

Sequence-specific ^1H , ^{15}N and ^{13}C resonance assignments of Art v 1: a proline-rich allergen of *Artemisia vulgaris* pollen

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Abstract Art v 1 is the major allergen of *Artemisia vulgaris*. The IgE raised against Art v 1 not only can cross-react with other proteins from the *Asteraceae* family members but also with components of various forms of food. Art v 1 is an important target for immunotherapy strategies, including vaccination with hypoallergenic derivatives or chimeras. We report the ^1H , ^{13}C , and ^{15}N resonance assignments of the recombinant Art v 1 and identification of secondary structures based on ^{13}C chemical shifts.

Keywords Art v 1 · NMR assignments · Proline-rich allergen · Defensin-like

Biological context

Allergens are proteins able to stimulate inappropriate IgE production in atopic individuals leading to clinical manifestation such as asthma, rhinitis and atopic dermatitis (Akdis 2006). To date, allergen immunotherapy is the only treatment that provides long-term clinical benefits. Novel strategies for development of allergen vaccines include the generation of low-affinity IgE-binding molecules (Wallner et al. 2007; Wild et al. 2007). Therefore, allergens structural

determination is a crucial step for the understanding of the molecular interactions between the allergen cells of the immune system, the development of allergen therapeutics and the biological function of the protein in the pollen.

Pollen of *Artemisia vulgaris* (mugwort) is one of the main causes of allergic reactions in late summer and autumn in Europe, parts of Asia and North America (Himly et al. 2003). More than 95% of mugwort pollen-allergic individuals are sensitized to Art v 1, the major allergen in mugwort pollen. Several other members of the *Asteraceae*, a family of flowering plants, can lead to clinically significant cross-reactions due to structural similarities between allergens (Bauer et al. 1996; Schmid-Grendelmeier et al. 2003). Natural Art v 1 is a 108 aa secreted protein and primary sequence analysis suggests that it is formed by two domains: a defensin-like and a proline-rich domain (Himly et al. 2003). Art v 1 shows similarity between ESTs recently sequenced from *Lactuca serriola* (DW116026, BU012812), *Cichorium intybus* (EH71010), *Taraxacum officinale* (DY835145) and *Helianthus annuus* (DY929461), all from *Asteracea* family (<http://www.ncbi.nlm.nih.gov>). The defensin domain reaches similarity values of 80% while the whole protein sequence exhibits scores around 60%.

Here we present, as the first step in the NMR structural analysis, the sequence-specific ^1H , ^{13}C , and ^{15}N resonance assignments of recombinant Art v 1 and secondary structure elements based on ^{13}C chemical shift. Remarkably, proline-rich domain was almost fully assigned.

Methods and experiments

The Art v 1 mature sequence (accession number AF493943) was cloned into pHIS parallel2 vector (Sheffield et al. 1999; Dedic et al. 2009), using *NdeI* and *XhoI* restriction sites to

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produce a non-fusion protein. Recombinant Art v 1 was over-expressed in *Escherichia coli* Rosetta-gami B(DE3) pLysS in minimal media (Silantes) with ^{13}C -labeled glucose and ^{15}N -labeled ammonium chloride, supplemented with 50 $\mu\text{g/ml}$ ampicillin. Cells were grown at 37°C to an optical density at 600 nm of 0.7, protein expression was induced with 0.4 mM IPTG and carried out at 20°C for 22 h. The cells were harvested and dissolved in 10 mM NH_4HCO_3 pH 7.8, 10 mM NaCl, incubated with lysozyme (0.02 mg/ml) and disrupted with repeated cycles of freeze and thaw.

The rArt v 1 protein was purified with a cation exchange chromatography using a HiTrap SP FF column with 50 mM sodium acetate pH 5.35 as initial condition and eluted in a gradient mode from 0 to 1 M sodium chloride. The purified fractions were desalted with a NAP-25 column, freeze-dried and stored at -20°C .

NMR samples (1.0–1.2 mM) of purified isotopically labeled (^{15}N or ^{15}N , ^{13}C) Art v 1 were prepared in 10 mM sodium acetate pH 5.5 containing 10% D_2O and 0.03% sodium azide. These conditions were found to be optimal for the combination of sample stability and spectra quality.

NMR spectroscopy

NMR data were acquired at 298 K using Bruker Avance DRX 600 or Avance III 800 spectrometers equipped with a three-axis gradient 5 mm triple resonance probe. Proton and ^{13}C chemical shifts were referenced to DSS, whereas the ^{15}N was referenced using the absolute frequency ratios (Wishart et al. 1995). A set of 3D triple-resonance HNCACB, HNCACB(CO)NH, HCC(CO)NH spectra were collected for the sequential backbone resonance assignments. Side-chain resonance assignments have been achieved using the following experiments: HBHA(CBCA)(CO)NH, 3D H(CC)(CO)NHTOCSY, and ^{15}N -edited 3D TOCSY-HSQC. The assignments were confirmed and completed with ^{15}N -edited NOESY-HSQC (120 ms mixing time) and ^{13}C -edited NOESY-HSQC spectra (120 ms mixing time). Proline sequential resonance assignments of Art v 1 was obtained using the correlations of intra- and inter-residual $^1\text{H}/^{13}\text{C}\alpha$, ^{15}N resonances (HCAN/NCACON, Kanelis et al. 2000). All spectra were processed using Topspin 2.0 and analyzed using CARI 1.8.4 (Keller 2004).

Fig. 1 ^1H - ^{15}N HSQC spectrum of uniformly [^{13}C , ^{15}N]-labeled Art v 1 recorded in a Bruker Avance III 800 MHz and pH 5.5 at 298 K. Number correspond to residue numbers in Art v 1 primary sequence. Horizontal lines connect the side-chains of Asn19 and Gln33. The spectrum shows two distinct groups of peaks: one well dispersed, related to the defensin domain (in black) and less dispersed ones related to the C-terminal proline-rich region (in red). Residues Gly59, Ala63, Ala68, Asp82 and Ser101 are doubled probably due to proline isomerization (indicated by arrows and circles)

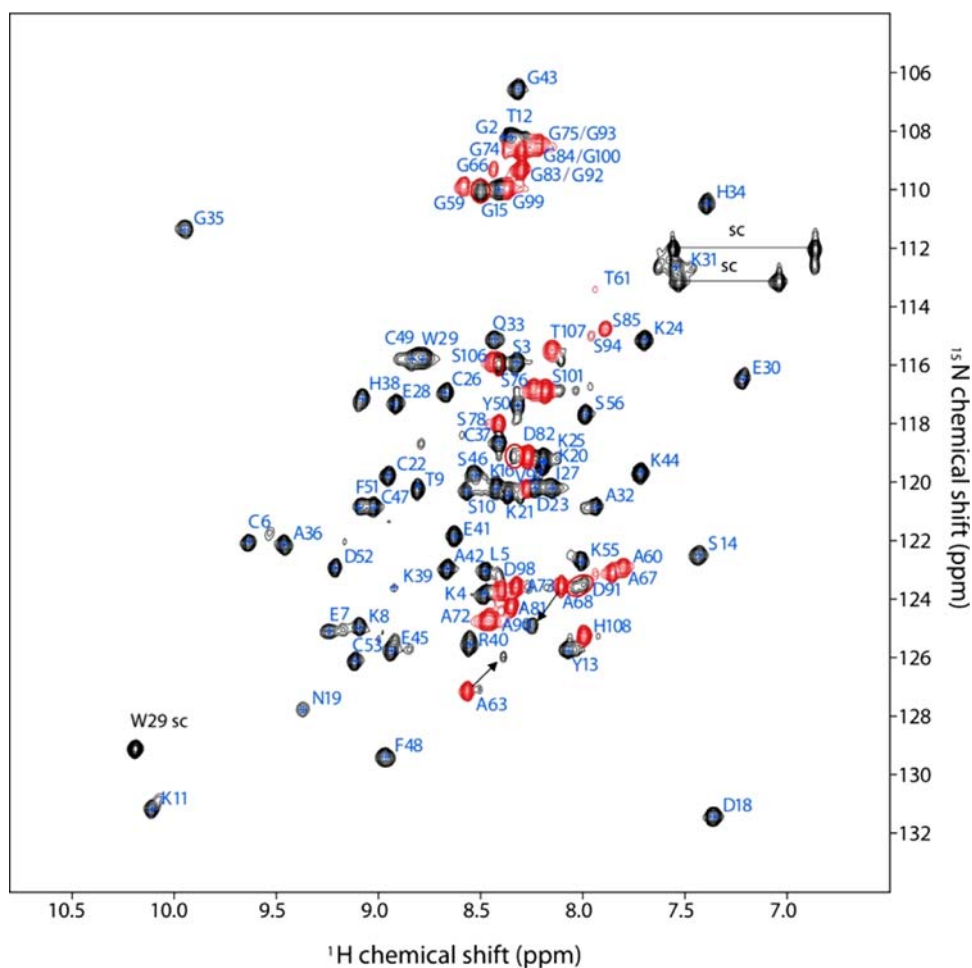
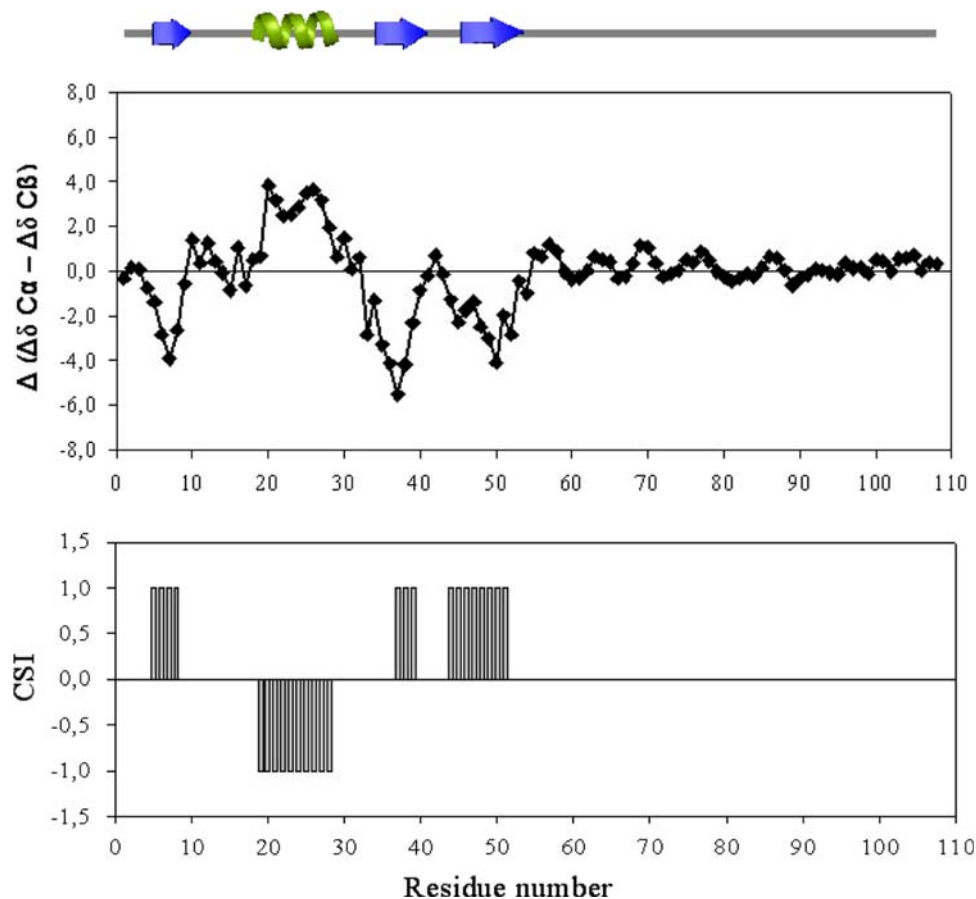


Fig. 2 Deviation of experimental of chemical shift with random coil values. **a** The difference in chemical shift between ΔC^α and ΔC^β carbons is presented for each residue. The region between residue 19 and 28 show positive values of C^α minus C^β that is indicative of α -helix. Three other regions (5–8; 34–38; 45–51) show negative values that are indicative of beta strand. All these regions belong to the defensin domain and are confirmed by the software CSI (Wishart and Sykes 1994)



Assignments and data deposition

The ^1H - ^{15}N HSQC spectrum of Art v 1 pH 5.5 and 298 K is shown in Fig. 1. Art v 1 resonances show two distinct properties in the spectrum: well dispersed peaks (in black), related to the defensin domain and less dispersed ones (in red) related to the C-terminal proline-rich region. The resonances from the C-terminal were grouped close to random coil chemical shift, although a clear definition of most of the peaks could be observed. These features were observed in all spectra recorded. Despite the smaller chemical shift dispersion, almost all residues of Art v 1 were assigned (98%) both in the defensin and proline-rich domains, reaching a completeness value of 90%. Unassigned systems were: two proline residues (79 and 102) could not be unambiguously assigned due to overlap; the amide proton of Cys17 was not observed, probably due to line broadening and some aromatic atoms of histidines could not be assigned: His34 (HE, HD1, CE1 and CG), His38 (HE2, HD1 and CG) and His108 (HD, HE, CG, CD and CE).

The ^1H - ^{15}N HSQC spectrum show that some peaks from the C-terminal region were duplicated, probably due to cis-trans isomerization of the adjacent prolines (Gly59,

Ala63, Ala68, Asp82, Ser101) indicated by red circle and arrow in Fig. 1. Remarkably, the majority of the prolines in the C-terminal tail are predominantly in trans-conformation. Only Pro103 and Pro95 are mainly in cis-conformation. The chemical shifts have been deposited in the BioMagRes- Bank (<http://www.bmrb.wisc.edu>) under the accession number 16111.

Identification of regular secondary structures from the ^{13}C chemical shifts

The deviation of the experimental C^α and C^β chemical shifts from random coil values was used to identify possible secondary structure elements (ΔC^α or β). Figure 2a shows the values of ΔC^α minus ΔC^β obtained for each residue and panel B shows the results obtained with the software CSI (Wishart and Sykes 1994). Positive values of the difference between ΔC^α and ΔC^β are indicative of α -helix and are clearly recognized between residues 19–28, in accordance to CSI results. Three regions showed high negative values of ΔC^α minus ΔC^β that are indicative of beta strands (residues 5–8; 37–39; 44–51). All these regions belong to the defensin domain.

Preliminary analysis of the NOEs and relaxation data suggested that part of the C-terminal is not completely flexible (data not shown). Full analysis of the data will be published in the future.

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