen for full activity, but does not respond to the isolated DH fragment. This observation represents the first, albeit indirect, evidence for a CYT-mediated activation of LPMO. Sygmund et al. (2012) later provided an assay-based indication for interaction, which showed that the reduction of the one-electron acceptor cytochrome *c* by CDH was slowed down considerably in the presence of *N. crassa* LPMO9C. The suggested reason was a competition for electrons between LPMO and cytochrome *c* at CDH's CYT domain. The strong inhibition of activity towards cytochrome *c*, which is an electron acceptor with a high bimolecular reaction rate, suggested a similarly fast interaction for CDH with LPMO. A direct spectroscopic approach published by Vu et al. (2014) indeed showed that fully reduced CDH re-oxidized considerably faster in the presence of the starch-active LPMO NCU08746 than by oxygen alone. The wavelength used for monitoring the redox state of CDH (430 nm) was specific for the heme *b*, which indicated that LPMO was a better electron acceptor for CDH than atmospheric oxygen. These important findings laid the foundation for further research. Tan et al. (2015) used the separated domains of *N. crassa* CDH IIA to show that direct electron transfer between CDH and LPMO occurs via CYT, but not via DH. Rapid-kinetics experiments revealed that the CYT-to-LPMO electron transfer proceeded with an apparent rate of 67 s^{-1}, which was about 100,000 fold faster than the rate measured for oxidation of CYT by atmospheric oxygen alone. No apparent interaction between DH and LPMO could be detected using the same experimental setup. These studies, however, were performed in homogenous solution in the absence of a suitable substrate for LPMO. The precise mechanistic and kinetic details of this interaction in a heterogeneous environment in the presence of cellulose await experimental clarification. A question of particular interest is how LPMO could receive electrons during catalysis. The ambiguity-driven docking experiments with the CYT domain of *N. crassa* CDH IIA and LPMO9C favored direct heme-to-copper interaction in solution (Tan et al., 2015). To date, the precise catalytic mechanism of LPMO is not known with certainty, but the suggested reaction pathways involve the formation of an intermediate oxyl radical (Kim et al., 2013) or a superoxo species (Phillips et al., 2011b; Beeson et al., 2012; 2015), both of which have a high oxidation potential. In both catalytic mechanisms, two separate single-electron

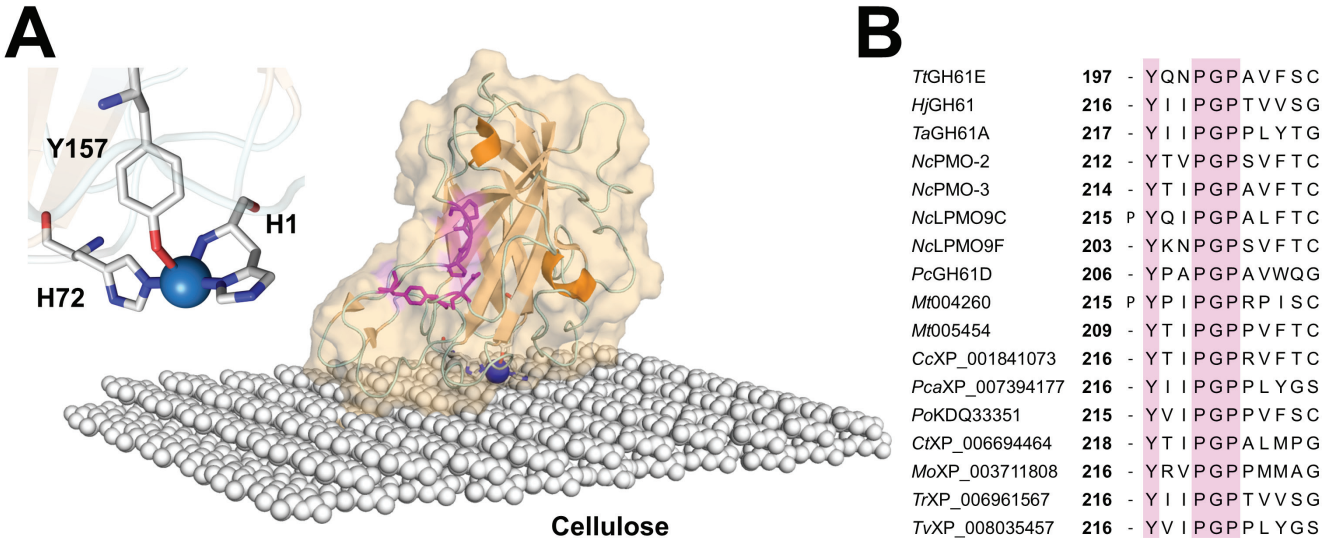

Figure 4 (Abbildung 4). Structure of lytic polysaccharide monooxygenases (Struktur von lytischen Polysaccharid-Monooxygenasen, LPMOs). (A) *N. crassa* LPMO9F modeled onto an artificial cellulose surface. The active-site copper is shown in blue. Conserved aromatic amino acids on the surface are indicated in pink. The inset shows the copper coordination with the typical "histidine brace" formed by His1 and His72. (B) Sequence alignment of 18 crystallized and putative LPMOs from basidiomycetes and ascomycetes. The suggested "PGP" docking patch for CDH is highlighted in pink. The sequence alignment was generated using MUSCLE.

transfers are required to facilitate hydroxylation and subsequent glycosidic bond cleavage in cellulose. While direct interaction between the CYT and LPMO cofactors in solution is plausible, the transfer of the second electron during catalysis would require the detachment of LPMO from the cellulose surface and subsequent re-attachment to cellulose to complete the reaction (Beeson et al., 2015). A single docking study predicted a CDH-binding site on the LPMO surface (Li et al., 2012) (Figure 4A, B) which is well-conserved in fungal cellulose-active LPMOs. Such interaction requires long-range electron transfer from the protein surface to the active site via conserved amino acid residues. In this scenario, CDH could donate electrons to LPMO during catalysis, while the active site of LPMO is oriented towards cellulose (Beeson et al., 2015). A high overall conservation of putative electron transferring amino acids, spanning the distance from the CDH binding site to the active-site copper (~20 Å) was noted (Beeson et al., 2015).

Several questions concerning the mechanistic context of this interaction remain to be addressed. For example, no structurally conserved surfaces on CYT domains are found; rather, the heme *b* propionate appears to be the only absolutely conserved surface feature among different CDHs. Finally, it remains to be elucidated how the charged propionate group of CYT could interact with the proposed (hydrophobic) "PGP" binding patch on the LPMO surface (Figure 4B). Also, two electron-donors such as ascorbic- or gallic acid were repeatedly shown to efficiently reduce the LPMO copper center and efficiently drive the oxidative cellulose degradation (Quinlan et al., 2011), which may indicate a 2-electron transfer to the copper before substrate binding. Mutational studies and further structural investigation of the CDH/LPMO couple will be needed to fully understand their interaction during oxidative cellulose degradation.

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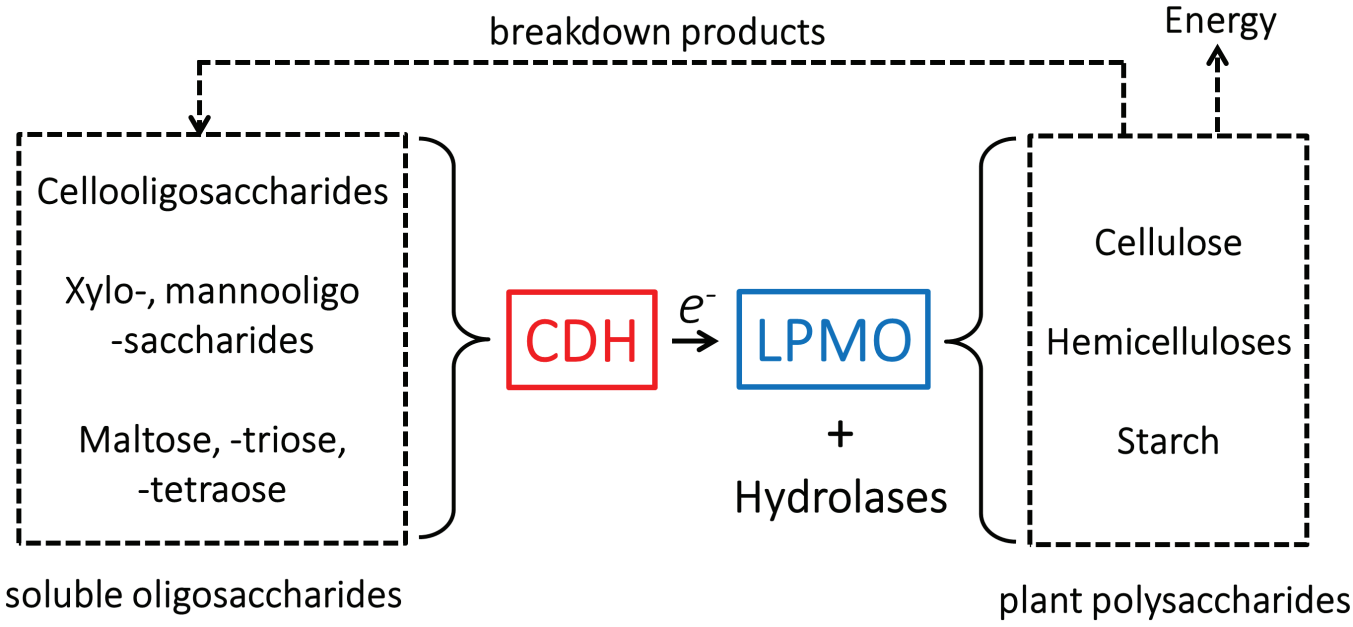

Figure 5 (Abbildung 5). Suggested substrate cycling during oxidative cellulolysis by wood decaying fungi (Substratkreislauf während des oxidativen Zelluloseabbaus durch holzabbauende Pilze). Cellulose-derived oligosaccharides, hemicellulose-derived xylo- and manno-oligosaccharides and starch-derived malto-oligosaccharides are substrates for a number of CDHs, and can be released by the action of LPMO and hydrolases.

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