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Letter to the Editor: ^{1}H, ^{13}C, ^{15}N NMR sequence-specific resonance assignment of a *Clostridium thermocellum* type II cohesin module

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Biological context

The cellulosome is a membrane-bound, extracellular multi-enzyme complex responsible for the degradation of crystalline cellulose by a number of organisms including anaerobic bacteria and fungi, and aerobic bacteria (Beguin and Lemaire, 1996). The organization of the catalytic enzymes into a single macromolecular machine is mediated by a calcium-dependent interaction between type I dockerin modules on the catalytic subunits and complementary type I cohesin modules on the non-catalytic, scaffolding subunit, termed CipA.

CipA of *Clostridium thermocellum* also comprises a C-terminal type II dockerin module, which via its interaction with type II cohesins of cell surface proteins, such as SdbA, is responsible for the attachment of the cellulosome to the cell surface (Leibovitz and Beguin, 1996). Subtle sequential differences exist between type I and type II dockerins and cohesins that are responsible for their distinct specificities.

Recent structures have been reported for both type I dockerins (Lytle et al., 2001) and cohesins (Shimon et al., 1997; Tavares et al., 1997; Spinelli et al., 2000). At present, there are no structures of type II dockerins, type II cohesins or dockerin/cohesin complexes. Here, we report the ^{1}H, ^{13}C, and ^{15}N chemical shifts and secondary structure assignments of the type II cohesin. These suggest that two inserts in the type II cohesin module sequence may be responsible for the specificity of the type II cohesin/dockerin interaction.

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Methods and experiments

A fragment corresponding to the SdbA type II cohesin module from *C. thermocellum* (residues 27 to 200) was subcloned from into the pQE-31 vector (Qiaexpress kit, Qiagen) giving rise to pCT1836, which was transformed into *E. coli* strain BL21. The 6xHis-SdbA cohesin II fusion protein was labelled with ^{15}N- and ^{13}C/^{15}N via bacterial expression and purified by Ni^{2+} affinity chromatography. NMR samples contained 1.2 mM SdbA cohesin II, 25 mM Tris-Cl pH 6.90, 25 mM KCl, 1 mM sodium azide in 90% H_2O/10% D_2O.

All NMR spectra were acquired at 30 °C. The 2D ^{1}H-^{15}N HSQC spectrum was collected on a Bruker AVANCE 600 NMR spectrometer equipped with a TXI Z-gradient inverse triple resonance cryoprobe. All other NMR datasets were acquired on a Bruker DRX 500 NMR spectrometer equipped with a pulsed field gradient probe. Backbone and side chain sequential assignments used the following experiments: 2D ^{1}H-^{15}N HSQC, ^{1}H-^{13}C HSQC; 3D HNHA, 3D HNCO, 3D HNCACB, 3D CBCA(CO)NH, ^{15}N-NOESY-HSQC, CC(CO)NH-TOCSY, HCC(CO)NH-TOCSY, HCCH-TOCSY. Spectra were processed and analyzed with Gifa, Version 4.2 (Pons et al., 1996) and XEASY (Bartels et al., 1995), respectively.

^{1}H, ^{13}C, ^{15}N chemical shifts were assigned using standard triple resonance techniques. Chemical shift index (Wishart and Sykes, 1994) indicated that the SdbA cohesin II module comprises nine β-strands (Ser^{19}-Lys^{29}; Ile^{35}-Asn^{43}; Ala^{49}-Asp^{58}; Val^{61}-Asp^{66}; Ile^{93}-Tyr^{111}; Val^{122}-Gln^{138}; Lys^{140}-Gln^{147}; Gly^{158}-Gln^{176}) and one short α-helical region (Ala^{115}-

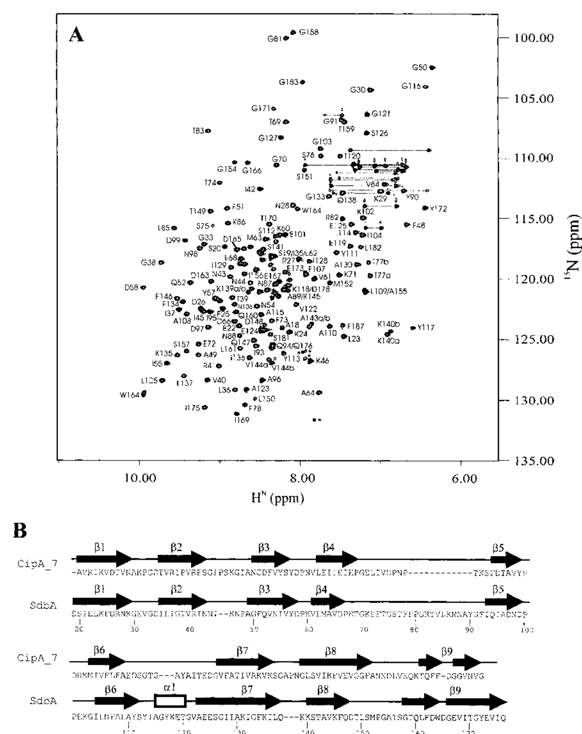

Figure 1. (A) 2D ^{1}H-^{15}N HSQC spectrum of the SdbA type II cohesin module at 600 MHz and 30 °C. The majority of peaks are labelled according to their position in sequence. Side chain amide proton resonances are connected by horizontal lines. Unidentified resonances are indicated by an asterisk. (B) Sequence and secondary structure alignment of SdbA type II cohesin module with a type I cohesin module (CipA_7). Gaps in sequence are shown as dashes and the residues numbered according to the SdbA sequence. β-strands and α-helices are displayed as arrows and boxes.

Thr^{120}). Figure 1B shows that the positioning of these secondary structural elements is very similar to those observed in the *C. thermocellum* type I cohesin module structures (Shimon et al., 1997, Tavares et al., 1997) with one notable exception. A previously unidentified α-helix in the type I cohesin modules is present between strands 6 and 7 of SdbA. This additional structural element may therefore contribute, at least in part, to the type II cohesin/dockerin interaction and specificity.

Extent of assignments and data deposition

Backbone ^{1}H, ^{13}C, and ^{15}N assignments of the SdbA cohesin II module have been completed, as illustrated by the ^{1}H-^{15}N HSQC spectrum in Figure 1A. Resonances were not observed for the nine proline residues (Pro^{59}, Pro^{67}, Pro^{79}, Pro^{80}, Pro^{92}, Pro^{100}, Pro^{153}, Pro^{177}, Pro^{186}) or for the seventeen N-terminus residues (Met^{1}-Lys^{17}). In addition, side chain H^{N} resonances corresponding to 9 asparagines and 6 glutamine residues were also observed. Duplicate resonances were observed for Thr^{77} and Phe^{78}, Lys^{139}/Val^{144}. *Cis-trans* isomerization of Pro^{79} is a potential cause of the doubling for the Thr^{77} and Phe^{78} resonances due to their close proximity while the region comprising Lys^{139}-Val^{144} is likely experiencing two different electronic environments. The ^{1}H and ^{13}C side chain assignments are complete with the exception of some longer side chains and aromatic side chains.

The ^{1}H, ^{13}C, ^{15}N chemical shifts for the *C. thermocellum* SdbA type II cohesin module have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under BMRB accession number 5267.

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