Journal of Biomolecular NMR, **23:** 333–334, 2002.
KLUWER/ESCOM

Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignment of *Bacillus agaradhaerens* family 11 xylanase

Marco Betz, Frank Löhr, Hans Wienk & Heinz Rüterjans*
Institut für Biophysikalische Chemie, Johann Wolfgang Goethe-Universität Frankfurt am Main, Biozentrum N230, 1. OG, Marie Curie-Strasse 9, D-60439 Frankfurt, Germany

Received 28 May 2002; Accepted 27 June 2002

Key words: NMR assignment, xylanase

Biological and industrial context

Xylanases belong to a large group of glycosyl hydrolases. They catalyze the degradation of xylan, one of the major constituents of plant cell walls. Xylans are heteropolysaccharides composed of β-1,4-linked xylopyranose units forming a backbone. They are typically branched with arabinose, glucoronic acid, and acetate substituents at 2- and 3-positions of xylose. The xylan fibers are in tight association with other biopolymers like cellulose, hemicellulose and lignin. Biologically, xylanases are synthesized by microorganisms and are secreted to degrade surrounding xylan as food supply.

In recent years, xylanases are increasingly used in biotechnological applications such as the bleaching of hardwood pulp for paper manufacture and the processing of vegetable matter in food industries (reviews: Subramaniyan et al., 2000; Kulkarni et al., 1999). Pretreatment of paper pulp with xylanases enhances the bleaching process. Lower amounts of bleaching chemicals are required thereby reducing environmentally toxic byproducts and economic costs. Desirable properties of a xylanase used in industrial scale include stability and activity at elevated temperatures and extreme pH values.

The familiy 11 xylanase (23 kDa) from *Bacillus agaradhaerens* (Sabini et al., 1999) carries out xylan hydrolysis via a two-step mechanism involving a covalent enzyme-glycosyl intermediate. Initially, carboxylate of Glu184 functions as a Brönsted acid, protonating the glycosidic bond to assist leaving-group departure. Glu94 performs a nucleophilic attack at the substrate anomeric center forming an intermediate bondage. In the second step hydrolysis proceeds in reversed order with water as the nucleophilic agent and Glu184 functioning as Brönsted base.

A complete ^{1}H, ^{13}C, ^{15}N assignment of this enzyme was obtained as a prerequisite to investigate the role of electrostatic interactions while following the pH dependence of catalytic activity.

Methods and experiments

The region of *B. agaradhaerens* xylanase encoding amino acids 1-207 was recloned into pET3a plasmid and overexpressed in *Escherichia Coli* strain BL21(DE3). Uniformly ^{15}N and ^{13}C double labelled xylanase was prepared from cells grown at 37 °C in minimal medium containing 1 g/l ^{15}N ammonium chloride, 1 g/l ^{13}C glucose and 2.5 g/l ^{13}C glycerol. The cell lysate was applied on a SP-Sepharose column operating at room temperature and equilibrated with 10 mM sodium acetate, pH 5.3. The enzyme was eluted using a gradient up to 0.6 M NaCl. The xylanase pool was concentrated and desalted on a Superdex G75 column buffered with 10 mM sodium acetate, pH 5.3. For NMR studies, the protein was rebuffered in 95% H_2O/5% D_2O with 10 mM perdeuterated sodium-acetate, pH 5.3, to a final concentration of 1.5 mM.

Experiments were carried out at 301 K on Bruker DMX 500 or DMX 600 spectrometers equipped with three-axis gradient ^{1}H{^{13}C^{15}N} triple resonance probes. The ^{1}H, ^{13}C, ^{15}N backbone and ^{13}C sidechain resonances were assigned using the following set of 3D triple resonance experiments: HNCO, (HCA)CO(CA)NH, HNCACB and

*To whom correspondence should be addressed. E-mail: hruet@bpc.uni-frankfurt.de

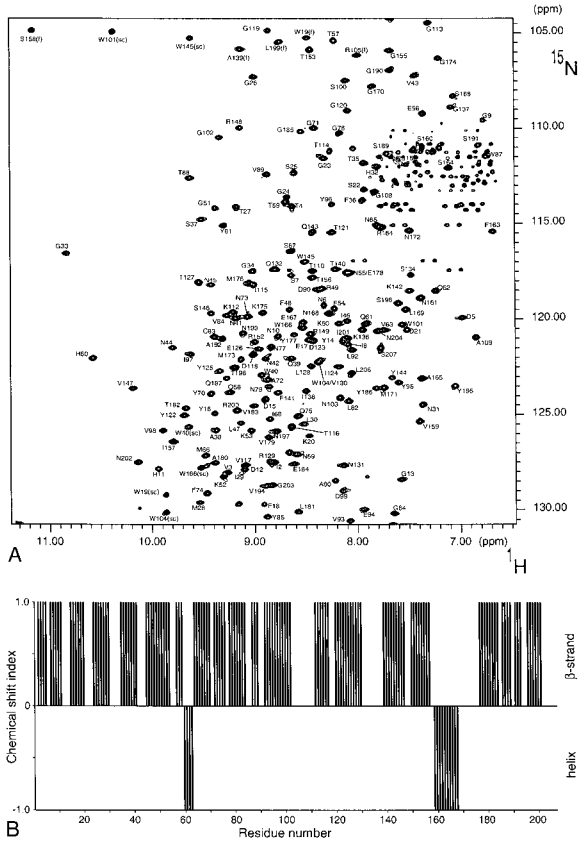

Figure 1. (A) [^{15}N-^{1}H] TROSY (500 MHz) spectrum (Pervushin et al., 1997) recorded of recombinant *B. agaradhaerens* xylanase. The H^N peaks of residues Trp19, Arg105, Ala139, Ser158 and Leu199 are folded (f) in the ^{15}N dimension. Tryptophan sidechain indole resonances are marked 'sc'. (B) Secondary structure prediction based on H^{α}, C^{α}, CO and C^{β} chemical shift diversions from random coil values. β-strands and helical structures are assigned +1 and −1, respectively.

CC(CO)NH-TOCSY spectra. ^{1}H aliphatic sidechain resonances were assigned by a combination of HBHA-CONH and H(CC)(CO)NH-TOCSY spectra. Time-domain data was Fourier transformed after zero-filling, apodization and linear prediction using XWIN-NMR 2.1 (BRUKER). To avoid errors, the sequential assignment trace was followed simultaneously using $^{13}C^{\beta}/^{13}C^{\alpha}$ and ^{13}CO resonances via the PRONTO (v.19990506) software (Kjaer et al., 1994). ^{1}H resonance positions were calibrated with internal DSS while indirect referencing according to the absolute frequency values was used for ^{15}N and ^{13}C chemical shifts (Wishart et al., 1995).

Extent of assignments and data deposition

For the 207 amino acid residues of recombinant *B. agaradhaerens* xylanase, the backbone assignment is essentially complete, as shown in Figure 1 for ^{1}H and ^{15}N resonances. All 200 non-proline residues were assigned, excluding two residues, Met1 and Lys151. Almost all sidechain proton and carbon shifts were assigned with the exception of Gln75, Thr110, Gln132, Ser150 and Ser207, where $^{1}H^{\beta}$ and/or the $^{1}H^{\gamma}/^{13}C^{\gamma}$ resonances could not be identified unambigously. A secondary structure prediction was performed, based on chemical shift differences between observed and random coil values for $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$ and ^{13}CO (Wishart et al., 1994). This prediction (data not shown) is in good agreement with expectations based on the crystal structure (Sabini et al., 1999). Additionally the aromatic resonances of all histidines and tryptophanes were assigned sequence-specifically, as reported previously (Löhr et al., 2002). The chemical shift values of proton, carbon and nitrogen resonances were deposited in the BioMagResBank (http://bmrb.wisc.edu) under the accession number 5352.

Acknowledgements

The recombinant enzyme was produced in collaboration with Novo-Nordisk A/S (Bagsvaerd, Denmark). H.W. is a recipient of a grant from the Alexander-von-Humboldt-Foundation.

References

Kjaer, M., Andersen, K.V. and Poulsen, F.M. (1994) *Meth. Enzymol.*, **239**, 288–307.

Kulkarni, N., Shendye, A. and Rao, M. (1999) *FEMS Microbiol. Rev.*, **23**, 411–456.

Löhr, F., Katsemi, V., Betz, M., Hartleib, J. and Rüterjans, H. (2002) *J. Biomol. NMR*, **22**, 153–164.

Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12366–12371.

Sabini, E., Sulzenbacher, G., Dauter, M., Dauter, Z., Jørgensen, P. L., Schülein, M., Dupont, C., Davies, G.J. and Wilson, K.S. (1999) *Chem. Biol.*, **6**, 483–492.

Subramaniyan, S. and Prema, P. (2000) *FEMS Microbiol. Lett.*, **183**, 1–7.

Wishart, D.S. and Sykes, B.D. (1994) *J. Biomol. NMR*, **4**, 171–180.

Wishart, D.S., Bigam, C.G., Yao, J., Abildgaard, F., Dyson, H.J., Oldfield, E., Markley, J.L. and Sykes, B.D. (1995) *J. Biomol. NMR*, **6**, 135–140.