The Structural Basis of the Thermostability of SP1, a Novel Plant (Populus tremula) Boiling Stable Protein*

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We previously reported on a new boiling stable protein isolated from aspen plants (Populus tremula), which we named SP1. SP1 is a stress-related protein with no significant sequence homology to other stress-related proteins. It is a 108-amino-acid hydrophilic polypeptide with a molecular mass of 12.4 kDa (Wang, W. X., Pelah, D., Alergand, T., Shoseyov, O., and Altman, A. (2002) Plant Physiol. 130, 865–875) and is found in an oligomeric form. Preliminary electron microscopy studies and matrix-assisted laser desorption ionization time-of-flight mass spectrometry experiments showed that SP1 is a dodecamer composed of two stacking hexamers. We performed a SDS-PAGE analysis, a differential scanning calorimetric study, and crystal structure determination to further characterize SP1. SDS-PAGE indicated a spontaneous assembly of SP1 to one stable oligomeric form, a dodecamer. Differential scanning calorimetric showed that SP1 has high thermostability i.e. \( T_m \) of 107 °C (at pH 7.8). The crystal structure of SP1 was initially determined to 2.4 Å resolution by multi-wavelength anomalous dispersion method from a crystal belonging to the space group I222. The phases were extended to 1.8 Å resolution using data from a different crystal form (P21). The final refined molecule includes 106 of the 108 residues and 132 water molecules (on average for each chain). The R-free is 20.1%. The crystal structure indicated that the SP1 molecule has a ferredoxin-like fold. Strong interactions between each two molecules create a stable dimer. Six dimers associate to form a ring-like-shaped dodecamer strongly resembling the particle visualized in the electron microscopy studies. No structural similarity was found between the crystal structure of SP1 and the crystal structure of other stress-related proteins such as small heat shock proteins, whose structure has been already determined. This structural study further supports our previous report that SP1 may represent a new family of stress-related proteins with high thermostability and oligomerization.

Stable protein 1 (SP1), which we previously isolated from aspen plants (Populus tremula) (2–4), is a boiling stable, stress-responsive protein with no significant sequence homology to other stress-related proteins (5–7). SP1 cDNA encodes a 12.4-kDa hydrophilic polypeptide having no cysteine or potential glycosylation sites (1). Amino acid analysis and the N-terminal sequence of SP1 revealed that SP1 is a homooligomeric protein composed of 12 subunits that are tightly bound to each other even under extreme conditions. Similar to native SP1, recombinant SP1 produced in both Escherichia coli and Pichia pastoris assembles spontaneously to a multimeric stable complex (8).

Environmental stresses, such as drought, salinity, or heat, induce specific genes in higher plants. Plants inherently possess various molecular-biochemical mechanisms by which they can cope with such stresses (6, 9, 10). One of the mechanisms that may confer such tolerance is the activation of a large set of genes that lead to the accumulation of specific proteins in the cells. Several families of proteins have been identified as stress-induced proteins (8). The small heat shock protein (Hsps) family is the major type of stress-induced proteins and is believed to exert cellular protection during stress (5, 11). Hsps are molecular chaperones that are characterized by their ability to selectively recognize and bind to misfolded proteins. They possess high thermostability and create oligomers spontaneously.

Attempts to identify the factors responsible for the high thermostability of proteins have included sequence and structural comparisons of homologous proteins from mesophile, thermophile, and hyperthermophile organisms (12–16). These comparisons have shown that proteins isolated from thermophile organisms have a better internal packing of non-polar groups, more hydrogen bonds, and more ion pairs than homologous proteins isolated from mesophiles.

Here we performed a thermostability study of SP1 using the differential scanning calorimetry (DSC) method, and we also determined the crystal structure of SP1 at 1.8 Å resolution. The DSC study showed a \( T_m \) of 107 °C for SP1, which supports our

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The abbreviations used are: SP1, stable protein 1; DSC, differential scanning calorimetry; EM, electron microscopy; sHsp, small Hsp.
| TABLE I | Data processing and merging statistics |
|-----------------|---------------------------------------|
| **MAD data set from Se-Met 1422 crystal** |
| Lambda 0.979 Å | Resolution (Å) | 40.83 - 2.4 |
| Number of reflections | 93175 |
| Number of unique reflections | 15619 |
| R-sym | R-anom | \( \frac{I}{\sigma(I)} \) | Completeness (%) | Multiplicity | Anomalous completeness (%) |
| Overall | 0.095 | 0.067 | 6.5 | 100 | 6.0 | 99.7 |
| Last resolution bin (2.57-2.4 Å) | 0.521 | 0.289 | 1.4 | 99.9 | 5.1 | 99.3 |
| Lambda 0.905 Å | Resolution (Å) | 40.83 - 2.4 |
| Number of reflections | 87157 |
| Number of unique reflections | 15570 |
| R-sym | R-anom | \( \frac{I}{\sigma(I)} \) | Completeness (%) | Multiplicity | Anomalous completeness (%) |
| Overall | 0.096 | 0.058 | 6.3 | 99.7 | 5.6 | 97.8 |
| Last resolution bin (2.57-2.4 Å) | 0.487 | 0.299 | 1.5 | 99.0 | 4.3 | 92.0 |
| Lambda 0.980 Å | Resolution (Å) | 25.410 - 2.4 |
| Number of reflections | 90275 |
| Number of unique reflections | 15590 |
| R-sym | R-anom | \( \frac{I}{\sigma(I)} \) | Completeness (%) | Multiplicity | Anomalous completeness (%) |
| Overall | 0.092 | 0.056 | 6.9 | 99.8 | 5.8 | 98.8 |
| Last resolution bin (2.57-2.4 Å) | 0.541 | 0.300 | 1.3 | 99.2 | 4.6 | 95.4 |
| **Native data set from 1422 crystal** |
| Lambda 0.954 Å | Resolution (Å) | 40.8 - 2.0 |
| Number of reflections | 125502 |
| Number of unique reflections | 18279 |
| R-sym | \( \frac{I}{\sigma(I)} \) | Completeness (%) | Multiplicity |
| Overall | 0.078 | 6.3 | 99.9 | 6.9 |
| Last resolution bin (2.45-2.27 Å) | 0.340 | 2.4 | 100.0 | 6.9 |
| **Native data set from P2₁ crystal** |
| Lambda 0.954 Å | Resolution (Å) | 48.5 - 1.8 |
| Number of reflections | 1219831 |
| Number of unique reflections | 35953 |
| R-sym | \( \frac{I}{\sigma(I)} \) | Completeness (%) | Multiplicity |
| Overall | 0.087 | 8.6 | 92.5 | 1.9 |
| Last resolution bin (1.9-1.8 Å) | 0.447 | 2.8 | 88.4 | 1.9 |
previous finding that the SP1 is a boiling stable protein. The crystal structure of SP1 was refined to a crystallographic R-factor of 16.4%. The SP1 monomer has a ferredoxin-like fold or an α/β structure of four β-strands and three α-helices. Monomers of SP1 assemble into a dimer via their hydrophobic interface. The hydrophobic inner core and dimer interface probably contribute to the enhanced thermostability of SP1 as well as its oligomerization. Sequence and structural comparisons between SP1 and other stress-related proteins, such as small Hsp (sHsp), suggest that SP1 represents a new class of stress-related proteins.

**EXPERIMENTAL PROCEDURES**

**SDS-PAGE of SP1**

SP1 samples (wild type and recombinant) were purified as reported earlier (1). Wild-type SP1 samples (10 mg/ml) were subjected to SDS-PAGE using sample buffer (SB, containing 2% SDS) with or without boiling treatment (10 min). The low molecular weight band (which corresponds to the monomeric form of SP1) was electro-eluted from the SDS-PAGE and dialyzed overnight against 25 mM Tris, 200 mM glycine, and 0.025% SDS. This low molecular band protein sample was subjected again to SDS-PAGE using SB with or without boiling treatment.

**DSC Study**

Solutions of recombinant SP1 (24 μmol in a sodium phosphate buffer, pH 7.8) were scanned with a Microcal VP-DSC-ER calorimeter (Microcal Inc., Northampton, MA) at a scan rate of 60 degrees/h. The data was analyzed using the 5.0 software supplied by the DSC manufacturer. The data was analyzed using Origin 5.0 software (MicroCal).

**Crystallization**

Crystallization experiments were carried out at room temperature using the hanging drop vapor-diffusion method with VDX-24 tissue culture plates (Hampton HB3–140). Droplets of the protein solution containing 7 mg/ml protein in 50 mM Tris, pH 7.5, 100 mM NaCl, 20 mM CaC2 were mixed with an equal volume of reservoir solution of 20% polyethylene glycol 3000, 0.1 M HEPES, pH 7.5, and 0.2 M NaCl. Crystals appeared within a few days and were grown to their maximal size in 2 weeks. These crystals belong to the space group P21 with unit cell $a = b = 90.50 \, \text{Å}, c = 186.30 \, \text{Å}$. New crystals with different morphology appeared within the same droplet after 3 weeks. These new crystals belong to the P21 space group with unit cell of $a / c = 97.03 \, \text{Å}, b = 94.75 \, \text{Å}, c = 168.03 \, \text{Å}$. Selenium-methionine crystals were also crystallized in the I422 space group with unit cell $a = b = 90.75 \, \text{Å}, c = 186.41 \, \text{Å}$. The structure factor amplitudes were calculated using the CCP4 program package (18). The data were scaled and merged using SCALA and monoclinic crystals.

**X-ray Diffraction Data Collection and Processing**

All the data used for solving and refining the structure were collected on the SSRL wiggle beamline 9-2, using a 2 × 2 area detector systems corporation charge-coupled device detector. Both the native and selenium-methionine crystals were transferred from the mother liquor to the cryoprotectant (13% glycerol and 87% mother liquor). After a few seconds, the crystals were scooped up in a cryoloop and exposed to a cold nitrogen stream (Oxford Instruments), where they were rapidly frozen to $-100 \, \text{K}$. Multiwavelength anomalous diffraction data were collected on selenium-methionine crystals belonging to the space group I422. The data were collected at three wavelengths around the selenium absorption edge (see Table I). Native data was collected from the tetragonal and monoclinic crystals.

All the data (three-wavelength multiwavelength anomalous diffraction and two native data sets) were integrated with the MOSFLM program package (18). The data were scaled and merged using SCALA (19, 20). The structure factor amplitudes were calculated using the TRUNCATE program (19, 21). Data statistics derived from data processing are shown in Table I.

**Structure Solution**

**Tetragonal Structure**—The phases for the selenium-methionine data were calculated with SOLVE-RESOLVE. Six selenium sites were located for three selenium-methionine found in SP1 sequence. Because the Mathews coefficient (22) was very high for two copies of the molecule in the asymmetric unit, it was assumed that there were three molecules and that the N-terminal methionine was disordered. A trimer in the asymmetric unit resulted in a Mathews coefficient of 2.8 for a solvent content of 58% of the unit cell volume. The mean experimental figure of merit was 0.34–2.4 Å. The non-proper non-crystallographic symmetry operations relating the monomers could be estimated from the selenium sites. The CCP4 program DM (23) was used for refining the non-crystallographic symmetry operations and averaging and extending the phases to the native data set resolution. The model was built into the map calculated with these modified phases.

**Monoclinic Structure**—The structure in the space group P21 was solved by molecular replacement using the structure of the I422 crystal form. Attempts to solve the structure using one to three copies of the molecule refined in the I422 space group as a search model failed. Previous EM studies showed that SP1 has a ring-like shape with a central cavity. EM studies also revealed oligomers possessing significant homogeneity in shape and size, which clearly demonstrated a 6-fold symmetry of the SP1 particle along the axis of the ring. It was then noticed that the dodecamer formed by the three molecules in the I422 asymmetric unit and nine additional molecules related to the first three by the I422 symmetry operations $-x, 1-y, z$; $y, -x+0.5, -z+0.5$ and $-x+0.5, -y+0.5, -z+0.5$ had a pseudo 6-fold axis and a shape and size resembling the particles in the EM studies. Using this dodecamer as a search model yielded a clear solution for the P21 structure using the program MOLREP (19, 24). Two dodecamers were found in the P21 asymmetric unit (Matthews coefficient 2.7).

**Model Refinement**—Both the tetragonal and monoclinic models were refined with the program REFMAC (Collaborative Computational Project, 1994) using a maximum likelihood target (24). The geometry was restrained to the standard Engh and Huber values (34), using the maximum likelihood target. The non-crystallographic symmetry and, in the case of the I422 structure, the multiwavelength anomalous diffraction experimental phases, were also used as additional restraints. Restricted refinement was preceded by translation, libration, and screw rotation parameters refinement (25). Hydrogen atoms were generated before refinement in their riding positions and used for geometry and structure factor calculations. Automatic water molecule searching was carried out using automated refinement of protein crystallography (26).

After refinement, the fit of the model to the electron density was

| TABLE II Summary of the refinement for the P2, and I422 models |
|-----------------------------------------------|
| I422 model                                    |
| Refined residues:                           |
| Solvent atoms:                               |
| Glycrol:                                     |
| Ramachandran most favored region:            |
| Ramachandran allowed region:                 |
| Ramachandran generously allowed region:      |
| Ramachandran disallowed region:              |
| R-factor (all reflections, work + free set):  |
| R-factor (highest resolution bin):            |
| Reflections in free set:                     |
| R-factor (free set):                         |
| R-factor (free set in highest resolution bin): |
| Correlation coefficient (all reflections):    |
| Correlation coefficient (free set):          |
| Overall coordinate e.s.u.:                   |
| Overall B e.s.u.:                            |
| P2, model                                    |
| Refined residues:                            |
| Solvent atoms:                               |
| Glycrol:                                     |
| Ramachandran most favored region:            |
| Ramachandran allowed region:                 |
| Ramachandran generously allowed region:      |
| Ramachandran disallowed region:              |
| R-factor (all reflections, work + free set):  |
| R-factor (highest resolution bin):            |
| Reflections in free set:                     |
| R-factor (free set):                         |
| R-factor (free set in highest resolution bin):|
| Correlation coefficient (all reflections):    |
| Correlation coefficient (free set):          |
| Overall coordinate e.s.u.:                   |
| Overall B e.s.u.:                            |

*a Based on maximum likelihood target $R = \sum (|F_a| - |F_c|)/|F_c|$, e.s.u., estimated standard uncertainties.
Results

SDS-PAGE Analysis and DSC Study of SP1

Fig. 1 shows the results of a SDS-PAGE analysis of wild-type SP1 isolated from aspen plants. Fig. 1, lane 1 shows wild-type SP1 without boiling treatment as one high molecular band, which may correspond to the SP1 dodecamer. After boiling for 10 min the SP1 dodecamer dissociates to a lower molecular weight band which may correspond to the monomeric form of SP1 (Fig. 1, lane 2). This monomeric form of SP1 was electroeluted and subjected again to SDS-PAGE. Fig. 1, lane 3 shows that the electro-eluted SP1 spontaneously reassembled to the same low molecular weight band after another boiling treatment in the presence of 2% SDS (lane 4).

Inspected using difference Fourier density maps 2mFo – DFc and mFo – DFc, where Fo and Fc are the scaled observed and calculated structure factors, m is the figure of merit, and D is an estimate of the error in the partial structure from coordinate errors (27). These coefficients were calculated by REFMAC. The results of the refinement and the stereochemical analysis of the model, carried out with PROCHECK (28), are summarized in Table II. The final refined models have been deposited in the PDB with the code 1SI9 for the I422 crystal form and the code 1TR0 for the P21 crystal form.

The Crystal Structure Description of SP1

The Monomer—The maximum average root mean square displacement between the monomers in the asymmetric unit is less than 0.20 Å (0.43 Å for the tetragonal model), which indicates an overall high similarity between all the SP1 molecules, although some side chains in surface loops could be modeled in different conformations in different molecules. The protein chain has an α and β folding with three α-helices, H1 (residues 23–39), H2a (residues 74–81), and H2b (residues 84–93), and a β-sheet formed by four antiparallel β-strands, B3 (residues 9–17), B1 (residues 45–50), B2 (residues 65–71), and B4 (residues 97–108). Secondary structure characterizations were carried out using the PROCHECK software package (28) (Fig. 3).

The Dimer—The dimer appears to be the smallest stable SP1 unit. The two molecules in the dimer are related by a 2-fold axis parallel to helix H1 and β-strands B3 and B4 (Fig. 5). The outer surface of the β-sheets of the two molecules forms a β barrel.
like structure, defining a central pore. At one end of the pore, the loop comprising the residues 51–64 bridges the gap between the two B4 strands in the dimer. The center of the pore is closed to the solvent by a barrier of hydrophobic side chains in B3 and B4 (Leu-14, Leu-101, Ile-103, and Tyr-105) and Tyr-63. The side chains of residues Asn-47, Ser-45, Thr-70, Leu-8, and Leu-107 occupy the surface at the end of the pore closer to the N termini.

Glu-68 is an important residue in creating stable dimers of SP1. Glu-68 side chain carboxylate forming hydrogen bonds to Lys-10 NZ and to the Tyr-105 side chain OH group of the opposite molecule (see Fig. 6). This residue is conserved in other proteins with the same fold as SP1. Water 5 mediates a dimeric contact bonding Phe-106 N and Thr-50 N in neighboring monomers. The complete list of direct dimer contacts is given in Table III.

The Dodecamer—The interdimer contacts predominantly involve hydrophilic side chains and charged groups or are mediated by water molecules. These contacts take place mainly along the B1, H1, and the N-terminal tails. The complete list of direct interdimeric contacts is given in Table IV. As a result of the interdimeric interactions six dimers create a ring-like structure around a pseudo 6-fold axis. The ring-like structure of the dodecamers has an outer radius of ~50 Å and an inner radius of ~15 Å (Fig. 7). The loop including residues 18–22 in each dimer protrudes toward the solvent, whereas the arms of the N-terminal are extending toward the inner part of the ring-like structure (Fig. 7). The 6-fold symmetry is broken, because the contacts between equivalent molecules in neighboring dimers are not identical.

In Fig. 8 the EM density map is superimposed on top of the crystal structure of a dodecamer. The high density regions of the EM map correspond to regions that are mainly α-helices and are related to each other by the same pseudo 6-fold axis. The similarity of the dodecamer to the particles seen in EM studies (Fig. 8) and the fact that this arrangement is observed in different crystal space groups with no intrinsic 6-fold symmetry suggest strongly that the dodecamer is the biological unit active form of SP1.
The Interdodecamer Contacts—In the P21 space group the dodecamers are stacked in perpendicular interleaving layers (see Fig. 9, a and b). The interdodecamer interactions take place between dodecamers in perpendicular planes. There are water molecules that mediated hydrogen bonds between solvent-facing side chains in loop 16–22 of one dodecamer and the external surface of the dimer defined by a β-barrel in a neighboring dodecamer. There are also hydrophobic interactions that involve aliphatic side chains in the external loops. These contacts involve different residues for each SP1 molecule in the dodecamer, which explains the breakdown of the 6-fold symmetry in the crystal forms.

**TABLE III**

| Molecule A | Molecule B | Distance (Å) |
|------------|------------|--------------|
| Lys-10 N-ζ | Glu-68 Oε-1 | 3.0 |
| Lys-10 N-ζ | Glu-68 Oε-2 | 2.9 |
| Trp-48 N   | Tyr-108 O   | 3.1 |
| Trp-48 O   | Tyr-108 N   | 2.8 |
| Leu-52 N   | Asp-104 O   | 3.0 |
| Leu-59 O   | Val-102 N   | 2.9 |
| Arg-61 Nε-1| Arg-100 O   | 2.9 |
| Arg-61 Nε  | Arg-100 O   | 2.8 |
| Glu-68 Oε-1| Lys-10 N-ζ | 3.1 |
| Glu-68 Oε-2| Lys-10 N-ζ | 2.9 |
| Arg-100 O  | Arg-61 Nε-1| 2.8 |
| Arg-100 O  | Arg-61 Nε  | 2.8 |
| Val-102 N  | Leu-59 O    | 2.6 |
| Asp-104 O  | Leu-52 N    | 2.9 |
| Tyr-105 OH | Glu-68 Oε-2| 2.7 |
| Tyr-105 N  | Trp-48 O    | 2.9 |
| Tyr-108 O  | Trp-48 N    | 3.1 |

**TABLE IV**

| Neighboring dimer contact | Distance (Å) |
|---------------------------|--------------|
| Molecule A                |              |
| Thr-5 N                   | Lys-44 O (mol. C) | 2.7 |
| Lys-7 N-ζ                 | Phe-46 O (mol. C) | 3.0 |
| Lys-7 N-ζ                 | Asn-47 Oε-1 (mol. C) | 2.7 |
| Lys-7 N-ζ                 | Tyr-108 O (mol. D) | 3.1 |
| Lys-7 N-ζ                 | Tyr-108 OXT (mol. D) | 2.9 |
| Tyr-108 O                 | Lys-7 N-ζ (mol. D) | 3.3 |
| Tyr-108 O                 | Lys-7 N-ζ (mol. D) | 3.1 |
| Asn-31 Oε-1               | Tyr-108 OH (mol. K) | 2.7 |
| Asn-31 Nε-2               | Thr-50 Oγ-1 (mol. L) | 3.1 |
| Thr-34 Oγ-1               | Tyr-108 OH (mol. K) | 3.1 |
| Leu-37 O                  | Arg-4 Nε-1 (mol. K) | 3.2 |
| Asp-38 Oε-1               | Ser-75 Oγ (mol. K) | 3.0 |
| Asn-47 Oε-1               | Lys-7 N-ζ (mol. K) | 2.9 |
| Thr-50 Oγ-1               | Asn-31 N δ-2 (mol. L) | 3.1 |
| Molecule B                |              |
| Lys-18 O                  | Arg-23 N ζ-2 (mol. C) | 3.0 |
| Ile-21 O                  | Arg-23 N ζ-1 (mol. C) | 2.8 |
| Asn-31 Nδ-2               | Thr-50 Oγ-1 (mol. C) | 3.1 |
| Asp-38 O δ-1              | Ser-75 Oγ (mol. D) | 2.9 |
| Lys-44 O                  | Thr-5 N (mol. D) | 2.7 |
| Phe-46 O                  | Lys-7 N-ζ (mol. D) | 2.9 |
| Asn-47 Oε-1               | Lys-7 N-ζ (mol. D) | 3.0 |
| Thr-50 Oγ-1               | Asn-31 Nδ-2 (mol. C) | 3.0 |
| Thr-3 Oγ-1                | Lys-44 N-ζ (mol. L) | 2.6 |
| Thr-5 N                   | Lys-44 O (mol. L) | 2.9 |
| Thr-5 Oγ-1                | Lys-44 O (mol. L) | 3.3 |
| Lys-7 N-ζ                 | Lys-7 N-ζ (mol. L) | 3.3 |
| Lys-7 N-ζ                 | Lys-7 N-ζ (mol. K) | 3.1 |
| Tyr-108 O                 | Lys-7 N-ζ (mol. K) | 3.2 |
| Tyr-108 O                 | Lys-7 N-ζ (mol. K) | 3.1 |
| Tyr-108 OXT               | Lys-7 N-ζ (mol. K) | 2.9 |

**FIG. 7.** View of the dodecamer along the pseudo 6-fold axis.

**FIG. 8.** Superposition of the crystal structure of a dodecamer and the electron density map obtained from EM studies.

**DISCUSSION**

The crystal structure of SP1 indicated strong hydrophobic interactions between two monomers that created a very stable dimer. With the exception of the N-terminal residues, the main chain of the protein was well defined into the electron density map, and no substantial differences, other than a better accuracy of the P21 model arising from the higher resolution of the diffraction and higher non-crystallographic symmetry, could be found between the molecules in the two different space groups.

Our results showed that SP1 belongs to the α+β protein class with ferredoxin-like fold composed of an α+β-sandwich with an antiparallel β-sheet. Two molecules of SP1 form a dimer. This dimer belongs to the superfamily of dimeric α+β-barrels in which the two β-sheets form a β-barrel. SP1 like other proteins in this family may assemble into higher order
oligomers. Proteins with the above mentioned dimeric \(\alpha+\beta\)-barrel folding include monoxygenase isolated from Streptomyces coelicolor (PDB entry 1LQ9) (ActVA-Orf6 (29)), TT1380 isolated from Thermus thermophilus (PDB entry 1IUJ), and Arabidopsis thaliana stress-related protein (PDB entry 1Q4R). In all of these cases, the B4 strand in one molecule creates hydrophobic interactions with the \(\beta-2\) strand of the neighbor monomer, which results in a stable dimer. It is reasonable to assume that strong dimer interactions contribute to protein stability under extreme conditions. However, monoxygenase isolated from S. coelicolor was not reported to have high thermostability and was found to be active in its dimeric form.

The structural similarity of SP1 to TT1380 from T. thermophilus together with the apparent high \(T_m\) of SP1 (107 °C) implies that these proteins may use a ferredoxin-like fold as a scaffold for hyperthermostability and that they are evolutionarily related. In addition to the thermostability of SP1 gained by creating dimers, it is possible that the assembly of dimers to dodecamers may also contribute to the high thermostability of SP1. Although the relationship between the degree of oligomerization and \(T_m\) is not yet clear (30), it is still possible that oligomerization contributes to the stability of SP1. Thermostable dodecamers as found for SP1 are rather rare for proteins with a ferredoxin-like fold. Most thermostable oligomers of this family of proteins are found in the range of monomers to tetramers (30, 31). Sequence comparisons of SP1 (Fig. 10) with proteins of ferredoxin-like fold such as A. thaliana At3g17210 stress-related protein and ActVA-Orf6 monoxygenase indicated a conserved region between SP1 and At3g17210 at residues 62–74 (GYTHXFESTFESK). This region corresponds to the region of the \(\beta-2\) strand, which borders the helices 2 and 3 deep cavity, and it may have an important role in the function of these two proteins by serving as the active site in a way similar to that found for ActVA-Orf6 monoxygenase (17). It should be noted that the sequence similarity in this region found between monoxygenase and the above mentioned proteins is low. However this loop is the interdimer contact region as found for SP1, and it may also play a role in preserving the stability of SP1 (see Glu-68 role in dimer contacts).

Because SP1 may have some functional similarity to the small heat shock proteins family (sHsp) (i.e. it is stress-related and has high thermostability), we compared its structure to the only two sHsp crystal structures analyzed thus far, sHsp16.9 isolated from wheat (32) and sHsp16.5 isolated from Methanococcus jannaschii (33). These two sHsps have high amino acids sequence similarity between them and belong to the \(\alpha\)-crystallin family. High structural similarity was found between the monomers of these two sHsps, and both monomers have a similar \(\beta\)-sandwich fold. In addition, both sHsps assemble to an oligomeric form. Hsp16.9 was found in an oligomeric form of a dodecamer composed of two stacking hexamers in which each hexamer is composed of three dimers, and the Hsp16.5 oligomer was found to be a hollow spherical complex composed of 24 units.

Nevertheless no amino acid sequence similarity exists between SP1 and these two sHsps. Radical differences also were found in the crystal structure of the SP1 and the two sHsps, indicating that SP1 does not belong to the conserved sHsps family. This may suggest that SP1 may be representing a different family of proteins with unique thermostability and a different function in stress protection.

Based on the present structural study of SP1 we can conclude that SP1 thermostability is a result of several factors. 1) SP1 has a ferredoxin-like fold, which was found in other studies to be a fold of several other thermostable proteins; 2) monomers of SP1 assemble into a stable dimer via a double hydrogen bond of residue Glu-68; 3) hydrophobic residues create interactions found in each inner core of the monomers and at the dimer interface; and 4) dimers form high oligomeric forms of SP1, such as a dodecamer, via additional interactions between the dimers.
REFERENCES

1. Wang, W. X., Pelah, D., Alergand, T., Shoseyov, O., and Altman, A. (2002) *Plant Physiol.* **130**, 865–875
2. Pelah, D., Shoseyov, O., and Altman, A. (1995) *Tree Physiol.* **15**, 673–678
3. Pelah, D., Shoseyov, O., Altman, A., and Bartels, D. (1997) *J. Plant Physiol.* **151**, 96–100
4. Pelah, D., Wang, W. X., Altman, A., Shoseyov, O., and Bartels, D. (1997) *Physiologia Plantarum* **99**, 153–159
5. Dure, L., III (1993) *Plant J.* **3**, 363–369
6. Ingram, J., and Bartels, D. (1996) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47**, 377–403
7. Thomashow, M. F. (1999) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50**, 571–599
8. Wang, W., Vinocur, B., Shoseyov O., and Altman, A. (2004) *Trends Plant Sci.* **9**, 244–252
9. Hoekstra, F. A., Golovina, E. A., and Buitink, J. (2001) *Trends Plant Sci.* **6**, 431–438
10. Chen, Q., Osteryoung, K., and Vierling, E. (1994) *J. Biol. Chem.* **269**, 13216–13223
11. Almog, O., Gallagher, D. T., Ladner, J. E., Strausberg, S., Alexander, P., Bryan, P., and Gilliland, G. L. (2002) *J. Biol. Chem.* **277**, 27553–27558
12. Almog, O., Gonzalez, A., Klein, D., Greenblatt, H. M., Braun, S., and Shoham, G. (2003) *J. Mol. Biol.* **332**, 1071–1082
13. Kim, K. K., Kim, R., and Kim, S. H. (1998) *Nature* **394**, 595–599
14. Cowtan, K. (1994) Joint CCP4 and ESF-EACBM Newsletter on Protein Crystallography **31**, 34–38
15. Winn, M. D., Isupov, M. N., and Murshudov, G. N. (2001) *Acta Crystalogr.* **57**, 122–133
16. Lamzin, V. S., and Wilson, K. S. (1997) *Methods Enzymol.* **277**, 269–305
17. Luzzati, P. V. (1953) *Acta Crystallogr.* **6**, 142–152
18. Matthews, B. W. (1968) *J. Mol. Biol.* **33**, 491–497
19. French, G. S., and Wilson, K. S. (1978) *J. Mol. Biol.* **122**, 100–106
20. Laskowski, R. A., Macarthur, M. W., Moss, D. S., and Thornton, J. M. (1993) *J. Appl. Crystallogr.* **26**, 283–291
21. Kumar, S., Tsai, C. J., and Nussinov, R. (2000) *Protein Eng.* **13**, 179–191
22. Clantin, B., Tricot, C., Loison, T., Stalon, V., and Villeret, V. (2001) *Eur. J. Biochem.* **268**, 3937–3942
23. van Montfort, R. L., Basha, E., Friedrich, K. L., Slingsby, C., and Vierling, E. (2001) *Nat. Struct. Biol.* **8**, 1025–1030
24. Leslie, A. G. W. (1991) in *Crystallographic Computing V* (Moras, D., Podjarny, A. D., and Thierry, J. C., eds) pp. 27–38, Oxford University Press, Oxford
25. Collaborative Computational Project 4 (1994) *Acta Crystalogr.* **50**, 760–763
26. Evans, P. R. (1999) *Acta Crystalogr.* **55**, 1771–1772
27. Pan, J., and Wilson, K. S. (1996) *Acta Crystalogr.* **52**, 100–106
28. Kim, K. K., Kim, R., and Kim, S. H. (1998) *Nature* **394**, 595–599
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