Journal of Biomolecular NMR, **17:** 357–358, 2000.
KLUWER/ESCOM

Letter to the Editor: Backbone ^{1}H, ^{13}C, and ^{15}N resonance assignments of the anti-dansyl antibody Fv fragment

Kazuyasu Shindo[a], Katsyoshi Masuda[b], Hideo Takahashi[a], Yoji Arata[c] & Ichio Shimada[a,*]
[a]*Graduate School of Pharmaceutical Sciences, The University of Tokyo, Hongo, Tokyo 113-0033, Japan;* [b]*Suntory Institute for Bioorganic Research, Mishima-gun, Osaka 618-8503, Japan;* [c]*Water Research Institute, Sengen, Tsukuba, Ibaraki 305-0047, Japan*

Received 24 April 2000; Accepted 19 June 2000

Key words: anti-dansyl antibody, Fv fragment, heteronuclear NMR, resonance assignments

Biological context

Immunoglobulin G (IgG), which is an important member of the group of glycoproteins that function as antibodies, consists of two identical heavy chains and two identical light chains. The heavy chains are composed of four homology units, the V_H, C_H1, C_H2, and C_H3 domains, whereas the light chains are divided into two homology units, the V_L and C_L domains. The Fv fragment, which is a heterodimer of V_H and V_L domains, is the smallest antigen-binding unit with a molecular weight of 25 000. In previous papers, we have shown that the Fv fragment can be prepared in high yield and with full antigen-binding activity by enzymatic proteolysis of a short-chain mouse IgG2a anti-dansyl antibody, in which the entire C_H1 domain is deleted (Igarashi et al., 1990; Takahashi et al., 1991).

Here we report the backbone assignments of the Fv fragment, which was prepared by using mouse hybridoma cells grown in the ^{15}N and ^{13}C,^{15}N-labeled amino acid mixtures. The assignments obtained from the present study will be used for the determination of the three-dimensional structure and further understanding of the antigen recognition mechanism.

Methods and results

The mouse hybridoma cell line, 1B10. 7, which produces the short-chain mouse IgG2a anti-dansyl monoclonal antibody (Dangl et al., 1982; Igarashi et al., 1990), was adapted to serum-free medium (Nissui NYSF 404) and then grown in the medium containing stable-isotope-labeled amino acid(s). All labeled analogues of the Fv fragment were prepared by clostripain digestion of the short-chain antibody, as described previously (Takahashi et al., 1991). To obtain uniformly ^{15}N-labeled and ^{13}C,^{15}N-labeled Fv fragments, amino acid mixtures labeled with ^{15}N (^{15}N: 96–98 atom%) and ^{13}C,^{15}N (^{13}C: 95–98 atom%; ^{15}N: 96–98 atom%) (Chlorella Industry Co., Ltd, Japan) were used, respectively (Hansen et al., 1992). Before their use in the medium, the labeled amino acid mixtures were purified by anion exchange chromatography to remove the components in the mixtures that are toxic to the hybridoma cells. The purified mixtures were subjected to amino acid analysis to determine the composition of each amino acid in the mixtures quantitatively.

On the basis of the results from the analysis, [U-^{15}N] Gln, [U-^{15}N] Arg, [α-^{15}N] Cys, [α-^{15}N] Phe, [α-^{15}N] Tyr and [α-^{15}N] Trp, were further added to the medium to prepare the [^{15}N] labeled Fv fragment. [U-^{15}N] Gln was synthesized from [α-^{15}N] Glu and [^{15}N] NH_4Cl by glutamine synthetase, since a large amount of Glu was required for the medium. These labeled amino acids and compounds, except for [U-^{15}N] Gln, were purchased from Cambridge Isotope Laboratories, Inc. In a similar way, the ^{15}N,^{13}C-labeled Fv fragment was prepared. The concentrations of the ^{15}N-labeled and ^{13}C,^{15}N-labeled Fv fragments were 1.5 mM and 1.2 mM, respectively. All of the sample solutions contained 5 mM phosphate buffer, 150 mM NaCl, and 0.05% NaN_3 in 90% H_2O/10% D_2O. The pH (uncorrected for deuterium isotope effects) was 6.0. NMR spectra were recorded at 37 °C on either a Bruker

*To whom correspondence should be addressed. E-mail: shimada@iw-nmr.f.u-tokyo.ac.jp

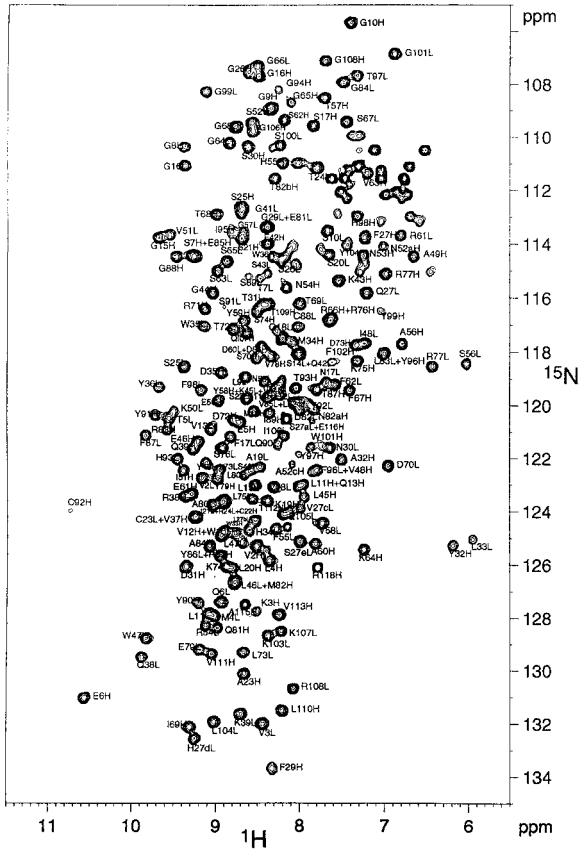

Figure 1. The amide region of the ^{1}H-^{15}N HSQC spectrum of the ^{15}N-labeled anti-dansyl Fv fragment (1.5 mM) at pH 6.0 and 310 K. Assignment of cross peaks is shown by the one-letter code for amino acids.

DRX400 or a DRX600 spectrometer equipped with a triple-resonance inverse probe with a field gradient unit.

For the sequential assignment of the labeled Fv fragment, HSQC, 3D ^{15}N-separated TOCSY (mixing time 50 ms), NOESY (mixing time 100 ms), CT-HNCA, and CT-HN(CO)CA experiments (Bodenhausen and Ruben, 1980; Kay et al., 1990, 1994) were performed. To identify the amino acid type assignments and the starting position of the backbone signal assignment, 11 specifically stable isotope labeled Fv samples were prepared by cultivating 1 B 10.7 in a medium containing labeled amino acids, Ala, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr, and Val. About 50% backbone amide resonances of the Fvs of specific labeling of amino acid type were identified in 2D CT-HNCO spectra by using the$^{13}C/^{15}N$-double labeling technique (Kainosho and Tsuji, 1982). The ^{1}H chemical shifts were referenced to external DSS in D_2O (0 ppm), while the ^{15}N and ^{13}C chemical shifts were referenced indirectly to the absolute frequency ratios $^{15}N/^{1}H = 0.101329118$ and $^{13}C/^{1}H = 0.251449530$ (Live et al., 1984).

Extent of assignments and data deposition

Figure 1 shows the ^{1}H-^{15}N HSQC spectrum observed for the ^{15}N-labeled Fv fragment in the absence of hapten with the resonance assignments. All of the resonances originating from the amide groups of the backbone could be observed and assigned in a residue-specific way with the exception of the N-terminal ends of the polypeptide chains for the V_H and V_L domains, Asp 1 in the V_L domain, Glu 1 in the V_H domain, and Ala 103 in the V_H domain. A previous study suggested that the H3 loop undergoes exchange among more than two conformations in the absence of the hapten, leading to significantly shorter T_2 relaxation times for the amide nitrogens of the residues in the H3 loop (Takahashi et al., 1993). Therefore, it is most likely that the resonance originating from Ala 103 in the V_H domain, which is located at the H3 loop, could not be detected due to chemical exchange broadening. The backbone resonance assignments, including the $^{1}H^{N}$, ^{15}N, and $^{13}C^{\alpha}$ chemical shifts, of the anti-dansyl Fv fragment without hapten have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) database under accession number #4580.

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