STRUCTURAL BIOLOGY
COMMUNICATIONS

ISSN 2053-230X

Functional characterization of heat-shock protein 90 from *Oryza sativa* and crystal structure of its N-terminal domain

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Received 20 February 2015

Accepted 2 April 2015

Edited by S. W. Suh, Seoul National University, Korea

Keywords: Hsp90; *Oryza sativa*; 17-AAG; ATPase activity; cross-seeding.**PDB reference:** heat-shock protein 90, 4x9I**Supporting information:** this article has supporting information at journals.iucr.org/f

Heat-shock protein 90 (Hsp90) is an ATP-dependent molecular chaperone that is essential for the normal functioning of eukaryotic cells. It plays crucial roles in cell signalling, cell-cycle control and in maintaining proteome integrity and protein homeostasis. In plants, Hsp90s are required for normal plant growth and development. Hsp90s are observed to be upregulated in response to various abiotic and biotic stresses and are also involved in immune responses in plants. Although there are several studies elucidating the physiological role of Hsp90s in plants, their molecular mechanism of action is still unclear. In this study, biochemical characterization of an Hsp90 protein from rice (*Oryza sativa*; OsHsp90) has been performed and the crystal structure of its N-terminal domain (OsHsp90-NTD) was determined. The binding of OsHsp90 to its substrate ATP and the inhibitor 17-AAG was studied by fluorescence spectroscopy. The protein also exhibited a weak ATPase activity. The crystal structure of OsHsp90-NTD was solved in complex with the nonhydrolyzable ATP analogue AMPPCP at 3.1 Å resolution. The domain was crystallized by cross-seeding with crystals of the N-terminal domain of Hsp90 from *Dictyostelium discoideum*, which shares 70% sequence identity with OsHsp90-NTD. This is the second reported structure of a domain of Hsp90 from a plant source.

1. Introduction

The heat-shock protein 90 (Hsp90) family of proteins form a highly conserved class of molecular chaperones which are involved in the activation of a wide range of proteins. They belong to the GHKL superfamily (gyrases, Hsp90, histidine kinase and MutL) of ATPases (Dutta & Inouye, 2000). Hsp90s form an essential component of various cellular processes such as signal transduction (by the activation of steroid hormone receptors, protein kinases and transcription factors; Picard *et al.*, 1990; Pratt, 1997; Jackson *et al.*, 2004), protein trafficking (Young *et al.*, 2003) and protein degradation (McClellan *et al.*, 2005), thereby aiding in maintaining protein homeostasis (Taipale *et al.*, 2010). They are ubiquitous, widespread and are found in all kingdoms of life from prokaryotes to eukaryotes with the exception of archaea. Hsp90 functions as a dimer in which each monomer consists of three functional domains: an N-terminal ATP-binding domain (NTD), a middle domain, which interacts with the client proteins and carries the catalytically essential residue required for ATP hydrolysis, and a C-terminal dimerization domain, which holds the dimer together and is also involved in interaction with cochaperones (Pearl & Prodromou, 2006). The roles of Hsp90 in diverse cellular functions and the nature of its interaction with other proteins have been extensively studied in bacteria, yeast, fungi and mammalian systems. In terms of structure, several

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structures of individual domains from various sources and three full-length structures from bacterial (PDB entry 2iop; Shiau *et al.*, 2006), yeast (PDB entry 2cg9; Ali *et al.*, 2006) and mammalian (PDB entry 2o1u; Dollins *et al.*, 2007) sources have been determined in native forms and in complexes with different ligands. We have previously reported the crystal structure of the NTD of Hsp90 from *Dictyosteleum discoideum* (Sawarkar *et al.*, 2008). However, knowledge of Hsp90 in plants is limited, as there are fewer reports on their physiological functions and structural aspects.

Studies of the physiological functions of Hsp90 in plants began much later compared with studies of those from animals and fungi. Hsp90 has been implicated to play a role in plant growth and development and in buffering genetic variation (Kadota & Shirasu, 2012). A number of Hsp90 genes have been identified in many plants, where Hsp90 is induced in response to environmental stress (reviewed in Xu *et al.*, 2012). In rice, a gene for an Hsp90 was observed to be upregulated under carbonate (NaHCO_3) stress, and Hsp90 mRNA accumulated in different tissues such as roots and leaves following exposure to several abiotic stresses, including salts (NaCl , NaHCO_3 and Na_2CO_3), desiccation, high pH and high temperature (Liu *et al.*, 2006). Overexpression of OsHsp90 in yeast led to a greater tolerance to high-salt and bicarbonate stress and in the case of *Escherichia coli* allowed growth at higher temperatures by protecting many bacterial proteins from denaturation (Liu *et al.*, 2009). Hsp90 was observed to form one or more protein complexes in rice cells with effector molecules involved in immune responses, suggesting that it has important roles in innate immunity in rice (Thao *et al.*, 2007). Although these studies provide insights into the role of OsHsp90 in various environmental stress responses and in plant disease resistance, the molecular mechanism of these stress responses is still unclear and there are very few reports on Hsp90 from plant sources from a structural perspective. To our knowledge, the crystal structure of the NTD of Hsp90 from *Hordeum vulgare* (barley) is the only structure available for this protein from a plant source (Zhang *et al.*, 2008, 2010). The sequence identity between the Hsp90 protein from rice (*Oryza sativa*; OsHsp90) and Hsp90 from barley is 95%. Here, we report structural and biochemical studies of OsHsp90, in which we analyzed the binding of Hsp90 to its substrate ATP and the inhibitor 17-(allylamino)-17-demethoxygeldanamycin (17-AAG, a derivative of geldanamycin) and also checked its ATPase activity. We have also solved the crystal structure of the NTD of OsHsp90 (OsHsp90-NTD) in complex with AMPPCP, which is the second crystal structure of NTD of Hsp90 from a plant source, the first being that from barley.

2. Materials and methods

2.1. Cloning of full-length OsHsp90 and OsHsp90-NTD

The OsHsp90 gene was identified from the KOME (Knowledge-based *Oryza* Molecular biological Encyclopedia) database and the full-length cDNA (KOME Accession No. AK243060) was obtained from the Rice Genome Research

Centre, Japan. The OsHsp90 insert of 2.1 kbp in length was cloned into pET-28a(+) vector with an N-terminal histidine tag. PCR amplification of OsHsp90 cDNA was performed using 5'-GCCATATGATGGCGTCGGAGACCGAGACG-3' as the forward primer and 5'-GCGCGGCCGCTTAGTCGACCTCCTCCATCTT-3' as the reverse primer, which contained NdeI and NotI sites (underlined), respectively. Owing to the larger insert size, the extension time and the number of cycles of the PCR was increased to 4 min and 35 cycles, respectively, compared with the conventionally used 1 min and 29 cycles. The amplified product and the vector were digested with restriction enzymes and the gene was subsequently ligated into the vector.

The OsHsp90-NTD construct of insert size 648 bp was cloned in similar manner in pET-28a(+) vector with an N-terminal histidine tag using the forward primer 5'-TGGGGATCCATGGCGTCGGAGACCGAG-3' and the reverse primer 5'-GAGCTCGAGTTACTCAGTTGTCTTCTCAGTCCA-3' containing BamHI and XhoI sites (underlined) in the forward and reverse primers, respectively. The sequences of all of the constructs were confirmed by DNA sequencing.

2.2. Protein expression and purification

Each clone was transformed into *E. coli* BL21 (DE3) cells. The transformed cells were grown at 37°C in Luria–Bertani medium in the presence of the antibiotic kanamycin [a selection marker for pET-28a(+)]. Protein expression was induced with 0.8 mM IPTG when the optical density of the culture at 600 nm reached 0.8. Cells were pelleted after 5 h of induction.

Purification of both the full-length construct and the NTD was performed by Ni–NTA affinity chromatography. The harvested cells were resuspended in lysis buffer consisting of 50 mM Tris, 200 mM NaCl pH 7.5. The cells were lysed by sonication and then centrifuged at 15 000g for 1 h. The supernatant was loaded onto a column containing Ni–NTA agarose beads. Subsequent washes were performed with lysis buffer containing an imidazole concentration gradient from 5 to 25 mM. The protein was finally eluted with buffer containing 150 mM imidazole. The eluted fractions were pooled and dialysed against a buffer consisting of 25 mM Tris, 150 mM NaCl pH 7.5. The protein was further purified by size-exclusion chromatography using a Sephacryl S-200 HR column. The protein was eluted and stored in 25 mM Tris, 150 mM NaCl buffer pH 7.5.

2.3. Fluorescence measurements

Fluorescence measurements were carried out in a Jasco FP6300 spectrofluorometer using 1 μM OsHsp90 in 25 mM Tris, 100 mM NaCl, 5 mM MgCl_2 pH 7.5. The sample was excited at 280 nm and emission was scanned between 300 and 400 nm at 100 nm min⁻¹. The slit widths were adjusted to 5 and 10 nm for excitation and emission, respectively. Fluorescence emission at 340 nm was used for all calculations. The difference in the fluorescence at 340 nm was plotted against different ligand concentrations. For the ATP-binding

Figure 1
Multiple sequence alignment of Hsp90s from different species. The symbols *, : and . indicate full conservation of residues, conservation between residues with strongly similar properties and conservation between residues with weakly similar properties, respectively. The green, blue and yellow underlined regions indicate the NTD, middle and C-terminal domains, respectively. The variable linker region is shown in a red box.

experiment, the concentration of ATP was varied from 0.05 to 5 mM. In order to prevent any contribution owing to the inner filter effect on the addition of nucleotide, control readings were measured with increasing concentrations of ATP in blank buffer, which were subtracted from the corresponding test readings with the protein. The binding of the inhibitor 17-AAG to OsHsp90 was measured by varying the concentration of 17-AAG between 0.25 and 100 μM . The data points were fitted using nonlinear regression analysis with single-site specific binding to obtain the value of the dissociation constant (K_d).

2.4. ATPase assay

An ATPase assay was carried out in a reaction buffer consisting of 40 mM HEPES-KOH, 5 mM MgCl_2 pH 7.5. The final concentration of the protein used was 1.2 μM , and the concentration of cold (nonradioactive) ATP was varied from 100 to 4000 μM in a final reaction volume of 10 μl . ($\gamma^{32}\text{P}$)-ATP with a specific activity of 0.55 Ci mmol^{-1} was used as a tracer. The reaction mixture was incubated at 37°C for 1 h and then

quenched by adding 1 μl 0.5 M EDTA. Thin-layer chromatography (TLC) was carried out on polyethyleneimine-cellulose F using a mobile phase consisting of 0.5 M LiCl, 0.5 mM EDTA, 2 N formic acid. The polyethyleneimine-cellulose sheets were dried and analyzed by phosphorimaging. The spots corresponding to phosphate and ATP were quantified using *ImageQuant* (Fujifilm). To ensure that the observed ATPase activity is not contributed by other contaminants such as kinases or ATPases during the procedure of Hsp90 purification, 150 μM 17-AAG was used to inhibit Hsp90-specific ATPase activity. The actual Hsp90 ATPase activity was calculated by subtracting the residual activity in the presence of 17-AAG from the total ATPase activity.

2.5. Crystallization of OsHsp90-NTD

The concentration of the protein used in crystallization trials ranged from 10 to 15 mg ml^{-1} . Crystallization screens were set up for OsHsp90-NTD using the microbatch-under-oil method with 2 μl protein solution and 2 μl crystallization condition (evaporation through silicone oil and paraffin oil in

Figure 2 Characterization of OsHsp90. (a) SDS-PAGE profile of purified OsHsp90 (calculated molecular mass 82.48 kDa). The left lane contains molecular-mass markers (labelled in kDa). (b, c) Change in the intrinsic fluorescence intensity of OsHsp90 with (b) increasing ATP concentration and (c) increasing 17-AAG concentration. (d) Michaelis-Menten plot representing the fractional cleavage of ($\gamma^{32}\text{P}$)-ATP versus ATP concentration. The inset graph is the representative Lineweaver-Burke plot for OsHsp90.

a 1:1 ratio), as well as the hanging-drop vapour-diffusion method, using screens from Hampton Research, Jena Bioscience and Molecular Dimensions. Several crystallization trials were attempted under native conditions (in the absence of ligand) as well as in the presence of various ligands. The protein was incubated with 5 mM concentrations of various ligands and MgCl_2 overnight and set up for crystallization at 16°C using the hanging-drop method. Crystals appeared in conditions consisting of 0.1 M Tris pH 7.0–8.5, 2 M ammonium sulfate, 10–15% PEG 400 with 5 mM AMPPCP and 5 mM

MgCl_2 by cross-seeding with crystals of the NTD of Hsp90 from *D. discoideum*, which had previously been crystallized in our laboratory. The sequence identity between OsHsp90-NTD and the NTD of Hsp90 from *D. discoideum* is 70%.

Cross-seeding was attempted with *D. discoideum* NTD crystals obtained in a condition similar to that described above. These crystals were crushed and suspended in 50 μl mother liquor, which was used as an additive in crystallization trials of OsHsp90-NTD in the same set of conditions. 0.2 μl seed solution was added to a drop consisting of 2 μl protein solution and 2 μl precipitant solution.

2.6. Data collection, structure solution and refinement

Preliminary characterization of the crystal was performed using diffraction data collected at the home source using a MAR 345 imaging plate. The X-ray beam (Cu $K\alpha$ radiation) from a Rigaku UltraX 18 rotating-anode X-ray generator operating at 50 kV and 80 mA was focused with an Osmic mirror system. The crystal diffracted to a resolution of 3.6 Å at the home source. Data were subsequently collected on beamline BM14 at the ESRF at 100 K with 0.5° oscillation per image, a crystal-to-detector distance of 307 mm and an exposure time of 10 s. The crystal diffracted to a resolution of 3.1 Å. A total of 283 frames were collected. The data were processed and merged using *MOSFLM* and *SCALA* from the *CCP4* suite (Winn *et al.*, 2011). The structure was solved by molecular replacement using *Phaser* (McCoy *et al.*, 2007) with the structure of the NTD of barley Hsp90 as a search model (PDB entry 2jki; Zhang *et al.*, 2008). Model building was performed using *Coot* (Emsley *et al.*, 2010) and the structure was refined using *phenix.refine* (Afonine *et al.*, 2012). Figures were produced with *PyMOL* (DeLano, 2002).

3. Results and discussion

3.1. Identification of Hsp90 from the genome of *O. sativa*

A *BLAST* search performed against the KOME database (which gives information on full-length cDNA clones that have been collected and completely sequenced) generated 11 hits with significant identity between 60 and 70% using the protein sequences of yeast Hsp90 and human Hsp90 α as queries. The cDNA with the highest sequence identity was selected for our study and was obtained from the Rice Genome Research Centre, Japan.

A sequence alignment of OsHsp90 and Hsp90s from barley, human and yeast is shown in Fig. 1. The sequences were found to be highly similar, especially the regions comprising the three domains. The main difference was observed in the linker region between the NTD and the middle domain, which consists of several charged residues such as glutamate and lysine. This region is known to be variable in size and composition among different species. OsHsp90 shares a sequence identity of 95% to barley Hsp90 and shows more than 60% identity to the Hsp90s from human and yeast.

Figure 3
OsHsp90-NTD. (a) SDS–PAGE profile of purified OsHsp90-NTD. The left lane contains molecular-mass markers (labelled in kDa). (b) Mass-spectrometric analysis of purified OsHsp90-NTD (calculated molecular mass 27.54 kDa).

Table 1

Comparison of the biochemical properties of OsHsp90 with those of Hsp90s from other organisms.

Organism	ATP			Geldanamycin	
	K_m (μM)	k_{cat} (min^{-1})	Catalytic efficiency k_{cat}/K_m ($\text{min}^{-1} \mu M^{-1}$)	K_d (μM)	K_d (μM)
<i>Oryza sativa</i>	467.8 ± 45	0.0237 ± 0.0002	5×10^{-5}	262 ± 22	8.8 ± 11 (17-AAG)
<i>Homo sapiens</i>	$840 \pm 60^\dagger$	0.0890 ± 0.004	10×10^{-5}	$240 \pm 14^\ddagger$	$4.4^\S$
<i>Saccharomyces cerevisiae</i>	$300^\P$	0.35	0.001	$132 \pm 47^\dagger\dagger$	$1.2^\ddagger\ddagger$

† McLaughlin *et al.* (2002). ‡ McLaughlin *et al.* (2004). § Pallavi *et al.* (2010). ¶ Weikl *et al.* (2000). †† Prodromou *et al.* (1997). ‡‡ Roe *et al.* (1999).

Table 2

Data-collection and refinement statistics.

Values in parentheses are for the last resolution shell.

Data-collection statistics	
Space group	<i>I</i> 422
Unit-cell parameters ($\text{\AA}$)	$a = 116.87, c = 105.34$
Resolution range ($\text{\AA}$)	$58.44\text{--}3.10$ ($3.27\text{--}3.10$)
Wavelength ($\text{\AA}$)	0.953
No. of molecules in asymmetric unit	1
Solvent content (%)	62
No. of observed reflections	75886 (11055)
No. of unique reflections	6890 (975)
Completeness (%)	100 (100)
$R_{\text{merge}}^\dagger$ (%)	13.3 (83.6)
Average $I/\sigma(I)$	12.8 (3.0)
Multiplicity	11.0 (11.3)
Refinement statistics	
No. of reflections used	
Total	6886
Working set	6557
Test set	329
$R_{\text{work}}^\ddagger$ (%)	24.51
$R_{\text{free}}^\ddagger$ (%)	29.17
No. of atoms	
Protein atoms	1577
Solvent atoms	20
Ligand atoms	
AMPPCP	31
PEG	13
Average B factor ($\text{\AA}^2$)	80
R.m.s. deviation from ideal values	
Bond lengths ($\text{\AA}$)	0.009
Bond angles ($^\circ$)	1.45
Residues in Ramachandran plot (%)	
Most favoured	92
Additionally allowed region	8
Disallowed region	0

$^\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$. $^\ddagger R_{\text{work}} = \frac{\sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}||}{\sum_{hkl} |F_{\text{obs}}|}$. R_{free} is calculated in the same way but for a subset of reflections that were not used in the refinement.

3.2. Binding of Hsp90 to nucleotide and inhibitor

To characterize OsHsp90, we cloned the OsHsp90 cDNA and expressed and purified the full-length protein to homogeneity (Fig. 2a). The binding of OsHsp90 to ATP was examined by fluorescence spectroscopy by monitoring the quenching of the internal tryptophan fluorescence as described in §2. A progressive decrease in the intrinsic fluorescence of Hsp90 was observed upon incubation with increasing concentrations of ATP. The saturation curve was obtained by plotting the difference in the intrinsic fluorescence against the concentration of ATP (Fig. 2b). The data points were analyzed using nonlinear regression for single-site specific binding. The K_d for ATP was determined to be

262 μM , which is in a similar range to those reported for Hsp90s from other organisms (McLaughlin *et al.*, 2004). The binding of 17-AAG to Hsp90 was measured in a similar manner and the value of K_d was determined to be 8.8 μM (Fig. 2c).

The members of the Hsp90 family exhibit slow ATPase activity, which is required for the chaperone function. The ATPase activity also varies substantially between homologues (Krukenberg *et al.*, 2011). To assess the biochemical parameters of the OsHsp90 ATPase activity, the purified protein was incubated with increasing concentrations of ATP along with ($\gamma^{32}\text{P}$)-ATP as a tracer and the rate of hydrolysis was analyzed by TLC. OsHsp90 bound to ATP with a K_m of 467 μM and was observed to be an active ATPase with a k_{cat} of $2.37 \times 10^{-2} \text{ min}^{-1}$ (Fig. 2d). The results show that OsHsp90 exhibits a lower ATPase activity compared with homologous proteins, as shown in Table 1.

3.3. Crystallization and structure solution of OsHsp90-NTD

Crystallization attempts with full-length OsHsp90 were unsuccessful owing to the flexible and dynamic nature of the protein. Since the NTD of Hsp90 is involved in interactions with the nucleotide and inhibitors, OsHsp90-NTD was cloned and the protein was expressed and purified. The purity and the mass of the protein were verified by SDS-PAGE and mass spectrometry (Figs. 3a and 3b).

Initial crystallization attempts with OsHsp90-NTD using various screens gave only crystalline precipitates in various conditions and did not translate into well formed crystals. Finally, diffraction-quality crystals were obtained by micro-seeding. Seeding is a method that is used for the separation of nucleation and growth. In this technique, previously nucleated crystals are used as seeds and introduced into new drops equilibrated at lower levels of supersaturation (Bergfors, 2003). The method can also be used to facilitate crystallization of similar related proteins. Crystals of the NTD of Hsp90 from *D. discoideum* were used as seeds to probe the crystallization of OsHsp90-NTD. In the initial stages, a mesh of crystals were observed that were fragile and had a tendency to dissolve. After several rounds of optimization by keeping the crystals at 4°C for 48 h and reducing the seed concentration twofold, well separated three-dimensional crystals with dimensions of $0.01 \times 0.02 \times 0.10 \text{ mm}$ were obtained (Fig. 4).

The crystals diffracted to a resolution of 3.1 $\text{\AA}$ at the ESRF. The crystals belonged to space group *I*422, with unit-cell parameters $a = b = 116.87, c = 105.34 \text{ \AA}$. Molecular replace-

ment gave a clear solution with a Z score and an LLG of 7.9 and 586, respectively. Rigid-body refinement of the solution yielded an R_{work} and an R_{free} of 42.2 and 43.0%, respectively. Mutation of the residues to those of OsHsp90-NTD followed by model building using *Coot* and restrained refinement using *phenix.refine* with default parameters gave an R_{work} and an R_{free} of 29.4 and 36.5%, respectively. Clear density was observed for the AMPPCP ligand in the electron-density maps. With the inclusion of the ligand and the addition of a PEG molecule and water molecules, followed by multiple model-building and refinement cycles, R_{work} and R_{free} converged to 24.5 and 29.1%, respectively. Table 2 gives the data-collection and refinement statistics.

3.4. Structure of OsHsp90-NTD

The overall structure of OsHsp90-NTD was observed to be similar to the other reported structures of NTD from homologous proteins, retaining the secondary-structural organiza-

tion of the two-layer α/β -sandwich motif with an antiparallel β -sheet forming one face and two layers of α -helices on the other face of the domain. The OMIT map clearly showed the presence of bound ligand (AMPPCP), and density was observed for the full ligand (Fig. 5a). The residues involved in interaction with the nucleotide were also conserved between OsHsp90, barley Hsp90 (PDB entry 2jki; Zhang *et al.*, 2008), yeast Hsp90 (PDB code 1am1; Prodromou *et al.*, 1997) and human Hsp90 (PDB entry 3t10; Li *et al.*, 2012) (Fig. 5b). The OsHsp90-NTD structure, however, lacked density for a stretch of eight amino-acid residues (Gly113–Gly120) which belong to a region of the NTD known as the ATP-lid segment (Gly96–Phe126; Fig. 5a).

Comparison of the structures of the AMPPCP complex in the cases of OsHsp90-NTD and human Hsp90 (PDB entry 3t10; Li *et al.*, 2012) shows that the nucleotide occupies a similar position in both proteins (Fig. 6a). The lid segment in the case of OsHsp90-NTD was observed to be disordered and density was only observed for part of the lid region. A segment

Figure 4
Optimization of the cross-seeding of OsHsp90-NTD with crystals of the NTD of *D. discoideum* Hsp90.

Figure 5
Structure of OsHsp90-NTD in complex with AMPPCP. (a) Cartoon representation of the protein. AMPPCP is shown in ball-and-stick representation. The missing segment of the lid region is indicated by a dashed line. The OMIT map contoured at 3σ for AMPPCP is shown as a grey mesh. (b) Residues involved in interaction with the nucleotide in OsHsp90-NTD (orange) and the NTDs of the Hsp90s from barley (green), yeast (grey) and human (blue) after overall structural superposition. The numbering corresponds to that of OsHsp90.

of this lid region is observed to be in a different conformation when compared with the lid region in the structure of the NTD of human Hsp90 (residues 108–138 of human Hsp90; Fig. 6*b*). Part of the lid segment from Thr97 to Glu105 in the case of OsHsp90-NTD has shifted and moved partially closer to the binding pocket, with the maximum C^α deviation observed at Ser101 (Ser113 in the human protein), which has moved by

about 5 Å when compared with the NTD of human Hsp90 (Fig. 6*c*). The only other structure of the NTD of Hsp90 available from a plant source is that from barley, which shares a sequence identity of 95% with OsHsp90-NTD. The structure of the NTD from barley Hsp90 (PDB entry 2jki; Zhang *et al.*, 2008) is similar to that observed for the human protein, with the lid in an open conformation, and hence also differs from

Figure 6
Comparison of the structure of OsHsp90-NTD with the structures of homologous proteins. (a) Superposition of AMPPCP from the OsHsp90-NTD complex (orange) and the human Hsp90 NTD complex (blue; PDB entry 3t10). (b) Superposition of OsHsp90-NTD (orange) on the NTD of human Hsp90 (blue) and the NTD of barley Hsp90 (green; PDB entry 2jki) representing the change in the conformation of the lid region. (c) Comparison of lid region of OsHsp90-NTD (orange) and NTD of human Hsp90 (blue).

Figure 7
Stereo image of comparison of the structure of OsHsp90-NTD (orange) with the open form (blue; PDB entry 1yes) and the closed form (green; PDB entry 1yer) of the NTD of human Hsp90.

the structure of OsHsp90-NTD in its lid conformation (Fig. 6*b*). The overall r.m.s.d.s between the structures of OsHsp90-NTD and the NTD of human Hsp90 and those of OsHsp90-NTD and the NTD of barley Hsp90 were calculated to be around 1 Å. The lid region alone of the OsHsp90-NTD showed an r.m.s.d. of more than 2 Å from the structures of the NTDs of human and barley Hsp90.

The ATP-lid region of Hsp90 is known to be flexible and essential for its ATPase activity. This region, which is common in the nucleotide-binding domains of GHKL ATPases, forms a helix-loop-helix motif and is present adjacent to the ATP-binding pocket (Pearl & Prodromou, 2006). A GxxGxxG motif at the C-terminal end of the lid segment interacts with the phosphates of the bound ATP and facilitates the positioning of the phosphate for ATP hydrolysis. Local structural rearrangements of the lid region have been reported previously that display different conformations in different crystal forms (Krukenberg *et al.*, 2011). Two apo-form structures of the NTD of human Hsp90 have been determined in two different crystal forms where the structure differs by a local conformational change in the lid region (Stebbins *et al.*, 1997). These were described as open and closed forms based on the pocket size and accessibility. However, in the previously solved structures of isolated NTDs (from different species) in complex with different ligands such as ATP, a nonhydrolyzable analogue and inhibitors, the lid region is observed to have an overall similar structure and to be present in the open conformation. However, in the present structure of OsHsp90-NTD the lid region is observed to be disordered even in the presence of the ligand AMPPCP, and this conformation is observed to be different from the open and closed apo forms of the NTD of human Hsp90 (Fig. 7). Comparison of the structure of OsHsp90-NTD with its homologues shows that although the overall structure is similar, local conformational changes are observed within the structure. OsHsp90-NTD crystallized in complex with AMPPCP with the lid segment in a conformation that was not observed in previously reported structures of isolated NTDs.

Acknowledgements

This research was supported by The Department of Biotechnology (DBT)–Indian Institute of Science (IISc) partnership program. Data were collected at the National X-ray Data Collection Facility at the Molecular Biophysics Unit, IISc funded by The Department of Science and Technology and at beamline station BM14 of the European Synchrotron Radiation Facility (ESRF) funded by the DBT. SR thanks the Indian Council of Medical Research for a research fellowship. The authors would like to thank Meetali Singh and Professor Utpal Tatu, Department of Biochemistry, IISc for their help with the ATPase assay.

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