An Aspergillus nidulans GH26 endo-β-mannanase with a novel degradation pattern on highly substituted galactomannans

The activity and substrate degradation pattern of a novel Aspergillus nidulans GH26 endo-β-mannanase (AnMan26A) was investigated using two galactomannan substrates with varying amounts of galactopyranosyl residues. The AnMan26A was characterized in parallel with the GH26 endomannanase from Podospora anserina (PaMan26A) and three GH5 endomannanases from A. nidulans and Trichoderma reesei (AnMan5A, AnMan5C and TrMan5A). The initial rates and the maximal degree of enzymatically catalyzed conversion of locust bean gum and guar gum galactomannans were determined. The hydrolysis product profile at maximal degree of conversion was determined using DNA sequencer-Assisted Saccharide analysis in High throughput (DASH). This is the first reported use of this method for analyzing galactomannooligosaccharides. AnMan26A and PaMan26A were found to have a novel substrate degradation pattern on the two galactomannan substrates. On the highly substituted guar gum AnMan26A and PaMan26A reached 35-40% as their maximal degree of conversion whereas the three tested GH5 endomannanases only reached 8-10% as their maximal degree of conversion. α-Galactosyl-mannose was identified as the dominant degradation product resulting from AnMan26A and PaMan26A action on guar gum, strongly indicating that these two enzymes can accommodate galactopyranosyl residues in the -1 and in the +1 subsite. The degradation of α-64-63-di-galactosyl-mannopentaose by AnMan26A revealed accommodation of galactopyranosyl residues in the -2, -1 and +1 subsite of the enzyme. Accommodation of galactopyranosyl residues in subsites -2 and +1 has not been observed for other characterized endomannanases to date. Docking analysis of galactomannooligosaccharides in available crystal structures and homology models supported the conclusions drawn from the experimental results. This newly discovered diversity of substrate degradation patterns demonstrates an expanded functionality of fungal endomannanases, than hitherto reported.

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