

The arabinohydrolases of *Chryso sporium lucknowense* mode of action and substrate specificities

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Conclusions

The C1 arabinohydrolases Abn1, Abn2, Abn4 and Abf3 act together on arabinan degradation. Endoarabinanase Abn1 can hydrolyze substrates that carry a double substitution at the -2 subsite and is therefore considered as side chain tolerant. The arabinohydrolases Abn4 and Abf3 release arabinose monomers from the non-reducing end of the molecule with different linkage specificities. The characterized enzymes allow the controlled and efficient degradation of arabinan to either monomers, or, by partial degradation, to branched arabinose oligomers.

Introduction

Biomass utilization for biorefinery and biofuels production is a hot research topic worldwide. Fungal enzymes can be used to degrade the plant cell wall to fermentable monosugars.

The ascomycetous fungus *Chryso sporium lucknowense* C1 expresses 14 arabinose releasing enzymes, of which four were purified and characterized[1]. Different combinations of three of these enzymes released a complex mixture of arabinose and arabinose oligomers from sugar beet arabinan, among them several branched isomers (Tab. 1)[2]. These oligomers were used together with a series of reduced arabinose oligomers for a detailed characterization of the C1 arabinohydrolases with regard to mode of action and substrate affinity.

| Component | Schematic structure |
|-----------|---------------------|
| 3.1 | |
| 4.1 | |
| 4.2 | |
| 5.1 | |
| 5.2 | |
| 6.1b* | |
| 6.2 | |
| 7.2** | |
| 8.1 | |

Tab. 1 Branched arabinose oligomers released by C1 arabinohydrolases. Structures were determined by NMR spectroscopy. *proposed structure based on digest of 8.1 with Abf3, **proposed structure based on digest of 8.1 with Abn4.

Results

The mode of action of C1 arabinohydrolases was determined toward reduced arabinose oligomers. Abn2, Abn4 and Abf3 have an exo-mode of action (Tab. 2). They release arabinose (Abn4 and Abf3) or arabinobiose from the non-reducing end of the substrate.

| | Abn1 | Abn2 | Abn4 | Abf3 |
|-----------------------|-----------------|-----------------|-------------------|---------|
| GH family | 43 | 93 | 43 | 51 |
| Mode of action | endo | exo | exo | exo |
| pH optimum | 5.5 | 4.5 | 5.5 | 5.0 |
| pH stability | 5-8 | 6-7 | 6-8 | 5-7 |
| Temp. optimum (°C) | 55 | 50 | 55 | 40 |
| Spec. Activity (U/mg) | 26.0 | 7.1 | 9.5 | 21.4 |
| Substrate | linear arabinan | linear arabinan | branched arabinan | pNP-Ara |
| Released product | ara+ara2 | ara2 | ara | ara |

Tab. 2 Biochemical properties of C1 arabinohydrolases. Specific activity determined toward preferred substrate at 30 °C and optimal pH (1 U = μmol min⁻¹), ara – arabinose, ara2 – arabinobiose.

Abn1 has an endo-mode of action and its activity increases stepwise with increasing length of the substrate (Fig. 1A). The stepwise activity increase can be explained with different binding affinities of the Abn1 subsites (Fig 1B). The subsites -2 and +2 seem to have a lower binding affinity than the neighboring subsites.

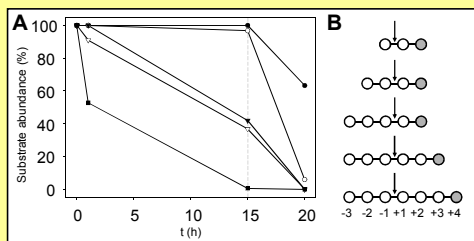


Fig. 1. Abn1 activity toward reduced arabinose oligomers. Left – time dependent degradation. Right – preferred hydrolyzed linkage. ● - reduced arabinotriose, ○ - reduced arabinotetraose, ▼ - reduced arabinopentaose, ▽ - reduced arabinohexaose, ■ - reduced arabinheptaose.

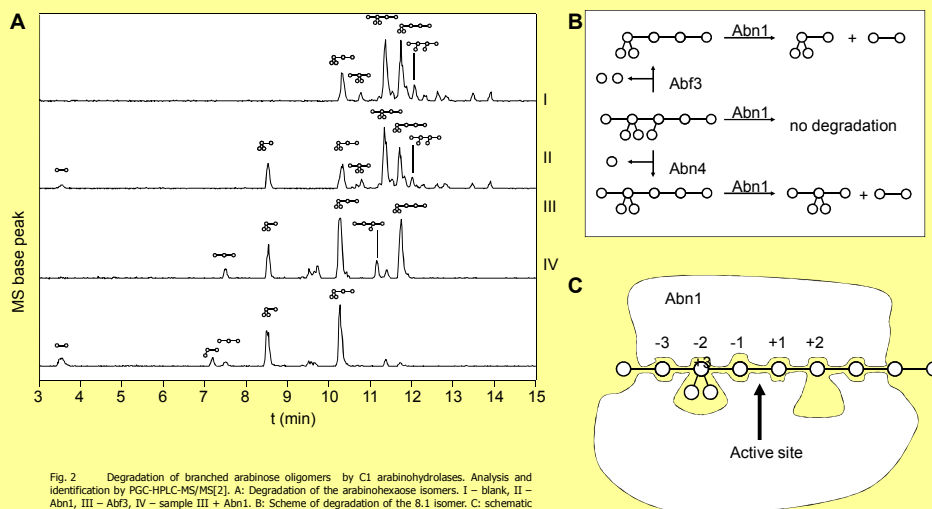


Fig. 2 Degradation of branched arabinose oligomers by C1 arabinohydrolases. Analysis and identification by PGC-HPLC-MS/MS[2]. A: Degradation of the arabinohexaose isomers. I – blank, II – Abn1, III – Abf3, IV – sample III + Abn1. B: Scheme of degradation of the 8.1 isomer. C: schematic model of Abn1 to illustrate side chain tolerance.

The specificity of C1 arabinohydrolases was studied toward branched arabinose oligomers (Fig. 2). Abn1 can solely degrade the 6.1b isomer (Fig. 2, line II), which suggests that it needs at least two unsubstituted arabinose residues at subsite -1 and +1 (Fig 2C). Furthermore, the subsite +2 needs to be occupied for Abn1 activity, irrespective of the nature of the residue. When the arabinose at subsite -2 is double substituted by to arabinose residues, Abn1 activity is reduced by factor five. Larger oligomers like the 8.1 isomer can be converted into smaller isomers by a sequential digest with Abn1, Abn4 and Abf3 (Fig. 2B). Abn4 and Abf3 differ in linkage specificity. Abn4 is hardly active the backbone of small oligomers and releases only the single side chain from 8.1. Abf3 liberates two arabinose residues and produces the isomer 6.1b that can be further degraded by Abn1 to the 4.1 isomer and arabinobiose.

1. Kühnel, S., et al., *Chryso sporium lucknowense* arabinohydrolases efficiently degrade sugar beet arabinan. *Bioresour. Technol.*, 2010. *in press*

2. Westphal, Y., et al., *Branched arabino-oligosaccharides isolated from sugar beet arabinan*. *Carbohydr. Res.*, 2010. **345**(9): p. 1180-1189.

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