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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N resonance assignments of the C-terminal domain of MutY: An adenine glycosylase active on G:A mismatches

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Biological context

MutY from *Escherichia coli* is a DNA mismatch repair enzyme involved in the base excision repair pathway. It is an adenine glycosylase which removes adenine when mispaired with guanine, cytosine or 7,8-dihydro-8-oxoguanine (8-oxoG). 8-oxoG is a common DNA oxidative damage lesion and mutant strains of *E. coli* that lack MutY activity have elevated rates of G:C to T:A tranversions (Nghiem et al., 1988). Trypsin produced an N-terminal domain of residues 1–225, p26, and a C-terminal domain of 226–350, p13 (Manuel et al., 1996). The catalytic activity of the enzyme was found solely in the N-terminal domain. Recent work has determined the crystal structure of the p26 domain; the protein has a helix-hairpin-helix structural motif in common with a number of DNA glycosylases and DNA glycosylase/AP lysases (Guan et al., 1998). The crystal structure suggests that MutY utilizes a nucleotide flipping mechanism, in which the adenine is moved to an extrahelical position within the DNA, into an active site pocket where it is excised.

Studies of intact MutY and the N-terminal domain show that the C-terminal domain affects substrate binding and mismatch repair activity. Manuel and Lloyd (1997) found that the largest differences between MutY and p26 in binding of natural substrates involved A:G and in mismatch activity A:C. Recent biochemical data suggest that the C-terminal domain is the principal determinant of 8-oxoG specificity (Noll et al., 1999).

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Methods and results

The protein was over-expressed in BL21(DE3) *E. coli* cells using the pET11a vector. Post-induction, the cells were subjected to osmotic shock to break the outer cell membrane. After 50% ammonium sulfate precipitation, resuspension of the precipitate and subsequent dialysis, the samples were passed over Q-Sepharose (Pharmacia) , SP-Sepharose (Pharmacia) and Affigel Blue (Bio-Rad) column matrixes. A Superdex-75 sizing column was used at the final purification stage. Centricon filters (Amicon) were used for the final concentration. Isotopically labeled samples were prepared in media containing ^{15}N-NH_4Cl and unlabeled or U-^{13}C-glucose (Cambridge Isotopes and Isotec).

The unlabeled and ^{15}N/^{13}C double-labeled NMR samples (0.7–1.0 mM protein) were prepared in buffer containing 18 mM phosphate (pH = 7.5), 90 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT, and 0.1 mM NaN_3 (in 90% H_2O/10% D_2O or 100% D_2O). All NMR spectra were collected at 25 °C on Varian UnityPlus 750 MHz and 600 MHz instruments using triple resonance probes equipped with pulsed field gradients. HNCA, HNCO, HN-CACB, HBCBCA(CO)NH, (HB)CBCACO(CA)HA, and HCCH-TOCSY experiments were acquired. ^{15}N and ^{15}N/^{13}C edited HSQC-NOESY spectra were also recorded with 100 ms mixing times. For the triple resonance experiments the ^{13}CO and $^{13}C_\alpha$ regions were simultaneously decoupled using the Double-WURST decoupling scheme (Zhang et al., 1996). Several modifications were made to the original sensitivity enhanced pulse sequences. These modifications

include the use of shaped ^{13}C pulses for the elimination of Bloch–Siegert shifts during the t_1 period (Zhang and Gorenstein, 1998). Carrier frequencies were 4.76 ppm for ^{1}H, 119.0 ppm for ^{15}N, 43 ppm for aliphatic ^{13}C, 56 ppm for $^{13}C_\alpha$ and 175 ppm for ^{13}CO. NMR data were processed using FELIX (version 97, Molecular Simulations Inc.,/Biosym). Forward linear prediction was used to extend the time-domain data in the indirectly detected dimensions. The time domain data were zero-filled to increase the spectral resolution in all dimensions. Phase shifted skewed sine-bell apodization functions were used in all three dimensions. The ^{1}H chemical shifts were referenced relative to DSS. The ^{15}N and ^{13}C chemical shifts were referenced indirectly using the $^{1}H/X$ frequency ratios. Post acquisition water signal suppression was achieved using a sine-bell convolution function (provided in FELIX). Two-dimensional ^{1}H NOESY (100 ms mixing time), and TOCSY(30 and 80 ms mixing times) data were also collected on the unlabeled protein sample.

Sequence-specific backbone resonance assignments were done using 3D HNCACB, HNCA, and HBCBCA(CO)NH experiments (Muhandiram and Kay, 1994). The carbonyl carbon and α-proton chemical shifts were determined using the HNCO and (HB)CBCACO(CA)HA experiments. The side chain proton and carbon spin systems were determined using the 3D HCCH-TOCSY experiment (Kay et al., 1993).

The chemical shift index method (CSI) has proven to be a useful indicator of secondary structure in proteins (Wishart and Sykes, 1994). We have calculated the CSI-derived secondary structure using the H_α, C_α, C_β, and CO chemical shifts, and well-defined areas of β-sheets and α-helices were determined. This is illustrated by the consensus CSI in Figure 1. The structural predictions based on the CSI data have been verified by the 100 ms ^{15}N edited NOE data.

Extent of assignments and data deposition

The chemical shift values of the H_N, H_α, C_α C_β, N, CO and the side chain ^{13}C and ^{1}H resonances are deposited in the BioMagResBank (accession number: 4353). Probably due to dynamics/mobility, the backbone ^{15}N, ^{N}H, and CO resonances for the first five residues on the N-terminal side were not assigned. We were not able to assign any of the resonances for the following 11 residues: Q226, L228, L238, P250, E268, S269, L270, S295, P307, V308, and P328.

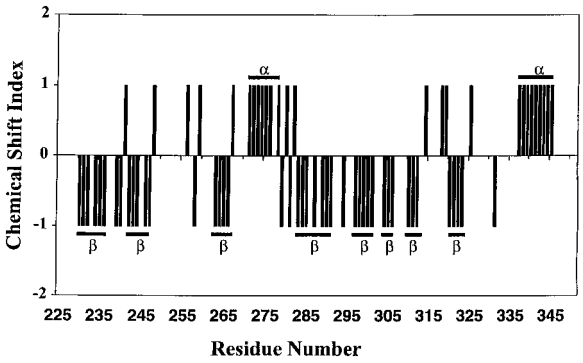

Figure 1. The consensus Chemical Shift Index (CSI) for the MutY p13 domain. H_α, C_α, C_β, and CO chemical shifts were included for the CSI analysis. Indexes of +1, 0 and −1 indicate α-helical structure, random coil structure, and β-sheet structure, respectively. The α and β secondary structure elements are indicated.

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