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Assignments of ^{1}H and ^{13}C resonances in the complex of palmitate and a non-specific lipid transfer protein (ns-LTP) isolated from barley seeds

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Biological context

Plant lipid transfer proteins (LTPs) are small 9–10 kDa basic proteins. They are named according to their capability of transferring lipid-like ligands between natural or artificial membranes. The plant ns-LTPs exhibit a strong sequence homology (40%–70%), and with a few exceptions they contain eight conserved cysteines that form four disulfide bonds. The ns-LTPs contain a signal peptide and have been localized in the epidermal cell wall, suggesting that the ns-LTPs are secreted. On this basis a possible role in the cutin formations seems more likely (Sterk et al., 1991). The three-dimensional structures of ns-LTPs are very similar, they all show ns-LTPs to be single compact domain structures comprising four α-helices and a long C-terminus with no regular secondary structure (Heinemann et al., 1996). From the structural studies it is evident that although the three-dimensional structures are very similar among plant ns-LTPs, the binding modes of these proteins differ. It may be that the observed flexibility of the binding sites is caused by different ns-LTPs being specific for different types of lipid-like ligands. In order to investigate this flexibility we have been studying the effect of ligand size on the ligand binding induced conformational changes in the barley ns-LTP structure and compared the binding modes of palmitate in two different ns-LTPs from barley and maize respectively. As a first step in this process we have used two-dimensional NMR spectroscopy to study the ^{1}H resonances of the complexed ns-LTP from barley and the ^{1}H and ^{13}C resonances of the complexed palmitate.

Methods and results

ns-LTP$_{barley}$ was isolated and purified as described previously (Lerche et al., 1997). ^{13}C-labelled palmitate was purchased from Cambridge Isotope Laboratories, Inc., USA. Two different samples were prepared from lyophilized material: [bLTP]/[C_{16}]:[2mM]/[6mM] and [bLTP]/[$^{13}C_{16}$]:[1.8mM]/[6.2mM] in 600 μl solutions. The pH in both samples was 7.2. All NMR spectra were recorded at 310 K on a Bruker AMX600 spectrometer operating at a frequency of 600 MHz for ^{1}H. A series of ^{1}H NMR spectra were recorded including DQF-COSY (Piantini et al., 1983), TOCSY (Braunschweier et al., 1983) and NOESY (Kumar et al, 1981). All spectra were recorded with 512 and 2048 complex points in the t_1 and the t_2 dimensions, and the spectral width was 7246 Hz. The FIDs were zero-filled to 1024 and 4096 complex points in t_1 and t_2 respectively, and multiplied with a phase-shifted sine-bell function in t_1 and a sine function in t_2. Two TOCSY spectra were recorded, with $\tau_m = 44$ ms and 30 ms. The TOCSY spectra were likewise zero-filled to 1024 and 4096 complex points in the t_1 and t_2 dimensions and subsequently multiplied with a phase-shifted sine-bell function in t_1 and an exponential function in t_2. Two types of heteronuclear spectra were recorded, a HSQC (Bodenhausen and Ruben, 1980) and a HSQC-NOE (Müller et al., 1987) experiment. In these experiments 256 complex points were recorded with 512 transients in t_1 and 2048 complex points in t_2. The spectral width in the ^{13}C-dimension was 4448 Hz. Two HSQC-NOE spectra were recorded with mixing times of 120 ms and 200 ms, respectively. The recorded data were processed using the MNMR software packages (Kjær et al., 1994).

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Extent of assignments and data deposition

The ^{1}H assignments of ns-LTP$_{barley}$ in complex with palmitate were performed using the Wüthrich-strategy combined with the PRONTO spin system identification tool (Kjær et al., 1994). The fingerprint region of the DQF-COSY spectrum revealed a total of 84 of the 93 expected (including the double peaks of the nine glycines) H^N-H^α cross-peaks. The missing cross-peaks of Ser^{40}, Ser^{41}, His^{59}, Asp^{86} and Cys^{87} were identified on the basis of NOE-connectivities. Resonance assignments were obtained for 87% of the protein ^{1}H atoms in the ns-LTP$_{barley}$-palmitate complex. A total of 127 dihedral angle restraints were determined. From NOE intensities, five out of six proline residues showed trans peptide bonds and one, Pro^{23}, was shown to have a cis peptide bond. The four disulfide bridges were identified by NOEs between the β protons in each pair: Cys^3-Cys^{50}, Cys^{13}-Cys^{27}, Cys^{28}-Cys^{73} and Cys^{48}-Cys^{87}.

The summary of the secondary structure elements present in the ns-LTP$_{barley}$ complexed with palmitate is shown in Figure 1. The four α-helices are defined as supported by the four areas of typical short range NOEs, comprising H_A (Cys^3-Gly^{19}), H_B (Glu^{26}-Gln^{37}), H_C (Arg^{44}-Gly^{57}) and H_D (Leu^{63}-Cys^{73}). The gaps in the sequential assignment are at the prolines for which the sequence connections are made by α-δ NOEs.

For the ligand, palmitate, three methylenes were assigned in the carboxylate end and likewise in the ω-end of the ligand, the methyl group and three additional methylene groups were assigned.

For the complex of palmitate and LTP there were specific changes of chemical shifts both in the ligand and in the protein. The observed perturbations were seen primarily in the C-terminal part of the LTP sequence, especially in the residues Val^{77}, Pro^{78}, Tyr^{79}, Thr^{80} and Ile^{81}.

^{1}H assignments of the complexed protein and ^{1}H and ^{13}C assignments of the ligand have been deposited in the BioMagResBank accession number 4142.

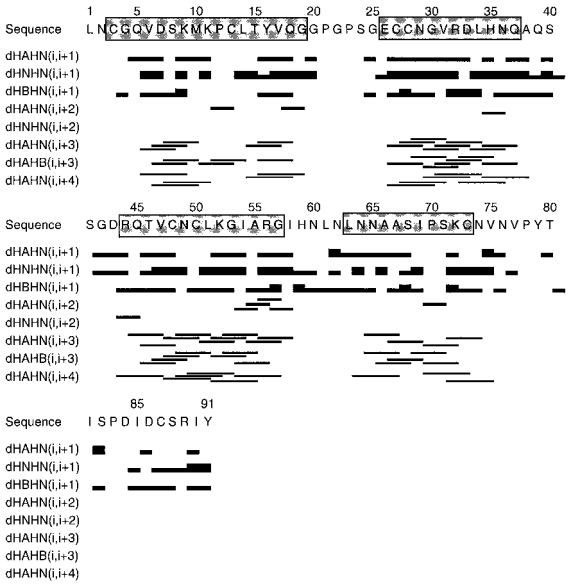

Figure 1. Summary of information applied in the sequential assignment and in the secondary structure analysis. The amino acid sequence is shown at the top. The framed regions indicating the four α-helices. The intensities of the sequential NOEs are categorized as either strong, medium or weak and shown accordingly by the thickness of the lines.

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