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Functional display of family 11 endoxylanases on the surface of phage M13

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Abstract

Two family 11 endoxylanases (EC 3.2.1.8) were functionally displayed on the surface of bacteriophage M13. The genes encoding endo-1,4-xylanase I from *Aspergillus niger* (ExlA) and endo-1,4-xylanase A from *Bacillus subtilis* (XynA) were fused to the gene encoding the minor coat protein g3p in phagemid vector pHOS31. Phage rescue resulted in functional monovalent display of the enzymes as was demonstrated by three independent tests. Firstly, purified recombinant phage particles showed a clear hydrolytic activity in an activity assay based on insoluble, chromagenic arabinoxylan substrate. Secondly, specific binding of endoxylanase displaying phages to immobilized endoxylanase inhibitors was demonstrated by interaction ELISA. Finally, two rounds of selection and amplification in a biopanning procedure against immobilized endoxylanase inhibitor were performed. Phages displaying endoxylanases were strongly enriched from background phages displaying unrelated proteins. These results open perspectives to use phage display for analysing protein–protein interactions at the interface between endoxylanases and their inhibitors. In addition, this technology should enable engineering of endoxylanases into novel variants with altered binding properties towards endoxylanase inhibitors.

Keywords: *Aspergillus niger* endoxylanase; *Bacillus subtilis* endoxylanase; Endoxylanase inhibitors; Phage display

Abbreviations: Ap, ampicillin; ExlA, endo-1,4-xylanase I from *A. niger*; Glc, D(+)-glucose; K_i, inhibition constant; Km, kanamycine; LB, Luria–Bertani (medium); LB(2× TY)/Ap(Km)/Glc, LB (2× TY) medium supplemented with 100 μg/ml Ap (35 μg/ml Km) and 2% Glc; PBS(T), phosphate buffered saline (supplemented with 0.1% Tween 20); TAXI, *Triticum aestivum* xylanase inhibitor; 2× TY, tryptone and yeast extract medium; XIP, xylanase-inhibiting protein; XynA, endo-1,4-xylanase A from *B. subtilis*

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1. Introduction

Endo-β-1,4-xylanases (EC 3.2.1.8) (further referred to as endoxylanases) are produced by several bacteria, fungi and plants, and hydrolyse the β-1,4-xylan linkages in the (arabino)xylan component of plant cell walls. They have been classified mainly into two classes, families 10 and 11 glycoside hydrolases (EC 3.2.1.8), based on amino acid sequence similarities (Henrissat, 1991). Family 11 endoxylanases are characterised by a jelly roll conformation and molecular masses close to 20 kDa (Törrönen and Rouvinen, 1997). Because of their ability to modify arabinoxylan functionality, endoxylanases are increasingly used in a number of industrially relevant processes including bread making (Courtin et al., 1999), gluten–starch separation (Christophersen et al., 1997), beer production (Viëtor et al., 1993) and paper production (Christov et al., 1999). They also improve the nutritional properties of agricultural silage and grain feed (Bedford and Schulze, 1998; Malathi and Devegowda, 2001).

Endoxylanase I from *Aspergillus niger* (further denoted as ExlA) has a molecular mass of approximately 20 kDa and a p*I* of 3.5 (Berrin et al., 2000). Due to its low pH optimum (3.5), this fungal enzyme is added as supplement in animal feed (Krengel and Dijkstra, 1996). It has also been studied for its role as a bread improver (Hessing et al., 1994; Debyser et al., 1999) and in wheat processing (Christophersen et al., 1997). The X-ray crystal structure of this family 11 endoxylanase from *A. niger* has been solved by Krengel and Dijkstra (1996). Endoxylanase A from *Bacillus subtilis* (further denoted as XynA) is a 20 kDa family 11 endoxylanase with a p*I* of 9.3 (Courtin and Delcour, 2001). This enzyme has many applications, especially in bread-making (Courtin and Delcour, 2001) and the paper and pulp industry (Kulkarni et al., 1999).

Two distinct classes of endoxylananase inhibitors, *Triticum aestivum* xylanase inhibitor (TAXI) (Debyser and Delcour, 1997) and xylanase inhibitor protein (XIP) (McLauchlan et al., 1999), have been discovered in cereals. The (TAXI)-type exhibits inhibitory activity towards fungal and bacterial family 11 endoxylanases (Gebruers et al., 2004), whereas the XIP-type inhibits fungal family 10 and 11 endoxylanases but not bacterial endoxylanases (Juge et al., 2004). Consequently, the *A. niger* endoxylanase (ExlA) is inhibited by TAXI-I and XIP-I, whereas the activity of the *B. subtilis* endoxylanase (XynA) is only inhibited by TAXI-I. Recent studies have shown that endoxylanase inhibitors considerably affect the functionality of endoxylanases in biotechnological processes (Sorensen et al., 2004).

The last decade filamentous phage display of proteins, protein fragments or peptides became an important tool to study protein–protein interactions (Smith and Petrenko, 1997) and to select derivatives of known proteins with altered properties. E.g. phage display has been used for the selection of amylase variants with improved low pH starch binding (Verhaert et al., 2002). The power of this technique is based on the unique feature of phage particles to provide a physical link between genotype (foreign DNA carried inside the particle) and phenotype (peptide or protein expressed on the surface). Besides, extremely large libraries can be generated and screened for peptides or proteins with a desired property. The sequence of selected (poly)peptides can easily be deduced from the sequence of the encapsulated DNA.

We aim to use phage display for studying the interaction between endoxylanases and endoxylanase inhibitors. Therefore, we explored the possibility of functional display of entire endoxylanase enzymes on the coat of M13 bacteriophage. Endoxylanase I from *A. niger* (ExlA) and endoxylanase A from *B. subtilis* (XynA) were chosen for this analysis, because of their importance in industrial applications and their diverse characteristics concerning TAXI and XIP mediated inhibition. Moreover, the dissimilarities of these enzymes with respect to p*I* and pH optimum may provide insight in the general applicability of phage display technology in the study of endoxylanases. In this report, we describe the functional monovalent exposition of both endoxylanases on the surface of phage M13-derived particles. We show that the displayed endoxylanases specifically bind endoxylanase inhibitor. In addition, we demonstrate that both endoxylanases were efficiently enriched from background phages displaying unrelated yeast proteins.

2. Materials and methods

2.1. Strains, plasmids, phagemid and helper phage

Escherichia coli XL1-Blue MRF′ (*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac*$^{-}$ *Δ*(*mcrA*)*183*