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Letter to the Editor: Backbone and side chain resonance assignments of a TRAV14-3 mouse T cell receptor domain

Jin-Shan Hu[a,*], Daniel Plaksin[b] & David H. Margulies[b]
[a]*Department of Chemistry and Biochemistry, and the Center for Biomolecular Structure and Organization, University of Maryland, College Park, Maryland, 20742, USA;* [b]*Molecular Biology Section, Laboratory of Immunology, NIAID, National Institutes of Health, Bethesda, MD, 20892, USA*

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Biological context

T cell receptors (TCR) are $\alpha\beta$ heterodimeric cell surface glycoproteins expressed clonally on mature T lymphocytes bearing the CD4 or CD8 coreceptor molecules. The major histocompatibility complex (MHC)-encoded class I or class II molecule, which is expressed on the surface of antigen presenting cells (APC), binds to an antigenic or self peptide to form an MHC/peptide complex. The interaction of a TCR with an MHC/peptide complex initiates a discriminatory binding event of the antigen-specific T cell response, and leads to T cell activation (Germain and Margulies, 1993). Our understanding of the structural basis of this molecular recognition event has been greatly enhanced by identification of peptide motifs for binding to MHC molecules, by measurements of the thermodynamics of interaction of MHC/peptide complexes with TCR, and by X-ray crystal structures of a number of MHC class I and class II/peptide complexes, of TCR, and of a limited number of MHC/peptide/TCR complexes (Rudolph et al., 2002). Recent structural studies that compare X-ray structures of unliganded TCR with TCR/MHC/peptide complexes provide evidence that conformational changes of the complementarity determining regions (CDRs) of the TCR play an important role in the functional recognition of MHC/peptide. NMR spectroscopy offers high-resolution information concerning the solution structure and real-time mobility of regions of proteins. We report here the NMR backbone and side chain resonance assignments of a TCR Vα domain, TRAV14-3*02; TRAJ38*01 (previously known as Vα2.6Jα38), derived from a TCR that has specificity for the human immunodeficiency virus envelope glycoprotein 120-derived peptide P18-I10 (RGPGRAFVTI) bound to a murine MHC class I molecule, H-2D^d. The extensive ^{1}H, ^{13}C and ^{15}N resonance assignments provide the basis for NMR structural and dynamic studies of Vα domain, to generate crucial information concerning the relative mobility of the unliganded CDR1, CDR2, and CDR3 loops.

Methods and experiments

The cloning, expression, refolding, and purification of the ^{15}N- and ^{13}C/^{15}N-labeled TRAV14-3*02; TRAJ38*01 mouse TCR domain proteins were performed as described previously (Plaksin, et al., 1996). NMR samples were prepared using 0.9 mM protein in 50 mM potassium phosphate buffer at pH 7.0 with 154 mM sodium chloride, 95% H_2O/5% D_2O. All NMR data were recorded at 27 °C on Bruker DMX-600 and DMX-750 spectrometers equipped with triple-axis pulse field gradients and ^{1}H, ^{13}C, ^{15}N triple-resonance 5 mm probes (Figure 1). All data were processed with the NMRPipe package (Delaglio et al., 1995) and analyzed using the programs PIPP, CAPP, and STAPP (Garrett et al., 1991). ^{1}H chemical shifts were referenced to the H_2O signal relative to 3-(trimethyl-silyl)propane-1,1,2,2,3,3-d_6-sulfonic acid, sodium salt (DSS), and ^{13}C and ^{15}N

*To whom correspondence should be addressed. E-mail: jh336@umail.umd.edu

chemical shifts were referenced indirectly to DSS, using the absolute frequency ratios.

The highly sensitive and simplified 2D spin-echo difference experiments, HNCG_arom and HN(CO)CG_arom, offered identification of $^{1}H^{N}$–^{15}N resonances of aromatic residues and following residues, in addition to providing χ_1 angle restraints for all aromatic residues (Hu et al., 1997; Hu and Bax 1997a). These $^{1}H^{N}$–^{15}N resonances of aromatic residues and of following residues were used as starting points for automatic sequential backbone resonance ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$) assignments when combined with 3D HNCACB, CBCA(CO)NH and HNCA experiments. To verify the backbone resonance assignments, to obtain backbone carbonyl (^{13}C) resonance assignments, and to simultaneously obtain backbone ϕ restraints, 3D HNCO and HNCOCO (Hu and Bax, 1996; Grzesiek and Bax, 1997) experiments were performed. 3D HNHA were used to provide $^{1}H^{\alpha}$ resonance assignments and to obtain ϕ restraints.

A 3D HN(CO)C experiment was used to provide side chain C^{γ} resonance assignments, in addition to generating χ_1 restraints (Hu et al., 1997). Other side chain resonance assignments were made by extension from the sequentially assigned amide resonances to the side chain carbon and proton resonances using 3D HBHA(CO)NH, DIPSI-H(CCO)NH, and DIPSI-C(CCO)NH experiments. Complementary information was obtained using 3D HCCH-TOCSY and HCCH-COSY experiments. Assignments for prochiral β-methylene protons and identification of the principal χ_1 rotamer were obtained by the quantitative *J*-correlation approach, including 2D HNCG-arom, 2D HN(CO)CG_arom, 2D HNCG (Hu and Bax, 1997b), 3D HNCOCO, and 3D HAHB.

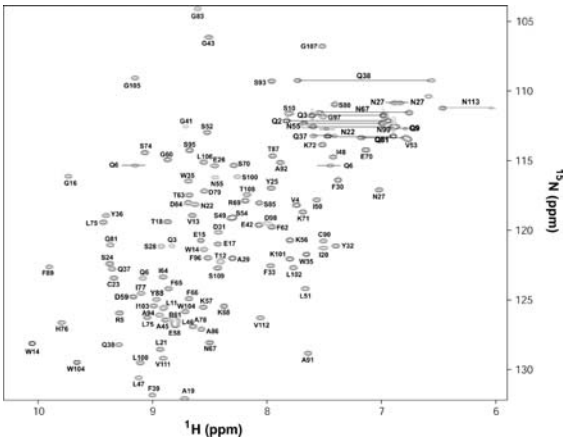

Figure 1. 2D $^{1}H^{N}$–^{15}N HSQC spectrum of ^{15}N-labeled TCR Vα domain. The data were acquired at 27 °C using a Bruker DMX 600 MHz spectrometer. The sample consisted of 0.9 mM Vα in 50 mM K_2HP_4 (pH 7.0), 154 mM NaCl, and 5% D_2O/95% H_2O.

Extent of assignments and data deposition

Extensive assignments for the backbone and side chain resonances of Vα were obtained. Almost all $^{1}H^{N}$ and ^{15}N resonances of native non-proline backbone residues (100/113) have been assigned. Definitive assignments for the non-native residue M1, and native residues, Q2, Q9 and N99, have not been obtained due to rapid amide proton exchange with water. ^{1}H–$^{13}C^{\alpha}$ resonances for all residues including M1 have been assigned. The resonances for 109 out of 114 possible backbone carbonyl groups have been assigned. ^{1}H–$^{13}C^{\beta}$ resonances for all non-Gly residues, ^{1}H–$^{13}C^{\gamma}$ resonances for all residues except R61 and the C-terminal P114, ^{1}H–$^{13}C^{\delta}$ resonances for all residues except R61, Y88, and F89, $^{1}H^{\epsilon}$–$^{13}C^{\epsilon}$ resonances for all residues except R5, F33, R61, R69, Y88, and F89, have been assigned. The side chain amide resonances for all seven Gln and five Asn residues have been assigned. All possible side chain resonances for Trp residues have been assigned. The ^{1}H, ^{13}C and ^{15}N chemical shifts have been deposited in the BioMagResBank Database (http://www.bmrb.wisc.edu) under BMRB accession number 6406.

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