Backbone and side-chain ^{1}H, ^{13}C, and ^{15}N chemical shift assignments for the *apo*-form of the lytic polysaccharide monooxygenase *Nc*LPMO9C

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Abstract

The *apo*-form of the 23.3 kDa catalytic domain of the AA9 family lytic polysaccharide monooxygenase *Nc*LPMO9C from *Neurospora crassa* has been isotopically labeled and recombinantly expressed in *Pichia pastoris*. In this paper, we report the ^{1}H, ^{13}C, and ^{15}N chemical shift assignments of this LPMO.

Keywords

lytic polysaccharide monooxygenase, LPMO, AA9, cellulose, xyloglucan

Biological context

Lignocellulosic biomass is primarily composed of cellulose, hemicellulose and lignin. Cellulose forms crystalline structures that make the polysaccharide resistant to enzymatic hydrolysis. The formation of co-polymeric structures with hemicelluloses such as xylan, glucomannan or xyloglucan may create additional barriers for enzymatic conversion. Biomass recalcitrance is a challenge for biomass conversion and the utilization of cellulose in biorefineries. Enzymatic biomass conversion normally requires several enzymes, including hydrolases and the recently discovered lytic polysaccharide monooxygenases (LPMOs). LPMOs comprise four families of carbohydrate-active enzymes (AA9, AA10, AA11 and AA13) (Levasseur et al. 2013; Hemsworth et al. 2014; Lo Leggio et al. 2015; Beeson et al. 2015; Hemsworth et al. 2015) that catalyze oxidative cleavage (Vaaje-Kolstad et al. 2010; Quinlan et al. 2011; Phillips et al. 2011; Kim et al. 2014). LPMOs boost the activity of the hydrolytic polysaccharide degrading enzymes and are thus of great importance for efficient biomass conversion.

Previous NMR investigations of LPMOs have been carried out solely on chitin-active members of the bacterial AA10 family (Aachmann et al. 2011; Aachmann et al. 2012; Courtade et al. 2014), whereas studies on the solution structures of the industrially more important fungal LPMOs in family AA9 are lacking. Here, we have focused on *Nc*LPMO9C, a C4-oxidizing AA9 LPMO from *Neurospora crassa* that has been shown to cleave β-1,4 glycosidic bonds in cellulose, cellulose oligomers and hemicellulose β-glucans such as xyloglucans (Kittl et al. 2012; Isaksen et al. 2013; Agger et al. 2014). The X-ray diffraction structure (PDB ID: 4D7U) of this protein has been published recently (Borisova et al. 2015). The structure displays the typical LPMO core composed of two β-sheets (one 3-stranded and one 4-stranded) that form a β-sandwich fold from which several loops protrude. The copper ion (a necessary cofactor for all LPMOs) is coordinated by the N-terminal histidine (His1), its side-chain ($N^{\delta 1}$) and the side-chain ($N^{\varepsilon 2}$) of His83. The hydroxyl group of a characteristic tyrosine, Tyr166, further shapes the copper-site by occupying one of the axial coordination positions. This copper coordination site is located in the center of a

flat surface, which is the putative substrate-binding site (Vaaje-Kolstad et al. 2005; Vaaje-Kolstad et al. 2010; Quinlan et al. 2011; Aachmann et al. 2012; Hemsworth et al. 2013).

The unique ability of *Nc*LPMO9C to act on soluble substrates makes it an attractive candidate to investigate substrate-binding of LPMOs in solution using NMR spectroscopy. The NMR assignment data presented here will allow future structural and functional studies on the *apo*-form of this LPMO.

Methods and experiments

The NMR assignment was performed on the *apo*-form of the recombinantly expressed catalytic domain of *Nc*LPMO9C (also known as NCU02916). Cloning was performed as described previously (Borisova et al. 2015). The production of the isotopically labeled catalytic domain of *Nc*LPMO9C was based on the protocol published by Pickford and O'Leary (Pickford and O'Leary 2004) for shake-flask cultures, with some modifications. In specific, the *Pichia* culture medium used in this study was ^{13}C, ^{15}N-labelled buffered minimal glucose medium (^{13}C, ^{15}N-BMD), composed of 0.34% (w/v) yeast nitrogen base (YNB) without amino acids or ammonium sulfate (Becton, Dickinson & Company, Sparks, MD 21152, USA), 4×10^{-5}% (w/v) biotin (Sigma-Aldrich, St. Louis, MO, USA), 1% (w/v) ^{15}N-labeled ammonium sulfate (Cambridge Isotope Laboratories, Tewksbury, MA, USA) and 0.5% (w/v) ^{13}C-labeled glucose (Cambridge Isotope Laboratories, Tewksbury, MA, USA) in 100 mM potassium phosphate buffer pH 6.0. After inoculation with single *P. pastoris* colonies, the culture was incubated with shaking (180 rpm) at 28ºC for 44 h. For the isolation of the isotopically labelled protein, the culture supernatant was recovered by centrifugation followed by sequential filtering through 0.45 µM and 0.22 µM polyethersulfone (PES) filters (Millipore, Billerica, MA, USA). A Vivaflow 200 tangential cross-flow protein concentrator (MWCO 5 kDa, Sartorius Stedim Biotech GmbH, Germany) was used to concentrate the supernatant and exchange buffer to 50 mM MES pH 6.5, 150 mM NaCl. The concentrated sample was loaded onto two Superdex 75 gel filtration columns (GE Healthcare Bio-Sciences, AB, Sweden), connected in series.

In order to obtain the *apo*-form of the protein, the sample was incubated in a 50 mM MES buffer pH 6.5 and 150mM NaCl containing 8 mM EDTA for 45 minutes at room temperature. Subsequently, the buffer was changed to 25 mM sodium phosphate buffer pH 5.5 and 10 mM NaCl in 90% H_2O/10% D_2O, using a Vivaspin 6 protein spin concentrators (MWCO 5 kDa, Sartorius Stedim Biotech GmbH, Germany). The protein concentration was determined by measuring the A_{280} of the protein solution using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and deducing the protein concentration based on the theoretical extinction coefficient (calculated using the ProtParam tool; http://web.expasy.org/tools/protparam/) (Gasteiger et al. 2005). The final samples contained 0.1-0.2 mM *apo*-*Nc*LPMO9C.

The NMR spectra were recorded at 25ºC on a Bruker Avance III 600 MHz spectrometer equipped with a 5 mm Z-gradient CP-TCI (H/C/N) cryoprobe or a 5 mm Z-gradient Prodigy TCI (H/C/N) cryoprobe at the NT-NMR-Center/Norwegian NMR Platform in Trondheim, Norway and at the Department of Chemistry and Biosciences, Aalborg University, Aalborg, Denmark, respectively. ^1H 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). Sequence-specific backbone and side-chain assignments of *Nc*LPMO9C were accomplished using ^{15}N-HSQC, ^{13}C-aliphatic HSQC, ^{13}C-aromatic HSQC, HNCO, HN(CA)CO, HNCA, CBCANH, CBCA(CO)NH, HBHA(CBCACO)NH, (H)CCH-TOCSY, H(C)CH-TOCSY, (HB)CB(CGCD)HD, CACO, CON, ^{15}N-edited NOESY-HSQC, and ^{13}C-edited aliphatic and aromatic NOESY-HSQC spectra. The NMR data were recorded and processed with Bruker TopSpin version 3.2/3.5 and spectral analysis was performed using CARA version 1.5.5 (Keller 2004). Secondary structure elements were analyzed using the web-based version of the TALOS-N software (http://spin.niddk.nih.gov/bax/software/TALOS-N/) (Shen and Bax 2013) using the N, C', H^N, C^α, C^β, H^α and H^β chemical shifts.

Assignment and data deposition

We report here the assignment of the backbone and side-chain resonances of *Nc*LPMO9C. The ^{15}N-HSQC spectrum of *Nc*LPMO9C, together with the assignment of the resonances is shown in Fig. 1. The backbone and side-chain assignments are essentially complete (H^N, H^α, N, C^α, C' > 98%; H and C side-chains > 90%). Because of fast-exchange, the amide group of His1 could not be found, whereas other nuclei of this residue (C^α/H^α, C', $C^{\varepsilon 1}/H^{\varepsilon 1}$) were assigned. Exchangeable side-chain protons were not assigned, nor were the amide side-chain protons of Asn and Gln. For the aromatic side-chains, assignment of the side-chain $C^{\varepsilon 1}/H^{\varepsilon 1}$ and $C^{\delta 2}/H^{\delta 2}$ histidine pairs was prioritized and successful, whereas other aromatic side-chains were not assigned. The chemical shift data has been deposited in the Biological Magnetic Resonance Data Bank (BMRB) under the accession number 26717.

Analysis of the secondary structure elements of *Nc*LPMO9C indicated the presence of one α-helix (in the so-called LS loop) and 7-8 β-strands. The length and position of these secondary structure elements was in excellent agreement with those observed in the X-ray crystal diffraction structure of *Nc*LPMO9C.

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Conflict of interest

The authors declare that they have no conflict of interest.

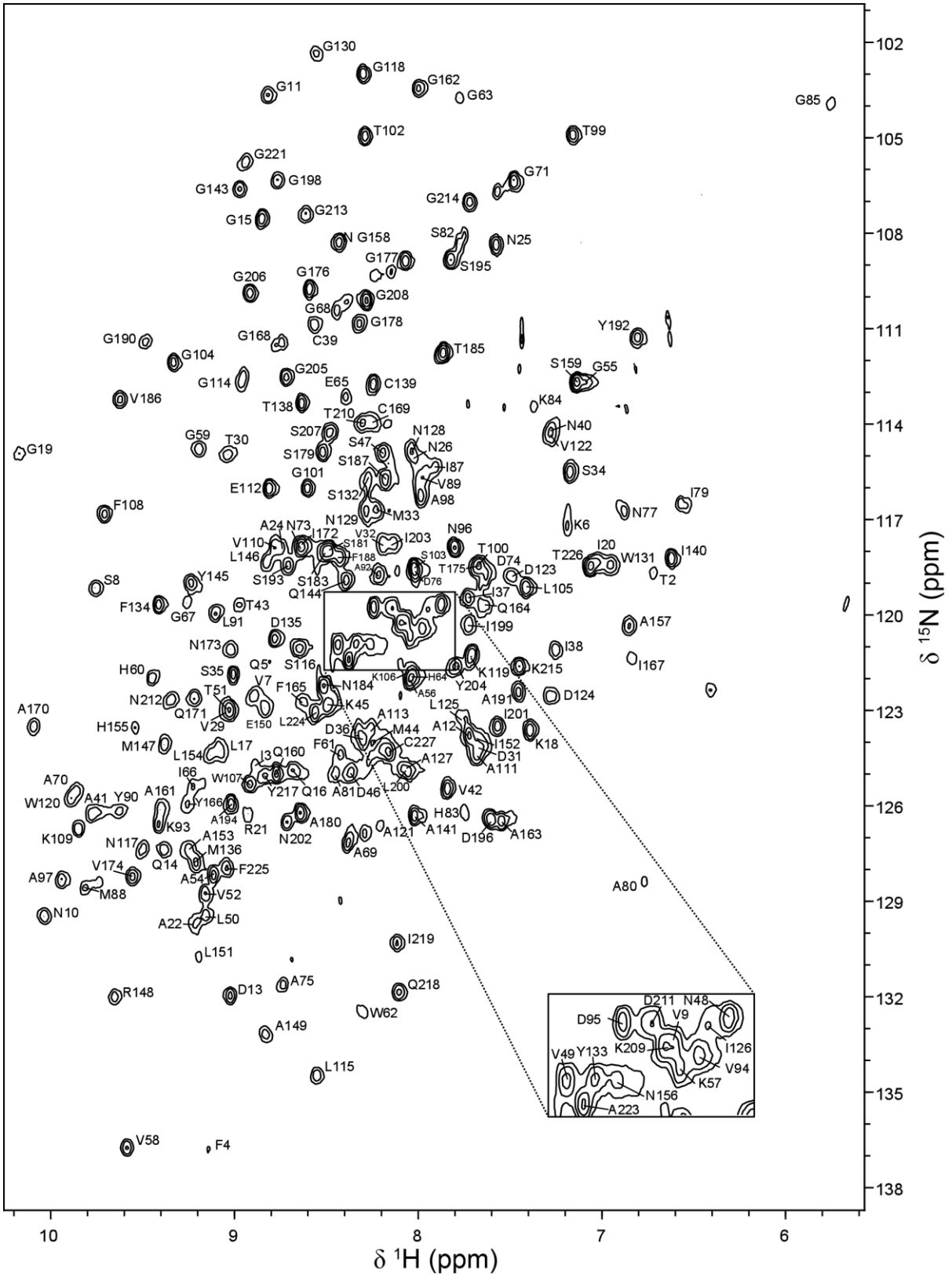

Fig. 1 ^{1}H, ^{15}N HSQC spectrum of ^{13}C, ^{15}N-labeled *apo-Nc*LPMO9C (0.2 mM) from *N. crassa* in (90:10) H_2O:D_2O at pH 5.5, 298 K. Residue types and numbers are indicated.

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