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Letter to the Editor: Assignment of ^{1}H and ^{15}N resonances and secondary structure of the recombinant RicC3 of 2S albumin storage protein from *Ricinus communis*

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Biological context

The 2S albumins are storage proteins widely distributed in plant seeds. They are small proteins (12 to 15 kDa) generally composed of two different polypeptide chains linked by disulphide bridges. Due to their amino acid compositions, their high content in the protein bodies of the seeds, and their mobilization during germination, a role as nitrogen and sulfur donor has been proposed for these proteins (Youle and Huang, 1978). However, other activities have been ascribed to the 2S albumins: they have been shown to act as antifungal, serine protease inhibitors, and calmodulin antagonists. Moreover, in recent years, several 2S albumins have been described as allergens, suggesting that this family of proteins is intrinsically allergenic. In addition to their biochemical interest, the 2S albumins have been used, by means of genetic engineering as carriers for the synthesis of biologically active peptides (Vandekerckhove et al., 1989), as well as to improve the nutritional properties of grain crops by increasing their content of essential amino acids. One of the products of the *Ricinus communis* 2S seed storage protein, termed RicC3 (Bashir et al., 1998) constitutes the peptidic component of the immunomodulator Inmunoferon®, a widely used pharmaceutical speciality (Varela et al., 2002).

The determination of the three-dimensional structure of RicC3 constitutes a fundamental first step for understanding its biological and pharmacological properties at a molecular level. Moreover, knowledge of the three-dimensional structure of RicC3 should provide firm bases for genetic manipulations aimed towards the improvement of its pharmacological applications. In addition, the determination of the three-dimensional structure of RicC3 should provide a detailed general picture of the structural properties of the broad family of the 2S albumin proteins. There is only a structural study of a 2S albumin, that of napin BnIb (Rico et al., 1996), in which the residue heterogeneity present in the natural protein isolated from rapeseed precluded the determination of a high-resolution three-dimensional structure, and only the global fold could be determined. Recently, the high yield synthesis of RicC3 using genetically engineered *E. coli* grown in defined culture media has been described (Fernández-Tornero et al., 2002), what prompted us to determine its high-resolution three-dimensional structure. Here we report the ^{1}H and ^{15}N chemical shifts and secondary structure of ^{15}N labeled recombinant RicC3.

Methods and experiments

The recombinant RicC3 was obtained using a new system for high level expression of heterologous proteins in native conformation using minimal medium cultures of *E. coli* (Fernández-Tornero et al., 2002) using $^{15}NH_4Cl$ as nitrogen source. The purified protein was lyophilized and kept at -20 °C until the NMR experiments.

RicC3 samples were prepared for NMR experiments at $\sim$2.0 mM concentration in 95% H_2O/5%

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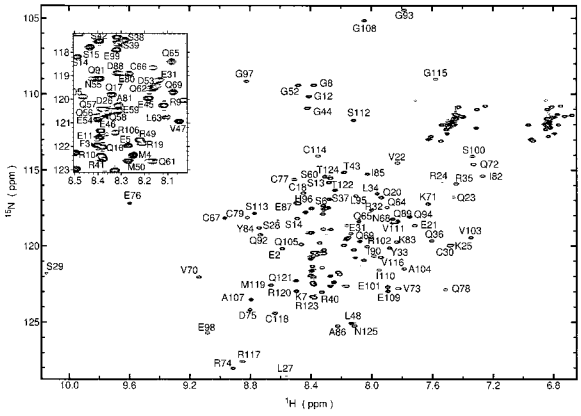

Figure 1. ^{1}H-^{15}N HSQC spectrum of 2 mM RicC3 in 10 mM phosphate buffer and TSP at pH 3.5 and 308 K, recorded on a Bruker-DMX 600 NMR spectrometer. Cross peaks are labeled with their single letter code and residue number.

D_2O or in D_2O solution containing 10 mM sodium phosphate and TSP at pH 3.5 in 5 mm NMR tubes. Proton chemical shifts were referenced to internal TSP and ^{15}N chemical shifts were referenced indirectly using the gyromagnetic ratios of ^{15}N:^{1}H. The experiments performed included 2D ^{1}H-^{15}N HSQC, ^{1}H NOESY (80 ms mixing time), ^{1}H TOCSY (65 ms mixing time), ^{1}H COSY and 3D ^{15}N-NOESY-HSQC (80 ms mixing time), ^{15}N-TOCSY-HSQC (65 ms mixing time), HNHA and HNHB. All NMR data were collected at 308 K on a Bruker AV 600 NMR spectrometer.

The programs NMRPipe (Delaglio et al., 1995) and NMRView (Johnson and Blevins, 1994) were used for spectral processing and data analysis, respectively. The resonance assignments of proton and ^{15}N resonances have been achieved by combination of 2D and 3D experiments using a standard strategy.

Extent of assignments and data deposition

The ^{1}H-^{15}N spectra were well dispersed as shown by the ^{1}H-^{15}N HSQC spectrum in Figure 1. Backbone ^{1}H and ^{15}N assignments were complete, except for Ala1 and Pro51. Further analysis of the NOE data permitted nearly complete ^{1}H side chain assignments with the exception of some distal side chain ^{1}H resonances in arginines and lysines and side chain NH2 resonances of asparagines and glutamines.

An analysis of the assigned chemical shifts using the CSI method (Wishart and Sykes, 1994) and short range NOEs indicated the presence of five helical regions: helix 1 (residues 18 to 23), helix 2 (residues 30 to 35), helix 3 (residues 63 to 72), helix 4 (residues 78 to 92) and helix 5 (residues 100 to 114). This spanning of the five helical segments was further supported by $^{3}J_{HNH\alpha}$ and $^{3}J_{H\alpha N}$ coupling constants and by the backbone torsion angles predicted using TALOS (Cornilescu et al., 1999) and is further confirmed by preliminary results on the three-dimenisonal structure now in progress.

The proton and nitrogen chemical shift values have been deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number BMRB-5374.

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