

Backbone assignment of the catalytic core of a Y-family DNA polymerase

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Abstract *Sulfolobus solfataricus* DNA polymerase IV (Dpo4), a model Y-family DNA polymerase, bypasses DNA lesions. Here, we report the assignments for the backbone nitrogen, carbon, and amide proton NMR signals of Dpo4's catalytic core consisting of the finger, palm, and thumb domains. Our work provides the basis for further NMR spectroscopic studies of the interactions among Dpo4, DNA, and an incoming nucleotide.

Keywords *Sulfolobus solfataricus* Dpo4 · Catalytic core · Y-family DNA polymerases · Backbone resonance assignments · Heteronuclear NMR

Biological context

Cellular DNA transactions involve various DNA polymerases which are phylogenetically grouped into the A-, B-, C-, D-, X-, and Y-families. The Y-family DNA polymerases bypass replication-stalling DNA lesions and rescue cells from apoptosis or senescence. As a model Y-family DNA polymerase, *Sulfolobus solfataricus* DNA polymerase IV (Dpo4) has been extensively studied both

biochemically and structurally. So far, the PDB database has more than 60 crystal structures of Dpo4 in the presence or absence of DNA (damaged or undamaged) and an incoming dNTP, *e.g.* apo Dpo4, Dpo4-undamaged DNA binary and Dpo4-undamaged DNA·dNTP ternary complexes (Wong, Fiala et al. 2008). Although those crystal structures have provided a structural basis for the catalytic function of Dpo4, they may not reveal how Dpo4 catalyzes translesion DNA synthesis in solution due to the limitation of crystallography, *e.g.* crystal packing and inability to capture unstable but kinetically interesting complexes. Our recent real-time fluorescence resonance energy transfer studies (Xu, Maxwell et al. 2009) have revealed that Dpo4 undergoes global conformational changes during catalysis, which is inconsistent with what has been predicted based on the overlay of the X-ray crystal structures of Dpo4·DNA and Dpo4·DNA·dNTP (Rechkoblit et al. 2006; Wong et al. 2008). NMR is a powerful method to investigate enzyme conformational dynamics in solution and at atomic resolution. However, there have been no reported NMR spectroscopic studies of Dpo4 and other Y-family DNA polymerases.

Here, we carried out the first solution-phase NMR study of Dpo4. Dpo4 possesses finger, palm, thumb, and little finger domains. The first three domains form the catalytic core (residues 1–230) which is connected to the little finger domain by a 14-residue linker. This core and the full-length Dpo4 were prepared as either [¹⁵N]-labeled or [¹³C, ¹⁵N]-labeled proteins. The overlay of the ¹H-¹⁵N HSQC spectra between the core and the full-length Dpo4 indicated that the core alone is structurally intact in the absence of the little finger domain and the linker (data not shown). Using triple-resonance NMR experiments on uniformly [¹³C, ¹⁵N]-labeled Dpo4 core, we obtained sequence-specific NMR assignments for the backbone signals.

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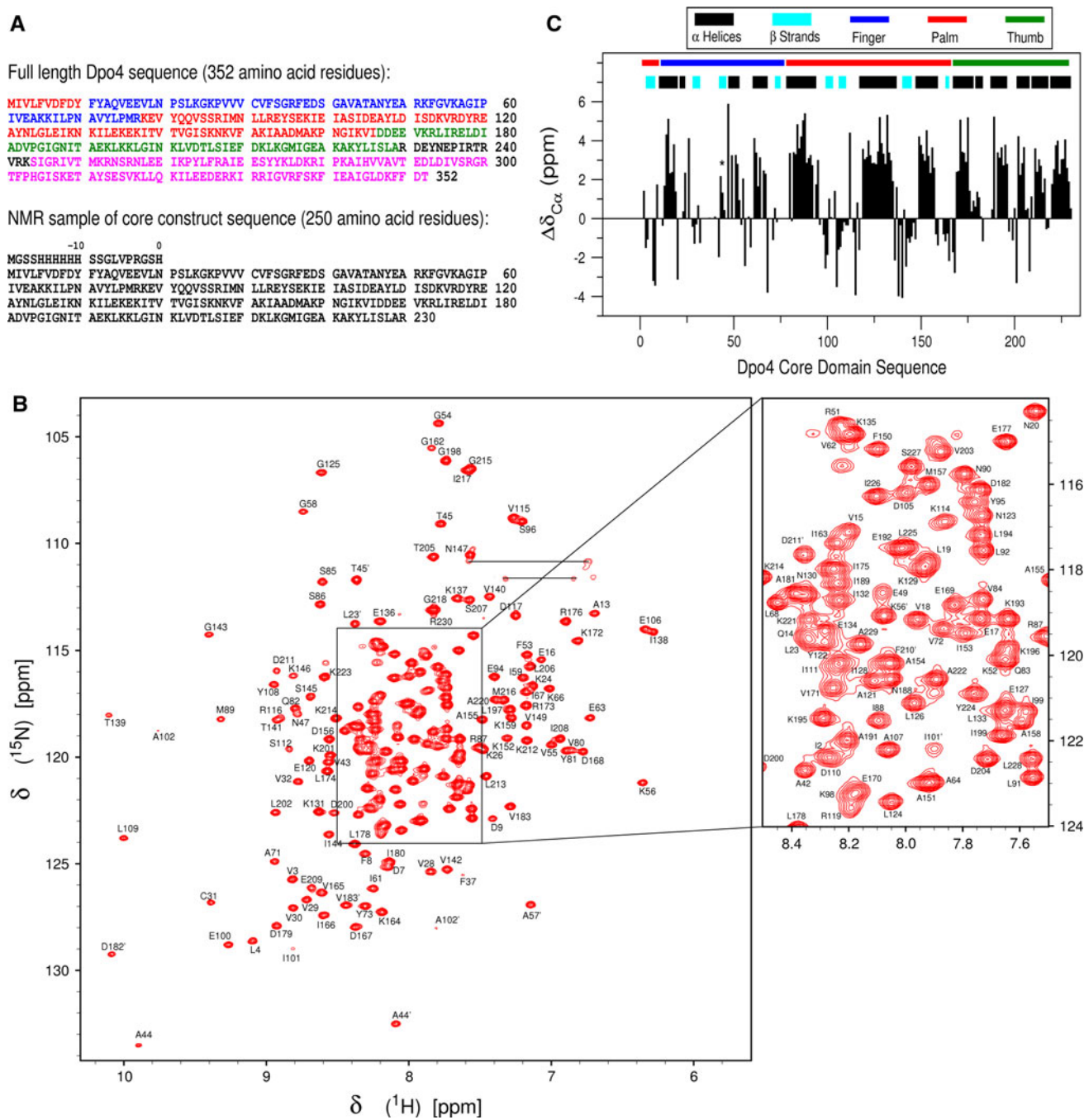


Fig. 1 **a** The primary sequences of the full-length Dpo4 and the NMR sample of the Dpo4 core construct. For the full-length Dpo4, the sequences of the finger, palm, thumb, and little finger domains are shown in *blue*, *red*, *green*, and *purple* colors, respectively, while the 14 amino acid residue linker is in *black*. The NMR sample of the Dpo4 core was expressed as a fusion protein between the N-terminal 20 foreign amino acid residues, numbered—19 to 0, and the native core for purification purposes; **b** 2D [^{15}N , ^1H]-HSQC spectrum of uniformly [^{13}C , ^{15}N]-labeled Dpo4 core. The spectrum was recorded on Bruker DRX-800 at 323 K with 256 increments in the ^{15}N dimension and 8 scans per increment. The backbone resonances assigned are labeled with one-letter-codes for amino acids and

sequential numbers. The 10 crosspeaks that are tentatively assigned to a second conformation are labeled with one-letter-codes for amino acids and primed sequential numbers. Only two pairs of side-chain NH_2 amide resonances were visible and are indicated by *horizontal lines*. The *right panel* is an enlarged portion from the *left panel*; **c** Chemical shift deviations of backbone $^{13}\text{C}\alpha$ atom with respect to the corresponding random coil values. Secondary structural features from the apo Dpo4 crystal structure (Wong, Fiala et al. 2008) are shown for the purpose of comparison. Small discrepancy between NMR and X-ray observations regarding the secondary structures is exemplified by the segment of V43, A44, and T45 residues marked by an *asterisk*

Methods and experiments

The Dpo4 core was expressed and purified in a similar manner as described previously (Fiala and Suo 2004). $^{15}\text{NH}_4\text{Cl}$ and [$^{13}\text{C}_6$]-D-glucose were the respective ^{15}N and ^{13}C sources to prepare a uniformly [^{13}C , ^{15}N]-labeled NMR sample using minimal media. The [^{13}C , ^{15}N]-labeled protein sample contained 170 mM NaCl, 20 mM sodium phosphate, pH 7.0, ~150 mM glycerol, and 95% $\text{H}_2\text{O}/5\%$ D_2O . The presence of glycerol and high salt concentration was necessary to keep 0.2 mM Dpo4 core soluble for the prolonged 3D NMR experiments. Notably, the Dpo4 core quickly precipitated at concentrations higher than 0.2 mM.

All NMR experiments were conducted at 323 K on Bruker DRX 800 MHz equipped with a cryogenic probe and z-axis gradient coil. For backbone resonance assignments, HNCO, HNCA, HN(CO)CA (Grzesiek and Bax 1992), and CBCA(CO)NH (Grzesiek and Bax 1993) were collected. 3D $^{15}\text{N}/^{13}\text{C}$ -simultaneously edited NOESY was also acquired to facilitate the assignment analysis (Sattler, Maurer et al. 1995). NMR spectra were processed using NMRPipe and NMRDraw software (Delaglio et al. 1995) and visualized as well as analyzed using Sparky 3 software (Goddard and Kneller, University of California, San Francisco). The assignment task was further assisted by using Mars software (Jung and Zweckstetter 2004).

Extent of assignments and data deposition

The primary sequences of Dpo4 and its catalytic core are shown in Fig. 1a. Both NMR samples of Dpo4 and its core were thermostable and well-folded at 323 K. The core construct (250 residues) contains 20 foreign residues at its N-terminus including a hexahistidine tag for the use of Ni^{2+} -affinity chromatography during protein purification. Interestingly, these 20 residues did not appear on the HSQC, and most of the side chain amide groups of 10 asparagines, 3 glutamines, and 10 arginines were also too weak to be detected. In addition, some residues from the presumably flexible loops were elusive despite of exhaustive search. All these observations were probably caused by the fast exchange of these resonances with the solvent H_2O under the current high experimental temperature considering the nature of their high flexibility. Although the viscosity effect caused by the presence of 150 mM glycerol did not affect the quality of our NMR data due to the high temperature used, its presence did introduce somewhat serious t_1 noise to the 3D ^{13}C -edited NOESY. These t_1 noise strips on the other hand had little effect on the analysis of triple resonance experiments. In summary, a total of 195 crosspeaks were detected on the HSQC, among which 185 were confidently assigned to the backbone amides of the Dpo4 core (Fig. 1 b).

Several lines of evidence indicated that some of the remaining crosspeaks could be attributed to a different backbone conformation. However, this warrants further investigation. The chemical shifts have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under the accession number 16869.

Identification of regular secondary structures from $^{13}\text{C}\alpha$ chemical shifts

The differences of the assigned backbone chemical shifts with respect to the corresponding random coil values can lead to the identification of regular secondary structural elements. As shown in Fig. 1c, although the NMR result generally agrees with the published crystal structure of apo Dpo4 (Wong et al. 2008), some disparities were also noticed, e.g. the interface region between the finger domain of Dpo4 and DNA. Specifically, our NMR data showed that the $^{13}\text{C}\alpha$ atoms of V43, A44, and T45 experience an average of ~1.3 ppm downfield secondary shift, which is inconsistent with the β -strand conformation adopted by the stretch of these residues in the crystal structure of apo Dpo4 (Wong et al. 2008).

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