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Letter to the Editor: ^{1}H(N), ^{15}N, ^{13}CO, ^{13}Cα, ^{13}Cβ assignment and secondary structure of a 20 kDa α-L-fucosidase from pea using TROSY

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Biological context

α-1,2-L-Fucosidase is a 20 kDa glycolytic enzyme which hydrolyzes fucosyl-terminal groups of oligosaccharides (Augur et al., 1993). This function is related to plant defense processes. The protein is synthesized in plant cells as a prepro-protein, reaching its mature form (Fuc-9) after the cleavage of a C-terminal pro-peptide of nine amino acids during its transit to the vacuoles. The C-terminal pro-peptide is necessary for fucosidase sorting to the vacuole. It is the first plant fucosidase cloned and it does not show apparent similarities with other fucosidases. Nevertheless, two new plant fucosidase genes have recently been identified in *Cicer arietinum* (EMBL CAB76906.1 and EMBL CAB76907.3). Moreover, several expressed sequence tags (EST) have been identified in other leguminous plants such as *Glycine max* (EST accession number AF128268) and *Medicago truncatula* (# AW126318). The deduced amino acid sequences show a high degree of homology (63–90%) and identity (49–82%) with the pea α-1,2-L-fucosidase. The five Cys residues (Cys64, 109, 127, 162 and 169) of the protein (Augur et al., 1995) and most of the residues surrounding these Cys residues are conserved in this family of fucosidases. It is unknown whether the Cys residues of the mature enzyme Fuc-9 are involved in a catalytic dyad.

Here we report the near complete ^{1}H(N), ^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ assignment and secondary structure of the pro-protein (Fuc). This is the first step of the structure determination and provides the basis for further studies of structure-activity between the pro-fucosidase (Fuc) and its mature form (Fuc-9).

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Methods and results

The uniformly ^{15}N,^{13}C-labeled Fuc with 85% deuteration was expressed with 6xHis tag at the N-terminus from the plasmid Pet-14b vector (Novagen) in the *E. coli* strain BL21(DE3). Cells were grown in M9 minimal medium (100% $^{2}H_2O$) supplemented with 1 g l^{-1} $^{15}NH_4Cl$, 2 g l^{-1} $^{13}C_6$-glucose, nitrogenate bases (10 mg l^{-1} adenine, guanine, cytosine, thymine and uracil), vitamins (1 mg l^{-1} biotin, folic acid, nicotinamide, pantotenate, piridoxal and 10 mg l^{-1} thiamine) and oligoelements (100 μmols l^{-1} $CaCl_2$, 1 μmol l^{-1} $FeCl_3$, 0.1 μmol l^{-1} $ZnCl_2$, 0.025 μmol l^{-1} $CuCl_2$ and 100 μmols l^{-1} $MnCl_2$). Cells were grown at 37 °C to an OD_{600} of 0.6 to 0.8 and induced with 0.4 mM IPTG (isopropyl β-D-thiogalactopyranoside). Six hours after induction, cells were harvested and resuspended under denaturing conditions. The sample was subsequently purified on a Ni-agarose column (Pharmacia). The purified protein was refolded *in vitro* removing progressively the denaturant (first) and the reducing (last) agents by a series of dialyses. After the oxidation of the sample, it was concentrated and prepared for NMR experiments. The final NMR sample contained 1 mM of Fuc in 100 mM phosphate buffer (pH 7.7), 100 mM NaCl and 5% v/v $^{2}H_2O$.

NMR data were acquired at 298 K using a Bruker DRX-800 NMR spectrometer equipped with

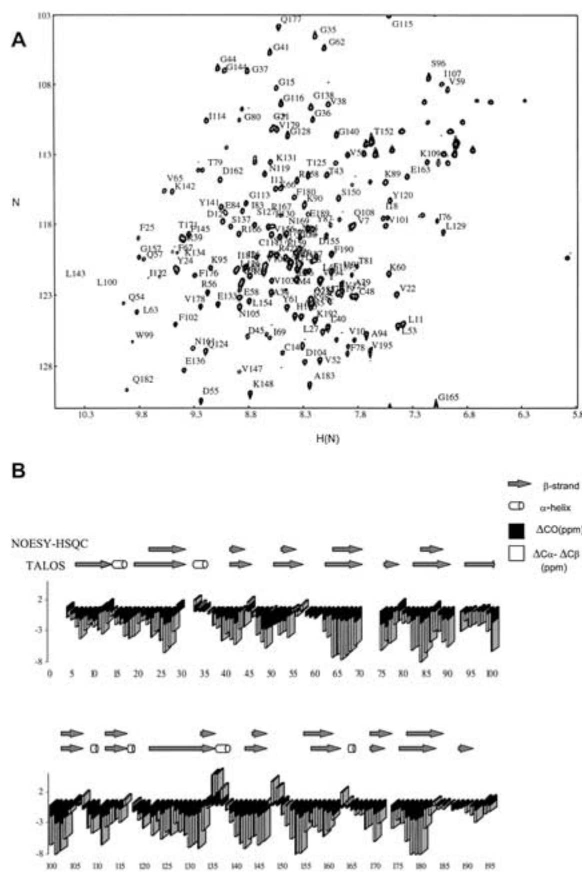

Figure 1. (A) 800 MHz ^{15}N-HSQC spectrum of Fuc. (B) A summary of the secondary structure elements based on ^{1}H(N)-^{1}H(N) connectivities, TALOS software and on ^{13}Cα, ^{13}Cβ and ^{13}CO chemical shifts.

an xyz-gradient ^{1}H,^{15}N,^{13}C triple resonance probehead. The sensitivity and resolution of triple resonance experiments was improved by employing the TROSY technique (Salzmann et al., 1998). ^{1}H chemical shifts were referenced to internal TSP (3-(trimethylsilyl)propionate-2,2,3,3-d_4 sodium salt) at 0.00 ppm. ^{15}N and ^{13}C chemical shifts were calibrated indirectly using the appropriate gyromagnetic ratios (Wishart et al., 1995). Spectra were processed using NMRPipe software package (Delaglio et al., 1995) and analyzed with nmrView program (Johnson et al., 1994).

^{1}H(N), ^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ assignments were obtained with HSQC (Figure 1A), TR-HNCO, TR-HN(CA)CO, TR-HNCA, TR-HN(CO)CA, TR-HNCACB and TR-HN(CO)CACB experiments. ^{15}N-edited NOESY-HSQC was used not only to confirm the assignment but also to determine the secondary structure of Fuc. The secondary structure elements were also identified based on the TALOS program by Delaglio and Bax (1999) and utilizing chemical shift differences from random coil values for ^{13}Cα, ^{13}Cβ and ^{13}CO (Venters et al., 1996, Wishart et al., 1994). A summary of the results is shown in Figure 1B. The data revealed that Fuc is a mostly-β protein containing at least twelve, probably anti-parallel, β-strands.

Extent of assignments and data deposition

Sequential assignment of ^{1}H(N), ^{15}N, ^{13}CO, ^{13}Cα and ^{13}Cβ of most of the Fuc residues were made (90% aprox.). Many of the non-assigned resonances can be attributed to ambiguities or to the fast exchange rates of the more flexible parts due, in turn, to the relatively high pH (7.7) necessary for solution stability of Fuc. The assignment has been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-5090. Chemical shifts from the deuterated protein spectra were corrected with a factor of + 0.23 in case of ^{15}N (Gardner et al., 1996) and according to the number of deuterons one and two bonds away from the ^{13}Cα and ^{13}Cβ nucleus (Venters et al., 1996). TROSY effects were corrected based on a J_{HN} of 93 Hz.

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