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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments for the perdeuterated 22 kD palm-thumb domain of DNA polymerase β

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Biological context

DNA polymerase β is a 39 kDa mammalian DNA repair enzyme which acts in the base excision repair (BER) pathway. The BER pathway functions at abasic sites generated by targeted excision of a damaged DNA base or abasic sites formed by spontaneous hydrolysis. The dual role of DNA polymerase β is to both remove the deoxyribose 5′-phosphate (dRP) from incised abasic sites and to polymerize DNA at the excised site (Beard & Wilson, 2000).

DNA polymerase β consists of two domains: the N-terminal, '8K' domain, and the C-terminal, '31K' domain formed by the 'fingers', 'palm', and 'thumb' subdomains. Proteolysis of the 31 kDa domain produces a stable palm-thumb domain. The N-terminal domain is responsible for binding to the abasic site and for dRP lyase activity. The NMR solution structure of the N-terminal domain has been solved and its dynamics characterized correlating flexibility with DNA binding and lyase activity (Maciejewski et al., 2000). The C-terminal fingers-palm-thumb domain is responsible for nucleotidyltransferase activity (Beard & Wilson, 1995). The palm-thumb domain forms the essential core of a template-directed nucleotidyltransferase as is seen by the recent structure of the homologous 20 kDa DNA polymerase X (Maciejewski, et al., 2001). In order to obtain well-resolved spectra, a ^{2}H/^{13}C/^{15}N analog of the palm-thumb domain with ^{1}H/^{13}C labeled methyl groups of Val, Ile, and Leu has been prepared.

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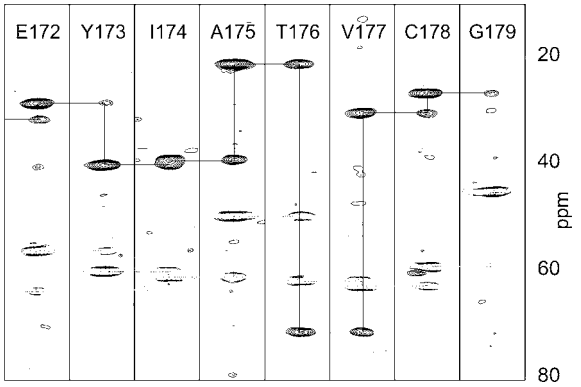

Figure 1. Illustration of the sequential connectivities for a representative sequence of NH strips from the HNCACB experiment. Strips shown are for residues 172 through 179. Each NH showed crosspeaks to the C_α (dotted) and C_β (bold) of both its own and the preceding residue.

Methods and experiments

DNA coding for the palm-thumb domains (149–335) of rat DNA polymerase β with an additional 5′ sequence coding for amino acids MGK was cloned into the pET-28a(+) vector (Novagen). Transformed BL21 cells were grown in LB media and stored as a glycerol stock at −80 °C. This stock was used to innoculate an overnight culture (2 ml) of minimal media containing 67% D_2O and supplemented with vitamins (Weber et al., 1992). This culture, in turn, was used to innoculate successive cultures (20 ml and 250 ml) of minimal media containing 100% D_2O supplemented with the same vitamins, $^{15}NH_4Cl$ as the sole nitrogen source, $^{13}C_6$ ^{2}H-D-glucose as the major carbon source, and 100 mg l^{-1} α-ketoisovaleric-U-$^{13}C_5$ acid-

3-d_1 and 50 mg l^{-1} α-ketoisobutyric acid-$^{13}C_4$-3,3-d_2 to provide ^1H,^{13}C labeling of the methyl groups of valine and leucine and the δ-methyl of isoleucine (Goto et al., 1999). The overnight culture was grown at 28 °C while successive growths were at 20 °C, taking 3 days to reach an OD of 0.8. Cells were induced with IPTG and harvested after 22 hours of further growth.

The expressed protein remained in the soluble fraction of the cell extract and was purified at pH 5.0 on a CM Sepharose ion exchange column (Pharmacia) using a buffer gradient containing 20 mM to 1 M NaCl, dialyzed into 50 mM phosphate buffer containing 150 mm NaCl and concentrated using Centricon-10 filtration devices (Millipore). The NMR sample contained 1 mM palm-thumb domain, 46 mM Na_2HPO_4, 135 mM NaCl, 0.002% NaN_3, 1 mM DTT, 100 μM AEBSF in 90% H_2O/10% D_2O, pH 6.8.

All NMR data used in resonance assignments were acquired at 25 °C on a four-channel Varian INOVA-600 spectrometer equipped with a pulse field gradient triple resonance probe. Backbone resonances as well as those of the beta carbons were assigned using a combination of TROSY-HNCACB, TROSY-HNCO and TROSY-HN(CA)CO experiments (Figure 1). HN and N resonances were back-corrected for TROSY J-coupling shifts by comparing decoupled and TROSY-selected ^{15}N HSQC spectra. Resonances of the methyl protons and sidechain carbons of I, L, and V residues were assigned using a combination of CCH-TOCSY, (HM)CMC(CM)HM (which correlates the ^1H and ^{13}C resonances of a protonated methyl group to its attached carbon) (Gardner et al. 1998), and ^{15}N, ^{13}C edited NOESY spectra. All pulse programs were standard Varian *Protein Pack* sequences (http://www.varianinc.com) with the exceptions of the CCH-TOCSY which was modified to begin on ^{13}C resonances and the (HM)CMC(CM)HM sequence which was generously provided by Dr Lewis Kay.

NMR spectra were processed with NMRPipe (Delaglio et al., 1995) using scripts generated from http://sbtools.uchc.edu. Spectra were analyzed and assigned using XEASY (Bartels et al., 1995). Spectra were referenced to the H_2O resonance relative to TSP either directly (^1H) or indirectly (^{13}C, ^{15}N) (Markley et al., 1998).

Extent of assignments and data deposition

Backbone assignments are essentially complete for residues 149-335 in β-Pol palm-thumb. A total of 95% of the HN and N resonances have been assigned (excluding the 11 proline ^{15}N resonances) with the following resonances remaining unassigned (S180, G189, S202, S205, K209, D276, R328 and E329). A total of 97% of the C′ resonances were assigned with S204, P208, S275, R328 and E329 unassigned. A total of 98% of the C_α resonances were assigned (S180, P208, R328 and E329 unassigned) as well as 96% of the C_β resonances (S180, P198, S202, P208, P261, R328 and E329 unassigned). H_α and H_β positions were deuterated for this study.

Side chain resonances for the C_γ and H_γ resonances of all 12 valines were assigned. Side chain resonances for the $C_{\gamma 1}$, C_δ, and H_δ resonances of all 12 isoleucine resonances were assigned. Side chain resonances for the C_γ, C_δ, and H_δ resonances of all leucines were assigned except for L211 and L218.

Chemical shifts have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under the accession number BMRB-5208.

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