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ARTICLE

^{1}H, ^{13}C, and ^{15}N NMR assignments of the *Pyrococcus abyssi* DNA polymerase II intein

Jiajing Liu · Zhenming Du · Clayton D. Albracht · Roshni O. Naidu · Kenneth V. Mills · Chunyu Wang

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Abstract Protein splicing is a precise post-translational process mediated by inteins. Inteins are intervening proteins that cleave themselves from a precursor protein while joining the flanking sequences. Here we report the ^{15}N, ^{13}C, and ^{1}H chemical shift assignments of the intein from DNA polymerase II of *Pyrococcus abyssi* (*Pab* PolII intein), which has been recombinantly overexpressed and isotopically labeled. The NMR assignments of *Pab* PolII intein are essential for solution structure determination and protein dynamics study.

Keywords Intein · NMR · Assignment · Hyperthermophile · DNA polymerase II · *Pyrococcus abyssi*

Biological context

Protein splicing is a precise post-translational process in which an in-frame intervening polypeptide, called an intein, is excised out of a precursor protein, while the flanking sequences, termed the N- and C-exteins, are joined together (Paulus 2000). Protein splicing is orchestrated in a precise sequence of bond rearrangements and is self-catalyzing, requiring no cofactors. Protein splicing has been harnessed as a valuable tool in biomedical research and biotechnology (Elleuche and Poeggeler 2010). Although intein structures have been characterized extensively by X-ray crystallography (Saleh and Perler 2006) and NMR methods (Du et al. 2010; Oeemig et al. 2009; Johnson et al. 2007; Frutos et al. 2010; Du et al. 2009), details of the protein splicing mechanism, including the the precise mechanistic roles of highly conserved residues or motifs, are still lacking. This is due, in part, to the spontaneous nature of protein splicing, making it difficult to obtain functional precursors for structural studies. *Pab* PolII intein is present in a hyperthermophilic organism and can yield a functioning precursor inactive at room temperature. Such an intein precursor, the equivalent of an enzyme-substrate complex, would be ideal for NMR studies of intein mechanism. In addition, the *Pab* PolII intein is only the second intein demonstrated to splice with a C-terminal glutamine (Mills et al. 2004). In contrast to *Chiloiridescent virus* (*CIV*) RNR intein (Amitai et al. 2004), the mutation of the C-terminal glutamine to an asparagine enhances protein splicing by three fold (Mills et al. 2004). As a first step towards carrying out structural and mechanistic studies at atomic resolution using solution NMR, we have determined the backbone and sidechain resonance assignments of the wild-type *Pab* PolII intein, which contains 185 residues and no homing endonuclease domain.

Methods and experiments

The *Pab* PolII intein gene cloned into the pETM-44 vector ppC1Q185 expresses a fusion protein with a N-terminal $(His)_6$ tag and maltose-binding protein (MBP). There is a single proline between the $(His)_6$-tag and MBP and a linker sequence TPGSLEVLKQGPM between MBP and the intein. Isotopically labeled ([U–^{15}N], [U–^{13}C; U–^{15}N] and

J. Liu · Z. Du · C. D. Albracht · R. O. Naidu · C. Wang (✉)
Biology Department, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
e-mail: wangc5@rpi.edu

K. V. Mills
Department of Chemistry, College of the Holy Cross, Worcester, MA 01610, USA

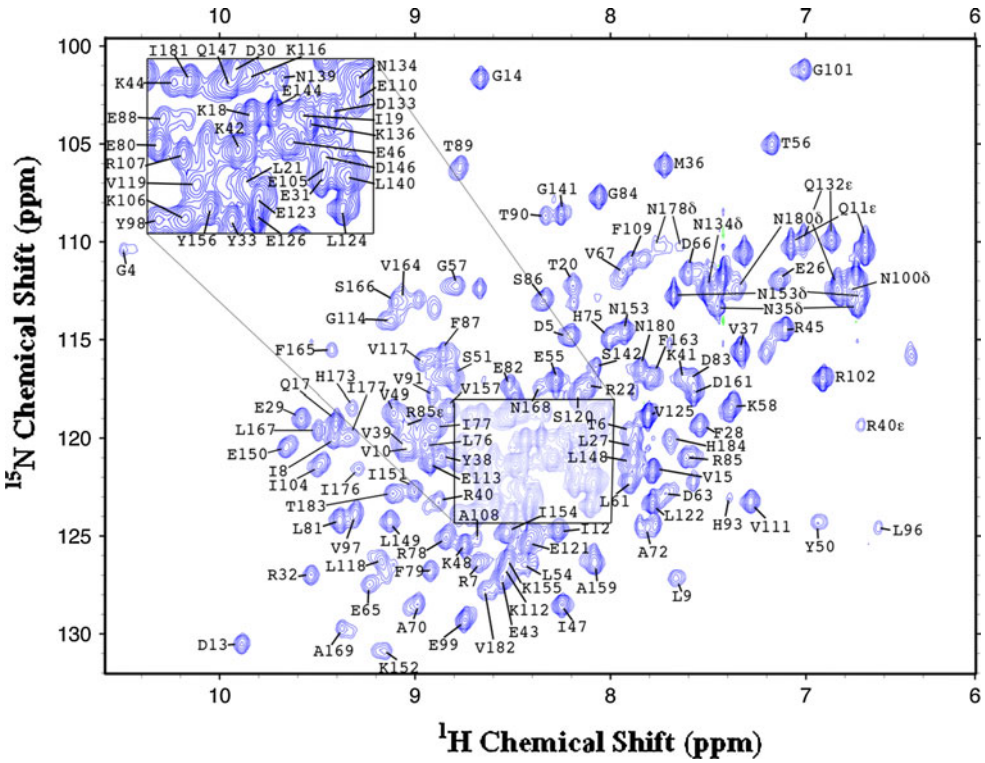

Fig. 1 ^{1}H–^{15}N HSQC spectrum of the *Pab* PolII intein backbone assignments. The *inset* at *upper left* is for labeling peaks in the crowded central regions of the HSQC. Spectrum is recorded at 800 MHz and 47°C

[∼70%-^{2}H; U–^{13}C; U–^{15}N]) proteins were obtained by transforming the plasmid into *E. coli* strain BL21(DE3) and over-expressing the fusion protein in M9 medium. The M9 cultures were incubated at 37 °C until OD_{600} reaches 0.6–0.8 and induced with 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) and incubated for additional 16 h at 20 °C. Cell lysate was purified by nickel-NTA affinity chromatography to obtain the fusion protein. The inteins were cleaved from the N-terminal affinity domain by incubation with 100 mM dithiothreitol (DTT) at 60 °C for 6 h. Affinity chromatography was utilized again to trap the $(His)_6$-tagged MBP and uncleaved fusion protein, with the *Pab* PolII intein collected in the flow through. The protein was then exchanged into buffer containing 20 mM sodium phosphate, 0.5 mM ethylenediaminetetraacetic acid (EDTA), and 0.05 mM sodium azide in 90% H_2O/10% D_2O or 99.9% D_2O at pH 6.5. The final concentrations of the NMR samples are between 1.2 and 2.0 mM.

All spectra were acquired at 47 °C on a Bruker 800 or 600 MHz (^{1}H) spectrometer each equipped with a cryogenic probe. Spectra were processed with nmrPipe software and analyzed using Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco, USA). The ^{1}H chemical shifts were referenced relative to DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) and the ^{15}N and ^{13}C chemical shifts were referenced indirectly using the frequency ratios between ^{15}N, ^{13}C, and ^{1}H (^{15}N/^{1}H = 0.101329118, ^{13}C/^{1}H = 0.251449530).

The backbone sequence-specific assignments of $^{1}H^{N}$, $^{13}C^{\alpha}$, $^{13}C'$, and ^{15}N for the *Pab* PolII intein were obtained using standard triple resonance NMR methods, such as HNCACB, HN(CO)CACB, HNCO, HN(CA)CO, HNCA, and HN(CO)CA, with deuterium decoupling when necessary. Nearly complete backbone assignments were obtained (∼97%) with the exception C1, Y172, H173, N174, and Q185. The backbone resonance assignments are illustrated in Fig. 1. Assignments for aliphatic side chains were achieved by using 3D H(CC)(CO)NH, (H)C(CCO)NH, HCCH-TOCSY, ^{15}N-NOESY and ^{15}N-TOCSY.

Extents of assignments and data deposition

The *Pab* PolII intein contains 185 amino acid residues. Assignments of backbone resonances $^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{1}H^{\alpha}$, and $^{13}C'$ were nearly completely assigned (97%) with the exception of the resonances of C1, Y172, H173, N174, and Q185 and ^{15}N resonances of seven proline residues P3, P16, P43, P71, P94, P135 and P143. Subsequently, all aliphatic side chain resonances were assigned to 96% complete with the exception of I52, L167, Y172, H173, N174, H184 and Q185. Most ^{13}C chemical shifts were derived from ^{13}C HCCH-TOCSY of a uniformly ^{13}C labeled sample without deuteration. One of the K69 H_{ε} has a chemical shift of 1.11 ppm, the lowest chemical shift of

lysine H_{ε} protons reported in the BioMagResBank (http://www.bmrb.wisc.edu). This could be due to the ring current effect of the nearby F162 and F165 in 3D structure of intein. The chemical shifts have been deposited in the BioMagResBank under accession number 17418.

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