

Solution Structure of Plant Nonspecific Lipid Transfer Protein-2 from Rice (*Oryza sativa*)*

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The three-dimensional structure of rice nonspecific lipid transfer protein (nsLTP2) has been solved for the first time. The structure of nsLTP2 was obtained using 813 distance constraints, 30 hydrogen bond constraints, and 19 dihedral angle constraints. Fifteen of the 50 random simulated annealing structures satisfied all of the constraints and possessed good nonbonded contacts. The novel three-dimensional fold of rice nsLTP2 contains a triangular hydrophobic cavity formed by three prominent helices. The four disulfide bonds required for stabilization of the nsLTP2 structure show a different pattern of cysteine pairing compared with nsLTP1. The C terminus of the protein is very flexible and forms a cap over the hydrophobic cavity. Molecular modeling studies suggested that the hydrophobic cavity could accommodate large molecules with rigid structures, such as sterols. The positively charged residues on the molecular surface of nsLTP2 are structurally similar to other plant defense proteins.

Plant nonspecific lipid transfer proteins (nsLTPs)¹ have been isolated from a number of plant species including wheat, rice, and barley (1). nsLTPs enhance the intermembrane exchange or transfer of lipid molecules *in vitro* (2). Biotic and abiotic stresses stimulate nsLTP gene expression (3–5). nsLTPs are known to be involved in the formation of a protective hydrophobic layer over the plant surfaces (5). Despite their ability to help plants to manage stress, the exact mechanism of transport is still unclear. nsLTPs are also involved in other biological activities such as flowering and transportation of cutin and suberin monomers (6). nsLTPs present in cereals play an important role in food chemistry. nsLTPs directly affect dough rheology and breadcrumb texture (6). Reports about the isolation of glycosylated and reduced nsLTP fragments from beer suggest that nsLTPs are involved in froth formation during the malting and brewing processes (7).

nsLTPs are divided into two subfamilies, nsLTP1 (molecular mass ~9 kDa) and nsLTP2 (molecular mass ~7 kDa) (2). nsLTP1 is found primarily in aerial organs, whereas nsLTP2 is expressed in roots. Interestingly, both nsLTP1 and nsLTP2 are

found in seeds. nsLTP1 is proposed to transport cutin monomers, whereas nsLTP2 is involved in the transport of the more rigid suberin monomers (6). Three-dimensional structures of nsLTP1 from various sources were determined by x-ray and NMR spectroscopic techniques (8). All nsLTP1s share a common structural fold stabilized by four disulfide bonds. The prominent four helices of nsLTP1 are packed against a flexible C-terminal arm formed by a series of turns. In contrast to many globular proteins, the hydrophobic side chains of nsLTP1 do not form a rigid hydrophobic core but instead form a hydrophobic cavity at the interior of the protein. Recently, we have purified nsLTP2 from rice. The amino acid sequence, disulfide bond pattern and stability have been determined (TrEMBL ID P83210) (9). Rice nsLTP2 contains 69 residues and has less than 30% sequence identity with nsLTP1. The disulfide bond pattern of nsLTP2 differs from nsLTP1. Although the size is smaller, nsLTP2 has comparable lipid transfer activity and greater structural stability than nsLTP1 (10). Plant nsLTP1 proteins from various sources have been well characterized, whereas the structure and the functionality of nsLTP2 is poorly understood (6, 8). Here, we report the three-dimensional structure of nsLTP2.

MATERIALS AND METHODS

Nuclear Magnetic Resonance Experiments and Structure Calculation—Rice nsLTP2 was purified from rice flour as described previously (11). All NMR experiments were carried out on a 3 mm sample of rice nsLTP2 dissolved in 50 mM phosphate buffer (90% H₂O and 10% D₂O, pH 6.4) containing sodium 3-(trimethylsilyl)[2,2,3,3-²H]propionate (*d*⁴-TSP) as the internal standard. A Bruker 600 MHz NMR spectrometer was used to record two-dimensional TOCSY and NOESY spectra with 512 *t*₁ increments and 2048 *t*₂ complex data points at 25 °C. All of the NMR spectra were processed using XWIN-nmr (Bruker) and analyzed using SPARKY (12). Distance constraints were derived from a *y* spectrum recorded with a 150-ms mixing time. A TOCSY spectrum was used to derive *phi* (*φ*) angle constraints (13). Hydrogen bonding information was obtained from amide exchange data. The experimental NOEs were classified as weak, weak medium, medium, strong medium, or strong according to standard procedures (14). Structure calculations were carried out using XPLOR, version 3.8 (15).

Lipid Transfer Assay—Fluorescence lipid transfer assays were performed as previously described (10, 16). 2 μ l of pyrPtdCho in ethanol, 0.118 mM, and 2 μ l of Myr₂PtdGro, 1 mg·ml⁻¹, were added to a cuvette containing 0.8 μ M protein in 2 ml of 20 mM Tris-HCl, 5 mM EDTA buffer (pH 7.4). Fluorescence intensities were monitored at 396 nm with excitation at 346 nm using an SLM 48000S spectrofluorometer at 25 °C.

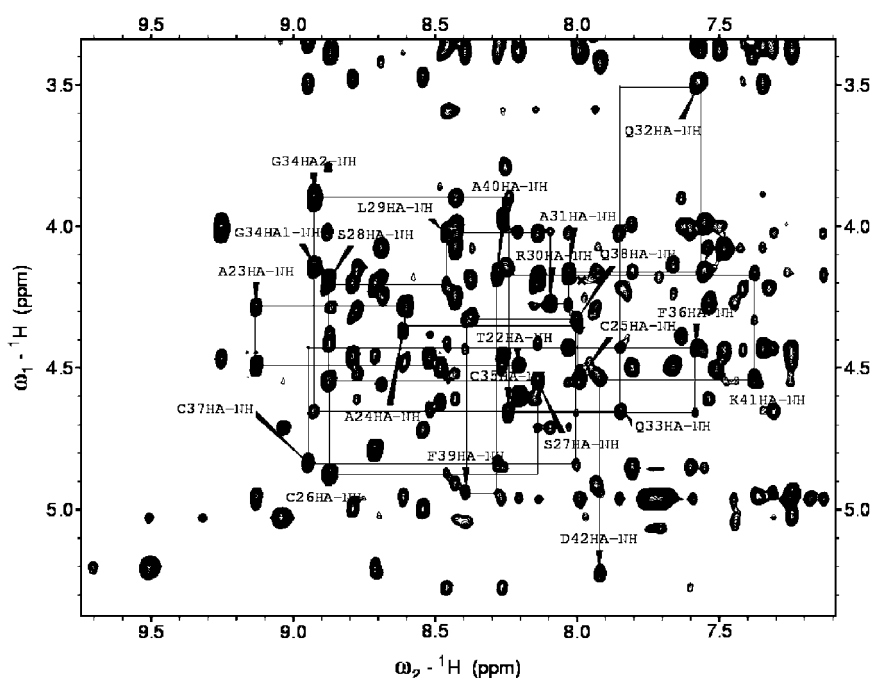
Molecular Modeling—A geometric recognition algorithm (gramm), was used to dock ligands with the protein (17). Grid steps of 1.7 Å for translation and 10° for rotation were used to dock the ligands in hydrophobic mode. Optimal values for the repulsion (*E*) and attraction double range were chosen as 10 Å and 0.5 Å, respectively. After initial docking, the best complex structures were chosen for further refinement using the Discover 3 in Insight II package (Molecular Simulation Inc.). 500 energy minimization steps were carried out with the steepest decent method using the consistent valence force field to attain the most stable conformation. The dielectric constant was set to 1.0. A program for studying cavities of proteins, VOIDOO, was used to evaluate the hydrophobic

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¹ The abbreviations used are: nsLTP, nonspecific lipid transfer protein; NOE, nuclear Overhauser effect; pyrPtdCho, L- α -phosphatidylcholine, β -(pyrene-1-yl)decanoyl- γ -palmitoyl; Myr₂PtdGro, L- α -phosphatidyl-DL-glycerol, dimyristoyl; r.m.s.d., root mean square deviation; PDB, Protein Data Bank.

A.



B.

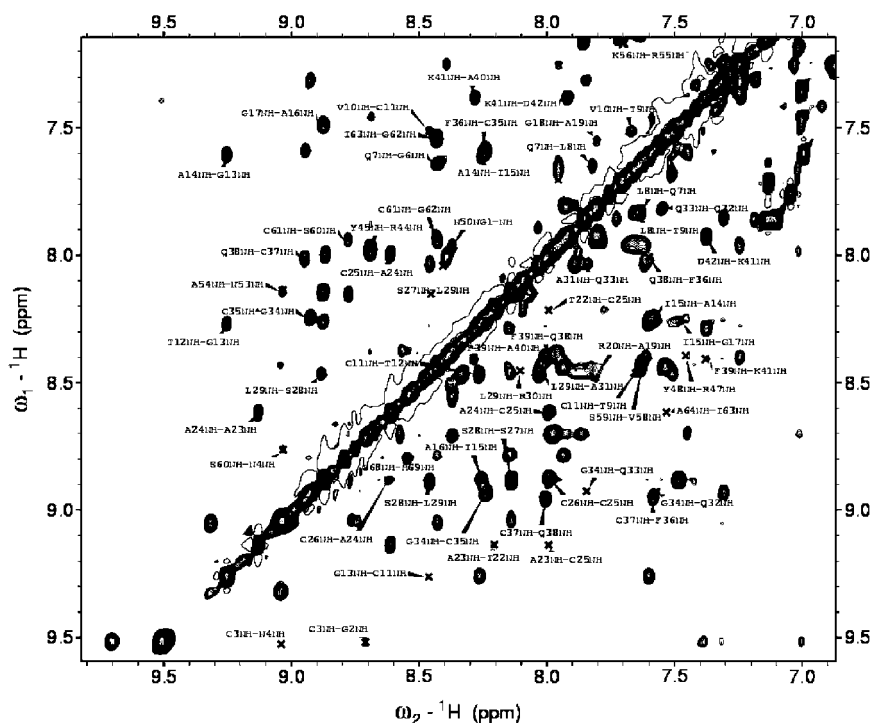


FIG. 1. NOESY spectrum of nsLTP2 measured at 25 °C with a mixing time of 150 ms. The protein sample was dissolved in 50 mM phosphate buffer (pH 6.4) containing 90% H₂O and 10% D₂O. A, sequential H α (i)-H N (i+1) connectivities of a portion (Thr²²-Asp⁴²) of the polypeptide chain in the fingerprint region. B, assignment of various cross-peaks in the H N -H N region.

cavities of nsLTPs in the complexed and noncomplexed forms (18). The van der Waals mode or the probe-occupied mode (probe radius 1.4 Å) was used to measure the volumes.

RESULTS AND DISCUSSION

Structure Calculation of nsLTP2—Resonance assignments for most protons were achieved through conventional methods

(14). Sequential assignments for a portion polypeptide chain are shown in Fig. 1A. Fig. 1B shows assignments for cross-peaks between various amide protons. The chemical shift data were deposited in the BioMagResBank (BMRB-5325). A total of 813 distance constraints were derived from cross-peaks in a 150-ms NOESY spectrum. Hydrogen bond constraints were

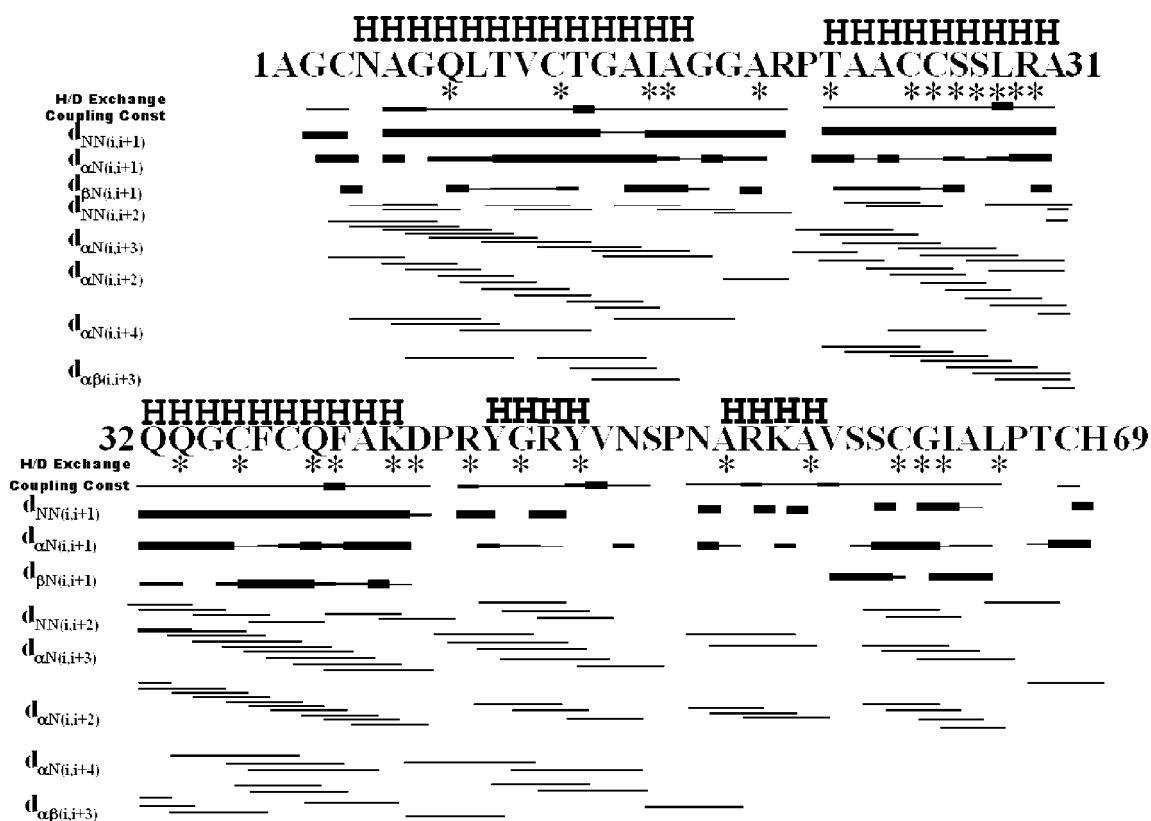


FIG. 2. **Amino acid sequence and survey of sequential connectivities of rice nsLTP2.** Differences in the NOE intensities are represented by block height. Various coupling constant values, 0–4, 4–8, and 8–12 Hz, are represented by different block intensities. *, indicates amide proton cross peaks present in the TOCSY spectrum recorded immediately after dissolving the lyophilized nsLTP2 in 99% D₂O at 25 °C. Various amino acids involved in the helical regions are denoted by the letter *H*.

FIG. 3. **A**, stereo representation of the 15 best superimposed NMR structures of rice nsLTP2 (only the backbone atoms are shown for clarity). **B**, solution structure of rice nsLTP2. Disulfide bonds involved in the three-dimensional structure are shown in ball-and-stick representation. Helix I (green) and helix II (red) are connected through a loop (Gly¹⁷–Pro²¹). Helix II and helix III (purple) are joined by a sharp 90° turn. The region containing two single turn helices and the C terminus are shown in orange and brown, respectively.

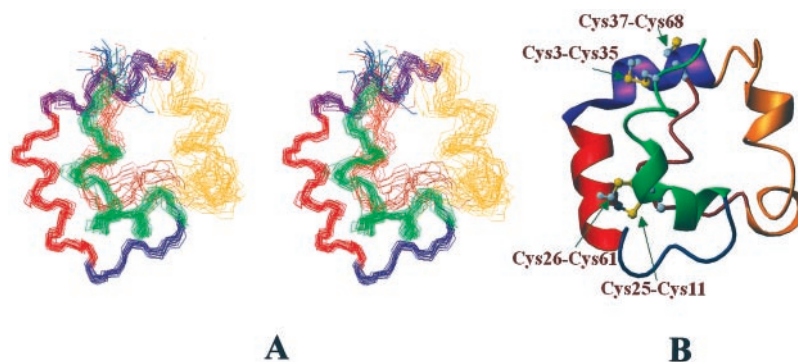


TABLE I

Restraint and structural statistics for 15 simulated annealing structures of rice nsLTP2 in aqueous solution at pH 6.4 and 298 K

Experimental constraints		
Total	862	
Intraresidue	353	
Sequential	197	
Medium range	203	
Long range	70	
Hydrogen bond restraints	30	
Dihedral angle restraint	19	
r.s.m.d. from the average structure (Å)		
Residues 1–69	1.54 ± 0.25	All heavy atoms
Residues 1–40	0.95 ± 0.15	1.09 ± 0.20
E_{total} measure from Xplor (KJ.mol ⁻¹)	300 ± 100	0.65 ± 0.10
ProCheck analysis ^a		
Residues in most favored regions		36 (65.5) ^b
Residues in additional allowed regions		16 (29.1)
Residues in generously allowed regions		1 (1.8)
Residues in disallowed regions		2 (3.6)

^a Result of minimized average structure through Ramachandran plot.

^b Numbers in parentheses are percent of total.

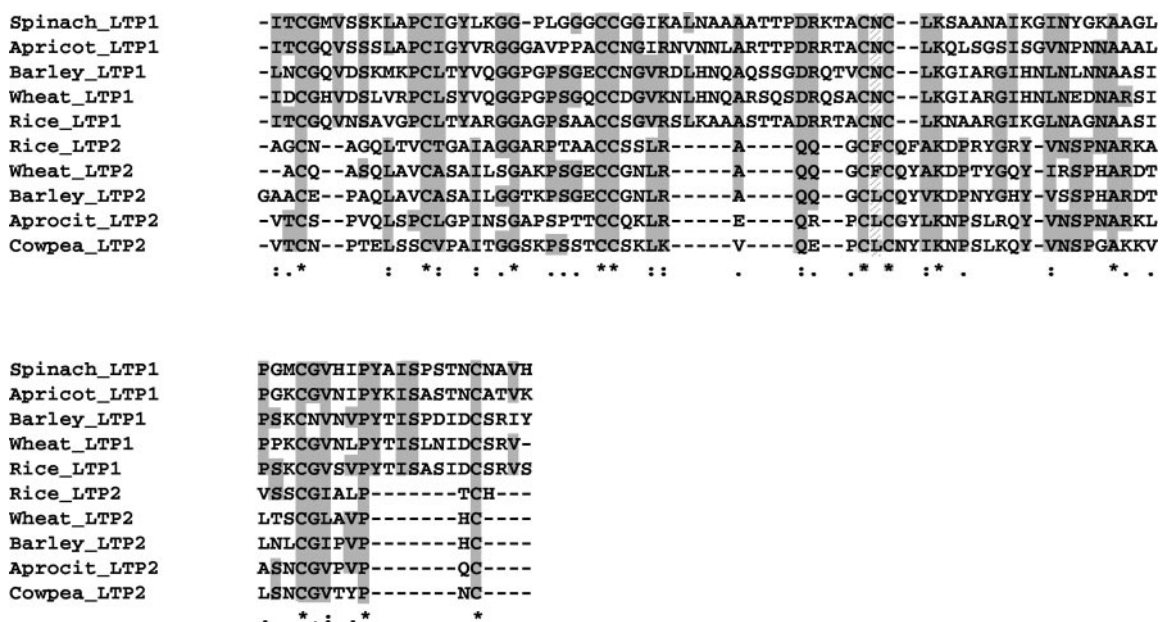


FIG. 4. A, sequence alignment of various plant nsLTPs with CLUSTAL W. *, denotes identical or conserved residues in all sequences; “:” and “.”, conserved and semiconserved substitutions, respectively. All of the cysteine residues and many of the hydrophobic residues are well conserved among the nsLTP family. A striking alignment difference between nsLTP1 and nsLTP2 is observed at the -CXC- motif.

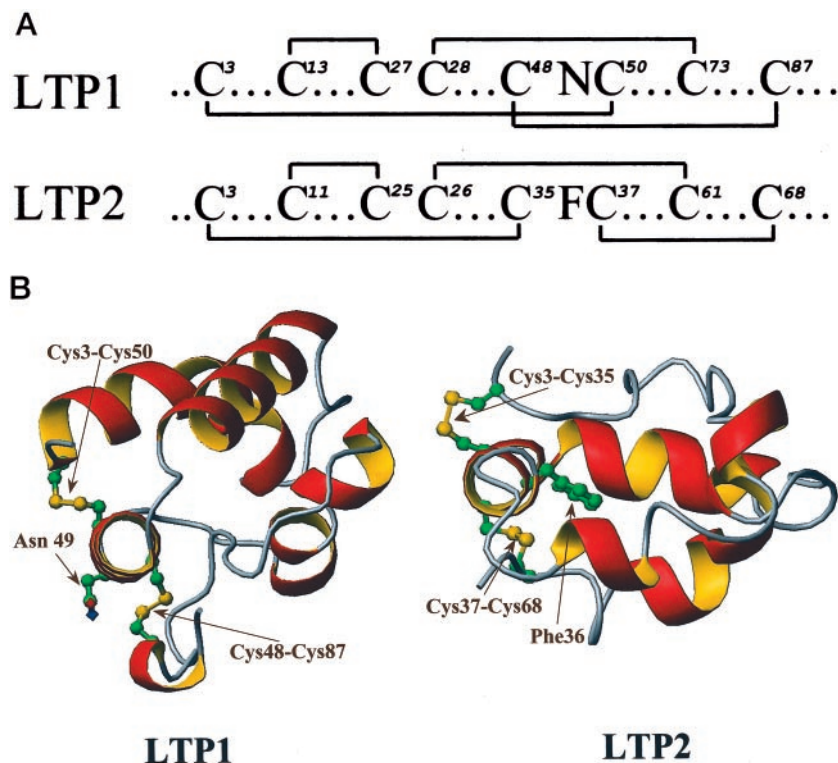


FIG. 5. A, schematic representations of the cysteine pairing patterns of nsLTP1 and nsLTP2 show high similarity except for the -CXC-motif. B, the side-chain orientations of nsLTP1 and nsLTP2 at the -CXC- motifs are shown with the ball-and-stick model. The hydrophilic Asn⁴⁹ present in nsLTP1 is projected to the periphery of the protein, whereas the hydrophobic Phe³⁶ of nsLTP2 is buried inside the molecule.

generated for 30 slowly exchanging backbone amides observed in a TOCSY spectrum measured immediately after dissolving the lyophilized sample in 99% D₂O, and 19 dihedral angle constraints were obtained from a TOCSY spectrum measured according to the procedure of Wang *et al.* (13). A total of 862 constraints were used for the structure calculations. Intensities of all sequential NOEs are summarized in Fig. 2. Because no crystal or theoretical structures are available for nsLTP2, ambiguous NOE constraints were added in an iterative manner over the course of the refinement. The final ensemble of 15 structures was chosen from 50 randomly simulated annealing structures (Table I). The set of final structures contains no vi-

lations greater than 0.5 Å for the NOE interproton distances or 5° for the dihedral angle constraints. The unminimized average structure was compared with the accepted structures to calculate atomic r.m.s.d. Although the disulfide bond constraints were available from biophysical studies, they were not incorporated into the structure calculations, to avoid biasing the process of refinement against experimental NMR data. Interestingly, in all 50 of the calculated structures, the pairs of cysteine residues that were near enough to form disulfide bonds were identical. We concluded that the disulfide bond pattern of the rice nsLTP2 is Cys³-Cys³⁵, Cys¹¹-Cys²⁵, Cys³⁷-Cys⁶¹, and Cys²⁶-Cys⁶⁸, which agrees with our enzymatic digestion results (9).

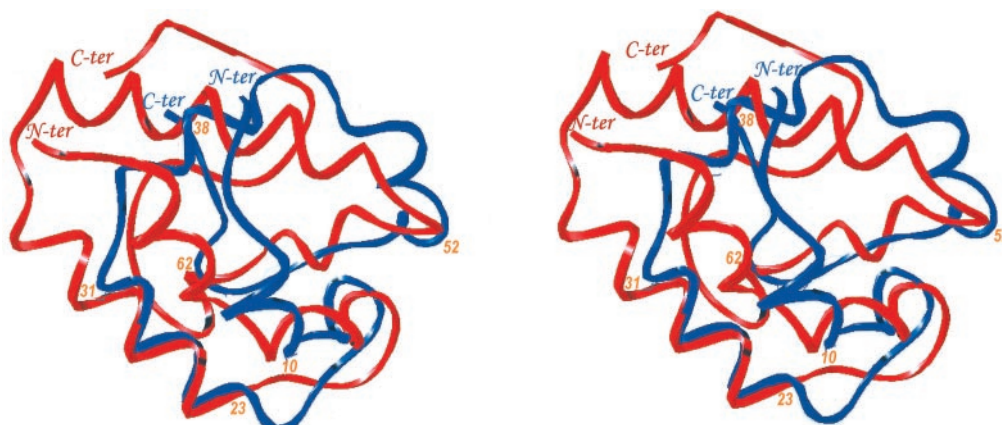
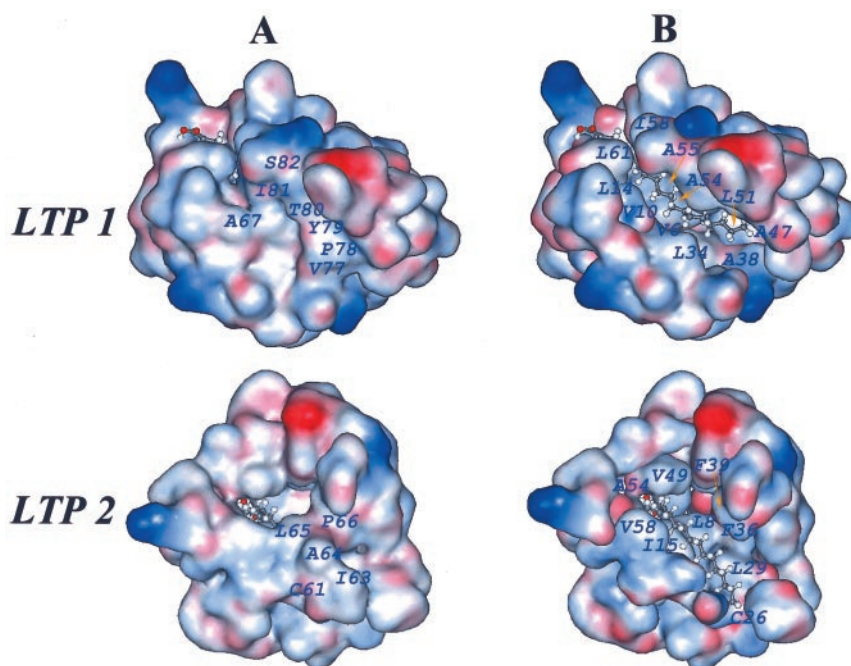


FIG. 6. Stereoview of the superimposed three-dimensional structures of rice nsLTP1 (red ribbon) and rice nsLTP2 (blue ribbon). Various positions of nsLTP2 are labeled according to their primary sequences. The N-terminal backbones of the residues of nsLTP2, Val¹⁰–Ala²² (1.10 Å) and Ala²³–Gln³¹ (0.52 Å), are closely aligned with their counterparts in nsLTP1. The residues Gln³⁸–Gly⁶² of nsLTP2 are aligned (~ 4.62 Å) with helices III and IV of nsLTP1. (The backbone r.m.s.d. values are within parentheses.)

FIG. 7. Web-Lab Viewer representation of the docking of C₁₈-stearic acid to the hydrophobic cavities of nsLTP1 and nsLTP2. The residues covering the hydrophobic cavities (labeled in A) are removed in B to allow the fatty acid orientation to be visualized clearly. Various residues constituting the hydrophobic cavities are labeled. The fatty acid is positioned compactly in the tunnel-like hydrophobic cavity of nsLTP1, and in the case of nsLTP2 the flexible triangular hollow box accommodates the fatty acid.



Structure of Rice nsLTP2—The rice nsLTP2 is a predominantly α -helical protein consisting of three prominent helices within the N-terminal 40 amino acids. The well conserved cysteine residues form four disulfide bonds to stabilize the three-dimensional fold of the protein. The C-terminal amino acid residues, Lys⁴¹–His⁶⁹, constitute a less structured region of the molecule with a high density of positively charged residues. The r.m.s.d. values for the backbone and all heavy atoms were 1.09 ± 0.20 and 1.54 ± 0.25 Å, respectively. The first 40 amino acids (Ala¹–Ala⁴⁰), constituting the rigid portion of the molecule, have r.m.s.d. values of 0.65 ± 0.1 Å for the backbone and 0.95 ± 0.15 Å for all heavy atoms. Superposition of the 15 NMR structures are shown as a stereo representation in Fig. 3A. Three helices of rice nsLTP2 positioned at Cys³–Ala¹⁶, Thr²²–Ala³¹, and Gln³³–Ala⁴⁰ are colored green, red, and purple, respectively. Helices II and III are connected by a 90° turn to form a very rigid and unique structural motif. The curved helix I accommodates two disulfide bonds (Cys³–Cys³⁵ and Cys¹¹–Cys²⁵). The flexible portion of the polypeptide contains two single-turn helices at positions Tyr⁴⁵–Tyr⁴⁸ and Ala⁵⁴–

Val⁵⁸. A series of hydrophobic residues distributed throughout the nsLTP2 sequence combine to form a hydrophobic cavity. A continuous stretch of hydrophobic residues, Cys⁶¹–Ile⁶⁵, near the C terminus forms a flexible cap over the hydrophobic cavity. The C-terminal region also contains two cysteines bridged to the rigid portion of the molecule (Cys²⁶–Cys⁶¹ and Cys³⁷–Cys⁶⁸). These two disulfide bonds help to maintain the correct orientation of the hydrophobic cap. The final energy-minimized average structure of rice nsLTP2 is shown in Fig. 3B. A ProCheck analysis of the three-dimensional structure revealed that only Ser⁵⁹ and Ser⁶⁰ are in the disallowed region, corresponding to 3.6% of the residues in the protein (19). These residues constitute a portion in the flexible C terminus that makes a very sharp turn to cover the hydrophobic cavity.

Comparison of nsLTP2 with nsLTP1—The biophysical properties of the two subfamilies of nsLTP are very different. A higher concentration of GdnHCl is required to denature nsLTP2 ($C_m \sim 4.2$ M) than nsLTP1 ($C_m \sim 3.0$ M). nsLTP1 has unusual thermal stability ($T_m \sim 95$ °C), but nsLTP2 could not be thermally denatured even at temperatures approaching

100 °C (data not shown). A primary sequence analysis using CLUSTAL W revealed a close relationship between these two subfamilies (20). The locations of cysteines, hydrophobic amino acids, and important positively charged residues are well conserved. There are, however, notable differences. In the -CXC- motif, an asparagine between the two cysteines in nsLTP1 is replaced by a hydrophobic amino acid, phenylalanine, in nsLTP2 (Fig. 4). The disulfide bond pattern in nsLTP2 differs from nsLTP1 at the -CXC- motif (Fig. 5A). The hydrophobic residue in the -CXC- motif of nsLTP2 is buried inside the molecule, whereas the hydrophilic residue of nsLTP1 is at the surface (Fig. 5B). These observations suggest that the central residue of the -CXC- motif may govern the cysteine pairing and influence the overall fold of the protein.

The backbone folds of nsLTP1 and nsLTP2 show structural similarities. The superimposed three-dimensional structures of nsLTP1 (red) and nsLTP2 (green) are represented with a ribbon diagram in Fig. 6. Portions of helix I and helix II of nsLTP2 are closely aligned with nsLTP1. Helices II and III of nsLTP1 are connected through a loop to form a gradual bend. In nsLTP2, helix III joins helix II with a 90° angle (the region labeled 31

and 38 in the Fig. 6). The backbone r.m.s.d. values for the Val¹⁰–Ala²² and the perfectly aligned helix II (Ala²³–Gln³¹) are 1.10 and 0.52 Å, respectively. The residues Gln³⁸–Gly⁶², constituting the flexible region of nsLTP2, are aligned with helix III and helix IV of nsLTP1 but are quite divergent (r.m.s.d. ~ 4.62 Å). The C-terminal residues of nsLTP1 and nsLTP2 are not aligned as they form a supple cap over their hydrophobic cavities. The interior hydrophobic cavity of nsLTP2 is significantly different from nsLTP1 and important for its biological functions. The hydrophobic cavity of nsLTP1 is a tunnel (19), whereas the hydrophobic cavity of nsLTP2 is a triangular hollow box (Fig. 7). The three faces of the hydrophobic cavity are designated as face I, formed by Ala¹⁴, Val⁴⁹, and Ala⁵⁴, face II, formed by Cys²⁵, Cys²⁶, Leu²⁹, and Cys¹¹, and face III, formed by Phe³⁶ and Phe³⁹. All three faces are covered at the bottom by Leu⁸, Ala¹⁴, and Ile¹⁵ and at the top by Val⁵⁸, Cys⁶¹, Gly⁶², Ile⁶³, Ala⁶⁴, and Leu⁶⁵ residues. In nsLTP1, helices II and IV do not contribute to the hydrophobic cavity directly except for the terminal Leu³⁴, Ala³⁸, and Ile⁶⁹, whereas in the case of nsLTP2 all of the helices, including single helical turns at positions Tyr⁴⁵–Tyr⁴⁸ and Ala⁵⁴–Val⁵⁸, contribute to the hydrophobic cavity.

Lipid Transferring Activity of nsLTP2—*In vitro* studies have shown that nsLTP1 could transfer and/or exchange various phospholipid and glycolipid molecules across membranes (2). Lipid transfer activity of nsLTP2 was tested *in vitro* with fluorescence spectroscopy (Fig. 8). A solution consisting of two populations of homogeneous vesicles, one population containing fluorescent lipid molecules (pyrPtdCho) and the other population containing nonfluorescent lipid molecules (Myr₂PtdGro) fluoresced very little. Because of the interactions between the pyrene moieties, fluorescence intensity was quenched (shown by an arrow in Fig. 8) (16). The fluorescence intensity increased after the addition of protein (nsLTP1 or nsLTP2, 0.8 μM) at 200 s. The protein molecules catalyzed the transfer of lipid molecules between vesicles. The fluorescence intensities plateaued when the lipid molecules containing the pyrene moiety were evenly distributed between the vesicles (16). The lipid transfer efficiency is monitored by the time required for the fluorescence intensity to plateau. The lipid transfer profiles of nsLTPs shown in Fig. 8 imply that the transfer efficiencies of both proteins are comparable. However, the size of the hydrophobic cavity in nsLTP1 and nsLTP2 are different. nsLTP2 efficiently transferred lipid molecules, despite its smaller hydrophobic cavity. A similar trend was also observed for wheat nsLTPs (10). The van der Waals volume and probe-occupied volume of the hydrophobic cavities measured by VOIDOO were 569.2 and 140 Å³ for nsLTP1 and 200 and 38 Å³

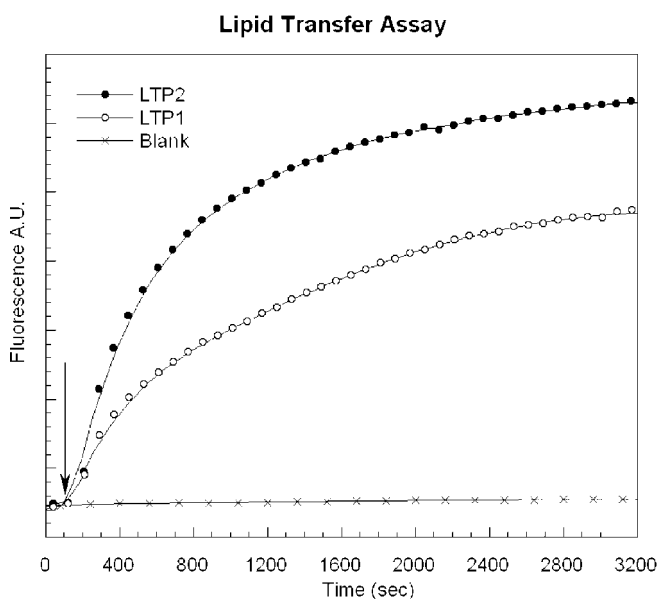
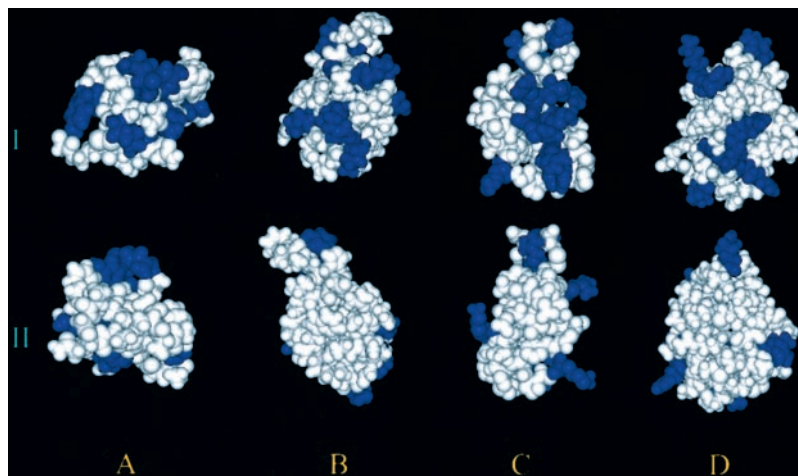


FIG. 8. Lipid transfer assay of nsLTP1 and nsLTP2. The proteins (0.8 μM) were injected at 200 s into a solution containing two vesicle populations (shown with an arrow). The fluorescence intensity increased as the protein catalyzed the shuffling of lipid molecules among the vesicles. The fluorescence intensity plateaued when the fluorescent lipid molecules were evenly distributed between the vesicles.

FIG. 9. Comparison of basic residue distribution of nsLTP2 with several known plant defense peptides. A, antifungal protein from poke weed (PDB code 1dkc); B, antifungal protein from radish (PDB code 1ayj); C, thionin from wheat (PDB code 1gps); D, nsLTP2. Panels I and II differ by a 180° rotation of the viewing angle.



for nsLTP2, respectively. Molecular modeling studies suggested that the hydrophobic cavity of nsLTP2 is more flexible than nsLTP1 (Fig. 7). When binding a molecule of stearic acid, cavity sizes of nsLTP1 and nsLTP2 increased, respectively, to 882.45 and 824.6 Å³ for the van der Waals volume and 419.9 and 1580.5 Å³ for the probe-occupied volume. The fatty acid molecule was oriented more compactly into the hydrophobic cavity of nsLTP1, but the triangular hollow box of nsLTP2 distorted to accommodate the fatty acid molecule. A molecular model of the nsLTP-ergosterol complex was analyzed to evaluate the flexibility of the hydrophobic cavity. The hydrophobic cavity of nsLTP1 was not flexible enough to accommodate the sterol molecule (21). However, the ergosterol molecule fit into the hydrophobic cavity of nsLTP2 without major changes in the secondary or tertiary protein structure. The cavity volume of nsLTP2 increased to 1099 and 1389 Å³ in van der Waals volume and probe-accessible volume, respectively. The plasticity of the nsLTP2 cavity might facilitate more rapid binding or release of the lipid molecule. The smaller molecular size may also increase the lipid transfer efficiency of the nsLTP2.

Probable Role of nsLTP2 as Plant Defensin—Plant defense peptides bind to membrane surfaces of microorganisms through a positively charged face and inhibit bacterial growth by blocking a key process or by lysing the cells (22, 23). Plants attacked by pathogens express large amount nsLTPs (24). NsLTP1 from maize, spinach, arabidopsis, radish, onion, and broccoli exhibit antimicrobial activity (25, 26). NsLTPs are complemented by thionin, a plant defense peptide, to impart antimicrobial action. NsLTPs are more active against bacterial pathogens, whereas thionins act on fungal pathogens (25). Although nsLTPs play an important role in plant response to pathogens, the mechanism of action is not known. The three-dimensional structure of nsLTP2 shows a high concentration of positively charged residues on the flexible face of the molecule. NsLTP1 also has a large number of positively charged residues, but these are evenly distributed over the entire molecular surface (27). Many plant defense peptides also have a large concentration of positively charged residues on one surface of the molecule. Examination of the charge distributions of structures of the antifungal protein from pokeweed (PDB code 1dkc), antifungal protein from radish (PDB code 1ayj), thionin from wheat (PDB code 1gps), and rice nsLTP2 (PDB code 1l6h) shows a concentration of basic residues on one molecular face (Fig. 9). The asymmetric distribution of positive charges may be necessary for effective antimicrobial activity in defense proteins. NsLTP2 may serve two functions for the plant. Initially the protein rapidly transports lipids to the plasma membrane, which may then remain at the cell surface to defend against pathogens.

CONCLUSION

The three-dimensional structure of nsLTP2 has been solved for the first time. Its unique structural features provide new insights into the biological function and significance of nsLTP2. The plasticity of the hydrophobic cavity suggests that the nsLTP2 could bind and transport a variety of molecular shapes and sizes. Although the nsLTP1 protein has been proposed for drug delivery, nsLTP2 may represent a more suitable candidate because of its flexible hydrophobic cavity (28). The identity

of the central residue of the -CXC- motif governs a variable cysteine pairing and may affect the overall fold of the protein. The influence of various amino acid substitutions at this position on the overall fold of the protein is presently under study in our laboratory. Despite the lack of experimental evidence, the distribution of basic residues in the nsLTP2 three-dimensional structure strongly suggests a determinant for antimicrobial activity. Currently investigations are under way to elucidate the relationship of nsLTP structure to the biological functions of nsLTP2.

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REFERENCES

- Poznanski, J., Sodano, P., Suh, S. W., Lee, J. Y., Ptak, M., and Vovelle, F. (1999) *Eur. J. Biochem.* **259**, 692–708
- Kader, J. C. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 627–654
- Dunn, M. A., Hughes, M. A., Zhang, L., Pearce, R. S., Quigley, A. S., and Jack, P. L. (1991) *Mol. Gen. Genet.* **229**, 389–394
- Molina, A., Segura, A., and Garcia-Olmedo, F. (1993) *FEBS Lett.* **316**, 119–122
- Trevino, M. B., and MA, O. C. (1998) *Plant Physiol.* **116**, 1461–1468
- Douliez, J. P., Michon, T., Elmorjani, K., and Marion, D. (2000) *J. Cereal Sci.* **32**, 1–20
- Jegou, S., Douliez, J. P., Molle, D., Boivin, P., and Marion, D. (2000) *J. Agric. Food Chem.* **48**, 5023–5029
- Sodano, P., Caille, A., Sy, D., de Person, G., Marion, D., and Ptak, M. (1997) *FEBS Lett.* **416**, 130–134
- Liu, Y.-J., Samuel, D., Lin, C.-H., and Lyu, P.-C. (2002) *Biochem. Biophys. Res. Commun.* **294**, 535–540
- Douliez, J. P., Pato, C., Rabesona, H., Molle, D., and Marion, D. (2001) *Eur. J. Biochem.* **268**, 1400–1403
- Yu, Y. G., Chung, C. H., Fowler, A., and Suh, S. W. (1988) *Arch. Biochem. Biophys.* **265**, 466–475
- Goddard, T. D., and Kneller, D. G. (1999) *SPARKY*, 3rd Ed., University of California, San Francisco
- Wang, Y., Nip, A. M., and Wishart, D. S. (1997) *J. Biomol. NMR* **10**, 373–382
- Wuthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, pp. 176–199, Wiley-Interscience, New York
- Brunker, A. T. (1992) *X-PLOR Software Manual*, Version 3.1, Yale University, New Haven, CT
- van Paridon, P. A., Gadella, T. W., Jr., Somerharju, P. J., and Wirtz, K. W. (1988) *Biochemistry* **27**, 6208–6214
- Katchalski-Katzir, E., Shariv, I., Eisenstein, M., Friesem, A. A., Aflalo, C., and Vakser, I. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89**, 2195–2199
- Kleywegt, G. J., and Jones, T. A. (1999) *Acta Crystallogr. Sect. D Biol. Crystallogr.* **55**, 941–944
- Morris, A. L., MacArthur, M. W., Hutchinson, E. G., and Thornton, J. M. (1992) *Proteins* **12**, 345–364
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) *Nucleic Acids Res.* **22**, 4673–4680
- Buhot, N., Douliez, J. P., Jacquemard, A., Marion, D., Tran, V., Maume, B. F., Milat, M. L., Ponchet, M., Mikes, V., Kader, J. C., and Blein, J. P. (2001) *FEBS Lett.* **509**, 27–30
- De Samblanx, G. W., Goderis, I. J., Thevissen, K., Raemaekers, R., Fant, F., Borremans, F., Acland, D. P., Osborn, R. W., Patel, S., and Broekaert, W. F. (1997) *J. Biol. Chem.* **272**, 1171–1179
- McManus, A. M., Nielsen, K. J., Marcus, J. P., Harrison, S. J., Green, J. L., Manns, J. M., and Craik, D. J. (1999) *J. Mol. Biol.* **293**, 629–638
- Broekaert, W. F., Cammue, B. P. A., De Bolle, M. F. C., Thevissen, K., De Samblanx, G. W., and Osborn, R. W. (1997) *Crit. Rev. Plant Sci.* **16**, 297–323
- Garcia-Olmedo, F., Molina, A., Segura, A., and Moreno, M. (1995) *Trends Microbiol.* **3**, 72–74
- Cammue, B. P., Thevissen, K., Hendriks, M., Eggermont, K., Goderis, I. J., Proost, P., Van Damme, J., Osborn, R. W., Guerbet, F., Kader, J. C., and Broekaert, W. F. (1995) *Plant. Physiol.* **109**, 445–455
- Han, G. W., Lee, J. Y., Song, H. K., Chang, C., Min, K., Moon, J., Shin, D. H., Kopka, M. L., Sawaya, M. R., Yuan, H. S., Kim, T. D., Choe, J., Lim, D., Moon, H. J., and Suh, S. W. (2001) *J. Mol. Biol.* **308**, 263–278
- Pato, C., Le Borgne, M., Le Baut, G., Le Pape, P., Marion, D., and Douliez, J. P. (2001) *Biochem. Pharmacol.* **62**, 555–560