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Letter to the Editor: Virtually complete ^{1}H, ^{13}C and ^{15}N resonance assignments of the second family 4 xylan binding module of *Rhodothermus marinus* xylanase 10A

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Biological context

Glycoside hydrolases are typically modular enzymes consisting of a catalytic module together with one or more non-catalytic modules, of which at least one is a carbohydrate binding module (CBM), whose function is to bring the enzyme into close proximity to its substrate. The xylanase Xyn10A from the thermophilic bacterium *Rhodothermus marinus* consists of two N-terminal CBMs, followed by a domain of unknown function, a catalytic module, and a second domain of unknown function (Nordberg Karlsson et al., 1997). The two CBMs are both from family 4 (Coutinho and Henrissat, 2001). This is an interesting family, because members of the family show diverse binding specificity. The CBM studied here, the second CBM (CBM4-2), is particularly interesting, not only because of its high thermal stability, but also because it binds to xylan and xylo-oligosaccharides with an unusually high affinity (the dissociation constant for xylohexaose is 10 μM at 65 °C and 5 μM at 30 °C: Contrast a typical mesophilic family 2b xylan binding module which has a K_d of 290 μM: Simpson et al., 1999), and because (uniquely) it also has a weak affinity for amorphous cellulose. It is therefore of interest to study the protein to understand how it can bind so strongly.

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Methods and experiments

The DNA encoding CBM4-2 (Nordberg Karlsson et al., 1997) was cloned into the *E. coli* expression vector pET-25b(+) (Novagen) (Abou Hachem et al., 2000). The resultant coding region consists of methionine, the CBM4-2 (163 residues) an alanine, and a 12-residue hsv-tag linked by 2 residues to a His_6-tag. For expression, the plasmid was freshly transformed into BL21(DE3). Shake flask cultures were grown at 37 °C either on LB medium or on minimal medium plus 0.1 mg ml^{-1} ampicillin, containing 2 g l^{-1} $(NH_4)_2SO_4$ and 5 g l^{-1} glucose. Cultures were induced at $[OD_{620}] = 0.5$ with 1 mM IPTG and continued until $OD_{620} = 2$. Cells were harvested, washed, resuspended and sonicated. The protein was purified using metal ion affinity chromatography (Abou Hachem et al., 2000), and eluted with 250 mM imidazole. For NMR, the buffer was exchanged to 50 mM $CaCl_2$, 50 mM sodium acetate-d_3, pH 6.0, containing 10% D_2O and 10 mM sodium azide, and the protein was concentrated to 1.5 mM, using a 5 kDa Vivaspin 20 ml concentrator (Vivascience).

All NMR spectra were recorded at 313 K on Bruker DRX 500 or 600 spectrometers equipped with z gradients, and analysed using Felix 2000, using a range of locally written macros. Backbone and C^{β} resonance assignments were obtained using sensitivity enhanced HNCACB, CBCA(CO)NH, HNCO, and HN(CA)CO spectra. Sidechain and H^{α} assignments were obtained from HNHA, HBHA(CBCACO)NH, CCH-TOCSY and HCCH-TOCSY spectra. Aro-

matic spin systems were identified by comparison of a ^{1}H,^{13}C-CT-HSQC with the 2D TOCSY spectrum. Approximately 60% of aromatic sidechains could be correlated with their C^{β} resonances via (HB)CB(CGCD)HD and (HB)CB(CGCDCE)HE spectra. The majority of remaining aromatics were correlated with their backbone assignments using 2D homonuclear NOESY and ^{13}C,^{15}N-resolved NOESY spectra with the ^{13}C offset centred on the aromatic region. Assignments were confirmed and in a few cases extended using 3D ^{15}N-dispersed TOCSY and NOESY, and using ^{13}C,^{15}N-resolved NOESY spectra. The N-terminal proline of the Pro_i-Pro_{i+1} sequence was identified through a H^{α}_{i}-H^{δ}_{i+1} NOE, and its sidechain was assigned using the CCH/HCCH TOCSY spectra, and confirmed by sequential NOEs. Stereospecific assignment of valine and leucine methyls was accomplished using a 10% uniformly ^{13}C labelled sample (Neri et al., 1989) observed using CT-HSQC with a mixing time of 26.4 ms, which also proved very useful in confirming the relative assignment of isoleucine Me^{γ} and Me^{δ} groups: because of the biosynthetic pathway for isoleucine, the Me^{δ} signal was weak and antiphase to alanine methyls, while the Me^{γ} signal had an intense signal in phase with alanine methyls and Valine $Me^{\gamma 1}$/Leucine $Me^{\delta 1}$ (Figure 1).

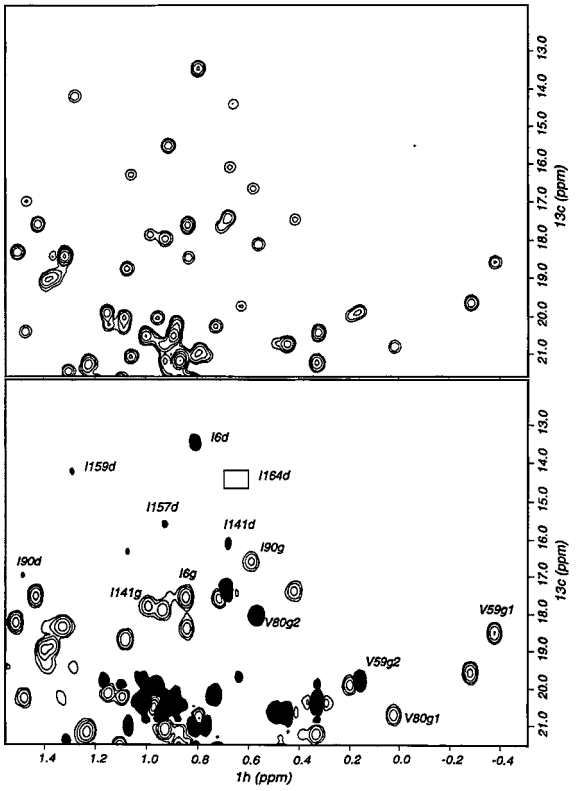

Figure 1. CT-HSQC spectra of (top) uniformly ^{13}C-labelled and (bottom) 10% U-^{13}C-glucose labelled CBM4-2. Negative peaks are coloured black.

Extent of assignments and data deposition

The high quality of the spectra allowed virtually complete assignments of all ^{1}H, ^{13}C and ^{15}N resonances, with (for residues 3 through 164) 100% assignment of backbone ^{1}H resonances (including H^{β}), 98% assignment of backbone ^{13}C and ^{15}N up to C^{β} (all unassigned resonances were from prolines), 92% assignment of sidechain ^{1}H and 81% assignment of sidechain ^{13}C and ^{15}N resonances (ignoring the ends of Lys and Arg), with the missing signals coming entirely from aromatic and Asn C^{γ}. Chemical shifts indicate that CBM4-2 is almost entirely β-sheet, as expected (Johnson et al., 1996). A table of the ^{1}H, ^{13}C and ^{15}N chemical shift assignments has been deposited with BioMagResBank (http://www.bmrb.wisc.edu) under accession number 4906.

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