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Letter to the Editor: Backbone and C^{β} assignments of the anti-gp120 antibody Fv fragment complexed with an antigenic peptide

Vitali Tugarinov, Rina Levy, Adi Dahan-Schokoroy & Jacob Anglister*
Department of Structural Biology, Weizmann Institute of Science, Rehovot 76100, Israel

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Biological context

The 0.5β is a potent HIV neutralizing antibody which was raised against the viral glycoprotein (gp120) of the HIV-1_{IIIB} strain and purified from infected cells (Matsushita et al., 1988). Like most HIV-1 neutralizing antibodies, 0.5β recognizes the principal neutralizing determinant (PND) located within the third hypervariable region (V3) of gp120. Measurements of the binding constants of 0.5β to the whole gp120 and to a 24-residue peptide from V3 revealed very similar affinities. The structures of both the V3 loop and 0.5β are not available. The antibody Fv fragment (∼25 kDa), consisting of the variable domains of the heavy (VH) and light (VL) chains generally retains the specificity and affinity of the parent antibody and is amenable to detailed NMR studies. We report the backbone assignments of the bacterially expressed and uniformly $^{13}C/^{15}N$-labeled 0.5β Fv (231 residues) in complex with the unlabeled 18-residue peptide (P1053) comprising the full epitope recognized by 0.5β. The results obtained in this work will be used for detailed heteronuclear NMR investigations of the Fv structure, dynamics and antibody-antigen interactions.

Methods and results

The 0.5β Fv was expressed in *E. coli* (Faiman et al., 1996). Celtone-CN rich medium (Martek Biosciences Corp., USA) supplemented with 0.5% ^{13}C-labeled glycerol was used for isotope labeling. The Fv was purified on a set of four columns, including Sepharose CL-4B, DE-52 anion exchange, Phenyl Sepharose CL-4B, and an affinity column prepared with P1053 cross-linked to Sepharose CL-4B. Two [U-^{13}C,^{15}N] samples in 95% H_2O/5% D_2O with complex concentrations of 0.6 and 1.1 mm and one [U-^{15}N] sample (1.7 mM) were used. All the samples contained 10 mm phosphate buffer (pH 7.2) and 0.05% NaN_3, and were inserted into Shigemi tubes. NMR spectra were recorded at 37 °C on either Bruker DMX600 or DMX500 spectrometers equipped with triple-resonance gradient probes. The spectra were processed using NMRPipe (Delaglio et al., 1995) and analyzed with the PIPP program. Mirror-image linear prediction was used to extend the time-domain data of the constant time dimensions.

The assignment procedure was similar to that used for calcineurin B (Anglister et al., 1993). 3D CT-HNCA, HN(CO)CA, CT-HNCO, CBCA(CO)NH, HNCACB, C(CO)NH, HBHA(CBCACO)NH and ^{15}N-separated TOCSY (see Clore and Gronenborn, 1994; Bax et al., 1994, and references therein) experiments were recorded and used simultaneously in the assignment process as shown in Figure 1. Although proton connectivities did not extend beyond H^{α} in most cases because of the low effectiveness of the TOCSY transfer, this simultaneous use of both ^{13}C and ^{1}H connectivities helped to avoid errors in the sequential walk. The C(CO)NH dataset was used extensively to aid in the amino acid type determination. The assignment performed on the whole Fv molecule was verified by preparing two additional samples with each Fv domain selectively labeled with ^{15}N using a chain recombination procedure (Anglister et al., 1985). ^{15}N-HSQC spectra recorded for each sample allowed fast verification of the resonance assignments to either the VL or the VH domain. The 3D

*To whom correspondence should be addressed.

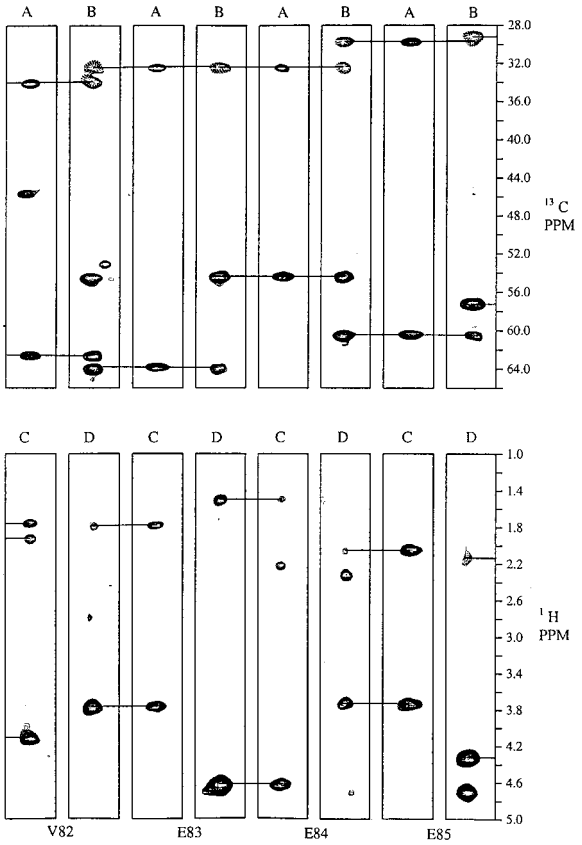

Figure 1. Strips along the ^{13}C and ^{1}H dimensions of the CBCA(CO)NH (panel A), HNCACB (panel B), HBHA(CBCACO)NH (panel C) and TOCSY-^{15}N-HSQC (panel D) spectra for residues Val^{82}-Glu^{85} of the Fv light chain. Sequential connectivities are drawn with lines connecting C^{α}, C^{β} and H^{α}, H^{β} resonances. Negative peaks are shown with dashed contours.

^{15}N-separated NOESY and HNHA spectra recorded for both these samples were almost free of overlap and allowed more reliable measurements of sequential NOEs and $^{3}J_{HNH\alpha}$ couplings.

Extent of assignments and data deposition

The incorporation of water flip-back pulses (Grzesiek and Bax, 1993b) and the use of radiation damping during the NOESY mixing period were necessary to avoid attenuation of the amide resonances due to exchange with the solvent at the neutral pH of the samples. However, this efficient technique does not help to detect protons that exchange faster than $\sim$30–50 s^{-1}. According to the crystal structure of 50.1 Fab (Rini et al., 1993), sharing 65% identity with 0.5β Fv, the amides that remained undetected almost exclusively belong to the residues located at the centers of turns. The only exception is Gln^{6}, which belongs to the outer β-strand of the VL domain and is not hydrogen-bonded in the 50.1 Fab. The amides of L-Ala^{9} and L-Gly^{45} were not found in either of the recorded spectra and correspond to sharp bends in the loops connecting two β-strands of the VL domain. As a consequence, all backbone resonances of L-Pro^{8} and L-Pro^{44} remain unassigned. Probably for the same reason, it was not possible to assign the amides of H-Thr^{28}, H-Phe^{29}, H-His^{41}, H-Gly^{42}, H-Ser^{75}, H-Ser^{88}, H-Asp^{89} and H-His^{99}. In addition, the backbone resonances of L-Pro^{47}, the carbonyl of H-Ser^{102} and the C^{β} resonances of H-Met^{106} and L-Gln^{94} remained unidentified. 97% of the backbone and C^{β} assignments have been obtained. The chemical shifts have been deposited in BioMagResBank under accession number 4171.

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