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Research review paper

GH11 xylanases: Structure/function/properties relationships and applications

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ABSTRACT

For technical, environmental and economical reasons, industrial demands for process-fitted enzymes have evolved drastically in the last decade. Therefore, continuous efforts are made in order to get insights into enzyme structure/function relationships to create improved biocatalysts. Xylanases are hemicellulolytic enzymes, which are responsible for the degradation of the heteroxylans constituting the lignocellulosic plant cell wall. Due to their variety, xylanases have been classified in glycoside hydrolase families GH5, GH8, GH10, GH11, GH30 and GH43 in the CAZy database. In this review, we focus on GH11 family, which is one of the best characterized GH families with bacterial and fungal members considered as true xylanases compared to the other families because of their high substrate specificity. Based on an exhaustive analysis of the sequences and 3D structures available so far, in relation with biochemical properties, we assess biochemical aspects of GH11 xylanases: structure, catalytic machinery, focus on their "thumb" loop of major importance in catalytic efficiency and substrate selectivity, inhibition, stability to pH and temperature. GH11 xylanases have for a long time been used as biotechnological tools in various industrial applications and represent in addition promising candidates for future other uses.

Contents

Abbreviations: AX, arabinoxylan; BCX, *Bacillus circulans* GH11 xylanase; CBD, cellulose-binding domain; CBM, carbohydrate binding module; CD, catalytic domain; Cel-12, GH12 cellulase; DP, degree of polymerization; GH, glycoside hydrolase; HX, heteroxylan; MW, molecular weight; XBD, xylan-binding domain; XOS, xylo-oligosaccharide; Xyl-10, GH10 xylanase; Xyl-11, GH11 xylanase.

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1. Introduction

Lignocellulosic materials account for approximately 50% of the biomass in the world, whose annual production through photosynthesis by plants is estimated to be 10–50×10^{12} tons (Claassen et al., 1999). Hemicellulose is the second most abundant renewable biomaterial available after cellulose inside lignocellulosic polymers, representing 20–35% overall (Saha, 2003). In interaction with a matrix of lignin, these heteropolysaccharides form a highly complex structure. For humankind, lignocellulosic biomass represents potentially a renewable and sustainable source of fuels, chemicals and other products that could replace their fossil-derived counterparts, whose availability is limited and use becomes a major concern (global warming, pollution…). But controlled destructuration and degradation processes of lignocellulosic materials are still challenging, because of their high ultrastructure and linkage complexity.

In nature, plant cell wall degradation is performed by fungi and bacteria that secrete a wide range of enzymes required for the complete breakdown of cellulose, hemicellulose and lignin which are then metabolized or stabilized in soil. Due to their intrinsic properties (high reaction yield, mild conditions of reaction, specificity), lignocellulosic enzymes are key partners for the set up of sustainable and reliable biomass upgrading processes, where they can advantageously replace or be used in combination with physical/chemical processes, for example to hydrolyse biomass to release monosaccharides to be fermented in bioethanol fuel in an economical way.

HXs are one of the most important hemicellulose components of the plant cell wall. They are made of a linear chain of β-D-xylopyranose residues linked in β-(1,4), variously substituted with sugars (arabinose, xylose, galactose, etc.), glucuronic acids, and other groups (acetyl, feruloyl, *p*-coumaryl). Inside the myriad of lignocellulosic enzymes, those acting on HXs are critical: they are called xylanases. Fig. 1 illustrates the growing interest for these enzymes in the past decade, through the increasing number of publications and the number of 3D structures solved.

Endo-β-1,4-xylanases (systematic name 1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) are the enzymes which cleave the β-xylosidic bond between two D-xylopyranosyl residues linked in β-(1,4). Xylan hydrolysis by micro-organisms is described since the end of the 19th century, but the first xylanase preparation and its partial purification from *Aspergillus foetidus* mycelium only dates back from half a century ago (Whistler and Masak, 1955). They were so-called "xylo-dextrinases", and became "pentosanases" when they were isolated from a rumen bacteria (Howard et al., 1960). Thereafter, numerous xylanases from bacteria, fungi, yeasts or plants were isolated, purified and characterized. Through the development of the molecular biology techniques in the 1970–1980s and the elaboration of the PCR technique, many of them have been cloned, over expressed, and many industrial applications were developed. For instance, xylanases are extensively employed in bread making to improve baking properties, in animal feed to increase digestibility, or in biobleaching of kraft pulps (see Section 5).

The first 3D structures were only solved in 1994 (Derewenda et al., 1994; Harris et al., 1994; Törrönen et al., 1994; White et al., 1994). Xylanases are highly diversified enzymes, at different levels: structural (catalytic domain, presence or absence of CBMs), biochemical (pH or temperature optima and sensibility), catalytic (substrate selectivity, catalytic efficiency, inhibitor sensitivity). Actually, a majority of micro-organisms produce many different types of xylanases: *Sporotrichum dimorphosporum* can release 16 different forms (Comtat, 1983) and *Aspergillus niger* 15 forms (Biely et al., 1985). This diversification would originate from some well identified factors: genetic redundancies (Wong et al., 1988) and post-translational modification through proteolytic cleavage and glycosylation (Biely, 1985), presence of a signal peptide or partial proteolysis (Wong et al., 1988). This strategy is probably the adaptive answer found by micro-organisms to optimize the biodegradation of xylosidic linkages of plant cell walls, whose environment is likely to change during their growth. Each enzyme produced probably fits and degrades a given substrate of given structure and environment at a given time.

Facing this abundance of xylanases, the classical substrate-based classification showed some limitations. A new classification based

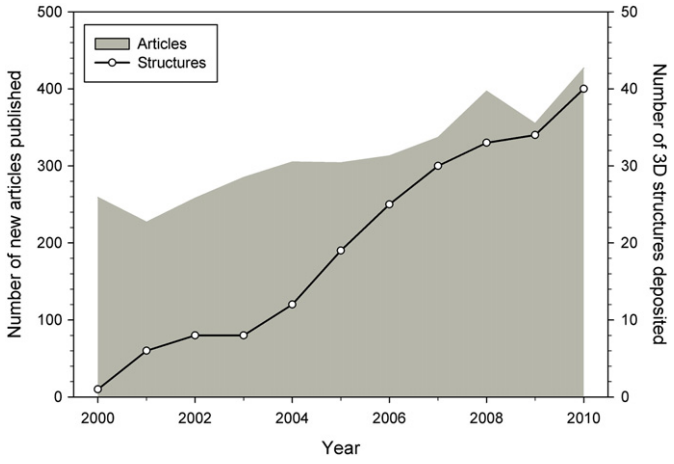

Fig. 1. Evolution on the 2000–2010 decade of the number of articles dealing with GH11 xylanases (data from Web of Sciences, Thomson Reuters, with "xylanase" as keyword in Topic field, early 2011) and of the GH11 xylanases structures solved and publically released (data from the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, early 2011).