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ARTICLE

NMR assignments of ^{1}H, ^{13}C and ^{15}N resonances of the C-terminal subunit from *Azotobacter vinelandii* mannuronan C5-epimerase 6 (AlgE6R3)

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Abstract The 19.9 kDa C-terminal module (R3) from *Azotobacter vinelandii* mannronan C5-epimerase AlgE6 has been ^{13}C, ^{15}N isotopically labelled and recombinantly expressed. We report here the ^{1}H, ^{13}C, ^{15}N resonance assignment of AlgE6R3.

Keywords Alginate · Mannuronan C5-epimerases · A and R-module

Abbreviations

DSS	4,4-Dimethyl-4-silapentane-1-sulfonic acid
DTT	Dithiothreitol
G	α-L-Guluronic acid
HEPES	*N*-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid
IPTG	Isopropyl β-D-1-thiogalactopyranoside
M	β-D-Mannuronic acid

Biological context

AlgE6 belongs to a family of seven structurally related alginate epimerases called AlgE1-7 produced by *Azotobacter vinelandii* (Ertesvåg et al. 1994; Ertesvåg et al. 1995). Alginate is initially produced as poly-β-D-mannuronic acid (M) and alginate epimerases introduce α-L-guluronic acid (G) into the polysaccharide (Hartmann et al. 2002; Campa et al. 2004). Each member of the AlgE-family produces a unique sequence of M and G subunits in the alginate polymer (Ertesvåg and Skjåk-Bræk 1999). All these epimerases consist of two types of structural modules, designated A (~385 amino acids) and R (~150 amino acids). All epimerases contain one N-terminal A-module, and AlgE1 and AlgE3 in addition contain a second such module internally in their sequences (Ertesvåg et al. 1998). The A-modules are the catalytically active parts of the epimerases, but the R-modules strongly enhance this activity although they don't possess any catalytic activity themselves (Ertesvåg and Valla 1999). The number of R-modules vary from 1 (AlgE4) to 7 (AlgE3), and AlgE6 contains three such modules. Compared to the other two R-modules of AlgE6, R3 has 69 and 64% sequence identity to R1 and R2, respectively. In addition, AlgE6R3 (located C-terminally) contains a predicted C-terminal signal peptide for secretion of the epimerase. The core structures of the R-modules are similar which is also reflected in some conserved chemical shift patterns found in ^{15}N HSQC fingerprint spectra. However, the individual R-modules show quite different affinity for different specifically tailored alginate polymers. Therefore structures of the three R-modules and their affinities to different alginates will allow us to gain a deeper insight into the role of the R-modules in epimerase functionality.

Methods and experiments

The gene coding for the AlgE6R3-module (residues 694–874) was synthesized *de novo* (GenScript, Piscataway, USA). The sequence was extended by one amino acid (Ala1) for optimal cleavage from the intein tag during purification.

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The DNA sequence corresponding to AlgE6R3 was cloned into pTYB12 (IMPACT-CN system, New England Biolabs.) using BsmI and XmaI sites, generating pFA13 which codes for a fusion protein consisting of AlgE6R3 and a chitin-binding domain. Uniform labelling of AlgE6R3 (181 amino acids) was achieved by overexpressing the protein in *Escherichia coli* ER2566 containing the plasmid pFA13. The cells were grown at 37°C to OD_{600} ~0.8 in M9-medium supplemented with $(^{15}NH_4)_2SO_4$ (1 g/L), $^{13}C_6$-D-glucose (2 g/L) (Sigma-Aldrich), 0.2 mM $CaCl_2$ and 200 µg/L ampicillin. Expression was induced by 1 mM ITPG at 15°C and allowed to continue over night. The cells were harvested and resuspended in 20 mM HEPES pH 6.9, 800 mM NaCl, 10 mM $CaCl_2$ and 0.1% Triton X-100 (Sigma-Aldrich). They were then lysed by sonification and the supernatant was loaded on a column with chitin beads (New England Biolabs). The column was washed with 20 mM HEPES pH 6.9, 800 mM NaCl and 5 mM $CaCl_2$. AlgE6R3 was cleaved from the chitin binding tag by incubating the column with the bound fusion protein with 50 mM DTT in 20 mM HEPES pH 6.9, 800 mM NaCl and 5 mM $CaCl_2$ at room temperature for ~16 h, whereafter it could be eluted from the column. The eluted AlgE6R3 was dialysed against 20 mM HEPES, pH 6.9, 25 mM $CaCl_2$.

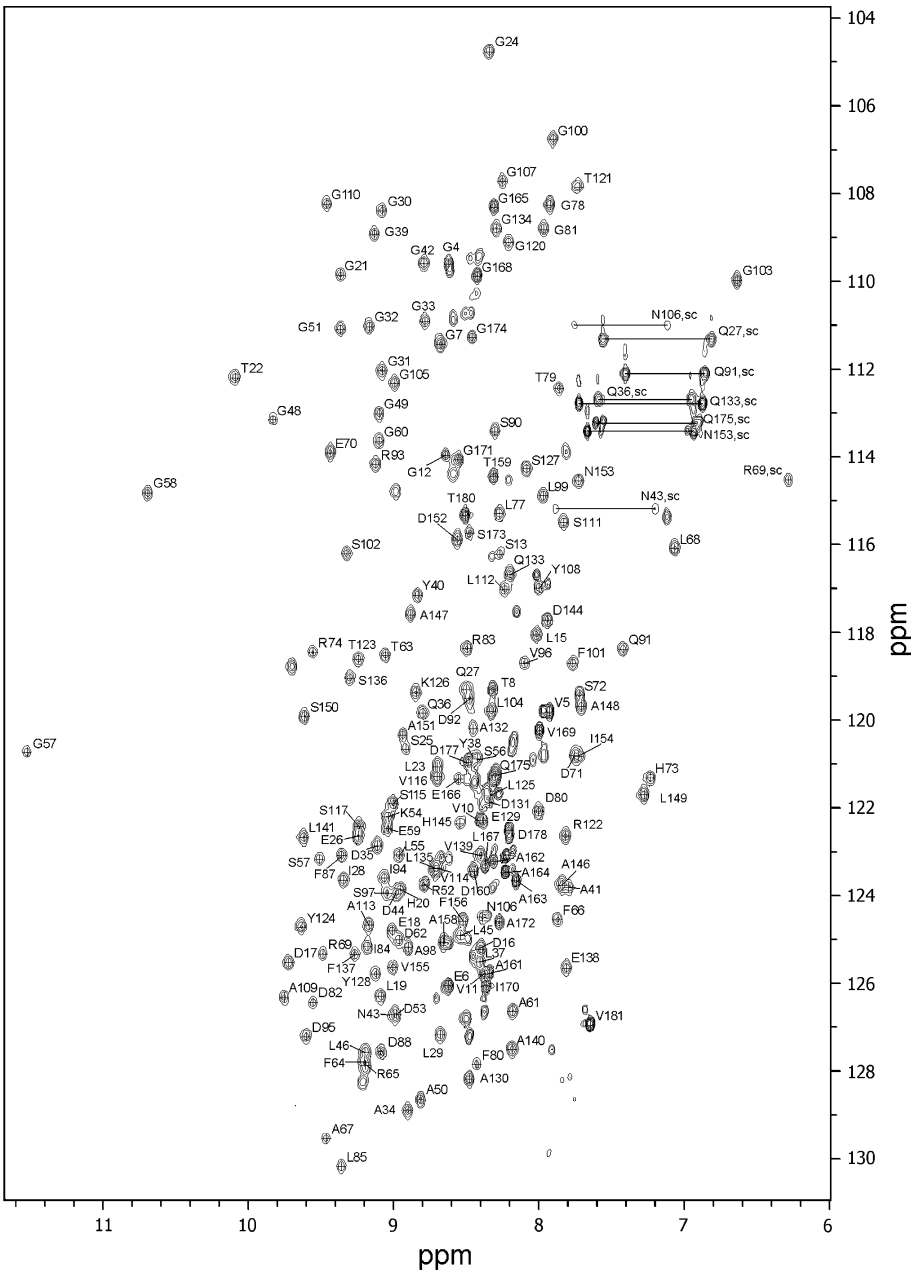

Fig. 1 ^{1}H, ^{15}N HSQC spectrum of the ^{13}C, ^{15}N-labelled AlgE6R3 subunit from *Azotobacter vinelandii* in 90:10 H_2O:D_2O at pH 6.9, 298 K. Residue numbers are indicated. Side-chain resonances of Asn and Gln residues are connected by *lines*. Other side-chain amine resonances are indicated with amino acid number and sc

Samples for NMR studies contained 1.0–1.4 mM AlgE6R3 in 20 mM HEPES buffer, pH 6.9 with 25 mM $CaCl_2$ dissolved in either 90% H_2O/10% D_2O or 99.9% D_2O.

The NMR measurements were performed on a Bruker Avance 600 spectrometer equipped with 5 mm z-gradient TXI (H/C/N) cryogenic probe and on a Bruker DRX 600 spectrometer equipped with 5 mm xyz-gradient TXI (H/C/N) probe. All experiments were performed at 298 K. Proton and carbon chemical shifts were referenced relative to internal 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS); ^{15}N chemical shifts were referenced indirectly to DSS, based on the absolute frequency ratios (Zhang et al. 2003). For the sequence specific backbone- and side-chain assignment, the following experiments were used: ^{15}N-HSQC, ^{13}C-HSQC, HNCO, HN(CA)CO, HNCA, HN(CO)CA, CBCANH, CBCA(CO)NH, HBHANH, HBHA(CO)NH, HCCH-TOCSY and HCCH-COSY. The assignment of aromatic side chains were based on 2D ^{13}C-HSQC and 2D-NOESY, 2D-COSY and 2D-TOCSY recorded on samples dissolved in D_2O. The NMR data were recorded and processed with Bruker XWinNMR version 3.5 or Bruker TopSpin 1.3 software and spectral analysis was performed using CARA version 1.4.1/1.8.4 (Keller 2004).

Assignment and data composition

We report here the backbone resonance assignments of the third R-module of AlgE6R3. The ^{15}N-HSQC spectrum of AlgE6R3, together with the assignments of the resonances, is shown in Fig. 1. The backbone and side chain assignment were essentially complete: 96.9% of the backbone H^N, H^{α}, C', C^{α} and N atoms, and 95.7% of the side-chain atoms has been assigned. A1 and D2 were not assigned. The amide groups (H^N, N) of D14, D47, D118, D119, E142, G143 and A157 could not be found, although other nuclei of these residues were assigned. Except for H^{ε} of R69, none of exchangeable side-chain protons of Arg and Lys residues were assigned. Side-chain amide protons of all Asn and Gln residues were assigned. All aromatic protons were assigned except H^{ζ} of F101. Protonated carbon atoms of aromatic residues were assigned to a large extent.The chemical shift data have been deposited in the BioMagResBank data-base under the accession number 16956.

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