Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 315 (2004) 113–118
BBRC
www.elsevier.com/locate/ybbrc

Role of the N-terminal region of the crenarchaeal sHsp, StHsp14.0, in thermal-induced disassembly of the complex and molecular chaperone activity☆

Keisuke Usui,[a] Omer Faruk Hatipoglu,[a] Noriyuki Ishii,[b] and Masafumi Yohda[a,*]

[a] *Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2-24-16, Naka-cho, Koganei, Tokyo 184-8588, Japan*
[b] *Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tsukuba Central 6, 1-1-1, Higashi, Tsukuba, Ibaraki 305-8566, Japan*

Received 12 December 2003

Abstract

Small heat shock protein is a ubiquitous molecular chaperone, which consists of a non-conserved N-terminal region followed by a conserved α-crystallin domain. To understand the role of the N-terminal region, we constructed N-terminal truncation mutants of StHsp14.0, the sHsp from *Sulfolobus tokodaii* strain 7. All the mutants formed a stable oligomeric complex similar to that of the wild type. Electron microscopy and size exclusion chromatography-multiangle light scattering showed that the N-terminal region should locate in the center of the oligomeric particle. The mutants exhibited reduced chaperone activity for the protection of 3-isopropylmalate dehydrogenase from thermal aggregation. This reduction correlates with lowered subunit exchange efficiency. The oligomeric structure was retained even after incubation at 90 °C. These results suggest that the N-terminal region of StHsp14.0 functions in the thermally induced disassembly of the complex.

Keywords: Molecular chaperone; *Sulfolobus*; Small heat shock protein; N-terminal region

Small heat shock proteins (sHsps) are ubiquitous molecular chaperones, which exist in all three domains, archaea, bacteria, and eukarya [1]. Their expression is induced by cellular stress [2,3]. They display chaperone activities in vitro, protecting model proteins from thermally induced aggregation and in some cases promoting renaturation [4–7]. In spite of a relatively low overall sequence homology, they have been grouped together based on a conserved domain, the α-crystallin domain, which is named after the α-crystallin of the vertebrate eye lens [8].

SHsps form large multimeric complexes of 9–40 subunits (12–42 kDa monomer size). The three-dimensional crystal structures of MjHsp16.5, a sHsp from the hyperthermophilic archaeon *Methanococcus jannaschii*, and TaHsp16.9, a sHsp from wheat, have been determined [9,10]. MjHsp16.5 forms a hollow spherical complex of 24 subunits, while TaHsp16.9 exists as a dodecameric double disk. The crystal structures show that the α-crystallin domain is composed of β-strands and the two sHsps use a similar dimer as a higher assembly building block but differ in their quaternary structure. In contrast, some members of the sHsp family like α-crystallin are reported to be remarkably polydispersed [11]. The quaternary structures of sHsps represent very dynamic systems. Rapid inter-subunit exchange between oligomeric complexes was observed in several sHsps [12,13]. It has been suggested that sHsps bind to their substrate proteins at denaturing temperatures and form a stable complex with folding intermediates [14]. It was reported that oligomers of TaHsp16.9 and Hsp26, a sHsp of *Saccharomyces cerevisiae*, underwent a reversible temperature-dependent dissociation

☆ *Abbreviations:* sHsp, small heat shock protein; SEC-MALS, size exclusion chromatography-multiangle light scattering; IPMDH, 3-isopropylmalate dehydrogenase from *Thermus thermophilus* HB8; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TEM, transmission electron microscopy.

* Corresponding author. Fax: +81-42-388-7479.
E-mail address: yohda@cc.tuat.ac.jp (M. Yohda).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.01.031

into smaller species, and can form high molecular weight complexes with a model substrate as the temperature increased [10,15].

One major characteristic of this protein family is the presence of a central α-crystallin domain, flanked by an N-terminal region and a C-terminal extension. The highest degree of amino-acid similarity is found within the α-crystallin domain, while the N-terminal region and the C-terminal extension are variable in length and sequence [8]. The N-terminal region appears to take a disordered structure in MjHsp16.5 [9]. In the crystal structure of the dimeric building block in TaHsp16.9, one N-terminal region is disordered and the other resolved to be the helices connected by a random coil [10]. From the finding of cryo-electron microscopy, it was suggested that the disordered N-terminal region is located inside the cavity [11]. Naturally occurring sHsps lacking these flanking regions exist as monomeric to tetrameric structures with poor or no chaperone activity [16,17]. A wide variety of truncations within the N-terminal region were reported to affect chaperone activity or complex formation [12,18–23]. However, the role of the N-terminal region in the chaperone activity and complex formation is poorly understood.

Recently, we have found that StHsp14.0, a sHsp from the thermoacidophilic crenarchaeon *Sulfolobus tokodaii* strain 7, binds unfolded proteins and prevents their thermal aggregation [24]. StHsp14.0 exists as a stable spherical oligomer, but subunit exchange between the oligomers was observed at elevated temperatures, which correlates well with the molecular chaperone activity [24].

In this study, we constructed N-terminal truncation mutants of StHsp14.0 and examined their characteristics. The results showed that the N-terminal region is required not for oligomerization but for the temperature-dependent complex dissociation and molecular chaperone activity.

Materials and methods

Enzymes and reagents. Ex *Taq* DNA polymerase and other reagents for gene manipulation were obtained from TAKARA Bio (Shiga, Japan). Citrate synthase (CS) from porcine heart was purchased from Sigma Chemical (MO). Wild-type StHsp14.0 and 3-isopropylmalate dehydrogenase from *Thermus thermophilus* HB8 (IPMDH) were expressed and purified as previously described [24].

Expression and purification of truncated StHsp14.0 variants. The N-terminal deletion mutants of StHsp14.0 (StHsp14.0ΔN8, StHsp14.0 ΔN15, and StHsp14.0ΔN21), whose N-terminal 8, 15, and 21 amino acids were replaced by a methionine residue, respectively, were expressed in *Escherichia coli* and purified.

The DNA fragments encoding truncated StHsp14.0 variants were amplified from the total DNA of *S. tokodaii* strain 7 by PCR using N-terminal primers (5′-GGG-AAT-TCC-ATA-TGA-AGA-GAA-GTG-AAG-AAC-3′, 5′-GGG-AAT-TCC-ATA-TGA-GAG-GTT-TCT-ATG-AGC-3′, and 5′-GGG-AAT-TCC-ATA-TGG-TCT-ATC-CAC-CAG-TTG-3′) in combination with a C-terminal primer (5′-GGG-ATC-CTC-ATT-CTA-TCT-TTA-TAA-CA-3′) and then subcloned into pT7Blue T vector. After sequence confirmation, the genes were excised with *Nde*I and *Bam*HI, and then introduced into the *Nde*I/*Bam*HI site of pET23a. The plasmids were introduced into *E. coli* BL21(DE3). All mutants were expressed in the soluble fraction of *E. coli*.

The soluble fractions of cells expressing StHsp14.0 variants were heated at 75 °C for 30 min to denature host proteins, and then the proteins were further purified by anion exchange and gel filtration columns using almost the same procedure as that for the wild type [24].

Size exclusion chromatography of StHsp14.0 variants. Size exclusion chromatography was performed using a TSKgel G4000SW_{XL} (Tosoh, Tokyo, Japan) with buffer (50 mM Tris–HCl, pH 7.3, 150 mM NaCl, and 1 mM dithiothreitol) at a flow rate of 0.8 ml/min with absorbance monitored at 280 nm. Standard proteins were obtained from Bio-Rad (CA).

Electron microscopy. The sample solution of StHsp14.0ΔN21 (0.1 mg/ml) or StHsp14.0 (0.1 mg/ml) was applied onto specimen grids covered with a thin carbon support film, which had been made hydrophilic by the ion spattering device (JEOL HDT-400), and then negatively stained with 1% uranyl acetate for 30 s. Images were recorded by making use of the slow scan CCD camera (Gatan Retractable Multiscan Camera) under low electron dose conditions at a magnification of 50,000× in the electron microscope (Philips Tecnai F20) operated at 120 kV. Images were analyzed on computers using Digital Micrograph. The electron microscopic specimen grids of both wild type and the mutant were prepared identically and examined with TEM at the same defocus value of −1.5 μm.

Size exclusion chromatography-multiangle light scattering. The purified sHsps complexes were analyzed using a size exclusion chromatography-multiangle light scattering (SEC-MALS) on Shodex PROTEIN KW-804 and KW-803 columns (Shoko, Tokyo, Japan) connected with a UV detector (Shodex UV-41, Shoko), a multiangle light scattering detector (Wyatt DAWN EOS), and a differential refractive index detector (Shodex RI-71, Shoko). A 50 mM Tris–HCl buffer (pH 7.3) containing 150 mM NaCl and 1 mM dithiothreitol was used as a mobile phase at 1.0 ml/min. An aliquot (100 μl) of sample was injected into the column. The molecular weight and protein concentration were determined according to the instruction manual [25].

Thermal aggregation measurements. The effect of the mutants of StHsp14.0 on the thermally induced aggregation of a 600 nM solution of IPMDH from *T. thermophilus* HB8 in 50 mM Tris–HCl (pH 8.0) buffer was monitored by measuring light scattering at 360 nm using a UV-1600PC spectrophotometer (Shimadzu Biotech, Kyoto, Japan) equipped with a thermostatted cell compartment preheated to 87 °C.

Labeling of StHsp14.0ΔN21 with fluorescent dye or biotin and measurements of subunit exchange. The labeling of StHsp14.0ΔN21 with a fluorescent dye or biotin probe was performed as described [24]. The labeling efficiency of Alexa Fluor 488 was estimated to be 0.3 mol/mol of subunit. That of biotin was calculated to be 1.7 and 1.9 mol of the wild and the mutant StHsp14.0 subunits, respectively.

To initiate the subunit exchange reaction, 3.6 μM (as monomer) each of fluorescence-labeled and biotin-labeled samples in buffer A (50 mM sodium phosphate, pH 7.5, 150 mM NaCl) was mixed together in a reaction tube at various temperatures for 20 min and cooled on ice. Magnetic beads covalently coupled with streptavidin (Dynabeads M-280 Streptavidin, DYNAL, Oslo, Norway) in an equivalent volume were added to the reaction solution. The suspension was kept on ice for 5 min with occasional pipeting. The magnetic beads were separated from the solution using a magnet, suspended in buffer A, and then transferred to a well on a microtiter plate. The fraction was subsequently excited at 532 nm, and the fluorescence emission cut-off below 526 nm by a filter was measured using a Typhoon 8600 (Amersham Bioscience).

Other methods. Proteins were analyzed by SDS–PAGE [26]. The separated proteins were visualized by staining with Coomassie brilliant blue R-250. The protein concentration was measured by the Bradford method with a protein assay kit (Bio-Rad) using bovine serum albumin as the standard [27].

Results

Construction of truncated StHsp14.0 derivatives

In order to study the role of the N-terminal region of StHsp14.0, we constructed a series of N-terminal truncated StHsp14.0 variants (StHsp14.0ΔN8, StHsp14.0 ΔN15, and StHsp14.0ΔN21) as described in "Materials and methods." In StHsp14.0ΔN21, approximately all the N-terminal region was eliminated. All StHsp14.0 variants were stable enough to remain soluble even after heat treatment at 75 °C for 30 min. The proteins were further purified to homogeneity and then analyzed by SDS–PAGE (data not shown).

Oligomeric structures of the N-terminal truncated StHsp14.0 variants

As previously demonstrated, wild-type StHsp14.0 exists as a spherical oligomer [24]. We examined the oligomeric characteristics of the truncated mutants by size exclusion chromatography. The results showed that the truncated mutants exist as oligomers similar to the wild-type StHsp14.0 (data not shown). The elution times were almost the same as that of the wild type in spite of the decreased molecular weight of the subunits.

Then, we determined the molecular weight of the oligomers of StHsp14.0 by SEC-MALS. StHsp14.0 exists as a uniform oligomer with a molecular weight of 349.3 kDa (Fig. 1A). Since the deduced molecular weight of the StHsp14.0 monomer is about 14.0 kDa, StHsp14.0 is a uniform complex of 24 subunits like other hyperthermophilic sHsps [3,9]. In spite of the elution time, the molecular weight of StHsp14.0ΔN21 was lower than that of the wild type and estimated to be 315.3 kDa (Fig. 1A). The elution time in gel filtration chromatography depends on the molecular configuration. Thus, deletion of the N-terminal region should cause a change not in the configuration of the oligomeric particle but in the interior of the particle.

Electron microscopy showed that StHsp14.0ΔN21, in which the N-terminal region was completely removed, existed as a spherical particle (Fig. 1B). The size of the particle was similar to that of wild-type StHsp14.0 [24]. The difference is the emergence of a cavity at around the center of the particle, which seems to correspond to the loss of the N-terminal region (Fig. 1C).

Thus, it is suggested that the N-terminal region of StHsp14.0 should be located inside of the StHsp14.0 spherical complex and that the region is not required for oligomerization.

The effect of N-terminal truncation on molecular chaperone activity and subunit exchange efficiency

StHsp14.0 possesses chaperone activity to prevent the thermal aggregation of IPMDH from the thermophilic bacterium *T. thermophilus* HB8 at around 90 °C [24]. We examined the effects of N-terminal truncations on the chaperone. StHsp14.0ΔN8 efficiently protected IPMDH

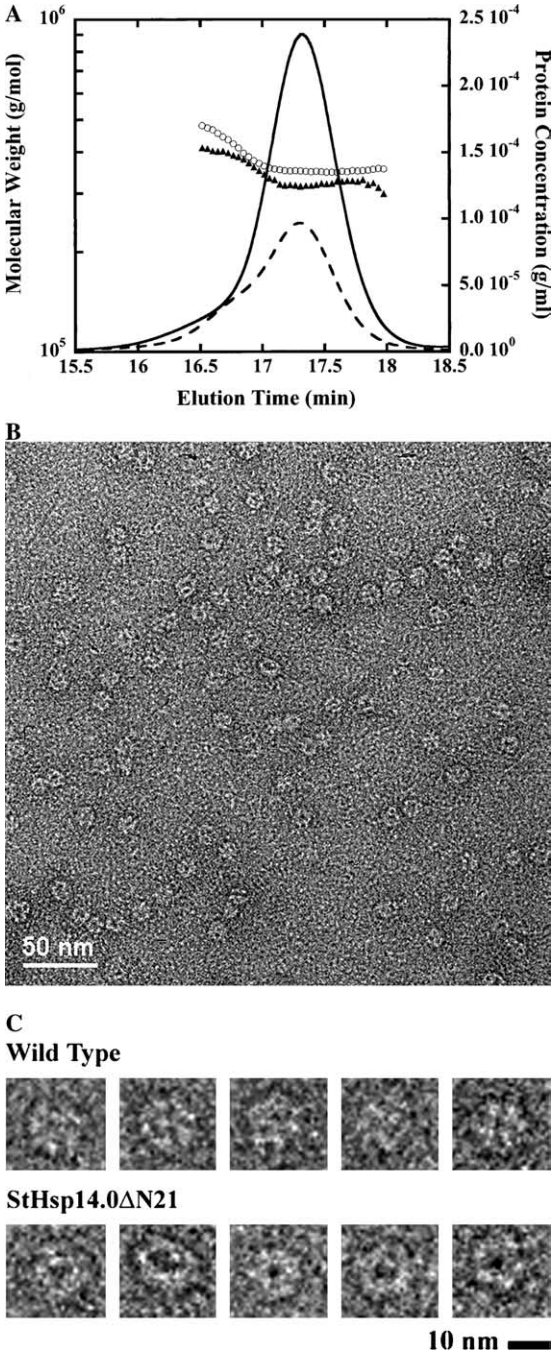

Fig. 1. Complex formation of N-terminal truncated StHsp14.0 variants. (A) The molecular weight and protein concentration were determined by SEC-MALS as described in "Materials and methods." The molecular weight of wild-type StHsp14.0 and StHsp14.0ΔN21 is indicated by open circles and closed triangles, respectively. The protein concentration of wild type and StHsp14.0ΔN21 is indicated by a solid line and dashed line, respectively. (B) Electron microscopic images of negatively stained StHsp14.0ΔN21. (C) Galleries of the enlarged electron micrographs of negatively stained single-particles typically seen for wild type and StHsp14.0ΔN21, respectively.

from thermally induced aggregation similar to wild-type StHsp14.0 (Fig. 2). However, StHsp14.0ΔN15 and StHsp14.0ΔN21 showed less chaperone activity (Fig. 2). Thus, the N-terminal region is likely to be required for the molecular chaperone activity.

In a previous report, subunit exchange between StHsp14.0 complexes and its relation with the molecular chaperone activity were demonstrated [24]. Then, we examined the subunit exchange of StHsp14.0ΔN21. Equimolar amounts of fluorescence-labeled StHsp14.0ΔN21 oligomers and biotin-labeled StHsp14.0ΔN21 oligomers were incubated at specified temperatures and captured with streptavidin-linked magnetic beads. The fluorescent intensity of the captured StHsp14.0ΔN21 gradually increased with the incubation temperature (Fig. 3A). However, whereas the subunit exchange efficiency of the wild type reached a maximum, of around 43% of the total intensity at 90 °C, that of StHsp14.0ΔN21 at 70, 80, and 90 °C was almost constant around 16% (Fig. 3A). These results suggested that StHsp14.0ΔN21 lost subunit exchange activity at elevated temperatures. These findings exhibit that the N-terminal region in StHsp14.0 is important for the exchange of subunits.

To confirm that the decrease in subunit exchange is not due to the instability of the complex at higher temperatures, we examined the oligomer of StHsp14.0ΔN21 after heat treatment at 90 °C by size exclusion chromatography. The elution profile of StHsp14.0ΔN21 did not change even after the heat treatment (Fig. 3B). There was also no significant change in CD spectra between before and after heat treatment (data not shown). These results suggest that StHsp14.0ΔN21 is thermally stable and the loss of subunit exchange activity is not due to the denaturation.

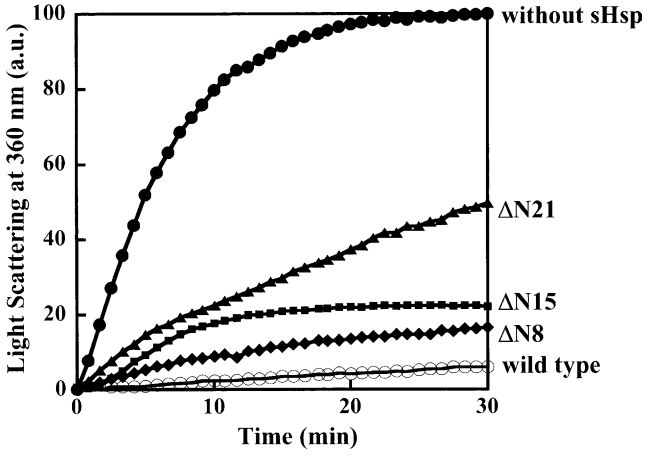

Fig. 2. Effect of N-terminal truncations on chaperone activity of StHsp14.0. IPMDH at 0.6 μM as a monomer was incubated at 87 °C with or without StHsp14.0 derivatives at 14.4 μM as a monomer. The aggregation of IPMDH was monitored by measuring apparent light scattering at 360 nm with a spectrophotometer.

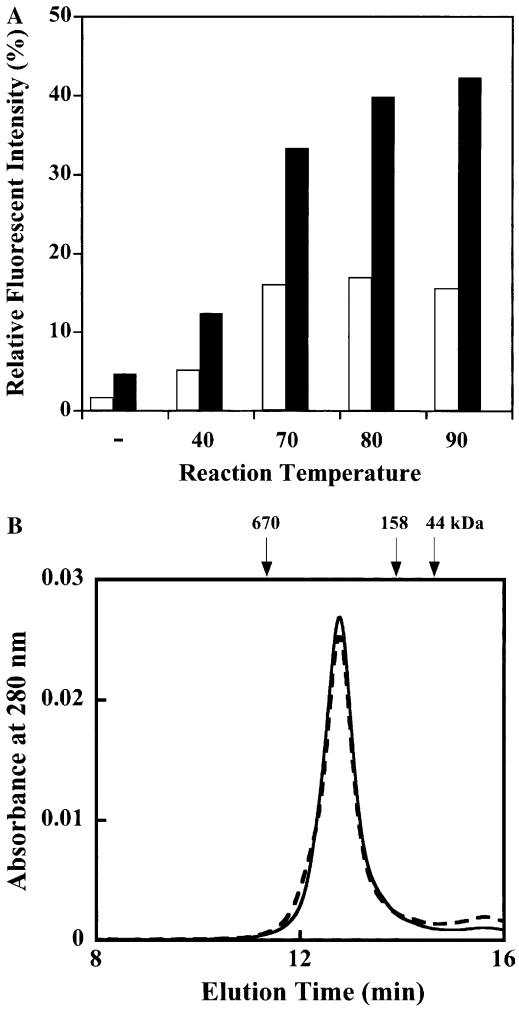

Fig. 3. Effect of temperature on subunit exchange and thermal stability of StHsp14.0ΔN21. (A) The subunit exchange reaction was performed with or without incubation at 45, 70, 80, or 90 °C for 20 min. The fluorescence intensity of the magnetic beads fraction was measured as described in "Materials and methods." The fluorescence intensities of the fluorescence-labeled StHsp14.0 and StHsp14.0ΔN21 captured by streptavidin-linked magnetic beads were designated in black and white bars, respectively. The percentage indicated is the ratio of the intensity of the magnetic beads fractions in the total intensity. (B) Size exclusion chromatography was performed using a TSKgel G4000SW$_{XL}$ column as described in "Materials and methods." StHsp14.0Δ21 was incubated for 20 min at 90 °C, cooled on ice for 5 min (dashed line) or not heat treated (solid line), and then centrifuged at 15,000*g* for 5 min at 4 °C. The supernatant was applied to the column. The column was standardized with the following markers as indicated above the figure: thyroglobulin (670 kDa); γ-globulin (158 kDa); and ovalbumin (44 kDa).

Discussion

SHsps contain a conserved α-crystallin domain and a non-conserved N-terminal region and C-terminal extension. We characterized N-terminal truncated StHsp14.0 variants. The truncated proteins formed oligomeric complexes indistinguishable from that of the wild type by size exclusion chromatography. SEC-MALS showed that

the wild-type StHsp14.0 exists as a 24 mer form. The molecular weight of the StHsp14.0ΔN21 oligomer was estimated to be a little smaller than that of the wild type. Although the molecular weight estimated by SEC-MALS was a little larger than 24 mer, we speculated that the deletion mutant formed a homologous complex with the wild type. In the electron microscopic images, StHsp14.0ΔN21 resembled the wild type except for a cavity in the oligomer. From the cryo-electron microscopic reconstruction of MjHsp16.5, the 32 disordered N-terminal residues that were not observed in the crystal structure extend into the central cavity of the assembly [11]. The six ordered N-terminal arms in the crystal structure of TaHsp16.9 were also located inside of the dodecamer [10]. Thus, we concluded that the N-terminal region of StHsp14.0 is also located in the center of the oligomeric particle.

The truncation mutants, especially StHsp14.0ΔN21, exhibited weak chaperone activity for the protection of IPMDH from thermal aggregation (Fig. 2). Although similar results have been reported for Hsp16-2 from *Caenorhabditis elegans* and sHsp from *Bradyrhizobium japonicum* [18,19], the role of the N-terminal region in the molecular chaperone activity could not be elucidated because of impairment of the oligomer formation.

As observed in our previous study, the molecular chaperone activity correlates with the subunit exchange efficiency [24]. The subunit exchange efficiency of StHsp14.0ΔN21 was found to be low, especially at elevated temperatures, compared with the wild type (Fig. 3A). Since the oligomeric structure of the mutant was not affected by heat treatment (Fig. 3B), the change in subunit exchange efficiency would not be due to instability or a problem with the reassembly, but to a decrease in the efficiency with which the oligomer is disassembled. The N-terminal region of sHsp is located in the center of the cavity and has a flexible conformation. Thus, the thermal fluctuation in the N-terminal region resulted in the disassembly of the oligomeric complex, which induced the molecular chaperone function of the sHsp.

The results are contradicting the previous observations with α-crystallin or other sHsps [12,18–23]. Among them, the deletion of the conserved RLFDQXFG sequence in the N-terminal region of α-crystallin reduced the higher order assembly and structural stability but increased the chaperone activity and rate of subunit exchange [23]. We think that the result should be caused by the instability of the mutant α-crystallin complex. The hydrophobic region of the sHsp is shielded from outside to suppress binding activity under unstressed condition. Thus, it is reasonable that the mutant exposing the hydrophobic region exhibits the higher chaperone activity. The subunit exchange activity should also be due to the non-specific interaction between the mutants. Another explanation is the difference between mammalian sHsp and archaeal sHsp. Although they are classified into sHsp family, they share only slight sequence homology in the α-crystallin region. Thus, it is also plausible that there is a difference in the role of N-terminal domain between them. Almost all of N-terminal deletion mutants of other sHsps also failed to assemble the native conformation [12,18–22]. Thus, we cannot exclude the importance of the N-terminal region in the assembly of the complex in other sHsps. Even though, the structural stability of StHsp14.0 and its N-terminal deletion mutants gave clear insights into the role of the region.

Acknowledgments

The work reported here is a part of the 21st Century COE (Center of Excellence) program of "Future Nano-Materials" research and education project, which is financially supported by the Ministry of Education, Science, Sports, Culture, and Technology through Tokyo University of Agriculture and Technology. This work was also supported by Grants-in-Aid for scientific research on priority areas (13033008 14037216, and 15032212), and a grant of the National Project on Protein Structural and Functional Analyses from the Ministry of Education, Science, Sports and Culture of Japan to M.Y.

References

[1] F. Narberhaus, Alpha-crystallin-type heat shock proteins: socializing minichaperones in the context of a multichaperone network, Microbiol. Mol. Biol. Rev. 66 (2002) 64–93.
[2] J. Horwitz, The function of alpha-crystallin in vision, Semin. Cell Dev. Biol. 11 (2000) 53–60.
[3] K. Usui, T. Yoshida, T. Maruyama, M. Yohda, Small heat shock protein of a hyperthermophilic Archaeum, *Thermococcus* sp. strain KS-1, exists as a spherical 24 mer and its expression is highly induced under heat-stress conditions, J. Biosci. Bioeng. 92 (2001) 161–166.
[4] J. Horwitz, Alpha-crystallin can function as a molecular chaperone, Proc. Natl. Acad. Sci. USA 89 (1992) 10449–10453.
[5] Z. Chang, T.P. Primm, J. Jakana, I.H. Lee, I. Serysheva, W. Chiu, H.F. Gilbert, F.A. Quiocho, *Mycobacterium tuberculosis* 16-kDa antigen (Hsp16.3) functions as an oligomeric structure in vitro to suppress thermal aggregation, J. Biol. Chem. 271 (1996) 7218–7223.
[6] P.J. Muchowski, J.I. Clark, ATP-enhanced molecular chaperone functions of the small heat shock protein human alphaB crystallin, Proc. Natl. Acad. Sci. USA 95 (1998) 1004–1009.
[7] R. Kim, K.K. Kim, H. Yokota, S.H. Kim, Small heat shock protein of *Methanococcus jannaschii*, a hyperthermophile, Proc. Natl. Acad. Sci. USA 95 (1998) 9129–9133.
[8] G.J. Caspers, J.A. Leunissen, W.W. de Jong, The expanding small heat-shock protein family, and structure predictions of the conserved alpha-crystallin domain, J. Mol. Evol. 40 (1995) 238–248.
[9] K.K. Kim, R. Kim, S.H. Kim, Crystal structure of a small heat-shock protein, Nature 394 (1998) 595–599.
[10] R.L.M. van Montfort, E. Basha, K.L. Friedrich, C. Slingsby, E. Vierling, Crystal structure and assembly of a eukaryotic small heat shock protein, Nat. Struct. Biol. 8 (2001) 1025–1030.
[11] D.A. Haley, M.P. Bova, Q.L. Huang, H.S. McHaourab, P.L. Stewart, Small heat-shock protein structures reveal a continuum from symmetric to variable assemblies, J. Mol. Biol. 298 (2000) 261–272.
[12] M.P. Bova, H.S. McHaourab, Y. Han, B.K. Fung, Subunit exchange of small heat shock proteins. Analysis of oligomer

formation of alphaA-crystallin and Hsp27 by fluorescence resonance energy transfer and site-directed truncations, J. Biol. Chem. 275 (2000) 1035–1042.
[13] M.P. Bova, Q. Huang, L. Ding, J. Horwitz, Subunit exchange, conformational stability, and chaperone-like function of the small heat shock protein 16.5 from *Methanococcus jannaschii*, J. Biol. Chem. 277 (2002) 38468–38475.
[14] G.J. Lee, A.M. Roseman, H.R. Saibil, E. Vierling, A small heat shock protein stably binds heat-denatured model substrates and can maintain a substrate in a folding-competent state, EMBO J. 16 (1997) 659–671.
[15] M. Haslbeck, S. Walke, T. Stromer, M. Ehrnsperger, H.E. White, S. Chen, H.R. Saibil, J. Buchner, Hsp26: a temperature-regulated chaperone, EMBO J. 18 (1999) 6744–6751.
[16] M.R. Leroux, B.J. Ma, G. Batelier, R. Melki, E.P. Candido, Unique structural features of a novel class of small heat shock proteins, J. Biol. Chem. 272 (1997) 12847–12853.
[17] B.P. Kokke, M.R. Leroux, E.P. Candido, W.C. Boelens, W.W. de Jong, *Caenorhabditis elegans* small heat-shock proteins Hsp12.2 and Hsp12.3 form tetramers and have no chaperone-like activity, FEBS Lett. 433 (1998) 228–232.
[18] M.R. Leroux, R. Melki, B. Gordon, G. Batelier, E.P. Candido, Structure–function studies on small heat shock protein oligomeric assembly and interaction with unfolded polypeptides, J. Biol. Chem. 272 (1997) 24646–24656.
[19] S. Studer, M. Obrist, N. Lentze, F. Narberhaus, A critical motif for oligomerization and chaperone activity of bacterial alpha-heat shock proteins, Eur. J. Biochem. 269 (2002) 3578–3586.
[20] R. Kim, L. Lai, H.H. Lee, G.W. Cheong, K.K. Kim, Z. Wu, H. Yokota, S. Marqusee, S.H. Kim, On the mechanism of chaperone activity of the small heat-shock protein of *Methanococcus jannaschii*, Proc. Natl. Acad. Sci. USA 100 (2003) 8151–8155.
[21] P. Laksanalamai, A. Jiemjit, Z. Bu, L. Maeder, F.T. Robb, Multisubunit assembly of the *Pyrococcus furiosus* small heat shock protein is essential for cellular protection at high temperature, Extremophiles 7 (2003) 79–83.
[22] J.C. Salerno, C.L. Eifert, K.M. Salerno, J.F. Koretz, Structural diversity in the small heat shock protein superfamily: control of aggregation by the N-terminal region, Protein Eng. 16 (2003) 847–851.
[23] S.Y. Pasta, B. Raman, T. Ramakrishna, M. Rao Ch, Role of the conserved SRLFDQFFG region of alpha-crystallin, a small heat shock protein. Effect on oligomeric size, subunit exchange, and chaperone-like activity, J. Biol. Chem. 278 (2003) 51159–51166.
[24] K. Usui, N. Ishii, Y. Kawarabayasi, M. Yohda, Expression and biochemical characterization of two small heat shock proteins from the thermoacidophilic crenarchaeon, *Sulfolobus tokodaii* strain 7, Protein Sci. 13 (2004) 134–144.
[25] P.J. Wyatt, Light scattering and the absolute characterization of macromolecules, Anal. Chim. Acta 272 (1993) 1–40.
[26] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[27] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Anal. Biochem. 72 (1976) 248–254.