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Letter to the Editor: Assignments of the ^{1}H, ^{13}C, and ^{15}N resonances of the winged helix domain of the proto-oncoprotein cQin (FoxG1B)

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Biological context

Qin is the oncogenic determinant of the retrovirus ASV 31 which causes fibrosarcomas in chickens (Vogt et al., 1997). The chicken homolog of viral Qin, termed cellular Qin (cQin), encodes a proto-oncoprotein that belongs to the 'winged helix' (WH) family of transcription factors (Li and Vogt, 1993). cQin is the avian ortholog of mammalian brain factor 1 (BF1), a telencephalon specific protein. The expression pattern of BF1 and its DNA binding site are very similar to those of cQin. Importantly, BF1 regulates the early development of cerebral hemispheres and BF1$^{-/-}$ mice die at birth.

ASV 31 proliferation involves expression of an altered form of cQin, termed viral Qin (vQin). Intriguingly, vQin is highly oncogenic in chickens while cQin is not. Mutation of Gly 233, located in the WH domain (WHD), to Asp has been identified as a molecular switch – 'the Qin molecular switch' – that converts Qin into an oncoprotein. To understand the biochemical functions of Qin in structural terms we have initiated a detailed study of its structure and dynamics using NMR spectroscopy. For the NMR studies reported here we have used a truncated 127-residue, 14.7 kDa construct, termed cQin-WHD, containing the DNA binding winged helix domain of cQin. The first four residues of this construct (GSHM) derive from the expression vector pET28a and, thus, Gly 5 and Arg 127 correspond to Gly 131 and Arg 253 of native cQin, respectively. One Cys residue (Cys 200) has been mutated to Ser to prevent oxidation and the formation of dimers. This mutation does not alter protein structure or DNA binding properties.

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Methods and experiments

The cDNA encoding cQin-WHD was subcloned into pET-28a, and was expressed in *E. coli*. Purification of cQin-WHD was carried out using three chromatographic steps: (1) Ni^{2+}-affinity chromatography, (2) cation-exchange chromatography, and (3) size-exclusion chromatography. NMR samples (2.0 mM) of cQin-WHD were prepared in 100 mM NaCl, 10 mM sodium acetate (pH 5.5) with 5% D_2O and 0.02% sodium azide.

NMR data were acquired at 40 °C using a Varian INOVA 600 MHz spectrometer using a 5 mm triple resonance probe equipped with x, y, and z axis pulsed magnetic field gradients (PFGs). Quadrature detection in the indirectly detected dimensions was achieved using either the States-TPPI method (Marion et al., 1989) or using echo-antiecho selection with PFGs (Kay et al., 1992). The software package Felix 98 (Accelerys) was used for all NMR data processing and analysis. Spectra were referenced indirectly to TSP at 0.00 PPM and the heteronuclear dimensions were referenced using the appropriate gyromagnetic ratios.

Backbone sequential assignments were obtained using 3D CBCA(CO)NH, HNCACB, HN(CO)CA, HNCA, HNCO and HN(CA)CO spectra. Aliphatic side-chain assignments were made through the analysis of 3D (H)C(CO)NH-TOCSY, H(CCO)NH-TOCSY, HBHA(CBCACO)NH, HCCH-COSY and HCCH-TOCSY spectra. Aromatic proton and carbon assignments were made based on correlation to

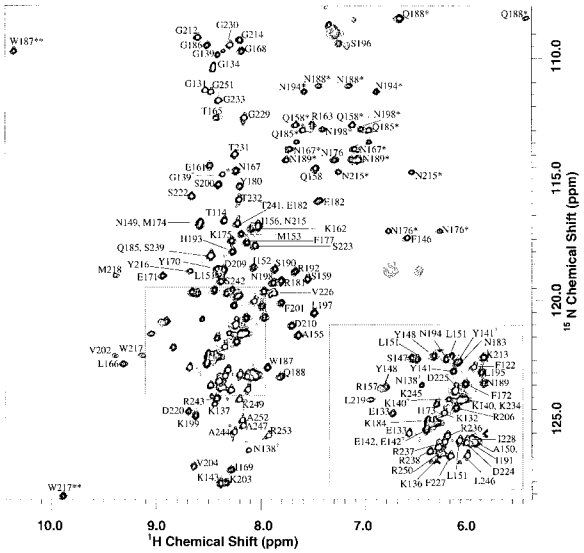

Figure 1. ^{1}H-^{15}N HSQC spectrum of the cQin-WHD in 100 mM NaCl, 10 mM sodium acetate (pH 5.5) with 5% D_2O and 0.02% sodium azide at 40 °C. Assignments for the backbone amides are indicated by their residue name and sequence number. Side chain amide groups of Asn and Gln are indicated by * and the indole groups of Trp by **. The resonance for Trp 187 is folded in the ^{15}N dimension; its true ^{15}N chemical shift is 131.1 ppm. The polypeptide segment from Asn 138 to Lys 143 exhibits an alternative conformation; resonances corresponding to this are marked by † (e.g., N138†). The residue numbering used herein corresponds to that of native cQin.

^{1}Hβ and ^{13}Cβ nuclei (Yamazaki et al., 1993) using the following 3D spectra: (HB)CB(CG)CDHD, HB(CBCG)CDHD, (HB)CB(CGCD)CEHE, and HB (CBCGCD)CEHE. Stereo-specific assignments of Val methyl groups were made through the analysis of two spin-echo difference CT-^{13}C HSQC spectra that allow the three-bond coupling constants between the methyl carbons and either the backbone ^{15}N or ^{13}C′ to be estimated (Bax et al., 1994). The methyl groups of Leu were stereo-specifically assigned using long-range ^{13}C-^{13}C J correlation (LRCC) experiments and long-range ^{1}H-^{13}C (LRCH) correlation experiments (Bax et al., 1992; Vuister et al., 1993).

Chemical shift index analysis and φ, ψ torsion angles obtained using TALOS (Cornilescu et al., 1999) identified four α helices spanning residues 148–157 (H1), 166–179 (H2), 179–182 (H4), and 185–195 (H3), and three short β strands between residues 162–164, 201–204, and 216–219. This secondary structure prediction is quite comparable to the classic WH fold consisting of 3 α-helices within a helix-turn-helix motif, and a three-stranded β-sheet. Wing 2 (residues 221–253) is highly disordered in the free protein and undergoes a binding-induced structural transition to an ordered conformation upon binding to DNA (data not shown).

Extent of assignments and data deposition

All the backbone resonances were assigned for all ^{1}H, ^{15}N, and $^{13}C_\alpha$ except the first three amino acids. The ^{1}H-^{15}N HSQC spectrum is shown in Figure 1. The ^{13}C′ chemical shifts are 80% complete. All aromatic side-chain assignments were obtained for HD and HE protons except for F248. A total of 57 Hβ methylene groups (58%) were stereo specifically assigned by analysis of 3D HNHB and ^{13}C- and ^{15}N-edited NOESY spectra. Methyl groups of Val residues (V202, V204, and V226) and Leu residues (L151, L164 and L166) were stereo specifically assigned. The side-chain amide resonances of Asn and Gln residues were also assigned except for N138 and N183. As indicated in Figure 1, two sets of resonances are observed for the segment from Asn 138 to Lys 143 due to cis/trans proline isomerism for Pro 144 and Pro 145, 143.

The ^{1}H, ^{15}N and ^{13}C chemical shifts for the cQin-WHD have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under BMRB accession number 5280.

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