Analysis of the Regulation of the Molecular Chaperone Hsp26 by Temperature-induced Dissociation

THE N-TERMINAL DOMAIN IS IMPORTANT FOR OLIGOMER ASSEMBLY AND THE BINDING OF UNFOLDING PROTEINS

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Small heat shock proteins (sHsps) are molecular chaperones that efficiently bind non-native proteins. All members of this family investigated so far are oligomeric complexes. For Hsp26, an sHsp from the cytosol of Saccharomyces cerevisiae, it has been shown that at elevated temperatures the 24-subunit complex dissociates into dimers. This dissociation seems to be required for the efficient interaction with unfolding proteins that results in the formation of large, regular complexes comprising Hsp26 and the non-native proteins. To gain insight into the molecular mechanism of this chaperone, we analyzed the dynamics and stability of the two oligomeric forms of Hsp 26 (i.e. the 24-mer and the dimer) in comparison to a construct lacking the N-terminal domain (Hsp26ΔN). Furthermore, we determined the stability of complexes between Hsp26 and non-native proteins. We show that the temperature-induced dissociation of Hsp26 into dimers is a completely reversible process that involves only a small change in energy. The unfolding of the dissociated Hsp26 dimer or Hsp26ΔN, which is a dimer, requires a much higher energy. Because Hsp26ΔN was inactive as a chaperone, these results imply that the N-terminal domain is of critical importance for both the association of Hsp26 with non-native proteins and the formation of large oligomeric complexes. Interestingly, complexes of Hsp26 with non-native proteins are significantly stabilized against dissociation compared with Hsp26 complexes. Taken together, our findings suggest that the quaternary structure of Hsp26 is determined by two elements, (i) weak, regulatory interactions required to form the shell of 24 subunits and (ii) a strong and stable dimerization of the C-terminal domain.

Molecular chaperones are a functionally related set of proteins whose basic property is to selectively recognize non-native proteins (1). Specifically, members of the groups of small heat shock proteins (sHsps) exhibit an astounding binding capacity for unfolded polypeptides (2–7). sHsps have been found in almost all organisms studied (8). Often, several members of this family are present in one cellular compartment, suggesting functional diversification (9–10).

sHsps are characterized by a conserved C-terminal α-crystallin domain and subunit masses between 15 and 40 kDa (2, 3, 5). This implies that the N-terminal parts are heterogeneous in size and sequence. A hallmark of sHsps is their tendency to assemble into large oligomeric complexes containing 9 to 24 monomers (11–14). There is ample evidence that oligomerization is a structural prerequisite for chaperone activity of the majority of sHsps (15–17). However, little is known about the correlation between structure and chaperone function in detail.

Some sHsps form assemblies with well-defined stoichiometries such as those from plants (14), whereas other sHsps, including the mammalian proteins, form a range of oligomeric sizes (12, 18). This polydispersity has limited the amount of structural information available. Two X-ray structures have been reported thus far for the hyperthermophilic archaeon Methanococcus janaschii Hsp16.5 (11) and for Hsp16.9 from wheat (14). The latter is composed of 12 subunits arranged into two hexameric rings. MJ/Hsp16.5 forms a hollow spherical complex of 24 subunits of octahedral symmetry with an inner diameter of 6.5 nm. In both structures, the α-crystallin domains form a dimeric building block with similar fold (4, 11, 14).

The dynamic exchange of subunits of the oligomeric complexes seems to be a common theme for sHsps (8, 19). The functional importance is not clear at present. An extreme case in this context is the sHsp Hsp26. Hsp26 is a cytosolic protein from Saccharomyces cerevisiae that exists as a hollow shell of 24 subunits under physiological conditions (20, 13). It has been shown to act as a chaperone and bind non-native proteins in a cooperative manner. Assays performed at different temperatures suggest that for efficient chaperone activity, the shell-like structure has to dissociate (13). This is achieved at heat shock temperatures (e.g. 43 °C). Here, the complex dissociates to a defined, dimeric species that binds non-native protein and then associates to large, homogeneous substrate-chaperone complexes. Electron microscopy revealed that this complex has a novel structure that is not based on the original shell-like structure of the Hsp26 oligomer (13).

A comparison of Hsp26 with Hsp25, a cytosolic sHsp from mouse, concerning the interaction with non-native proteins showed that the general chaperone properties are conserved although the mechanisms of activation are completely different (21). As mentioned above, Hsp26 requires dissociation for activation, whereas Hsp25 did not show any detectable dissociation. Nevertheless, the resulting complexes of non-native protein and sHsps are surprisingly similar in shape and size for a given non-native protein.

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1 The abbreviations used are: sHsp, small heat shock protein; Hsp26, sHsp from S. cerevisiae; Hsp25, sHsp from mouse; CD, circular dichroism, CS, citrate synthase; SEC, size exclusion chromatography.

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To gain further insight into the mechanism of Hsp26, we analyzed the association and dissociation reactions of Hsp26 and a variant lacking the N-terminal domain. We show that the quaternary structure of Hsp26 is regulated by two elements, a weak association of the 24-mer complex and a stable interaction of the dimeric building blocks.

**EXPERIMENTAL PROCEDURES**

**Materials**—Bovine serum albumin was obtained from Roche Applied Science. Bovine insulin and bovine Rhodanese were from Sigma.

Insulin was stored in 20 mM sodium phosphate, 0.1 M NaCl, pH 6.5, and Rhodanese in 50 mM Tris-HCl, 20 mM dithioerythritol, 50 mM sodium thiosulfate, pH 7.7. All protein concentrations in the text refer to monomers.

**Expression and Purification of CS**—Mitochondrial citrate synthase (CS) from pig heart (EC 4.1.3.7) was purified as described elsewhere (22). CS was stored in 50 mM Tris-HCl, 2 mM EDTA, pH 8.0.

**Expression and Purification of Hsp26**—The Hsp26-overexpressing yeast strain was a kind gift from Dr. S. Lindquist (Whitehead Institute, Boston, MA). Hsp26 was expressed and purified according to Haslbeck et al. (13). Hsp26 was stored in 40 mM Hepes/KOH, 50 mM NaCl, 1 mM EDTA, 1 mM dithioerythritol, pH 7.5.

**Expression and Purification of Hsp26ΔN—Hsp26ΔN** was amplified by PCR from genomic yeast DNA via 2×GAPDH (a primer pair TGACCGTGTCCTTAGTAGGG-CGGATCCTGATCCTCACTACCC-AAGGTAAAC-3′ and AGTTTGGACCATGACAACAACTA, TGACCATCTGCTTAGTTACCC-TGTAGGTTTCTCGGGATC). The PCR product was inserted into the BamHI site of pET28b (Novagen, Madison, WI), yielding plasmid pET28b-Hsp26. Bacteria were grown in Luria Bertani to A600 of 2.5 and induced with 2 mM isopropyl-β-D-galactopyranoside (IPTG). After 3.5 h, cells were harvested, washed once with buffer A (40 mM sodium phosphate, 150 mM NaCl, 10 mM imidazole, pH 7.5) and lysed with a BasicZ cell disrupter (Constant Systems, Warwick, UK). The cell lysate was centrifuged (46,000 g, 45 min, 4 °C), and the soluble extract was applied to a 1-mL nickel-nitrilotriacetic acid fast flow column (Qiagen, Hilden, Germany) equilibrated in buffer A and eluted with a linear gradient of 10–300 mM imidazol. Hsp26ΔN-containing fractions were pooled and further purified on a 26/10 Superdex 75-5 pg gel filtration column (Amersham Biosciences). After dialysis against buffer B (40 mM HEPES/KOH, 50 mM NaCl, 1 mM EDTA, pH 8.0), the protein was loaded on a 6-ml Resource Q ion-exchange column (Amersham Biosciences) and eluted with a linear gradient from 50–500 mM NaCl. Hsp26ΔN-containing fractions were pooled, dialyzed against buffer B, and concentrated by ultrafiltration. Hsp26ΔN was stored in 40 mM Hepes/KOH, 50 mM NaCl, 1 mM EDTA, pH 8.0. The correct size of Hsp26ΔN was confirmed by matrix-assisted laser desorption ionization mass spectrometry.

**Circular Dichroism Measurements—Far-UV circular dichroism (CD) measurements were performed in a J-715 spectropolarimeter with a PTC343 peltier unit (Jasco, Grossumstadt, Germany). The proteins were dialyzed overnight against 10 mM sodium phosphate, pH 7.5. If not indicated otherwise, spectra were recorded from 250–190 nm at a constant temperature of 20 °C in 0.1-cm quartz cuvettes. All spectra are base line-corrected, and molecular ellipticities were calculated according to the corresponding mean residue weight. The secondary structure of the respective proteins was calculated using the CD spectra deconvolution software CDNN (www.bioinformatik.biochemtech.uni-halle.de).

To monitor differences in the chemical stability of Hsp26 and Hsp26ΔN, urea unfolding transitions were performed. 0.25 mg/ml of the respective protein were incubated for 24 h at 25 °C in 40 mM Hepes/KOH, pH 7.5, containing different urea concentrations. For urea transitions at 43 °C, proteins were additionally incubated at 43 °C for 6 h.

To monitor the thermal unfolding transition of Hsp26 and Hsp26ΔN, the proteins were incubated in 10 mM sodium phosphate, pH 7.5, in 1-mm cuvettes at a concentration of 0.3 mg/ml. The heating rate was 30°/h, and the ellipticity at 220 nm was recorded. The unfolding transitions were analyzed according to Pace and Scholtz (23) using the Scientist software (Micromath).

**Aggregation Assays—**To induce aggregation, CS was diluted in 40 mM Hepes/KOH, pH 7.5, and equilibrated at 43 °C (24). Rhodanese (30 μM) was diluted 1:100 into 40 mM sodium phosphate, pH 7.7, at 44 °C (25). Assays were performed in the presence and absence of Hsp26 or Hsp26ΔN. Assays in the presence of bovine serum albumin served as a control for unspecified protein effects (concentrations given in the figure legends). To monitor the kinetics of thermal aggregation in the presence of Hsp26 and Hsp26ΔN, light scattering was measured in a FluoroMax
I (Spex, Edison, NJ) fluorescence spectrophotometer in stirred and thermostatted quartz cells. During the measurements, both the excitation and emission wavelength were set to 360 nm with a spectral bandwidth of 4 nm.

Size Exclusion Chromatography—To analyze the stability of Hsp26 substrate complexes, size exclusion HPLC (SEC) was performed using a TosoHaas TSK3000PW column (30 cm × 0.75 cm; separation range 0.5–500 kDa). Chromatography was carried out at 25 or 43 °C in 0.1 M Hepes/KOH, 150 mM KCl, pH 8.0, with a flow rate of 0.75 ml/min. The sample volume was 100 µl. Hsp26 was detected by fluorescence at an excitation wavelength of 280 nm and an emission wavelength of 323 nm using a Jasco FP 920 fluorescence detector (Jasco).

To analyze the stability of the Hsp26 oligomer in contrast to Hsp26-substrate complexes at different urea concentrations, Hsp26 alone, Hsp26-CS or Hsp26-Rhodanese complexes were incubated at increasing urea concentrations (0–3 M) as described in the figure legends and separated by SEC at the respective urea concentration with a flow rate of 0.5 ml/min. To analyze the dissociation further, the peak area of the complex in the absence of urea was determined using the Sigma Plot software and set to 100%. The peak areas in the presence of urea are expressed in comparison to the value obtained in the absence of urea.

Analytical Ultracentrifugation—Determination of the native molecular weight of Hsp26ΔN was performed using a Beckman XL-1 analytical ultracentrifuge and a Beckman Ti-60 rotor equipped with a UV/Vis detector. Centrifugation was performed at 4 °C and 22,500 rpm until the sedimentation and 22,500 rpm until the sedimentation equilibrium was reached. Complete protease inhibitors (Roche Applied Science) were added to avoid proteolytic degradation. Protein concentrations were varied between 0.2 mg/ml and 0.8 mg/ml in a buffer containing 40 mM Hepes/KOH, 150 mM KCl, 1 mM EDTA, pH 7.5. Data analysis followed the one-species model included in the Origin software package, available from Beckman. As shown in Equation 1,

\[
c = c_0 \frac{e^{-\omega T} - e^{-\omega r}}{1 - e^{-\omega r}} + \text{base line (Eq. 1)}
\]

where \(c_0\) equals the signal at the reference radius \(r_0\), \(\omega\) is the angular velocity, \(R\) the gas constant, \(T\) the temperature in Kelvin, and \(M\) the molecular mass. \(\rho\) was assumed to be \(\rho = 1.015 \text{ g/ml}\), and the specific volume of the protein was 0.74 ml/g. The fit of \(c\) (detection signal) against \(r\) (rotation radius) resulted in values for the molecular mass \(M\) that were found to be independent of the detection system used. Generally the fits agreed very well with the one-species model.

RESULTS

Structural and Functional Analysis of Hsp26 and Its Isolated C-terminal Domain Hsp26ΔN—Hsp26 forms a globular complex of 24 identical subunits under physiological conditions. At higher temperatures, the complex dissociates to dimers and becomes active as a chaperone (13). This would imply that the underlying mechanism, we created a fragment of Hsp26 comprising the C-terminal-conserved α-crystallin domain, Hsp26ΔN (Fig. 1) with a view to compare its structure and stability to that of the full-length protein. Hsp26ΔN was produced in E. coli and purified to homogeneity. The analysis of the secondary structure of this fragment by CD spectroscopy showed a minimum at 204 nm with an ellipticity of \(-8,500 \theta\) (Fig. 2A). In comparison, the minimum of Hsp26 is at 216 nm and 8,000 \(\theta\). The analysis of the secondary structure elements indicated for both proteins a large amount of \(\beta\)-sheet structure (around 45%) and a low \(\alpha\)-helix content (18 and 12% for Hsp26 and Hsp26ΔN, respectively). This is consistent with the notion that the C-terminal α-crystallin domain is predominantly a \(\beta\)-sheet structure (27), whereas the N-terminal domain contains \(\alpha\)-helical elements.

To determine the quaternary structure of Hsp26ΔN, analytical ultracentrifugation was employed. The equilibrium sedimentation analysis of Hsp26ΔN resulted in a native molecular mass of 27,500 ± 1,500 Da, indicating that the fragment represents a dimer (Fig. 2B).

We had shown previously that Hsp26 efficiently prevents the aggregation of CS at 43 °C (13, 21 and Fig. 2C). Next, we were interested whether Hsp26ΔN exhibits chaperone activity, using CS as a model substrate. Hsp26ΔN, however, did not show any influence on CS aggregation even when added at a 30-fold excess (Fig. 2C). Furthermore, again in contrast to the full-length protein, complexes between the Hsp26ΔN and CS could not be detected by SEC or electron microscopy (data not shown). Thus, we conclude that although Hsp26ΔN is in the same oligomeric state as the dissociated full-length Hsp26, it is not able to perform detectable chaperone function.

Stability of Hsp26 and Hsp26ΔN—To analyze the intrinsic stability of full-length Hsp26 and Hsp26ΔN, we performed thermal unfolding experiments monitored by CD spectroscopy (Fig. 3). The CD spectra recorded at different temperatures showed that at 43 °C Hsp26 exhibits a spectrum significantly different from that recorded at 25 °C (Fig. 3A). The amplitude between 235 and 210 nm decreased, and the shape of the spectrum changed. For Hsp26ΔN, the spectra recorded at 25 °C remained unaltered, indicating that Hsp26ΔN is thermodynamically more stable than the full-length protein.
and 43 °C are essentially identical (Fig. 3B). For both proteins the spectra at 80 °C are very similar (Fig. 3, A and B). They indicate a loss of structure although the proteins did not seem to be completely unfolded. There was no visible aggregation, but unfolding at 80 °C was irreversible (see below). To determine the temperature transition of the proteins, the samples were heated at a constant rate and the signal at 220 nm was recorded (Fig. 3C). For Hsp26, two unfolding transitions could be detected. The first transition started at 29 °C and had a midpoint at 35 °C. The temperature range in which this transition occurred corresponds to that required for dissociation of the large oligomer. Above 43 °C, the signal reached a plateau until 70 °C. The second transition was between 70 and 80 °C with a midpoint at 77 °C. The analysis of the first transition of Hsp26 resulted in a stabilization energy at 25 °C of 4.3 ± 0.6 kJ/mol. Because the thermal transition observed above 70 °C was irreversible, no quantitative analysis was performed for this unfolding process.

For Hsp26ΔN, a completely different picture emerged (Fig. 3C). The transition in the heat shock region was absent. Only one transition in the high temperature range, 55–80 °C, with a midpoint of 70 °C was observed. Here again thermal unfolding proved to be irreversible (Fig. 3C). Taken together, these experiments suggest that structural changes in the heat shock temperature range that are absent in Hsp26ΔN are associated with the N-terminal domain. Hsp26ΔN reveals a remarkable stability against thermal unfolding that corresponds to the second transition of full-length Hsp26.

Next, we analyzed the stability of Hsp26 and Hsp26ΔN against unfolding by urea (Fig. 4). When the unfolding transitions were monitored by CD spectroscopy at 25 °C, Hsp26 exhibited two unfolding transitions (Fig. 4A). The first started at the lowest urea concentration tested and exhibited a midpoint at 0.4 M urea. The second transition was from 3 to 6 M urea and had a midpoint at 4.2 M urea. Hsp26ΔN, in contrast, showed only one transition between 3 and 6 M urea, with a midpoint at 4.2 M urea (Fig. 4A). In contrast to the heat-induced unfolding, the urea-induced unfolding was completely reversible.

A quantitative analysis to determine the corresponding stabilization energies resulted in values of 5.5 ± 1.1 kJ/mol and 13.3 ± 1.5 kJ/mol for the first and second transition of Hsp26, respectively, and 11.4 ± 1.2 kJ/mol for the single transition of Hsp26ΔN. The values of the stabilization energies for Hsp26ΔN and the second transition of Hsp26 indicate that the observed structural changes of the proteins are similar. To confirm this with a second set of experiments, we determined the stability of the two proteins at 43 °C, where both are in their dimeric forms (Fig. 4B). Again, the urea-induced unfolding at 43 °C of both proteins was completely reversible. Here, the transition of Hsp26ΔN is almost identical to the transition at 25 °C, resulting in stabilization energies of 14.9 ± 2.5 kJ/mol. In contrast, the unfolding of Hsp26 changed dramatically between 25 and 43 °C. Although the first transition was completely absent, the second transition occurred still at about 4 M with a stabilization energy of 19.8 ± 2.9 kJ/mol, which is even slightly more stable than the corresponding transition at 25 °C. Thus the urea-induced unfolding transitions confirmed that the unfolding process of Hsp26 is a two-step process at room temperature and a one-step process at heat shock temperatures. Furthermore, the experiments showed that the stability of Hsp26ΔN corresponds to that of the dissociated species of Hsp26 at 43 °C.

Stability of Hsp26 Substrate Complexes—A striking feature of the chaperone mechanism of Hsp26 is its ability to form large, well-defined complexes with substrates (13, 21). Analysis of electron micrographs suggested that the structural organization of the substrate complexes is different from that of the Hsp26-24-mers. The finding that substrate complexes can be isolated by SEC already suggested a stable interaction (13, 21). In the context of this study, we were interested to compare the stability of the substrate complexes with that of the native Hsp26 complex. To this end, we incubated the Hsp26 complex or the Hsp26-substrate complex with increasing concentrations of urea and analyzed the complex by SEC. The column was pre-equilibrated at the respective urea concentrations of the samples.

The analysis of the Hsp26 complex showed that it already started to dissociate at low urea concentrations, consistent with the results obtained for the unfolding transitions monitored by CD spectroscopy (Fig 5, A and B and compare Fig 4A). The remaining complex peak did not change its elution behavior. Dissociation was complete at urea concentrations higher than 0.5 M.

In the absence of urea, the Hsp26-substrate complexes eluted at the position expected (21). When the samples were incubated in the presence of urea, a decrease in the fluorescence intensity of this peak was observed; concomitantly, peaks at the positions of the components of the substrate complex appeared. No intermediate-sized peaks were observed. This behavior allows determination of the stability of the complex by plotting the fluorescence signal of the substrate complex versus urea concentration.

![Graph A](image1.png)

![Graph B](image2.png)

**Fig. 4. Urea-induced unfolding transitions of Hsp26 and Hsp26ΔN.** A, far-UV-CD unfolding transitions of Hsp26 at 25 °C (○) and 43 °C (▲). Refolding transitions of Hsp26 at 25 °C (▲) and 43 °C (■). B, far-UV-CD unfolding transitions of Hsp26ΔN at 25 °C (○) and 43 °C (▲). Refolding transitions of Hsp26ΔN at 25 °C (▲) and 43 °C (■). Proteins were incubated in 40 mM Hepes/KOH, pH 7.5, and increasing urea concentrations (0–8.5 M) for 20 h at 25 °C. The change in signal was calculated from the mean value ellipticity at 222 nm. For urea transitions at 43 °C, proteins were additionally incubated at 43 °C for 6 h. For refolding assays, denatured samples of Hsp26 were diluted into 40 mM Hepes/KOH, pH 7.5, containing the indicated urea concentrations and incubated for 20 h at 25 or 43 °C. The stabilization energy was calculated as described under Experimental Procedures.
For the Hsp26-CS complex, dissociation started at the lowest urea concentration used (Fig. 5, A and C). At 0.5 M urea, however, 20% of substrate complexes were still present. To dissociate the complexes completely, 2 M urea were required. To test whether the stabilization of the Hsp26-substrate complex compared with the Hsp26 complex is a specific property of the...
CS complex, we performed the experiment with Rhodanese, another protein that had been shown previously to form well-defined complexes with Hsp26 (21). The stabilization of this complex was even more dramatic than that of the Hsp26-CS complex (Fig. 5, A and D). Up to 0.5 M urea, 90% of the substrate complexes were stable. At higher urea concentrations, the amount of complexes decreased slightly. However, at 2 M urea, 65% of complexes could still be detected. Thus, the complex between substrate protein and Hsp26 is intrinsically more stable than the Hsp26 complex.

**DISCUSSION**

sHsps differ from other molecular chaperones in several aspects. The most important are the efficient binding of several non-native polypeptide chains in one chaperone complex and the dynamics of the quaternary structure (3, 8).

A key feature of Hsp26 is the existence of two distinct and well-defined oligomeric states. The 24-mer is a globular sphere that exists at physiological temperatures and is largely inactive as a chaperone (13). The dimer is the stable structure at higher temperature. The interconversion between the two species is completely reversible. For other sHsps, a defined transition between two oligomeric states has not been observed yet. However, in many cases the large oligomer exhibited unusual dynamic properties that seem to allow the association and dissociation of single subunits without apparent changes in the size of the oligomer. This has been demonstrated for α-crystallin, several sHsps from *Bradyrhizobium japonicum*, PsHsp18.1 and TaHsp16.9 from plants, and Hsp16.5 from *M. janaschii* (28, 14, 29). It is therefore reasonable to assume that this dynamic behavior is generally required for the chaperone function of sHsps. Which domains are involved in these processes is not clear yet (16, 30–34).

To analyze which parts of Hsp26 are responsible for association and chaperone activity, we created a variant consisting of the α-crystallin domain of Hsp26 and the C-terminal extension. This construct turned out to be dimeric and lacks chaperone activity. Therefore it can be concluded that regions that are both important for the assembly of the 24-mer and for the interaction with non-native proteins reside in the N-terminal part of the protein. This view is supported by thermal unfolding experiments that show that full-length Hsp26 exhibits two transitions, one in the heat shock temperature range and one at much higher temperatures. Only the high temperature transition is observed in the case of Hsp26ΔN. Furthermore, these results suggest that less energy is required for the dissociation of the 24-mer than for dissociation and unfolding of the dimer. Quantitative data for the changes in energy involved in these processes were obtained by analyzing urea-induced unfolding transitions at 25 and 43 °C. These data are in agreement with the thermal unfolding transitions and support the hypothesis of a thermolabile oligomeric assembly of Hsp26 that dissociates to stable dimers at elevated temperatures. For Hsp26ΔN, this transition was not observed, suggesting that the N-terminal domains of Hsp26 are involved in these processes. Thus, there are two contributions to the quaternary structure of Hsp26: the 24-mer as a regulatory component that allows changing the activity by dissociation and the dimer as a basic building block of high stability.

In summary, the following picture emerges for the structural organization of Hsp26 (Fig. 6). The stability of the dimeric species (Hsp26ΔN and Hsp26 after dissociation) is not influenced significantly by temperature shifts between 25 and 43 °C. This is deduced from the basically identical urea transitions for the unfolding of the dimer at these temperatures. In contrast, the energy of the oligomer is very sensitive to temperature, as can be seen from the dissociation of the oligomer at higher temperatures and the lack of the first phase of the urea transition at 25 °C. Because the first transition was found to be reversible in all cases, we performed quantitative analysis and obtained a stabilization energy of the oligomer at 25 °C of about 5 kJ/mol.

The strong dependence of the reaction pathway on temperature for the interconversion of dimeric and oligomeric species and the observed dissociation of oligomeric species at higher temperatures suggest that the primary driving force for the dissociation of the oligomeric complexes may be of entropic nature, i.e. a gain of entropy during formation of dimers, potentially because the N-terminal domains become unstructured.

In principle, complexes of Hsp26 with non-native proteins can be regarded as a third oligomeric species of Hsp26. We had shown previously that the morphology of these complexes depends on the nature of the non-native protein incorporated in the complex (21). We assume that parts of Hsp26 that, at lower temperature, are involved in contacts stabilizing the 24-mer participate in the interaction with non-native protein at higher temperature in a way that allows cooperative binding of unfolding proteins and the formation of regular complexes. In the presence of urea, the sHsp-substrate complexes also appear to dissociate in a cooperative reaction because no intermediate-sized species were detected. Surprisingly, the stability of the complexes between Hsp26 and non-native CS or Rhodanese was found to be much higher than that of the Hsp26 complex alone. The stability seems to depend on the non-native protein.
bound in the complex, suggesting that the affinity of Hsp26 for a given protein is a decisive factor in this context. Substrate recognition of sHsps may involve charged and hydrophobic amino acids (5, 14).

Taken together, our data suggest that in the case of Hsp26, the N-terminal domain is of critical importance both for the assembly of the 24-mer from dimers and for the interaction with non-native proteins, including the formation of the chaperone-substrate complex. It is tempting to speculate that some of the weak interactions between the dimers in the Hsp26 complex are replaced by stronger chaperone-substrate interactions. How exactly this results in the cooperative formation of defined complexes remains to be seen.

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