^{1}H, ^{13}C and ^{15}N backbone and side-chain assignment of a carbohydrate binding module from a xylanase from *Roseburia intestinalis*

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Abstract

The N-terminal domain (residues 28-165) from the glycoside hydrolase family 10 from *Roseburia intestinalis* (*Ri*CBMx), has been isotopically labeled and recombinantly expressed in *Escherichia coli*. Here we report ^{1}H, ^{13}C and ^{15}N NMR chemical shift assignments for this carbohydrate binding module (CBM).

Keywords

Carbohydrate binding module (CBM), xylan binding module, xylanase, *Roseburia intestinalis*, gut microbiota

Biological context

Most of the dietary fibers in the human diet comes from the plant cell walls present in fruits and vegetables. Here we find complex polysaccharides such as, cellulose, pectin and xylan (Koropatkin et al. 2012). The latter has a β-1,4-linked xylose backbone with varying degrees of polymerization and side-chain substitution (Rennie and Scheller 2014). Xylan can be substituted with carbohydrate side-chains, e.g. arabinofuranosyl and glucuronosyl residues or acetyl groups. A variety of linkages to side-chain residues necessitate the deployment of different enzymes for xylan degradation.

Members of the human gut microbiota (HGM) are able to ferment xylan into short-chain fatty acids (SCFAs) e.g. butyrate, propionate and acetate. Butyrate is known to have a beneficial effect on the host's health by being an energy source for colonocytes as well as reducing the risk of colon cancer and enteric colitis (Donohoe et al. 2012; Morrison and Preston 2016; Xu et al. 2017). As the population of butyrate-producers are more abundant in healthy individuals, there is a particular interest in the role they play in the HGM (Sheridan et al. 2016). One of the key known butyrate-producers from the HGM is *Roseburia*, a common genus in the clostridial cluster XIVa within Firmicutes (Louis and Flint 2009). *Roseburia intestinalis* has shown xylanolytic activity, and is together with species from *Bacteroides*, one of the few taxa that can utilize xylan (Chassard et al. 2007; Mirande et al. 2010). The ability of *Bacteroides* to degrade xylan have been investigated in detail (Martens et al. 2011; Rogowski et al. 2015; Zhang et al. 2014), but insight into the strategy used by Firmicutes to harvest energy from xylan has until recently been lacking.

A recent study (Leth et al. 2018) showed that *R. intestinalis* is able to breakdown xylan through a modular cell-attached xylanase of glycoside hydrolase family 10 *(Ri*GH10A) that is conserved within the species. This enzyme is highly upregulated when *R.intestinalis* is grown on xylan and comprises of four xylan-binding modules: Two carbohydrate binding modules (CBMs) of family 9 (CBM9), one from family 22 (CBM22) and an N-terminal of a previously unknown family (CBMx). This representative of a new CBM family possesses low affinity for xylan, but displays selectivity for arabinoxylan, which makes it an interesting candidate for both structural and functional characterization studies by nuclear magnetic resonance (NMR).

Methods and experiments

Protein expression and purification

^{13}C, ^{15}N and ^{15}N samples were expressed in *Escherichia coli* BL21 (DE3). Pre-culture was grown in LB medium (10 g/L tryptone, 5 g/L yeast extract and 5 g/L NaCl) supplemented with 10 µL kanamycin (50 mg/mL) in a shaking incubator at 30 °C, 225 rpm overnight. From the pre-culture, 1 % (v/v) was used to inoculate 450 mL M9 media (6 g/L Na_2HPO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl) supplemented with 500 µL kanamycin (50 mg/mL), 1 mL 1 M $MgSO_4$, 10 mL Trace Metal solution (0.1 g/L $ZnSO_4$, 0.8 g/L $MnSO_4$, 0.5 g/L $FeSO_4$, 0.1 g/L $CuSO_4$, 1 g/L $CaCl_2$), 5 mL Gibco™ MEM Vitamin Solution (100x), 10 mL ^{15}N Bioexpress Cell Growth Media (Cambridge Isotope Laboratories, Tewksbury, MA; USA), 2 g glucose (^{15}N label)/ 98 % $^{13}C_6$-D-glucose (^{13}C, ^{15}N label) in 20 mL milliQ. After inoculation the medium was supplemented 1 mL anti-foam and the cells were grown using Lex-24™

(Epiphyte) at 30 °C until OD_{600} = 0.8. The expression was induced with IPTG (isopropyl-1-thio-β-D-galactopyranoside) to a final concentration of 1 mM, and incubated with Lex-24™ (Epiphyte) at 16 °C over night. The cells were harvested by centrifugation (Sorvall) at 4 °C, 6000 ×g, 5 min. The pellet was resuspended in lysis buffer (50 mM NaH_2PO_4, 50 mM NaCl and 0.05 % TritonX-100) and ½ tablet cOmplete™ ULTRA protease inhibitor (Roche) in 20 mL milliQ, and sonicated using a Branson Sonifier equipped with a microtip for 10 minutes. Isolation of the lysate was done by centrifugation (Eppendorf) at 4 °C, 15 000 ×g, for 2.5 h.

An Econo-Column® (Bio-Rad) containing 2 mL Ni-NTA Agarose (QIAGEN) was equilibrated with 20 column volumes WEB (50 mM Na_2HPO_4, 300 mM NaCl), pH 8.0. The lysate was incubated in the column for 45 minutes, and eluted with WEB with an increased amount of imidazole: 10 mM, 15 mM, 20 mM, 100 mM, 200 mM and 400 mM. Fractions containing CBMx were collected and purity confirmed with SDS-PAGE. To remove imidazole, the fractions were dialysed (MWCO 3.5 kDa) against 5 mM NaH_2PO_4, pH 5.5 over night.

To cleave the His-tag of the fraction containing CBMx, TEV-protease was added in 1/100 (w/w) and dialysed (MWCO 3.5 kDa) against 20 mM sodium phosphate, 1 mM DTT, 0.5 mM EDTA, pH 8.0. Purification of CBMx was done using a gravity flow column containing 2 mL cOmplete His-tag purification resin (Roche) equilibrated with the dialysis buffer. The dialyzed sample was applied to the column and the flow through was collected. The resin was washed with 1-5 column volumes of the same buffer and the sample was collected by combining this fraction with the flow through. Regeneration of the column was done with dialysis buffer containing 50 mM imidazole. An SDS-PAGE was run to confirm the separation and purity of the mature CBMx.

The CBMx containing fractions were concentrated and buffer exchanged into the NMR-buffer (50 mM sodium phosphate, pH 6.5. Samples for NMR was made with CBMx in NMR-buffer with D_2O added to a final ratio of 90 % H_2O/10 % D_2O) by centrifugation using Vivaspin® 6 protein spin concentrators (MWCO 5 kDa, Sartorius) at 10 °C, 7000 ×g.

NMR spectroscopy

All CBMx NMR samples were prepared in 50 mM sodium phosphate buffer and 10 % D_2O, pH 6.5.
All spectra were recorded at 25 °C on a Bruker Ascend 800 MHz spectrometer Avance III HD Bruker Biospin equipped with a 5 mm Z-gradient CP-TCI (H/C/N) cryoprobe at the NV-NMR-Centre/Norwegian NMR Platform at NTNU Norwegian University of Science and Technology, Trondheim, Norway. ^{1}H shifts were referenced internally to HDO, while ^{13}C and ^{15}N chemical shifts were referenced indirectly to HDO, based on the absolute frequency ratios (Zhang et al. 2003). Backbone and side-chain assignments of CBMx were elucidated using ^{15}N-HSQC, ^{13}C-HSQC, HNCA, HNcoCA, HNCO, HNcaCO, HNCACB, HNcoCACB, HNHAHB, HNcoHAHB and HcCH-TOCSY. The NMR data were recorded and processed with TopSpin version 3.5 and the data was analyzed with CARA version 1.5 (Keller 2004). Secondary structure elements were evaluated using TALOS-N (https://spin.niddk.nih.gov/bax/software/TALOS-N/) (Shen and Bax 2013) and chemical shits of N, H^N, C^α, C^β, H^α, H^β and C'.

Assignment and data deposition

Here we report the backbone and side-chain assignment of CBMx. Fig.1 shows the ^{15}N-HSQC spectrum of CBMx together with the assigned resonances. The backbone assignment is essentially complete (N, HN, C^α, H^α and C' > 98 %). The mature protein contains two extra amino acids (Gly-Ala) at the N-terminus (for purification purpose) that were only partially assigned. Side-chain assignment is almost complete (H and C side-chains >78 %). The overall percentage of completion is affected by the missing assignment of exchangeable side-chain protons of Arg, Lys, Asn and Gln as well as aromatic residues. Chemical shift data have been deposited at the Biological Magnetic Resonance Data Bank (BMRB) under the accession number 27536.

Analysis of the secondary structure elements (Fig. 2) indicates three α-helices and ten β-sheets. A high degree of β-sheets is consistent with previously reported structures of carbohydrate binding modules. The typical β-sheet scaffold support the evolution of a variety of binding specificities and affinities in xylan-specific CBMs which merits further analyses.

NOTES

Ethical standards

The authors declare that the experiments described in this publication were done in compliance with the ethical standards of the countries in which they were performed.

Conflict of interest

The authors declare that they have no conflict of interest.

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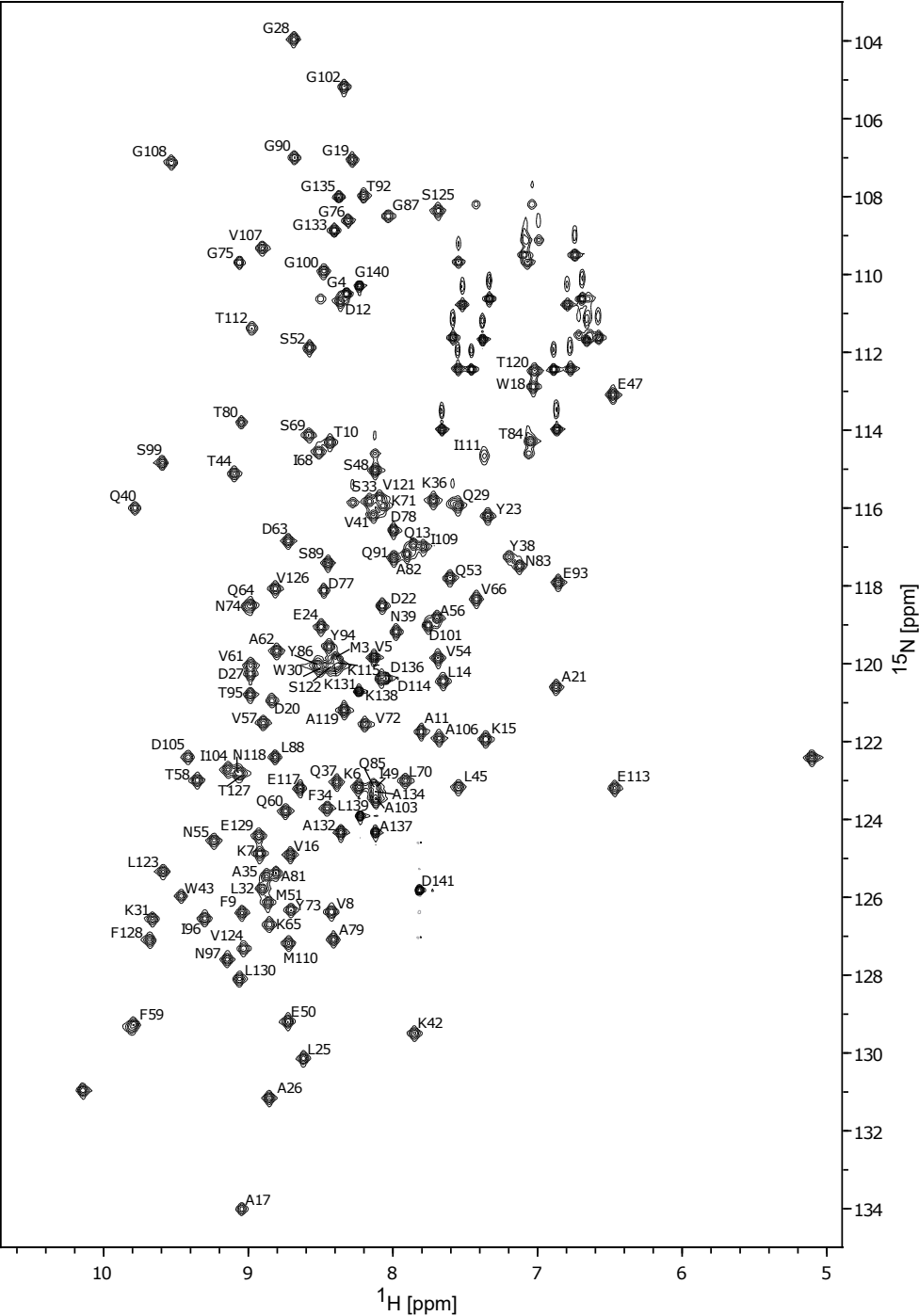
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D12
T112
S52
T120
W18
E47
T80
S69
T10
T84
I111
S99
T44
I68
S48
S33
V121
K36
Q40
K71
Q29
Y23
V41
D78
D63
Q13
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S89
Q91
Y38
N83
A82
Q53
E93
Q64
V126
D77
V66
N74
D22
A56
E24
N39
Y94
D101
A62
Y86
M3
V5
V54
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T127
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A137
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F128
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N97
M110
L130
F59
E50
K42
L25
A26
A17
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106
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110
112
114
116
118
120
122
124
126
128
130
132
134
10
9
8
7
6
5
^{15}N [ppm]
^{1}H [ppm]

Fig. 1

^{1}H, ^{15}N HSQC spectrum of ^{13}C, ^{15}N-labeled CBMx (1.7 mM) from the glycoside hydrolase family 10 xylanase from *Roseburia intestinalis* (*Ri*Xyn10A) in 50 mM sodium phosphate, pH 6.5 with D_2O added to a final ratio of 90 % H_2O/10 % D_2O. Residue number and type are indicated on the figure

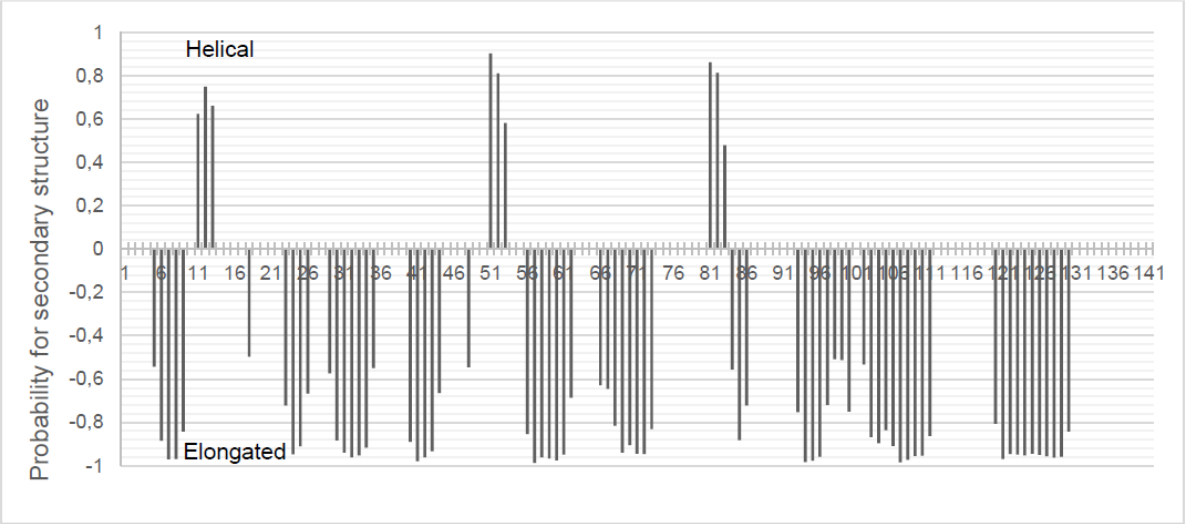

Fig. 2
Secondary structure probability of CBMx using TALOS-N. The probability of helical structure is shown as positive values, while negative values are used for the probability of an elongated structure