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Letter to the Editor: Sequence-specific ^{1}H, ^{13}C and ^{15}N resonance assignments of Ara h 6, an allergenic 2S albumin from peanut

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Biological context

In many countries peanuts contribute significantly to the total dietary protein and peanuts or peanut byproducts are widely used as supplements in many different types of foods. Despite the eminent nutritional properties of this legume, peanuts can cause severe, type I hypersensitivity reactions (Bochner and Lichtenstein, 1991). Peanut allergy is a significant health problem because of the prevalence and severity of the reaction and is more often associated with fatal-induced anaphylaxis than any other food allergy (Yunginger et al., 1988; Sampson et al., 1992). Due to the life-threatening character of this reaction the development of therapeutic and prophylactic strategies are of extreme importance. For a detailed understanding of the molecular mechanisms of food-induced allergies and for the development of therapeutic strategies, knowledge of high-resolution three-dimensional structures of allergenic proteins is essential. Although several different major and minor peanut allergens have been isolated, only very few structural data for allergenic peanut proteins exist. The 2S albumin Ara h 6 (Kleber-Janke et al., 1999) represents one of three 2S albumin type allergens among at least six allergenic components in peanuts and shows a sequence identity of 58,5% to the well investigated major allergen Ara h 2 (Burks et al., 1992). 2S albumins are storage proteins widely distributed in plant seeds. These proteins include several disulfide bridges, the cysteines forming a conserved pattern (Shewry et al., 2001). In addition to their role as nitrogen and sulfur donor that has been proposed from their amino acid composition, activities as antifungal, serine protease inhibitor, and calmodulin antagonists have been ascribed for several 2S albumins (Shewry et al., 2001). Moreover, the 2S albumin family is an important class of food allergens of well recognized clinical importance. Several allergenic 2S albumins were identified in different seeds and nuts such as mustard, sesame, Brazil nut, walnut, and peanut (Pastorello et al., 2001). This interesting protein group contains many highly potent allergens and many studies explored the allergenic character of this proteins using immunological as well as mutational methods. Nevertheless, only one structural study of a 2S albumin, that of napin BnIb from rape seeds (Rico et al., 1996), was carried out so far. This BnIb structure only presents the global fold of the protein due to experimental restrictions arising from the heterogeneity of the protein sample isolated from rapeseed (Rico et al., 1996). Recently we reported a recombinant strategy for the efficient large-scale preparation of properly folded and disulfide bridged Ara h 2 for structural studies (Lehmann et al., 2003). Exploiting *E. coli* strains with an oxidizing cytoplasm, we used this strategy for the preparation of the highly homologous Ara h 6. Here we report the majority of assignments of the ^{1}H, ^{13}C and ^{15}N resonances of Ara h 6 and its secondary structure based on multidimensional heteronuclear NMR data.

Methods and results

Recombinant Ara h 6 was obtained using a new bacteria based strategy (Lehmann et al., 2003) for a high level expression of authentically folded and disulfide bridged 2S albumins using M9 minimal medium with $^{15}NH_4Cl$ and $^{13}C_6$ glucose. The purified protein was partially digested with trypsin and chymotrypsin and subsequently purified using size exclusion and reversed phase chromatography. Due to the very stable nature of the protein, digestion occurs only at two distinct regions in the protein: the aminoterminus and

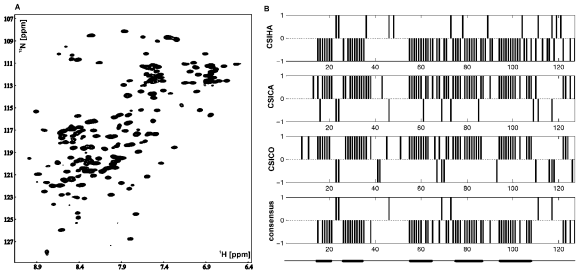

Figure 1. (A) $^{1}H,^{15}N$-HSQC spectrum of $^{13}C/^{15}N$-labeled Ara h 6, showing the well-dispersed amide proton resonances. (B) Secondary chemical shift indices for HA, CA and CO nuclei and a consensus index. The artwork at the bottom indicates the deduced secondary structure.

one internal position. The resulting protease resistant protein core lacks seven aminoterminal residues (G1–R7) and consists of two disulfide bridged subunits due to an internal protease cleavage. As evidenced by NMR, the folding of the protease-resistant core is nearly identical to the undigested protein. The protease cleavage strongly reduced the amount of broad unresolved signals observed in the middle of the spectrum of uncleaved protein, caused by conformational heterogeneity and dynamics on a ms-μs timescale due to an internal flexible region without regular secondary structure.

For NMR studies samples of 0.8 mM Ara h 6 in 50 mM potassium phosphate (pH 7.0) in H_2O/D_2O (9:1) were prepared. All NMR spectra were acquired on a Bruker DRX 600 MHz and DMX 750 MHz spectrometer at a temperature of 25 °C. The following 3D and 2D NMR spectra were recorded for the backbone, aliphatic, and aromatic side chain resonance assignment: HNCO, HNCA, HNCACB, CBCA(CO)NH, H(CCO)NH, C(CO)NH, (H)CCH-COSY, HBHA(CO)NH, HNHA, ^{15}N-NOESYHSQC, ^{13}C-NOESYHSQC, $^{15}N/^{15}N$-HMQC-NOESYHSQC, $^{13}C/^{13}C$-HMQC-NOESYHSQC, $^{13}C/^{15}N$-HMQC-NOESYHSQC, and 2D [$^{1}H,^{1}H$] aromatic ^{13}C-edited NOESY (Sattler et al., 1999). The NMR data were processed using in-house written software and analyzed with the program packages NMRView (B.A. Johnson, Merck, Whitehouse Station, NJ) and NDEE (SpinUp Inc., Dortmund, Germany).

Extent of assignment and data deposition

The $^{1}H,^{15}N$-HSQC spectrum was well dispersed (Figure 1A). ^{1}H and ^{15}N backbone assignments were complete, except for a protease digested aminoterminal segment of seven residues (G1-R7), prolines and, residues D44, D47, N93, R92. The assignment of the latter four residues was seriously hampered by spectral overlap. For all assignable residues 99.2% of the C^{α} and C^{β} chemical shifts, 96.1% of the H^{α}, and 75.0% of the H^{β} chemical shifts could be assigned. The assignment of ^{1}H and ^{13}C chemical shifts of aliphatic side-chain resonances was obtained to a degree of 78.6% with the exception of side chain NH_2 resonances of asparagines, glutamines, arginines, and lysines. The assignment of aliphatic resonances was hampered by high chemical shift similarities caused by an excessive amount of glutamine (15,7%) and arginine (12,6%) residues in the protein. Due to the special properties of the expression system leucine residues were only ^{15}N and not ^{13}C labelled. Thus, no ^{13}C chemical shifts for leucine residues were assigned.

Analysis of the H^{α}, C^{α}, and CO chemical shifts (Figure 1B) together with an assessment of sequential and medium-range NOEs indicated the existence of five helical regions: helix 1 (residue 15 to 21), helix 2 (residue 25 to 35), helix 3 (residue 55 to 65), helix 4 (residue 74 to 87), and helix 5 (residue 94 to 108). The existence of these five α-helices was further supported by $^{3}J_{HNH\alpha}$ coupling constants and exchange-protected backbone amide protons.

^{1}H, ^{13}C, and ^{15}N chemical shifts have been deposited with BioMagResBank (access code: 5871).

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