Published in final edited form as:

Biomol NMR Assign. 2015 October ; 9(2): 317–319. doi:10.1007/s12104-015-9600-8.

^1H , ^{13}C and ^{15}N resonance assignment of the anti-HIV lectin from *Oscillatoria agardhii*

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Abstract

Lectins from different sources are known to interfere with HIV infection. The antiviral activity is mediated by binding to high mannose sugars present on the viral envelope, thereby inhibiting cell entry. The lectin from *Oscillatoria agardhii* (OAA) specifically recognizes a unique substructure of high mannose sugars and exhibits broad anti-HIV activity. Here we report the assignment of backbone and side-chain ^1H , ^{13}C and ^{15}N resonances of free OAA.

Keywords

anti-HIV lectins; *Oscillatoria agardhii* agglutinin; NMR assignment

Biological context

In the last decade lectins from different sources have been found to prevent infection by HIV. In general, lectins block virus entry into the host cells by binding with high specificity and affinity to high-mannose carbohydrates (Man-9) present on the viral envelope. Interestingly, the binding epitopes on Man-9 vary for different lectins (Balzarini 2007; Huskens and Schols 2012), and application of such lectins as anti-HIV agents requires detailed characterization of their biophysical properties. Here we focus on the 14 kDa *Oscillatoria agardhii* agglutinin (OAA) (Sato et al. 2000; Sato et al. 2007). Compared to other carbohydrate binding agents with anti-HIV activity, OAA was found to specifically recognize a unique substructure of the Man-9 glycan found on the viral protein gp120 and to inhibit infection on a broad range of HIV-1 clinical isolates (Férrir et al. 2014; Koharudin and Gronenborn 2011).

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Conflict of Interest

The authors declare that they have no conflict of interest.

Methods and experiments

OAA was expressed and purified as described by (Koharudin et al. 2011). The NMR samples consisted of 2mM ^{15}N or ^{15}N , ^{13}C labeled OAA in 20 mM sodium acetate, 20 mM sodium chloride, 3 mM sodium azide, 90/10 % $\text{H}_2\text{O}/\text{D}_2\text{O}$ (pH 5) or 100 % D_2O . Sequence specific assignments of the backbone resonances were obtained using 2D [^{15}N , ^1H]-HSQC and 3D HNCA (Bax et al. 1990; Grzesiek and Bax 1992) measured on a Bruker 800 MHz AVANCE III spectrometer. Ambiguities were resolved using sequential NOEs from a 3D ^{15}N -resolved [^1H , ^1H] NOESY (Marion et al. 1989a; Marion et al. 1989b; Zuiderweg and Fesik 1989), measured on a Bruker 800 MHz AVANCE III spectrometer, using a mixing time of 60 ms. Complete ^1H and ^{13}C assignments of aliphatic side-chains were achieved using 2D [^1H , ^{13}C]-HSQC, 3D hCCH-TOCSY (Bax et al. 1990), measured on a Bruker 600 MHz AVANCE III spectrometer and 3D $^{13}\text{C}_{\text{aliphatic}}$ -resolved [^1H , ^1H] NOESY (Muhandiram et al. 1993), measured on a Bruker 800 MHz AVANCE III spectrometer with a mixing time of 80 ms. Nearly complete assignments of aromatic side-chains were accomplished using 2D [^1H , ^{13}C]-HSQC and 3D $^{13}\text{C}_{\text{aromatic}}$ -resolved [^1H , ^1H] NOESY (Muhandiram et al. 1993) measured on an Oxford 700 MHz AVANCE III spectrometer with a mixing time of 80 ms. Amide side-chains were assigned using the 2D [^{15}N , ^1H]-HSQC, 2D [^1H , ^{13}C]-HSQC, 3D ^{15}N -resolved [^1H , ^1H] NOESY and 3D $^{13}\text{C}_{\text{aliphatic}}$ -resolved [^1H , ^1H] NOESY. The spectrometers operating at 600 and at 700 MHz were equipped with 5 mm TXI room-temperature probes and the spectrometer operating at 800 MHz was equipped with a 5 mm TCI cryogenic probe. Backbone carbonyl resonance assignments were obtained using 3D HNCO (Grzesiek and Bax 1992; Kay et al. 1994; Schleucher et al. 1993) measured on a Bruker 900 MHz AVANCE spectrometer equipped with a 5 mm TCI cryogenic probe. All experiments were performed at 298 K and all spectra were processed using NMRPipe (Delaglio et al. 1995) and analyzed with CARA (Keller 2004).

Assignments and data deposition

Complete backbone assignment was achieved (Fig. 1). Of note are the unusual proton chemical shifts of the G26 and G93 amide resonances caused by ring current effects from the aromatic side chains of W90 and W23, respectively (Koharudin et al. 2011). All ^1H , ^{13}C and ^{15}N side-chain assignments were obtained, except for the aromatic side-chain and backbone carbonyl resonances of Y71, and the carbonyl backbone resonance of W23. Based on the chemical shifts, 10 β -strands were predicted using the program TALOS-N (Shen and Bax 2013) and the positions in the sequence are consistent with the published crystal structure (Koharudin et al. 2011), as shown in Fig. 2. It should be noted that the OAA homolog lectin family have an unique fold (Koharudin et al. 2012) compared to other lectins and thus, only intimate correlations among OAA homolog lectin family in the chemical shifts of $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ and their secondary structural elements are expected.

The chemical shifts have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) database under the accession number 25306.

Acknowledgements

This work was supported by the Max Planck Society, the EU (ERC grant agreement number 233227 to CG), and the National Institute of Health grant GM080642 (to AMG).

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Figure 1.

2D ^1H - ^{15}N HSQC spectrum of uniformly ^{15}N -labeled OAA in 90% H_2O /10% D_2O recorded at 800 MHz and 298 K. Backbone resonance assignments are indicated by the one-letter amino acid code and the sequence number. The amide resonances of G26 and G93 exhibit unusual proton chemical shifts and are displayed in the inset. All side chain resonances are labeled in red and amino groups of N and Q are connected with a dotted line. Arginine side chain resonances are folded in the ^{15}N dimension and contoured in grey.

Figure 2. Secondary structure elements predicted by TALOS-N (Shen and Bax 2013). The locations of predicated β -strands are indicated by arrows.