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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C resonance assignments for a 20 kDa DNA polymerase from African swine fever virus

Mark W. Maciejewski, Borlan Pan, Ronald Shin, Adam Denninger and Gregory P. Mullen*
Department of Biochemistry, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3305,U.S.A.

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Biological context

The African Swine Fever Virus (ASFV) is known to cause a lethal disease in domestic pigs. The virus contains two DNA polymerases; a eukaryotic-like DNA polymerase involved in viral DNA replication and a 174 amino acid DNA polymerase that is a member of the polymerase X family (Pol X). This family includes DNA polymerase β (β-Pol), terminal transferase (TdT), DNA polymerase μ (Pol μ) (Dominguez et al., 2000), and DNA polymerase λ (Pol λ) (Aoufouchi et al., 2000). One notable difference between Pol X and the Pol X family is the absence of N-terminal DNA binding domains that are found in β-Pol, TdT, Pol μ, and Pol λ and the absence of BRCT domains that are found in Pol μ and Pol λ. In comparison to β-Pol, Pol X comprises only the palm and C-terminal subdomains. Based on biochemical analysis, Pol X was shown to be similar to β-Pol with respect to template-directed DNA synthesis, preference for deoxynucleotides versus ribonucleotides, and repair of a single nucleotide gap DNA (Oliveros et al., 1997). This would suggest that Pol X acts in base excision repair of the viral DNA inside the cytoplasm of the host cell. In addition, Pol X catalyzes the formation of a G:G mismatch with relatively high catalytic efficiency (Showalter and Tsai, 2001) and may provide mutase function to ASFV. As the smallest DNA polymerase, Pol X represents the minimal core needed for a protein to act as a template directed nucleotidal transferase. Here we report on the nearly complete backbone and side chain ^{1}H, ^{15}N, and ^{13}C resonance assignments for the 20 kDa Pol X.

*To whom correspondence should be addressed. E-mail: gmullen@bambam.uchc.edu

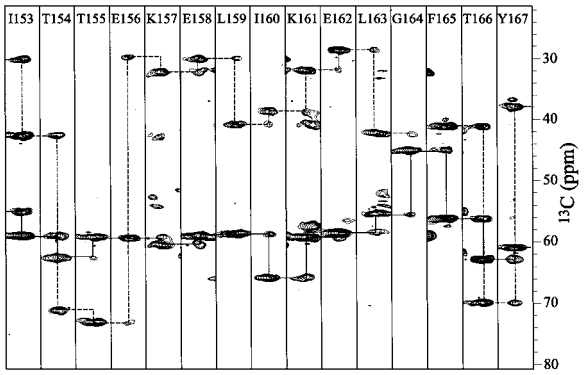

Figure 1. Strip plot from the HNCACB spectra of Pol X showing sequential $^{13}C_{\alpha}/^{13}C_{\beta}$ connectivities for residues Ile 153–Tyr 167. Connectivities between adjacent $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ nuclei are shown with a solid and dashed line, respectively.

Methods and results

The open reading frame containing the Pol X gene was inserted into the plasmid pET23a (Novagen). This construct (pAD1) was transformed into the BL21(DE3) cell line containing the pLysS vector. The ^{15}N and ^{15}N/^{13}C-labeled Pol X was produced from a cell culture grown in minimal media (Weber et al., 1992) by inducing the cell culture with 1 mM IPTG at an OD_{600} of 0.8. The cell culture was incubated after induction for 3 h, and cells were harvested. The Pol X enzyme was purified from the cell lysate in buffer containing 20 mM phosphate (pH 6.5), 500 mM NaCl, 2 mM BME, 5 mM EDTA, 0.01% lysozyme, and 0.5 mL of a protease inhibitor cocktail (Sigma), by selective ammonium sulfate fractionation, collecting the supernatant at 45% and the pellet at 77% saturation. The pellet was dissolved in 10 mL column loading buffer containing 20 mM phosphate (pH 6.5),

500 mM NaCl, 2 mM BME, 5 mM EDTA, and 0.02% sodium azide, and dialyzed versus 4 L of the same buffer. The dialysate was diluted with an equivalent volume of the column loading buffer containing no NaCl, and the solution was loaded onto a CM-Sephadex cation exchange column equilibrated in the same buffer. The column was washed with 150 mL of buffer, and the flow-through discarded. Purified Pol X was obtained from the column by using a salt gradient from 250 mM to 1 M NaCl in 20 mM phosphate (pH 6.5), 2 mM BME, 5 mM EDTA, and 0.02% sodium azide. Pure fractions were pooled, and concentrated by centrifugal ultrafiltration. The protein solution was exchanged with buffer containing 20 mM PIPES (pH 6.5), 500 mM NaCl, 10 mM DTT, 0.5 mM AEBSF, and 0.02% sodium azide by centrifugal ultrafiltration. After the final buffer exchange, the sample (1 mM) was made 10% D_2O, or was lyophilized and dissolved in 100% D_2O.

NMR experiments were performed at 25 °C on a Varian INOVA 500 or 600 MHz spectrometer. The data were processed using nmrPipe (Delaglio et al., 1995) with scripts generated from our web site http://sbtools.uchc.edu/nmr/. Data analysis was performed with the program XEASY (Bartles et al., 1995). ^{1}HN, ^{15}N, $^{13}C_{\alpha}$, and $^{13}C_{\beta}$ resonance assignments were made using connectivities in the HNCACB and CBCA(CO)NH spectra and HNCA and HN(CO)CA spectra. $^{1}H_{\alpha}$ resonance assignments for ~50% of the residues were obtained from the HNHA spectra. Side chain ^{1}H and ^{13}C assignments were obtained from the HCC(CO)NH-TOCSY, CC(CO)NH-TOCSY, HCCH-TOCSY, ^{15}N-edited NOESY (τ = 100 ms), and ^{13}C-edited HSQC-NOESY (τ = 100 ms) spectra. Aromatic assignments were obtained using 2D TOCSY (τ = 50 ms), 2D NOESY (τ = 150 ms), ^{15}N-edited NOESY, ^{13}C-edited NOESY (τ = 100 ms), and aromatic ^{1}H-^{13}C HSQC spectra. ^{13}C carbonyl resonances were assigned using a HNCO spectra.

Extent of assignments and data deposition

Backbone ^{1}H and ^{15}N assignments were made for all residues except M1, S39, L66, R70, V80, and K109. All $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ assignments were determined with the following exceptions. For M1, L53, I54, L66, R70, C81, F114, L142, and P170, the $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ assignments were not determined, and for L99, N100, and E108, the $^{13}C_{\beta}$ resonance assignments were not determined. Except for Tyr, Phe, Trp, and His residues, $^{13}C_{\gamma}$, $^{13}C_{\delta}$, and $^{13}C_{\epsilon}$ resonance assignments were determined for 131/148, 93/110, and 24/26 nuclei, respectively. Carbonyl ^{13}C resonance assignments were determined for all residues except for G38, V65, I69, K79, E108, L137, V56, L66, E109, G118, V149, and I169, six of which precede a proline. All $^{1}H_{\alpha}$ and $^{1}H_{\beta}$ resonance assignments were determined except for residues M1, L66, R70, and F114, for which $^{1}H_{\alpha}$ and $^{1}H_{\beta}$ assignments are missing, and for L53, L99, and L142, for which $^{1}H_{\beta}$ assignments are missing. With the exception of aromatic residues, $^{1}H_{\gamma}$, $^{1}H_{\delta}$, and $^{1}H_{\epsilon}$ resonance assignments were determined for 138/148, 98/110, and 24/26 nuclei, respectively. All ^{1}H resonance assignments for aromatic residues were determined except for the H_{ζ} nuclei of F76, F116, and F165. Side chain ^{1}H and ^{15}N assignments were made for 7/8 Asn and 4/5 Gln residues.

The ^{1}H, ^{15}N, and ^{13}C chemical shifts have been deposited at the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5010.

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References

Aoufouchi, S., Flatter, E., Dahan, A., Faili, A., Bertocci, B., Storck, S., Delbos, F., Cocea, L., Gupta, N., Weill, J.C. and Reynaud, C.A. (2000) *Nucl. Acids Res.*, **28**, 3684–3693.

Bartles, C., Xia, T., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6**, 1–10.

Delaglio, F., Grzesiek, S., Vuister, G.W., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Domínguez, O., Ruiz, J.F., Laín de Lera, T., García-Díaz, M., González, M.A., Kirchhoff, T., Martínez, A.C., Bernad, A. and Blanco, L. (2000) *EMBO J.*, **19**, 1731–1742.

Oliveros, M., Yanez, R.J., Salas, M.L., Salas, J., Vinuela, E. and Blanco, L. (1997) *J. Biol. Chem.*, **272**, 30899–30910.

Showalter, A.K. and Tsai, M.-D. (2001) *J. Am. Chem. Soc.*, **123**, 1776–1777.

Weber, D.J., Gittis, A.G., Mullen, G.P., Abeygunawardana, C., Lattman, E.E. and Mildvan, A.S. (1992) *Proteins*, **13**, 275–287.