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Letter to the Editor: Backbone resonance assignments for the Fv fragment of the catalytic antibody NPN43C9 with bound *p*-nitrophenol

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Biological context

The catalytic monoclonal antibody NPN43C9 catalyzes the hydrolysis of a variety of phenyl ester compounds and *p*-nitroanilide (Janda et al., 1988). The smallest domain of the antibody capable of catalytic activity is the 25 kDa Fv fragment, which consists of a heterodimer of a 117-residue heavy chain variable region (V_H) and a 113-residue light chain variable region (V_L). Each of the two polypeptides has three complementarity-determining regions (CDRs), which are grafted onto the outer end of the immunoglobulin fold to give the antigen-binding pocket, which is also the site of catalytic activity in NPN43C9. The three-dimensional structure of the Fv NPN43C9 and its binary complex with *p*-nitrophenol have recently been solved (Thayer et al., 1999). As a preliminary towards an investigation of the role of local dynamics in the binding/catalytic site, we report the backbone resonance assignments for the binary complex of the Fv fragment of NPN43C9 with *p*-nitrophenol.

Methods and results

Protein samples uniformly labeled with ^{15}N, $^{15}N/^{13}C$ and $^{15}N/^{13}C/^{2}H$ were used to obtain the backbone resonance assignments. The protein was over-expressed in *Escherichia coli* BL21(DE3) containing plasmid pUBS520 with the ArgU gene (Brinkmann et al., 1989) and pJK34 containing a dicistronic gene for the Fv preceded by the pelB peptide signal sequence.

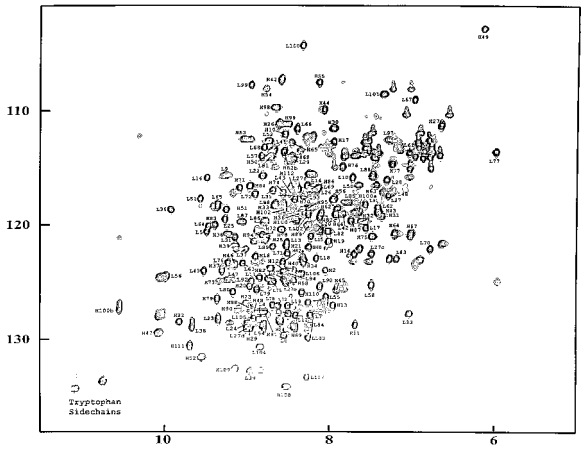

Figure 1. The assigned ^{1}H-^{15}N HSQC spectrum of 0.5 mM $^{2}H/^{13}C/^{15}N$-labeled Fv NPN43C9 at 25 °C obtained on a Bruker 600 spectrometer. The residues are numbered according to Kabat et al. (1991).

For the incorporation of isotopic labels, the cells were grown in M9 minimal medium containing 4 g/L glucose (Sigma) or 2 g/L $^{13}C_6$-glucose (Isotec), 1 g/L $(^{15}NH_4)_2SO_4$, 1 g/L $^{15}NH_4Cl$ (Isotec), 10 ml/L of a 100x Basal Medium Eagle vitamin solution (Gibco BRL), 0.1 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 0.5 g/L NaCl, in the presence of 100 μg/ml ampicillin and 30 μg/ml kanamycin. The triply labeled sample was prepared by growing the cells in M9 medium in 99% D_2O (Isotec). The cells were grown at 37 °C until A_{600} reached 0.7. At this point the temperature was lowered to 25 °C and IPTG added to a final concentration of 1 mM. The culture was then allowed to grow for 16 h before harvesting.

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Folded Fv from the periplasmic fraction was isolated using cation exchange (Pharmacia SP-Sepharose) followed by a *p*-nitrophenol affinity column to separate V_L homodimer from the Fv. For the triple-labeled protein, the amide deuterons were exchanged back to protons after the final purification step, by dialyzing against 10 mM Tris, 100 mM NaCl and 80 μM *p*-nitrophenol at pH 8.0 for 14 days at room temperature.

The NMR samples were concentrated and exchanged into 10 mM d_{11} Tris, 100 mM NaCl and 80 μM *p*-nitrophenol at pH 6.8 using an Amicon Centriprep membrane filter with a 3 kDa cutoff. The final NMR samples contained 0.5 mM protein estimated from the A_{280} (extinction coefficient 48000 $M^{-1}.cm^{-1}$) (Gibbs et al., 1991).

The ^{15}N-labeled protein was used to collect ^{1}H-^{15}N HSQC, ^{1}H-^{15}N NOESY-HSQC and ^{1}H-^{15}N TOCSY-HSQC spectra on Bruker DRX600 and DRX800 spectrometers. The ^{13}C/^{15}N labeled protein was used to collect the HNCACB and HN(CO)CACB, and the ^{2}H/^{13}C/^{15}N labeled protein for the HNCA, HN(CO)CA, HNCACB and HN(CO)CACB data. The latter two experiments were optimized for the observation of Cβ resonances. All triple resonance experiments were recorded on a Bruker DRX600 spectrometer at 25 °C. The data were processed using NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed using NMRVIEW (Johnson and Blevins, 1994) in combination with seq_prob (Grzesiek et al., 1992) on a Silicon Graphics O2.

Due to the long correlation time of the molecule (13.7 ns), only 50% of the Cα and Cβ resonances were observed in the double-labeled sample. The data obtained on the triple labeled protein contained 90% of the Cα and Cβ resonances and enabled resonance assignments to be made for most of the protein.

Extent of assignments and data deposition

The backbone assignments for the V_L chain are complete except for the first two residues, K45, L46 and the prolines. Residues Q38, K39 and R96 were only observed in the ^{1}H-^{15}N HSQC-NOESY and ^{1}H-^{15}N HSQC-TOCSY spectra.

The missing backbone assignments for the V_H are the first residue, residues E6 to G8, H35 in CDR H1, residues Y102 to G106 in CDR H3 and the prolines. Residues L4, L5, W36, Y102 and W103 were only observed in the ^{1}H-^{15}N HSQC-NOESY and ^{1}H-^{15}N HSQC-TOCSY spectra. The well resolved ^{1}H-^{15}N HSQC shown in Figure 1 makes this catalytic Fv very useful for studying ligand binding and backbone dynamics.

The assignments have been deposited in the BioMagResBank (accession number 4349).

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