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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignment of xylan-binding domain from *Streptomyces olivaceoviridis* E-86 β-xylanase

Hikaru Hemmi[a,*], Atsushi Kuno[b], Shigeyasu Ito[b], Satoshi Kaneko[a] & Tsunemi Hasegawa[b]
[a]*National Food Research Institute, Tsukuba, 305-8642 Ibaraki, Japan;* [b]*Department of Material and Biological Chemistry, Yamagata University, Yamagata, 990-8560 Yamagata, Japan*

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Biological context

Xylanases have been classified primarily to either family 10 or family 11 of the glycoside hydrolases, on the basis of amino acid sequence similarities of their catalytic domain. The family 10 xylanase from *Streptomyces olivaceoviridis* E-86 contains a $(\beta/\alpha)_8$-barrel as a catalytic domain, a family 13 carbohydrate binding module (CBM13) as a xylan binding domain in the Henrissat classification of carbohydrate binding modules (see http://afmb.cnrs-mrs.fr/CAZY/), and a Gly/Pro-rich linker between them. CBM13 consists of three repeat subdomains (α, β, and γ) of about 40 amino acid residues in length. The subdomains are homologues to each other and appear to contain possible sugar-binding sites, which could be predicted from the amino acid sequence of the ricin toxin B chain (Kuno et al., 2000). Recently, both the crystal structure of the xylanase containing CBM13 and the crystal structures of the sugar complexes of the xylanase have been reported (Fujimoto et al., 2000, 2002). In the complex structures, bound sugars were identified in two subdomains (α and γ) and the binding manners for some sugars were revealed. However, the sugar binding site in the β subdomain was buried in the crystal packing interface and bound sugars were not observed. Recently, sugar binding characteristics of CBM13 from *S. lividans* xylanase (Sl CBM13) with over 80% sequence similarity to that from *S. olivaceoviridis* xylanase, were identified (Notenboom et al., 2002; Schärpf et al., 2002). Three sugar binding sites (α, β, and γ) of Sl CBM13 have different association constants for a series of sugars and are differentiated from each other by chemical shift perturbation mapping using ^{1}H-^{15}N HSQC spectroscopy (Schärpf et al., 2002).

*To whom correspondence should be addressed. E-mail: hemmi@nfri.affrc.go.jp

Here we report chemical shift assignments for CBM13 from *S. olivaceoviridis* xylanase. The chemical shift mapping perturbation of CBM13 with sugar by ^{1}H-^{15}N HSQC spectroscopy will provide equilibrium association constants for each of three sugar-binding sites of CBM13. Furthermore, this work will provide sugar-protein interactions at the β subdomain in the sugar-CBM13 complex.

Materials and experiments

The CBM13 coding gene of the family 10 xylanase from *S. olivaceoviridis* (residues 305–436) was amplified by the polymerase chain reaction (PCR). An amplified fragment was subcloned into the plasmid pGEM-T Easy vector (Promega, WI). After digestion of the plasmid by two restriction enzymes (*Nco*I and *Xho*I), the target fragment was ligated into a pET27 vector (Novagen, WI) using DNA Ligation Kit version 2 (Takara, Kyoto, Japan). The resulting expression vector was transformed into *Escherichia coli* cells, Epicurian Coli® BL21-CodonPlus™ Competent Cell (Stratagene, CA) and was expressed as a C-terminal HSV- and His_6-tagged fusion protein. Uniformly ^{15}N and ^{13}C double labeled CBM13 was expressed in doubly labeled C.H.L. medium (Chlorella Co., Japan) containing kanamycin (25 μg/ml), and the soluble protein was purified as described previously (Kuno et al., 1998).

NMR samples contained 0.9 mM CBM13 in 50 mM phosphate buffer (pH 6.1, 90:10 v/v H_2O/D_2O) and 50 mM KCl. NMR experiments were recorded at 303 K with a Bruker Avance-500 spectrometer. Sequence-specific assignments of the polypeptide backbone were made from ^{1}H-^{15}N HSQC, HNCA, HNCO, HN(CO)CA, HACACONH, CBCACONH, and HNCACB spectra, while assignments of the side chain resonances were made from CCONH, HCCONH, HCCH-COSY, and HCCH-

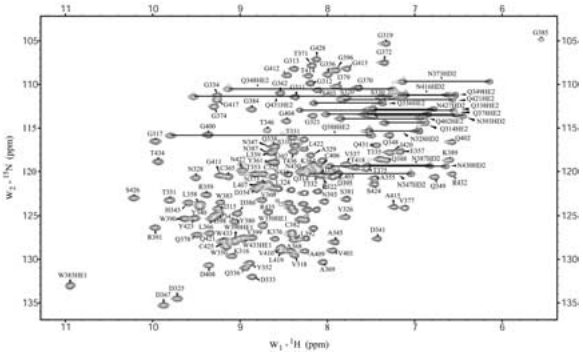

Figure 1. A 500 MHz 2D ^{1}H-^{15}N HSQC spectrum of 0.9 mM CBM13 at pH 6.1 and 303 K. Cross-peaks are labeled upon the basis of an analysis of through-bond connectivities. Side chains of NH_2 resonances of asparagines and glutamine are connected by horizontal lines. Cross-peaks for the amino acids in the HSV- and His_6-tags region of the fusion protein (near the center of the spectrum) have been omitted for clarity.

TOCSY spectra. Aromatic side chain resonances were assigned from 2D ^{1}H-NOESY and TOCSY, CT-^{13}C-HSQC, NOESY-CT-HSQC, and TOCSY-CT-HSQC spectra recorded on natural abundance and [*U*-^{13}C, *U*-^{15}N]-labeled samples in the aromatic carbon region.

NMR data were processed using Felix2000 and analyzed using Sparky (T.D. Goddard and D.G. Kneller, SPARKY 3, University of California, San Francisco). All ^{1}H dimensions were referenced to internal 4,4-dimethyl-4-silapentane-1-sulfate (DSS), and ^{13}C and ^{15}N were indirectly referenced to DSS (Wishart et al., 1995).

Extent of assignments and data deposition

The final product contains the full length 132 amino acid CBM13 sequence (residues 305–436 in *Streptomyces olivaceoviridis* E-86 β-xylanase) with C-terminal HSV- and His_6-tags. All ^{1}H and ^{15}N backbone resonances were assigned except for the first two residues of CBM13, the residues S344, S394, and S429, and five amino acids from the His_6-tag. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum for CBM13, and reveals 123 assigned ^{1}H-^{15}N backbone cross-peaks of CBM13 (96% complete) and 4 assigned ^{1}H-^{15}N side-chain cross-peaks of Trp in CBM13.

Of the 656 backbone resonance signals expected from CBM13, a total of 627 were observed and assigned. Resonance assignments were also made for 725 of the 873 expected side-chain atoms (83% complete) from the protein atom table created by the BMRB NMR-STAR atom table generator for amino acid chemical shift assignments (http://www.bmrb.wisc.edu).

Because the sequence of CBM13 has a high similarity to that of Sl CBM13, the HSQC spectrum of CBM13 is similar to that of Sl CBM13, as expected. However, the amide and amide ^{1}H chemical shifts of some residues in CBM13, such as Asn328, His343 and Gln388, are different from those of the corresponding residues in Sl CBM13. We now find that the binding specificities of the subdomains (α, β, and γ) in CBM13 are in part different from those of Sl CBM13, as evidenced by a mutagenic study (S. Ito and A. Kuno, unpublished results). This indicates that differences in the chemical shifts between the two CBM13s may be due to differences in the binding specificities of the two subdomains.

A secondary structure prediction was performed, using the CSI method (^{1}Hα, ^{13}Cα, ^{13}Cβ and ^{13}CO) (Wishart et al., 1994) and TALOS (^{1}Hα, ^{13}Cα, ^{13}Cβ, ^{13}CO and ^{15}N) (Cornilescu et al., 1999). The secondary structure of CBM13 determined by X-ray crystallography (Fujimoto et al., 2000) has 12 short β-strands and 4 small 3_{10}-helices, but the prediction indicates only 10 β-strands and 2 helices. However, the pattern of the secondary chemical shift changes ($\Delta\delta = \delta_{observed} - \delta_{randomcoil}$) for the ^{1}Hα shifts in CBM13 showed the characteristic β-trefoil fold formed by 12 short β-strands with three repeated structural subdomains (α, β, and γ).

The chemical shifts for CBM13 from *S. olivaceoviridis* xylanase have been deposited in the BioMagResBank database under the accession number 5679.

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