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Sequence-specific ^1H , ^{13}C and ^{15}N backbone resonance assignments of the 34 kDa *Paramecium bursaria* Chlorella virus 1 (PBCV1) DNA ligase

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Abstract

Chlorella virus DNA ligase (ChVLig) is a minimal (298-amino acid) pluripotent ATP-dependent ligase composed of three structural modules – a nucleotidyltransferase domain, an OB domain, and a β -hairpin latch – that forms a circumferential clamp around nicked DNA. ChVLig provides an instructive model to understand the chemical and conformational steps of nick repair. Here we report the assignment of backbone ^{13}C , ^{15}N , ^1H resonances of this 34.2 kDa protein, the first for a DNA ligase in full-length form.

Keywords

Chlorella virus; DNA ligase; DNA nick repair; DNA binding

Biological context

DNA ligases recognize and seal nicked duplex DNA by catalyzing the formation of a phosphodiester bond between the 3'-OH and the 5'-phosphate ends of the nick. Ligation is a three-step reaction fueled either by ATP or NAD^+ . The first step involves the formation of a phosphoamide bond between the enzyme and AMP through a reactive lysine side chain. Upon DNA binding, the AMP is transferred to the 5' end of the nick to form a high-energy DNA-adenylate intermediate that is instantly attacked by the nick 3'-OH to restore the phosphodiester backbone and release AMP.

Ligases are encoded in the genomes of all species and many viruses. They play a central role in DNA replication (by sealing Okazaki fragments), repair and recombination (Ellenberger et al., 2008). Complete loss of ligase activity is inevitably lethal. Partial genetic deficiencies in human DNA ligases have been associated with clinical syndromes marked by immunodeficiency, radiation sensitivity, and developmental abnormalities.

Paramecium bursaria Chlorella virus 1 (PBCV1), a DNA virus that infects green algae, encodes the smallest known eukaryal ATP-dependent DNA ligase, referred to hereon as

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ChVLig (Sriskanda et al., 1998a; Sriskanda et al., 1998b; Odell et al., 1999). Although ChVLig lacks the large accessory domains found in cellular DNA ligases, it can sustain mitotic growth and DNA repair in *Saccharomyces cerevisiae* (budding yeast) when it is the only ligase available. It is thought that ChVLig represents a stripped-down “pluripotent” ligase owing to its intrinsic nick sensing function, the basis of which was illuminated by the crystal structure of ChVLig-AMP bound to a nicked duplex DNA (Nair et al., 2007). ChVLig consists of an N-terminal nucleotidyltransferase domain and a C-terminal OB domain, which form the core of all DNA ligases. The nick-bound structure showed that ChVLig-AMP forms a C-shaped protein clamp around the duplex, via a novel beta-hairpin “latch” module that fits into the major groove. The latch is critical for clamp closure and is a key determinant of nick sensing.

DNA nick recognition by ChVLig involves large domain movements relative to the free ligase-adenylate (Nair et al., 2007). These large conformation changes, and subtle remodeling of the adenylate-binding pocket, are thought to orchestrate progression through the multi-step ligation pathway. Due to the inherent mobility of the enzyme domains and the flexibility of key protein segments such as the latch and the interdomain linker, the dynamic aspects of the pathway are not illuminated by the static crystal structures. Among the outstanding issues are: the nature of the interactions with ATP during the ligase-adenylation step; the spectrum of domain orientations in solution in the absence of DNA; the small-scale changes at the active site; and the number, location, and catalytic role of the metal cofactors. Solution-state NMR spectroscopy affords a powerful biophysical method to answer these mechanistic questions. Solution NMR spectroscopy is also well suited for the screening and identification of potential ligase inhibitors. For this reason we undertook the assignment of the NMR resonances of ChVLig in solution (as the ligase-adenylate) and we were successful in assigning a majority of the backbone resonances. This represents the first instance of the assignment of backbone resonances of a DNA ligase in full-length form.

Methods and experiments

A pET plasmid encoding the 298-amino acid ChVLig polypeptide fused to an N-terminal His₁₀ tag (MGHHHHHHHHSSGHIEGRH) was transformed into *Escherichia coli* BL21 (DE3). Uniformly ¹³C, ¹⁵N, ²H-labeled ChVLig was prepared by growing the cells in medium (0.5 L M9 prepared in 100% D₂O) supplemented with 3 g/L of ²H, ¹³C glucose and 1 g/L of ¹⁵N ammonium chloride. Cells were pre-adapted in small volumes (20 mL) to increasing amounts of D₂O (10%, 50%, 95% and 98%). Subsequently, a 20 mL culture prepared in labeled M9 in 100% D₂O was grown overnight and added to the 0.5 L labeled M9 media. The culture was incubated at 37° C until the A₆₀₀ reached 0.5, then placed on ice for 30 min, adjusted to 0.4 mM IPTG, and then incubated at 17° C for 24 h with constant shaking. The bacteria were harvested by centrifugation at 7000g for 20 minutes, resuspended in 25 mL of buffer A (50mM Tris-HCl, pH 7.5, 1.0 M NaCl, 10% glycerol, 10 mM MgCl₂, 500 μM ATP) and passed three times through a French press to achieve cell lysis. Insoluble material was removed by centrifugation at 16400g for 20 min, and the soluble supernatant fraction was incubated for 1 h with Co⁺² resin (Talon) equilibrated in buffer A. The resin was washed serially with buffer A, then buffer B (50 mM Tris-HCl, pH 7.5, 300 mM NaCl) with 5 mM imidazole. The bound material was eluted with 300 mM imidazole in buffer B. After an overnight dialysis in the NMR buffer (20 mM MES, pH 6.5, 300 mM NaCl, 1 mM EDTA, 2 mM DTT), the protein was subjected to gel filtration chromatography on a Supedex-75 (GE Healthcare) column in NMR buffer, during which the ligase eluted as a single monomeric species (data not shown). Using a similar protocol, ²H, ¹⁵N labeled samples (6 g/L unlabelled glucose) were prepared for 3D-TROSY-NOESY experiments.

All NMR experiments were performed at 25° C on Bruker Avance spectrometers operating at 800 or 900 MHz or on a Varian Inova spectrometer operating at 600 MHz, all equipped with

cryogenic probes capable of applying pulsed field gradients along the z-axis, using ~300 μ M samples (in 90% H_2O , 10% D_2O) in 4 mm H_2O -susceptibility matched tubes (Shigemitsu Inc.). ^{15}N , ^1H TROSY and TROSY-based HNC(O), HNCA, HN(CO)CA, HNCACB and HN(CO)CACB were performed (Salzmann et al., 1998). Additionally, a 3D NOESY-TROSY spectrum with a 180 ms mixing time was collected at 900 MHz to help with the assignment process. Proton chemical shifts were calibrated to DSS, and indirect referencing was used for the ^{13}C and ^{15}N dimensions. All spectra were processed using NMRPipe (Delaglio et al., 1995) and analyzed using Sparky (Goddard & Kneller, University of California, San Francisco).

Extent of assignment and data deposition

87.4% of ^{15}N , ^1H assignments were obtained for backbone amide resonances (250 of 286 excluding 12 prolines). Also assigned were 89.2% (255 of 286), 83.2% (228 of 274) and 78% (223 of 286) of the non-proline C^α , C^β and C' resonances, respectively. Of the 12 prolines, 10 C^α , 8 C^β and 11 C' resonances were assigned. Of the 41 amides for which resonance assignments were missing, 15 had no visible electron density in the crystallographic analysis of ChVLig-AMP. Most of these absent resonances correspond to amino acid residues located in loops or regions without defined secondary structure, suggesting that conformational exchange rather than spectral crowding resulted in the missing resonances.

Additionally, ^{15}N , ^1H -HSQC spectra of samples grown in media prepared in 100% H_2O did not display additional peaks compared to those grown in 100% D_2O , which confirmed that incomplete back-exchange of amide protons was not responsible for the missing resonances. All amide resonances in regions of well-defined secondary structure elements were successfully identified, with the exception of the segment encompassing residues Leu149 to Arg153. This region corresponds to an α -helix located at the interface between the two domains. Significantly, the extensive line broadening and doubling of resonances observed for the amino acids immediately flanking this segment seems to suggest chemical exchange, possibly due to structural rearrangement of the two domains. Extensive interdomain flexibility was not a surprise given that a major reorientation of the two domains occurs upon DNA binding (Nair et al., 2007). Moreover, the two crystal structures available for the free ChVLig-AMP (PDB codes 1FVI and 1P8L) (Odell et al., 2000; Odell et al., 2003) reveal slightly different relative positions of the nucleotidyltransferase and OB domains.

In general, we observed excellent agreement between the secondary structure obtained from NMR chemical shifts and that seen in the crystal structures of ChVLig in the absence of DNA (Fig. 2). It was notable that we could not fully assign resonances for the peptide segment from Val203 to Leu232, which comprises the latch module essential for DNA binding, and for which no electron density was evident in the crystal structures of the free ChVLig-AMP. NMR chemical shifts and backbone ^{15}N relaxation data (not shown) confirmed that this portion of the protein was disordered on the fast (ps-ns) timescale.

The backbone ^1H , $^{13}\text{C}^\alpha$, $^{13}\text{C}'$ and ^{15}N and sidechain $^{13}\text{C}^\beta$ chemical shifts have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 16059.

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Fig. 1. ^{15}N , ^1H TROSY spectrum of uniformly ^{13}C , ^{15}N , ^2H -labeled Chlorella virus DNA ligase (300 μM) in 20 mM MES, pH 6.5, 300 mM NaCl, 1 mM EDTA, 2 mM DTT. The spectrum was at 25 $^\circ\text{C}$ on an 800 MHz spectrometer; 128 and 512 complex points were acquired in the indirect and direct dimensions respectively with sweep-widths of 35 ppm and 12 ppm, and a recycle delay of 1.5 s. The amide NHs from the two W side chains are labeled as Win. Peaks from the N-terminal His₁₀ tag are not labeled. The congested central region is expanded in the insert on the top-left corner.

Fig. 2.

Comparison of the secondary structure predicted from NMR chemical shifts and that seen in the crystal structure (PDB ID: 1P8L). All the secondary structure elements visible in the crystal structure were detected in solution. The NMR chemical shifts hint at an additional helical segment between residues 179 and 183. Secondary structure prediction using measured chemical shifts was performed using the PECAN software (<http://bija.nmrfam.wisc.edu/PECAN/>). Secondary structure classification of the crystal coordinates was obtained using the ss.tcl script from the NMRPipe suite.