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Backbone resonance assignment of Alt a 1, a unique β -barrel protein and the major allergen of *Alternaria alternata*

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Abstract

Alt a 1 is the major allergen of the fungus *Alternaria alternata* and can be found in the cell wall of its spores. It is a cysteine linked homodimeric protein with a unique β -barrel fold as recently revealed by X-ray crystallography. Despite the elucidation of its structure, its biological function remains unknown. For *Alternaria*-sensitized patients, contact leads to respiratory allergy and in severe cases to asthma-related death. Here we report the sequence-specific Alt a 1 backbone ^1H , ^{15}N and ^{13}C chemical shift assignment.

Keywords

Alternaria alternata; Alt a 1; Unique β -barrel fold; Allergen; NMR backbone assignment

Biological context

The prevalence of allergenic diseases is increasing rapidly, with 30–40 % of the world's population being affected currently, as reported by the World Allergy Organization

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(Pawankar et al. 2012). Among airborne allergens, exposure to spores of the fungus *Alternaria alternata* is known to be one of the most common reasons for respiratory allergies like asthma and rhinitis (Andersson et al. 2003; Salo et al. 2006). Its major allergen, the highly allergenic protein Alt a 1, leads to IgE-mediated hypersensitivity in more than 95 % of *Alternaria*-sensitized patients (Twaroch et al. 2012a; Simon-Nobbe et al. 2008). The allergenic inflammation is a result of allergen induced cross linking of IgE, which leads to the degranulation of mast cells and basophils and in turn to a release of histamine and prostaglandins amongst others (Larche et al. 2006).

Alt a 1 forms a cysteine linked “butterfly-like” homodimer in nature, additionally stabilized by polar and hydrophobic interactions at the interface (Chruszcz et al. 2012). While allergen dimerization is certainly not a prerequisite for allergenicity, it could enhance the allergenic potential of the protein, as only one type of IgE antibody is needed for cross linking (Rouvinen et al. 2010). The recently reported crystal structure of Alt a 1 revealed a unique eleven stranded β -barrel structure, where all cysteines, except for the most N-terminal one, form intramolecular disulfide bonds contributing to the high temperature stability of this protein (Chruszcz et al. 2012). Although its biological function is still unknown, it has been hypothesized to be a transporter of small molecules due to its structure and/or to be important for spore germination. The latter is supported by the finding that Alt a 1 mainly localizes to the cell wall of spores (Twaroch et al. 2012b).

The presented NMR resonance assignment will allow the further biophysical characterization of Alt a 1 in particular with regards to its redox and dimerization properties. Furthermore it enables fast and straightforward ligand screenings, which will be helpful to elucidate its biological function.

Methods and experiments

For expression of recombinant $^{13}\text{C}/^{15}\text{N}$ -Alt a 1, *Escherichia coli* BL21 competent cells (Stratagene, La Jolla, California) were transformed with the rAlt a 1-pET27b(+) (GenScript, USA) construct and grown in *E. coli*-OD2 CN medium (Silantes, Munich, Germany) at 37 °C. Protein expression was induced at an $\text{OD}_{600\text{nm}}$ of 0.6 with 1 mM isopropyl- β -D-thiogalactoside (Calbiochem, Merck, Darmstadt, Germany). Expression was carried out for 5.5 h and bacterial cells were harvested by means of centrifugation (10,000 $\times$ g for 30 min at 4 °C). The bacterial pellets were re-suspended in 8 M urea, 40 mM imidazole and 1 mM EDTA (pH 7.4). Cells were disrupted by Ultraturax (Janke & Kunkel-IKA Labortechnik, Staufen, Germany) and stirred for 5 h at 4 °C. Insoluble debris was removed by means of centrifugation (10,000 $\times$ g for 20 min at 4 °C). The supernatant was dialyzed against 10 mM NaH_2PO_4 (pH 8) and applied to a HiTrapTM DEAE FF column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The recombinant protein was eluted with a linear gradient (0–500 mM NaCl) and fractions containing $^{13}\text{C}/^{15}\text{N}$ -labeled rAlt a 1 as the major component were pooled and dialyzed against 10 mM NaH_2PO_4 (pH 6.5). Afterwards the protein solution was applied to a pre-equilibrated (10 mM Na_2HPO_4 , 150 mM NaCl, pH 6.5) Superdex 200 gel filtration column. Fractions, containing Alt a 1, were pooled and concentrated to 500 μl using Millipore Centricons (MWCO 3500). Finally, the concentrated

protein was diluted four times with 3 ml of NMR Buffer (10 mM Na₂HPO₄, pH 6.5) and concentrated again to 500 µl (0.4 mM Alt a 1), in order to remove the sodium chloride.

All NMR measurements were carried out on a Bruker Avance III 700 MHz spectrometer equipped with a 5 mm triple-resonance TCI cryoprobe at 298 K. Sequence-specific backbone assignment was achieved by the combined use of the following standard triple-resonance spectra: HNCA, HN(CO)CA, HNCACB, HN(CO)CACB, HNCO and HN(CA)CO (Cavanagh et al. 2007). The spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed with CcpNmr Analysis (Vranken et al. 2005).

Assignments and data deposition

The combination of the above-mentioned backbone spectra allowed the assignment of 97 % of the ¹H^N, ¹⁵N, ¹³C' and ¹³C^{α/β} resonances. Met25 could not be assigned at all, as well as the amide signals of the five proline residues and Asp26. A second, lower populated conformation could be observed for the amino acids Asp26-Cys30, Tyr46, Leu58-Asn61, Asp71-Phe72, Cys74-Gln77, Phe99 and Lys111 and a third conformation for Phe60. Asp26 could be completely assigned in the second conformation, which also enabled the assignment of Met25 carbon atoms. Due to extensive line broadening or overlapping peaks Cys30 C', Cys89 C^β, Ser101 C^β, Pro135 C^β and Ser157 C' could not be assigned. An assigned ¹H, ¹⁵N HSQC is shown in Fig. 1.

The oxidized homodimeric state of Alt a 1, found in the crystal structure is in agreement with the C^β shifts of the assigned cysteines and can further be confirmed by gel filtration and SDS-PAGE. The program TALOS+ (Shen et al. 2009) was used to predict the secondary structure elements (Fig. 2a), which are in good agreement with the crystal structure (Fig. 2b), consisting mainly of eleven beta strands. For the second, lower populated conformation the TALOS predictions are almost identical, but a slightly lower β-strand probability is found for residues F45, C74 and S75 indicative of a slightly enhanced flexibility of these residues in the second conformer.

The assignment of the ¹H^N, ¹⁵N, ¹³C' and ¹³C^{α/β} chemical shifts of Alt a 1 has been submitted to the BMRB and can be found under the accession number 18993.

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Fig. 2.
a TALOS+ secondary structure prediction using Alt a 1 chemical shifts. To aid in visualization α -helical regions would correspond to negative confidence values. **b** Calculation of secondary structure content of the Alt a 1's crystal structure using STRIDE (Heinig and Frishman 2004)