J. Biochem. 2011;150(4):403–409 doi:10.1093/jb/mvr074

JB THE JOURNAL OF BIOCHEMISTRY

StHsp14.0, a small heat shock protein of *Sulfolobus tokodaii* strain 7, protects denatured proteins from aggregation in the partially dissociated conformation

Received March 28, 2011; accepted May 5, 2011; published online June 9, 2011

Tetsuya Abe[1], Toshihiko Oka[2], Atsushi Nakagome[1], Yoshihiro Tsukada[3], Takuo Yasunaga[3] and Masafumi Yohda[1,*]

[1]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei-shi, Tokyo 184-8588; [2]Department of Physics, Faculty of Science, Shizuoka University, 836 Ohya, Suruga, Shizuoka, 422-8529; and [3]Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, 680-4, Kawazu, Iizuka, Fukuoka, 820-8502, Japan

*Masafumi Yohda, Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16 Naka-cho, Koganei, Tokyo, 184-8588, Japan. Tel/Fax: +81 42 388 7479, email: yohda@cc.tuat.ac.jp

The small heat shock protein (sHsp), categorized into a class of molecular chaperones, binds and stabilizes denatured proteins for the purpose of preventing aggregation. The sHsps undergo transition between different oligomeric states to control their nature. We have been studying the function of sHsp of *Sulfolobus tokodaii*, StHsp14.0. StHsp14.0 exists as 24meric oligomer, and exhibits oligomer dissociation and molecular chaperone activity over 80°C. We constructed and characterized StHsp14.0 mutants with replacement of the C-terminal IKI to WKW, IKF, FKI and FKF. All mutant complexes dissociated into dimers at 50°C. Among them, StHsp14.0FKF is almost completely dissociated, probably to dimers. All mutants protected citrate synthase (CS) from thermal aggregation at 50°C. But, the activity of StHsp14.0FKF was the lowest. Then, we examined the complexes of StHsp14.0 mutants with denatured CS by SAXS. StHsp14.0WKW protects denatured CS by forming the globular complexes of 24 subunits and a substrate. StHsp14.0FKF also formed similar complex but the number of subunits in the complex is a little smaller. These results suggest that the dimer itself exhibits low chaperone activity, and a partially dissociated oligomer of StHsp14.0 protects a denatured protein from interacting with other molecules by surrounding it.

Keywords: assembly and dissociation/chaperone/small angle X-ray scattering/small heat shock protein/*Sulfolobus tokodaii*.

Abbreviations: CS, Citrate synthase from porcine heart; EM, electron microscopy; SAXS, small angle X-ray scattering; sHsp, small heat shock protein; StHsp14.0, small heat shock protein of *Sulfolobus tokodaii* with a molecular weight of 14.0 kDa; StHsp14.0WKW, StHsp14.0 with the mutations of I120W and I122W; StHsp14.0FKF, StHsp14.0 with the mutations of I120F and I122F; StHsp14.0IKF, StHsp14.0 with the mutation of I122F; StHsp14.0FKI, StHSp14.0 with the mutation of I120F.

Small heat shock proteins (sHsps) belong to the members of the ubiquitous molecular chaperones. They exist in all types of organisms, including archaea, bacteria, and eukarya (*1*). They endow cells with thermotolerance (*2*, *3*), protect proteins from thermal aggregation, and in some cases, promote refolding of proteins (*4*–*6*). Compared with other chaperones, the overall amino acid sequence homology among sHsps is rather low. Their common feature is the α-crystallin domain, which is named after the α-crystallin of the vertebrate eye lens (*7*). The α-crystallin domain is flanked by a highly variable N-terminal region and a short, partially conserved C-terminal extension (*8*, *9*). All sHsps exist as large oligomeric complexes composed of 9–40 subunits (*10*). Crystal structures of two sHsps in large oligomeric states have been reported (*11*, *12*). The sHsp from *Methanocaldococcus jannaschii* (MjHsp16.5) forms a spherical oligomer consisting of 24 subunits with the diameter of ~120 Å (PDB-ID: 1SHS). On the other hand, the sHsp from wheat (wHsp16.9) forms a double-ring-shaped oligomer consisting of 12 subunits (PDB-ID: 1GME). The oligomers consist of dimerized sHsps irrespective of difference in oligomeric structures as well as in amino acid sequences. The α-crystallin domain folds into a compact β-sandwich fold. The N-terminal regions are packed in the centre of oligomeric structures. The conserved short IXI/V motif at the C-terminus has a propensity to participate in inter–subunit interactions. These interactions are important in the structure and function of sHsps (*13*).

Under physiological temperatures, sHsps exist as large oligomeric structures and do not interact with denatured proteins. When sHsps are exposed to elevated temperatures, the large oligomeric structure dissociates into small oligomers, in most cases dimers, and exhibit chaperone function. Therefore, the hydrophobic sites are thought to be controlled by transitions between different oligomeric states (*14*, *15*). Basha *et al*. (*16*) have shown that substrate-binding sites comprise both the newly exposed hydrophobic surfaces on the α-crystallin domain of the sHsp, along with hydrophobic residues of the N-terminal arm. The exposed hydrophobic surfaces interact with the hydrophobic

surfaces of denatured proteins to protect them from aggregation.

Our previous study has shown that a sHsp of *Schizosaccharomyces pombe*, SpHsp16.0, interacts with denatured proteins as dimers at elevated temperatures, suggesting that sHsps protect denatured proteins from aggregation through transient interactions in the dissociated state (*17*). However, cross-linking experiments using Hsp26 have shown inconsistent results (*18*). The cross-linked Hsp26 does not dissociate into dimers at higher temperatures, but its chaperone activity remains unaffected. Basha *et al*. (*16*) has also shown that stability of the oligomer does not correlate with substrate protection ability.

The sHsp of the acidothermophilic archaeon *Sulfolobus tokodaii* with a molecular mass of 14 kDa (StHsp14.0) protects denatured proteins from aggregation formation at high temperatures around 87°C (*19*). Mutations of the IXI/V motif of StHsp14.0 (IKI) affect the stability of oligomers (*20*). In particular, the FKF variant, StHsp14.0 with the mutations of I120F and I122F, is defective in oligomer formation. Contrary to our expectations, the FKF variant did not exhibit chaperone function at the same temperature at which they exist primarily as dimers. The result suggests that the dimer is not the real functional state of StHsp14.0.

Here, we report further characterization of StHsp14.0 C-terminal variants of StHsp14.0 and discuss the relationship between function and oligomeric states of the protein.

Materials and Methods

Preparation of StHsp14.0 variants and substrates

The wild-type and variants of StHsp14.0 were constructed, expressed and purified as described previously (*19*, *20*). Citrate synthase (CS) from porcine heart and α-lactalbumin were obtained from Sigma.

Thermal aggregation measurements

The aggregation of CS was monitored by measuring light scattering at 500 nm with a spectrofluorometer (FP-6500, Jasco) at 50°C. CS was diluted to a final concentration of 150 nM (as a monomer) in the assay buffer (50 mM Tris–HCl, pH 8.0), with or without StHsp14.0 variants, at the specified monomeric concentrations. The reaction mixtures were continuously stirred throughout the experiment.

Aggregation of bovine α-lactalbumin was also monitored by measuring light scattering at 500 nm with a spectrofluorometer (FP-6500) at 25°C. α-Lactalbumin (2,400 nM, monomer) was incubated in the assay buffer (50 mM Tris–HCl, pH 6.8, 2 mM CaCl2) with or without StHsp14.0 variants (2,400 nM as monomers). After preincubation for 5 min, aggregation was induced by the addition of DTT (final concentration 360 nM). The reaction mixtures were continuously stirred throughout the experiment.

SAXS measurements

All SAXS data were measured at the BL40B2 in the SPring-8 (*21*). Scattering profiles were collected by a detector system using imaging plates, R-axis VII (Rigaku). X-ray wavelength and camera length were 1.000 Å and 2,116 mm, respectively. Concentrations of StHsp14.0 in the sample solution were kept at 1.0 mg/ml, regardless of CS addition. Before data collection, sample solutions were incubated in a sample cell of 3 mm path length at 25 and 50°C. The data collection time was 60 s. The two-dimensional data was circularly averaged after buffer subtractions and divided by the relative concentration of total protein. The innermost portion of $I(Q)$ was fitted under a Guinier approximation (*22*) to the equation $I(Q) = I(0)\exp[-\mathrm{Rg2Q2/3}]$, where I(0) and Rg are the forward scattering intensity ($Q = 0$) and the radius of gyration, respectively. The data in the range of $Q_{\min} = 0.008$–0.01 to $Q_{\max} = 1.3/\mathrm{Rg}$ Å-1.were used for calculation.

The $I(0)$ value is proportional to the averaged molecular weight of the particles in solution (*22*).

$$I(0) = \alpha CM = \beta NM^2$$

where C is concentration (weight/volume), N is the number of particles, M is the molecular weight and α, β is constant. The average molecular weight of wild-type was calculated from the $I(0)$ value using ovalbumin as a standard. Radius of gyration, Rg, is the z-average value (*22*), thus Rg of polydisperse solution, including the solution of a complex of StHsp14.0 variants and CS, is difficult to interpret. The low-resolution model was constructed from the SAXS data of the wild-type (5.0 mg/ml) in the Q range of 0.010–0.112 Å^{-1}, by DAMMIF (*23*) without symmetrical constrains. Ten independent models were averaged by DAMAVER (*24*).

According to Guiner and Fournet (*22*), the I(0) of a multi-component system is described by the summation of the forward scattering intensity of all components. If StHsp14.0 consists of both 24-mers and dimers, the I(0) is, therefore, described as follows:

$$I(0) = A\left[\frac{N}{24}P(24M_{\mathrm{mono}})^2 + \frac{N}{2}(1-P)(2M_{\mathrm{mono}})^2\right]$$

where A is a constant, N is the number of StHsp14.0 monomers, M_{mono} is the molecular weight of the monomer, and P is the probability of StHsp14.0 to form the 24-mer. Because the probability P of wild-type StHsp14.0 is estimated to be 1 at 25°C, the probabilities of other variants and temperatures were calculated.

Electron microscopy

Complexes of StHsp14.0 variants with CS were adsorbed onto a thin carbon film covering a grid at the room temperature and stained with 2% uranyl acetate. The carbon film was augmented for greater hydrophilicity with n-pentylamine. The grid was then observed in a transmission electron microscope (EF-2000, Hitachi) operated at an accelerating voltage of 200 kV. The electron micrographs were taken using a CCD camera with a pixel size of 24 μm at direct magnification of 140,000; the sampling steps were 0.17 nm. The electron dose was ~1,500 electron/nm$_2$. Then the image contrast was modified by defocusing (0.7–2 μm underfocus) and corrected by Eos (*25*), using contrast transfer functions calculated following their defocusing values, accelerating voltage, etc.

Results

Molecular chaperone activity of StHsp14.0 variants

Our previous results show that StHsp14.0FKF could not protect CS (citrate synthase from porcine heart) from thermal aggregation at 50°C (*20*). This result contradicts the hypothesis that the dimer is the active conformer. Then we examined whether chaperone function remains in StHsp14.0FKF at all by optimizing the assay conditions. Unlike previous observations, StHsp14.0FKF was capable of preventing CS aggregation (Fig. 1a). The chaperone activity of StHsp14.0FKF was the weakest among the tested variants (data not shown), and a >32-fold excess amount was required to show recognizable effect. The amount of StHsp14.0FKF used in the previous experiment (24-fold molar excess) seems not to be enough to observe a significant effect (*20*). At 25°C, neither the wild-type nor any of the StHsp14.0WKW (StHsp14.0 with the mutations of I120W and I122W), StHsp14.0IKF (StHsp14.0 with the mutation of I122F) and StHsp14.0FKI (StHsp14.0 with the mutation of I120F) variants were able to protect against aggregation of α-lactoalbumin, but only StHsp14.0FKF exhibited protection ability (Fig. 1b). These results suggest that

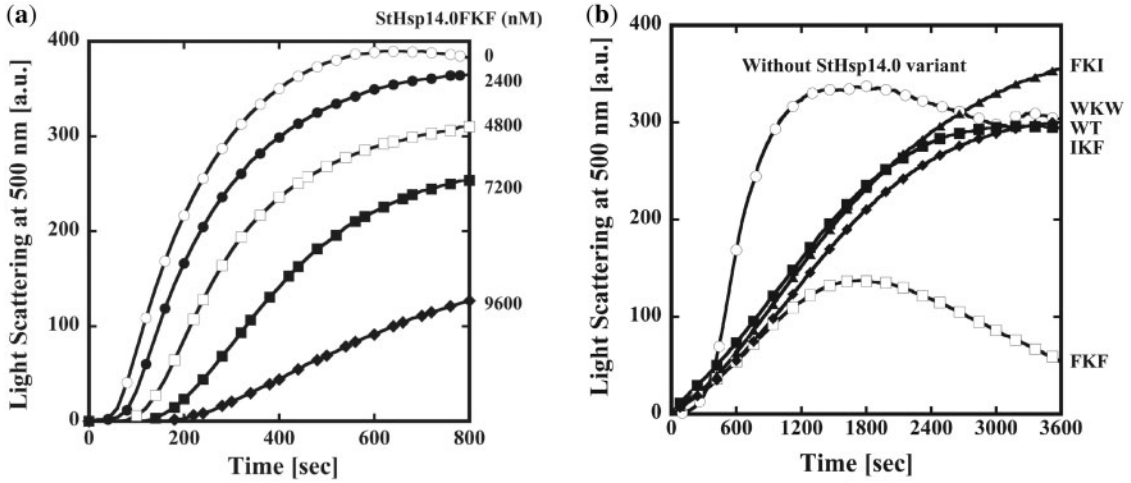

Fig. 1 Effects of StHsp14.0 variants on aggregation of CS and α-lactalbumin. Thermal aggregation of CS from porcine heart was monitored by measuring light scattering at 500 nm using a spectrofluorometer at 50°C with continuous stirring. (a) Effect of StHsp14.0FKF on thermal aggregation of CS. Open circle, without StHsp14.0FKF; closed circle, 2,400 nM; open square, 4800 nM; closed square, 7,200 nM; closed diamond, 9,600 nM. (b) Aggregation of α-lactalbumin was also monitored at 25°C using a spectrofluorometer. Open circle, without StHsp14.0; closed circle, the wild-type StHsp14.0; open square, StHsp14.0FKF; closed square, StHsp14.0WKW; closed diamond, StHsp14.0IKF; closed triangle, StHsp14.0FKI.

the partially dissociated complex of StHsp14.0 is a more active conformer than the dimer.

Oligomer structures examined by small angle X-ray scattering

Subsequently, these oligomeric conformations of StHsp14.0 variants were examined by small angle X-ray scattering (SAXS) (Fig. 2a–c). The scattering profiles of the wild-type at both 25 and 50°C have a clear peak and a valley, which were typical of spherical particles (*26*) (Fig. 2a). The average molecular weight of the wild-type from its $I(0)$ value at 25°C were estimated to be 317 kDa, which is close to 336 kDa, the molecular weight of an oligomer composed of 24 subunits (*19*). A low-resolution structure of the wild-type was constructed from the diffraction data of SAXS by DAMMIF (*23*). It is a distorted spherical structure, and fits well with the predicted structure of the StHsp14.0 oligomer (Fig. 2d). This result supports the idea that StHsp14.0 wild-type exists as the 24-mer similar to the modelled structure.

StHsp14.0WKW takes almost the same profile as the wild-type at 25°C (Fig. 2b). But a change was observed at 50°C (Fig. 2b), which indicated some portion of the oligomers dissociate into dimers. StHsp14.0FKF also formed spherical oligomers mainly at 25°C, but they dissociated into dimers at 50°C (Fig. 2c). Table I shows the forward scattering intensities, $I(0)$s, of the wild-type and the variants at 25 and 50°C. $I(0)$ represents a measure of the averaged molecular weight. Assuming that the wild-type exists as a spherical oligomer composed of 24 subunits at 25°C, the contents of the spherical oligomers of the variants at 25 and 50°C were calculated (Table I). In the case of StHsp14.0WKW, ~20% has dissociated into dimers at 50°C. Although 72% of StHsp14.0FKF exists as spherical oligomers at 25°C, most of the oligomers dissociated into dimers at 50°C. This result partially contradicts the previous observation that StHsp14.0FKF is eluted as small oligomers (probably dimers) during gel filtration at 25°C (*20*) and is a dimer in the crystal structure (*27*). As gel filtration experiments were done at a concentration lower than that used for SAXS measurements, StHsp14.0FKF should have dissociated into dimers during gel filtration at 25°C. Only StHsp14.0FKF in the dimeric conformation was likely to crystallize under the crystallization condition (*27*).

Complex of StHsp14.0 variants with denatured proteins

Next, we examined oligomeric states of StHsp14.0 variants complexed with denatured CS using SAXS. When CS was mixed with a 24-fold molar excess of the wild-type StHsp14.0 at 50°C, a sharp peak appeared at very low angles and increased with time (Fig. 3a). Since a similar peak was observed in the experiment without StHsp14.0 (data not shown), it should correspond to aggregates of CS. On the contrary, in the presence of StHsp14.0WKW, no such sharp peak appeared, presumably from its chaperone activity (Fig. 3b). Similar results were observed with StHsp14.0FKI and StHsp14.0IKF (data not shown). In the case of StHsp14.0FKF, no sharp peak appeared, as in the case for other variants, but the profile significantly changed from those without CS (Fig. 3c). After 10 min, the forward scattering increased and reached a plateau, suggesting the generation of a complex of StHsp14.0FKF and CS. $I(0)$ values of the complexes were calculated based on the data after 30 min (Table I). The $I(0)$ of StHsp14.0WKW with CS was a little smaller than that of the wild-type in the initial state, and gradually increased up to about 176, reflecting complex formation with CS. The value is almost the same as the calculated value for the complex of the 24-mer and one CS molecule, 181. The $I(0)$ of FKF with CS significantly increased up to 131. Although the

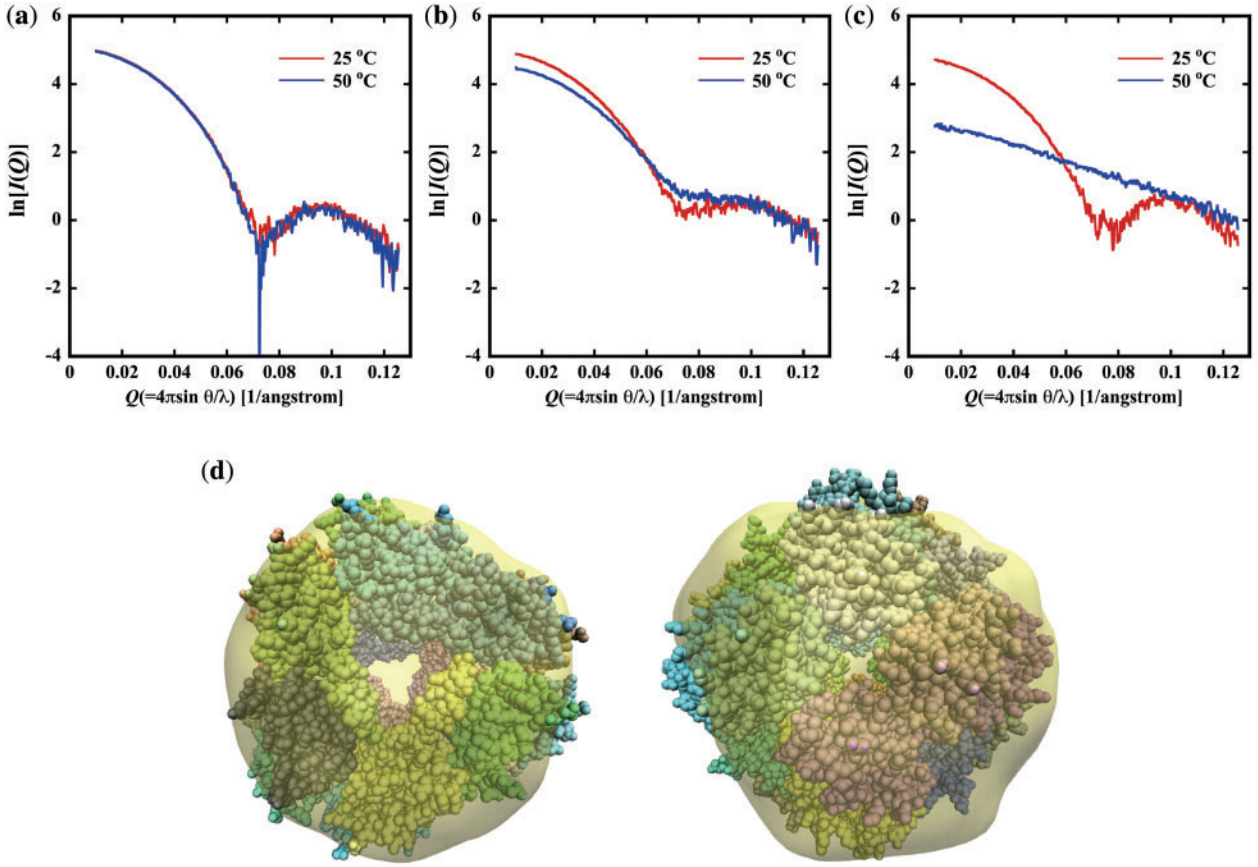

Fig. 2 SAXS profiles of StHsp14.0 variants at 25 and 50°C. SAXS curves of StHsp14.0 variants at 25°C (Red) and 50°C (Blue). (a) wild-type StHsp14.0, (b) StHsp14.0WKW, (c) StHsp14.0FKF. (d) A smooth envelope of a low-resolution structure of StHsp14.0 wild-type without N terminal and C-terminal regions (1–25 and 115–123) superposed on the oligomeric structure of StHsp14.0 at atomic resolution reconstituted from the dimeric structure (*27*). The envelope was calculated with the SITUS package (*28*). This figures (Left, the view from the 3-fold symmetrical axis; Right, the view from the 4-fold symmetrical axis) were prepared using VMD (*29*).

Table I. Forward scattering intensities, *I*(0)s and Radii of gyration, *Rg*s, of the wild-type and the mutants of StHsp14.0 at 25 and 50°C and calculated dimer contents.

	Wild-type		StHsp14.0WKW		StHsp14.0FKF	
	I(0)	*Rg* (Å)	*I*(0)	*Rg* (Å)	*I*(0)	*Rg* (Å)
25°C	158 (1.00)	48.3	155 (0.98)	48.8	120 (0.72)	45.9
50°C	156 (0.99)	48.2	127 (0.79)	48.5	16 (0.02)	31.0
50°C (+1/24 CS)	–	–	176	61.5	131	61.2

The values in parentheses are the ratio of subunits existing as 24-meric oligomers.

value is smaller than that of the 24-meric oligomer of StHsp14.0, formation of StHsp14.0FKF–CS complex is suggested. The electron microscopic images of the complexes of StHsp14.0WKW and StHsp14.0FKF with denatured CS prepared in the same manner (Fig. 3d and e). The complexes look round in shape in both cases, but differ in size. The image also supports the idea that StHsp14.0FKF protects denatured CS from aggregation by forming a similar complex to that of StHsp14.0WKW.

Discussion

The fact that StHsp14.0FKF is capable of preventing aggregation of CS at 50°C and α-lactoalbumin at 25°C supports the idea that the dimer is an active conformer of sHsp. However, activity of StHsp14.0FKF to protect CS from aggregation at 50°C was lower than other mutants that dissociate only partially at that temperature. These results coincide with previous reports that dissociation does not correlate with chaperone activity (*18*). SAXS experiments showed that StHsp14.0FKF was almost completely dissociated but StHsp14.0WKW was only partially dissociated at 50°C (Table I). At 25°C, StHsp14.0FKF was only partially dissociated (Table I). These results suggest that dissociation of the oligomer is required for chaperone activity but the activity of the StHsp14.0 dimer is small (*20*). These results coincide well with previous observations and support the idea that the partially dissociated oligomer is likely to be the active conformer of StHsp14.0 (*27*).

Oligomeric states of the complexes of StHsp14.0 variants with denatured CS were examined by SAXS and EM. StHsp14.0WKW formed complexes with denatured CS, and these complexes are larger than the complex between StHsp14.0 24-mer and denatured

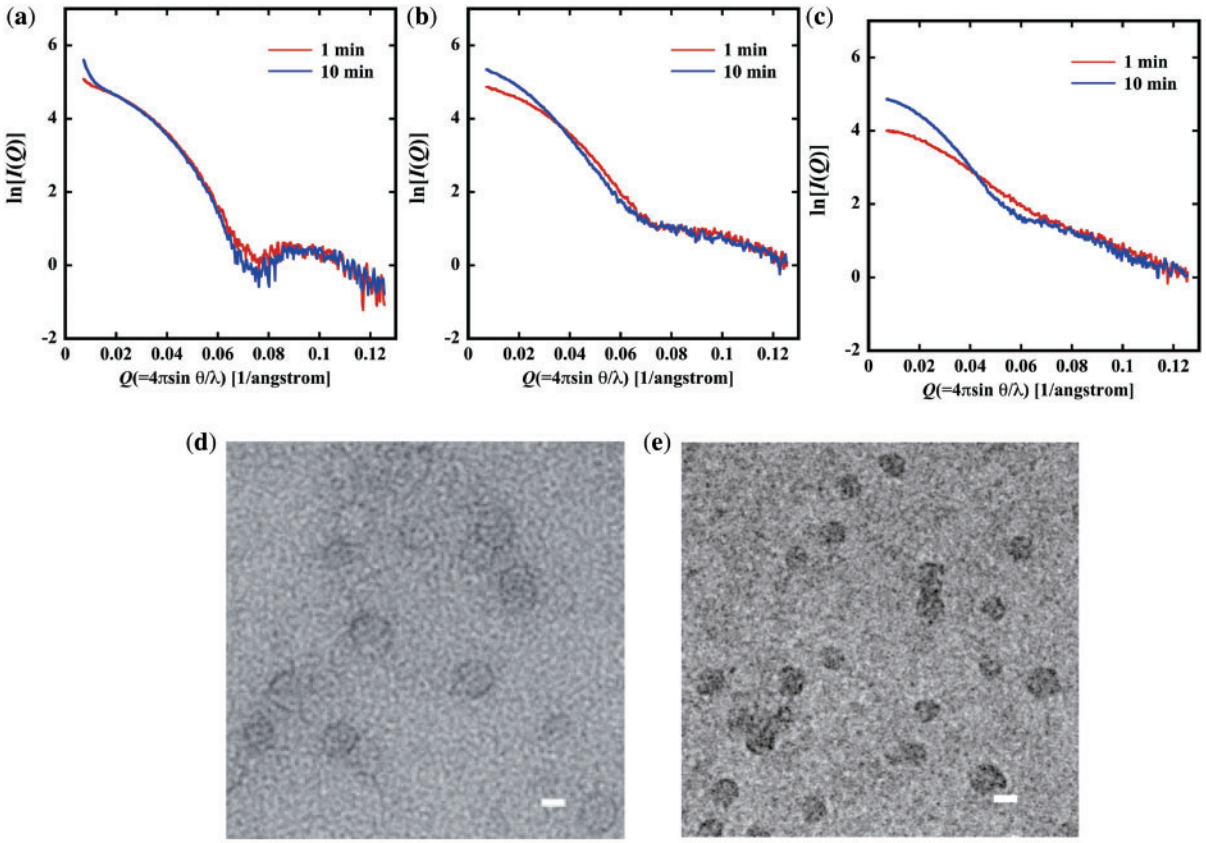

Fig. 3 Structures of the complexes of StHsp14.0 variants with CS. StHsp14.0 variants were incubated with CS at the molar ratio of 24 : 1 at 50°C and SAXS data were taken after 1 min (Red) and 10 min (Blue). (a) wild-type, (b) StHsp14.0WKW, (c) StHsp14.0FKF. Electron microscopic images of StHsp14.0WKW-CS complex (d) and StHsp14.0FKF-CS complex (e). The complexes (white) were negatively stained with uranyl acetate (black). Scale bar =10 nm.

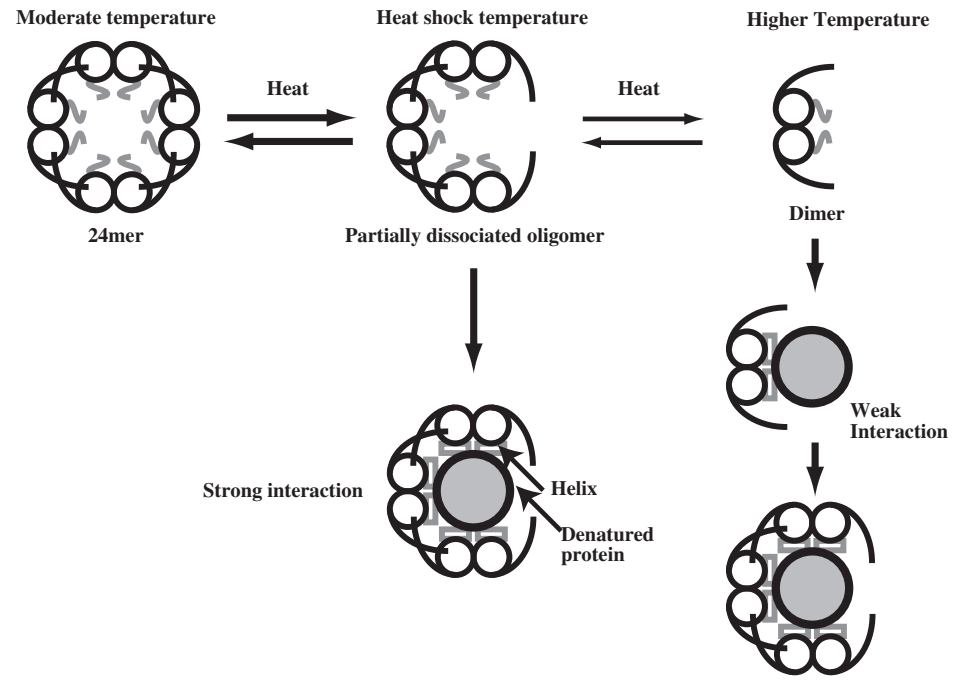

Fig. 4 Schematic model for chaperone function of StHsp14.0. At the moderate temperature, StHsp14.0 s exist as 24-meric oligomers, which have no affinity for denatured proteins. At the elevated temperature, StHsp14.0 oligomers dissociate and some dissociate completely into dimers. The partially dissociated oligomers expose their N-terminal regions, which have high affinity to denatured protein. The N-terminal region changes its conformation to an amphiphilic helix by the interaction with the denatured protein. The complex between StHsp14.0 and the denatured protein is strong due to multiple interactions. The dimer has affinity with the denatured protein, but the complex is unstable. The interaction is stabilized by formation of a large complex via the interaction between dimers.

CS (Table I). StHsp14.0FKF protected denatured CS from aggregation in the dissociated conformation in the beginning, but, interestingly, StHsp14.0FKF also gradually changes its oligomeric state from dimers to large complexes in the presence of denatured CS. EM images show that the complex is similar to that of StHsp14.0WKW and denatured CS.

Figure 4 shows the model for conformational change and chaperone function of StHsp14.0. At physiological temperature, StHsp14.0 exists as a spherical complex composed of 24 subunits (*19*), and does not have chaperone function. At elevated temperatures, it partially dissociates to expose its hydrophobic surface. A denatured protein is captured by the partially dissociated StHsp14.0 complex and protected from aggregation. The N-terminal region should be responsible for the interaction with denatured proteins in the amphiphilic helix conformation (*27*).

At the more elevated temperatures, StHsp14.0 completely dissociated into dimers. The dimer itself has the ability to interact with denatured proteins. However, the interaction between the dimer and the denatured protein is not strong enough to protect the substrate from aggregation. StHsp14.0 dimers gradually formed globular complexes with denatured proteins. The interaction with the denatured protein might induce the formation of globular complexes via an interaction at the C-termini. Thus, interaction between dimers was enhanced by the presence of denatured proteins. From these results, we concluded that StHsp14.0 protects denatured protein molecules from interacting with other denatured protein molecules by surrounding it in the partially dissolved conformation that is maintained by the interaction between dimers through their C- termini. Multiple interactions with denatured protein in the complex seem to be important for stability of the complex.

Acknowledgements

SAXS experiments were performed at the BL40B2 in the SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (Proposal No. 2008A1866, 2008B1266, 2009A1874). The authors thank Drs Noboru Ohta, Katsuaki Inoue and Hiroyasu Masunaga of JASRI for their support with beamline alignment. The authors also thank Drs Kazuki Tkaeda and Kunio Miki of Kyoto University for reading the manuscript. The work reported here is part of the support program for improving graduate school education by the 'Human Resource Development Program for Scientific Powerhouse', which is financially supported by the Ministry of Education, Culture, Sports, Science and Technology in Japan through Tokyo University of Agriculture & Technology. This study was also supported by grants-in-aids for scientific research (21370067 and 22020011) from the Ministry of Education, Science, Sports, and Culture of Japan to M.Y.

Funding

Grants-in-aids for scientific research (grant numbers 20059013, 21370067 and 22020011 to M.Y.); and a grant from the National Project on Protein Structural and Functional Analyses from the Ministry of Education, Science, Sports, and Culture of Japan (to K.M. and M.Y.).

Conflict of interest

None declared.

References

1. Narberhaus, F. (2002) Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network. *Microbiol. Mol. Biol. Rev.* **66**, 64–93
2. Van den IJssel, P.R., Overkamp, P., Knauf, U., Gaestel, M., and de Jong, W.W (1994) Alpha A-crystallin confers cellular thermoresistance. *FEBS Lett.* **355**, 54–56
3. Plesofsky-Vig, N. and Brambl, R (1995) Disruption of the gene for hsp30, an alpha-crystallin-related heat shock protein of Neurospora crassa, causes defects in thermotolerance. *Proc. Natl Acad. Sci. USA* **92**, 5032–5036
4. Horwitz, J (1992) Alpha-crystallin can function as a molecular chaperone. *Proc. Natl Acad. Sci. USA* **89**, 10449–10453
5. Jakob, U., Gaestel, M., Engel, K., and Buchner, J (1993) Small heat shock proteins are molecular chaperones. *J. Biol. Chem.* **268**, 1517–1520
6. Muchowski, P.J. and Clark, J.I (1998) ATP-enhanced molecular chaperone functions of the small heat shock protein human alphaB crystallin. *Proc. Natl Acad. Sci. USA* **95**, 1004–1009
7. Caspers, G.J., Leunissen, J.A., and de Jong, W.W (1995) The expanding small heat-shock protein family, and structure predictions of the conserved "alpha-crystallin domain". *J. Mol. Evol.* **40**, 238–248
8. de Jong, W.W., Caspers, G.J., and Leunissen, J.A (1998) Genealogy of the alpha-crystallin–small heat-shock protein superfamily. *Int. J. Biol. Macromol.* **22**, 151–162
9. Leroux, M.R., Melki, R., Gordon, B., Batelier, G., and Candido, E.P (1997) Structure-function studies on small heat shock protein oligomeric assembly and interaction with unfolded polypeptides. *J. Biol. Chem.* **272**, 24646–24656
10. Buchner, J., Ehrnsperger, M., Gaestel, M., and Walke, S (1998) Purification and characterization of small heat shock proteins. *Methods Enzymol.* **290**, 339–349
11. Kim, K.K., Kim, R., and Kim, S.H (1998) Crystal structure of a small heat-shock protein. *Nature* **394**, 595–599
12. van Montfort, R.L., Basha, E., Friedrich, K.L., Slingsby, C., and Vierling, E (2001) Crystal structure and assembly of a eukaryotic small heat shock protein. *Nat. Struct. Biol.* **8**, 1025–1030
13. Pasta, S.Y., Raman, B., Ramakrishna, T., and Rao Ch, M (2004) The IXI/V motif in the C-terminal extension of alpha-crystallins: alternative interactions and oligomeric assemblies. *Mol. Vis.* **10**, 655–662
14. Shashidharamurthy, R., Koteiche, H.A., Dong, J., and McHaourab, H.S (2005) Mechanism of chaperone function in small heat shock proteins: dissociation of the HSP27 oligomer is required for recognition and binding of destabilized T4 lysozyme. *J. Biol. Chem.* **280**, 5281–5289
15. Yang, H., Huang, S., Dai, H., Gong, Y., Zheng, C., and Chang, Z (1999) The Mycobacterium tuberculosis small heat shock protein Hsp16.3 exposes hydrophobic surfaces at mild conditions: conformational flexibility and molecular chaperone activity. *Protein Sci.* **8**, 174–179
16. Basha, E., Friedrich, K.L., and Vierling, E (2006) The N-terminal arm of small heat shock proteins is important for both chaperone activity and substrate specificity. *J. Biol. Chem.* **281**, 39943–39952
17. Hirose, M., Tohda, H., Giga-Hama, Y., Tsushima, R., Zako, T., Iizuka, R., Pack, C., Kinjo, M., Ishii, N., and Yohda, M (2005) Interaction of a small heat shock protein of the fission yeast, Schizosaccharomyces pombe, with a denatured protein at elevated temperature. *J. Biol. Chem.* **280**, 32586–32593

18. Franzmann, T.M., Wuhr, M., Richter, K., Walter, S., and Buchner, J (2005) The activation mechanism of Hsp26 does not require dissociation of the oligomer. *J. Mol. Biol.* **350**, 1083–1093
19. Usui, K., Ishii, N., Kawarabayasi, Y., and Yohda, M (2004) Expression and biochemical characterization of two small heat shock proteins from the thermoacidophilic crenarchaeon Sulfolobus tokodaii strain 7. *Protein Sci.* **13**, 134–144
20. Saji, H., Iizuka, R., Yoshida, T., Abe, T., Kidokoro, S., Ishii, N., and Yohda, M (2008) Role of the IXI/V motif in oligomer assembly and function of StHsp14.0, a small heat shock protein from the acidothermophilic archaeon, Sulfolobus tokodaii strain 7. *Proteins* **71**, 771–782
21. Inoue, K., Oka, T., Miura, K., and Yagi, N (2004) Present Status of BL40B2 and BL40XU at SPring-8 (Beamlines for Small Angle X-ray Scattering). *AIP Conf. Proc.* **705**, 336–339
22. Guinier, A. and Fournet, G (1955) *Small-Angle Scattering of X-Rays*., John Wiley & Sons, Inc, New York
23. Franke, D. and Svergun, D.I (2009) DAMMIF, a program for rapid ab-initio shape determination in small-angle scattering. *J. Appl. Cryst.* **42**, 342–346
24. Volkov, V.V. and Svergun, D.I (2003) Uniqueness of ab initio shape determination in small-angle scattering. *J. Appl. Cryst.* **36**, 860–864
25. Yasunaga, T. and Wakabayashi, T (1996) Extensible and object-oriented system Eos supplies a new environment for image analysis of electron micrographs of macromolecules. *J. Struct. Biol.* **116**, 155–160
26. Serdyuk, I.N., Zaccai, N.R., and Zaccai, J (2007) *Methods in Molecular Biophysics Structure, Dynamics, Function*., Cambridge University Press, Cambridge, UK
27. Takeda, K., Hayashi, T., Abe, T., Hirano, Y., Hanazono, Y., Yohda, M., and Miki, K (2010) Dimer structure and conformational variability in the N-terminal region of an archaeal small heat shock protein, StHsp14.0. *J. Struct. Biol.* **174**, 92–99
28. Wriggers, W. and Chacon, P (2001) Using Situs for the registration of protein structures with low-resolution bead models from X-ray solution scattering. *J. Appl. Cryst.* **34**, 773–776
29. Humphrey, W., Dalke, A., and Schulten, K (1996) VMD: Visual molecular dynamics. *J. Mol. Graph* **14**, 33–38