Model Peptide Studies Demonstrate That Amphipathic Secondary Structures Can Be Recognized by the Chaperonin GroEL (cpn60)*

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The molecular chaperone cpn60 binds many unfolded proteins and facilitates their proper folding. Synthetic peptides have been used to probe the question of how cpn60 might recognize such a diverse set of unfolded proteins. Three hybrid peptides were synthesized encompassing portions of the bee venom peptide, apamin, and the sequence KWLAESVRAGK from an amphipathic helix in the NH2-terminal region of bovine rhodanese. Two disulfides connecting cysteine residues hold the peptides in stable helical conformations with unobstructed faces oriented away from the disulfides. Peptides were designed to present either a hydrophobic or hydrophilic face of the amphipathic helix that is similar to the one near the amino terminus of rhodanese. Aggregation of these peptides was detected by measuring 1,1'-biss(4-anilino)naphthalene-5,5'-disulfonic acid (bisANS) fluorescence at increasing peptide concentrations, and aggregation was not apparent below 2 μM. Thus, all experiments with the peptides were performed at a concentration of 5 μM. Reducing agents cause these helical peptides to form random coils. Fluorescence anisotropy measurements of fluorescein-labeled peptide with the exposed hydrophobic face yielded a $K_d = 106 \mu M$ for binding to cpn60, whereas there was no detectable binding of the reduced form. The peptide with the exposed hydrophilic face did not bind to cpn60 in either the oxidized or reduced states. Fluorescence experiments utilizing bisANS as a probe showed that binding of the helical hydrophobic peptide could induce the exposure of hydrophobic surfaces on cpn60, whereas the same peptide in its random coil form had no effect. Thus, binding to cpn60 is favored by a secondary structure that organizes and exposes a hydrophobic surface, a feature found in amphipathic helices. Further, the binding of a hydrophobic surface to cpn60 can induce further exposure of complementary surfaces on cpn60 complexes, thus amplifying interactions available for target proteins.

It has been accepted for some time that the amino acid sequence of a protein contains all the information to specify its tertiary conformation (1). However, in vitro refolding is often inefficient due to competing kinetic pathways that lead to aggregation or the formation of non-native conformations (2). It is believed that aggregation is a result of the association of hydrophobic surfaces that are normally hidden in the core of a folded protein but are exposed in partially folded states. The yields of properly folded protein in these in vitro experiments can be improved by carefully selecting conditions such as lower temperature (3), lower protein concentration (4), or the addition of “nondenaturing” detergents (5), all of which favor folding. It is of interest to consider how aggregation can be avoided in vivo, where temperatures remain relatively constant and high local concentrations of interactive nascent polypeptides are often present.

Molecular chaperones are a group of cellular proteins known to be mediators of in vivo folding. Molecular chaperone proteins are mainly found in one of three highly conserved groups, Hsp60, Hsp70, and Hsp90, ranging from prokaryotes to mammals. Some molecular chaperones are expressed during heat shock, whereas others are expressed constitutively and are necessary for cell survival at nonstressed physiological conditions (6). A characteristic property of molecular chaperones is that they can bind non-native forms of proteins and release them in a controlled manner.

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was a random coil in solution, appeared when bound to adopt a helical conformation. It was conjectured that the sequence that could form an amphipathic helix might bind along the hydrophobic face. A second example, the capture of an all β-sheet Fab fragment by cpn60 (16), indicates that the specific secondary structure is not the determinant for the recognition by cpn60.

To investigate the structural and chemical nature of cpn60-substrate interactions, we chose to study constrained peptides. Apamin is a peptide component of bee venom that contains 18 residues with two disulfide bonds bridging Cys3 to Cys15. These disulfides lock the COOH-terminal half of apamin (residues 9–16) into a helical conformation (18, 35).

The NH2-terminal sequence of bovine rhodanese inserted into the apamin COOH terminus (KWLAESVRAGK), which has been shown to bind in a helical conformation to cpn60. These peptides were then tested for their ability to bind to cpn60. The peptides have the following sequences: C1NC3K(FITC)APETALC11 (APA08), C1NC3KAPETALC11, WLA15SVRAGK-NH2, C1NC3KAPETALC11, WLA15SVRAGK-NH2 (APA09), and C1NC3K(FITC)APETKWC11AES15RAGK-NH2 (APA14), with bold letters representing the rhodanese amino acids. The acronyms APA09 and APA14 are used in describing the hydrophobic peptides, and APA08 is used in describing the hydrophilic peptide. APA09 and APA08 were both synthesized with a FITC-labeled lysine at residue 4. Using these peptides, we demonstrate that an amphipathic α-helix with hydrophobic residues on its exposed helical face can bind to cpn60, and this binding can lead to exposure of hydrophobic surfaces buried within cpn60.

**EXPERIMENTAL PROCEDURES**

**Reagents and Proteins—**All reagents were analytical grade. 1,1'-Bis[4-anilino]naphthalene-5,5'-disulfonic acid (bisANS) was obtained from Molecular Probes (Junction City, OR). The chaperonin, cpn60, was purified from lysates of cells containing the multicycoplasmid pGroE5L (20). Following purification, cpn60 was dialyzed against 50 mM Tris-Cl, pH 7.5, containing 1 mM DTT. Then glycerol was added to 10% (v/v), and aliquots were frozen in liquid nitrogen and stored at −80°C. The monomer concentration of cpn60 was measured at 280 nm with an extinction coefficient of 1.22 × 10^3 M^-1 cm^-1 as determined by quantitative amino acid analysis, assuming a molar mass of 60 kDa (21).

**Preparation of Peptides—**The peptides were synthesized on para-

methyl benzhydridamine resin utilizing t-butoxycarbonyl chemistry with rapid manual cycles as described previously (22). The peptides were prepared with t-butoxycarbonyl-Lys (Fmoc) in the lysine 4 position. The Fmoc was removed with 20% piperidine in DMA after step-wise synthesis and prior to hydrogen fluoride cleavage. Fluorescein was incorporated into APA08 and APA09 by the addition of 4-fold excess FITC and diisopropylethylamine in DMA. The final t-butoxycarbonyl group was removed, the peptides were cleaved from the resin, and the side chain protecting groups were removed by treatment with anhydrous lithium hydrogen fluoride at 0°C for 1 h (10 ml of hydrogen fluoride, 1.0 ml of anisole, 0.2 ml of ethyl methylsulfide, 0.1 ml of thioanisole, 0.1 g of para-thiocresol/g of resin). The resultant peptide was dialyzed against 50 mM Tris-Cl, pH 7.5, containing 1 mM DTT, and the pH was adjusted to 7.5 by addition of 6.6 M NaOH; 50 mM CD3CN (final concentration, 1.9 M) was added to improve solubility. All spectra were acquired at 25°C on a Bruker AMX-500 spectrometer. Two-dimensional phase sensitive correlation spectroscopy (24), NOE spectroscopy (25–27), jump-return NOE spectroscopy (28), and total correlation spectroscopy (29, 30) spectra were acquired with low power coherent irradiation of the water resonance for 1.5 s prior to the pulse sequence and during the NOE spectroscopy mixing time. Total correlation spectroscopy mixing was achieved with a clean DIPSI-2rc sequence applied for 90 ms (31), and mixing times of 300 ms were used in both NOE spectroscopy experiments. The spectra were processed and analyzed using Felix (Biovyn Technologies, San Diego, CA), and assigned using established techniques (32). The NH-NH, NH-NH, and NH-CO coupling interactions were identified by using J-resolved spectra obtained with line fitting of the correlation spectroscopy spectrum processed with very high digital resolution. A second set of NMR spectra were acquired after the addition of 10 μM of 2 μM DTT to allow the uncyclized (reduced) peptide to be studied. A rotating frame Overhauser spectroscopy spectrum (33) of the uncyclized peptide failed to identify any additional dipolar coupling interaction.

**Fluorescence Spectroscopy—**Fluorescence of bisANS was measured using an excitation wavelength of 397 nm and an emission wavelength of 500 nm. Anisotropy measurements on APA08 and APA09 peptides, each of which had Lys4 labeled with fluorescein, were performed with an excitation wavelength of 492 nm and emission wavelength at 519 nm. Fluorescence studies were carried out on a SLM model 500C fluorometer with an excitation slit width of 2.5 nm and emission slit width of 10 nm.

**Pepptide Binding Measurements—**Binding experiments were carried out on both APA08 and APA09 peptides in the oxidized and reduced states as follows. To a cuvette containing 50 mM Tris-Cl, pH 7.8, a given peptide was added to a concentration of 1 μM, and the anisotropy values were recorded at 20°C. To this solution, cpn60 that had been dialyzed against 50 mM Tris-Cl, pH 7.8, was added to the cuvette to a final concentration of 10 μM, and the anisotropy value was measured.

**Peptide Sequences—**The hydrophobic peptide APA08 has the sequence C1NC3K(FITC)APETALC11, and APA09 and APA14 have the sequences C1NC3KAPETALC11, WLA15SVRAGK-NH2, and C1NC3KAPETALC11, WLA15SVRAGK-NH2, respectively. The bold characters in the above sequences represent the NH2-terminal portions of bovine rhodanese inserted into the apamin peptide. Disulfide bonds form between cysteine residues 11 and 12 and between cysteine residues 13 and 15 in the oxidized form of each peptide. The cyclized (oxidized) peptides are designated as APA08c, APA08r, and APA14c, with the lowercase c denoting the peptides in the oxidized form. The linear (reduced) peptides are designated as APA08r, APA09r, and APA14r, with the lowercase r denoting the peptides in the reduced form.

**Circular Dichroism Measurements—**Each peptide was reconstituted in 5 mM phosphate, pH 7.5, and placed in a 0.05-cm pathlength quartz cell. All samples were filtered. The final concentrations were determined by absorbance measurements. The concentrations of each peptide were as follows: 0.165 mg/ml for APA08 cyclized, 0.020 and 0.031 mg/ml for APA09 linear and cyclized, respectively, and 0.30 mg/ml for APA14 linear and cyclized. The samples were analyzed with an Aviv Circular Dichroism Spectrometer (model DS/60, Aviv Associates, New York, NY) using a constant bandwidth of 1.0 nm. Spectra were taken from 190 to 250 nm at 0.2-nm intervals with an averaging time of 3.0 s/data point. All spectra were acquired in triplicate and averaged. The spectrum of an appropriate buffer control sample was then subtracted from each of the sample spectra. The final spectral data were converted to mean residue weight ellipticities for determination of secondary structure content (mean residue weight factors: APA08, 129.9; APA09, 124.1; and APA14, 105.6). The mean residue weight ellipticities were used to calculate the relative contribution of secondary structural components (α-helix and β-sheet) to the overall spectra. The spectral deconvolution method used for these calculations has been described previously (23).

**NMR—**The peptide APA14 (2.0 mg) was dissolved in 440 μl of 95% H2O/D2O, and the pH was adjusted to 6.8 by microliter additions of 1 μl of NaOH; 50 mM of CD3CN (final concentration, 1.9 M) was added to improve solubility. All spectra were acquired at 25°C on a Bruker AMX-500 spectrometer. Two-dimensional phase sensitive correlation spectroscopy (24), NOE spectroscopy (25–27), jump-return NOE spectroscopy (28), and total correlation spectroscopy (29, 30) spectra were acquired with low power coherent irradiation of the water resonance for 1.5 s prior to the pulse sequence and during the NOE spectroscopy mixing time. Total correlation spectroscopy mixing was achieved with a clean DIPSI-2rc sequence applied for 90 ms (31), and mixing times of 300 ms were used in both NOE spectroscopy experiments. The spectra were processed and analyzed using Felix (Biovyn Technologies, San Diego, CA), and assigned using established techniques (32). The NH-NH, NH-NH, and NH-CO coupling interactions were identified by using J-resolved spectra obtained with line fitting of the correlation spectroscopy spectrum processed with very high digital resolution. A second set of NMR spectra were acquired after the addition of 10 μM of 2 μM DTT to allow the uncyclized (reduced) peptide to be studied. A rotating frame Overhauser spectroscopy spectrum (33) of the uncyclized peptide failed to identify any additional dipolar coupling interaction.

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A dissociation constant was estimated for oxidized APA09, which was the only fluorescently labeled peptide that displayed detectable binding to cpn60. The approach used was to titrate a 1 mM solution of the oxidized APA09 peptide with increasing amounts of cpn60. Titrations using increasing peptide concentrations were not possible because of strong self-association, as expected with amphipathic structures and demonstrated with these peptides (see "Results"). The dissociation constant was estimated as follows:

$$K_d = \frac{[C][F]}{[CF]}$$  \hspace{1cm} (Eq. 1)

where [C], [F], and [CF] are the concentrations of binary complexes of cpn60, APA09, and cpn60-APA09, respectively. If only the fluorophore-protein complexes ([CF]) result in significant anisotropy (r), then at a fixed total concentration of fluorophore ([F]T) and increasing concentrations of protein ([CT]), the anisotropy of bound (r_bound) can be determined from a plot of 1/r_bound versus 1/[CT], in which the y intercept is 1/r_bound.

$$r_{obs} = f_B r_B + f_F r_F$$  \hspace{1cm} (Eq. 2)

In Equation 2, r_{obs} is the measured anisotropy at a given protein concentration, f_B and f_F are the fractions of fluorophore bound and free, respectively, and r_B and r_F are the anisotropy values when the fluorophore is all bound or all free, respectively. Because r_free is small, Equation 2 can be simplified to:

$$r_{obs} = f_B r_B$$  \hspace{1cm} (Eq. 3)

or

$$f_B = \frac{r_{obs}}{r_{max}}$$  \hspace{1cm} (Eq. 4)

Thus, at any given protein concentration, assuming one binding site per
monomer, the fraction of fluorophore bound can be determined using Equation 4.

\[
[C_p] = \frac{f}{f + [C_p]}
\]  
(Eq. 5)

\[
[F] = [F_p] - [C_p]
\]  
(Eq. 6)

\[
 [C] = [C_p] - [C_p]
\]  
(Eq. 7)

\[
 [C_p] = [C_p] \left( \frac{1}{K_d} \right)
\]  
(Eq. 8)

Plotting Equation 8 as \([C_p] \) versus \([C][F]\), the slope of the fitted line is \(1/K_d\).

\textit{bisANS Binding Experiments}—To follow hydrophobic exposure on \(cpn60\), 10 \(\mu\)M \(bisANS\) was added to a cuvette containing 1 \(\mu\)M \(cpn60\), and the emission spectrum was recorded. Subsequently, 1 \(\mu\)M oxidized APA14 was added to the cuvette, and the \(bisANS\) emission was recorded. \(bisANS\) fluorescence was insignificant in the presence of 1 \(\mu\)M oxidized APA14 alone (data not shown). The increase in \(bisANS\) fluorescence in the presence of \(cpn60\) was attributed to hydrophobic exposure induced on the chaperonin by the oxidized APA14 peptide. To follow loss of hydrophobic sites following reduction of the peptide, 2 \(\text{mM}\) DTT was added to the samples above. Emission spectra were collected from 0 to 40 min.

\section*{RESULTS}

\textbf{Apamin Hybrid Peptide Structural Characterization}—The CD spectra of the oxidized peptides, APA09c (Figs. 1A), APA14c (Fig. 1B), and APA08c (Fig. 1C), indicate a high degree of helical secondary structure, whereas the spectra of reduced peptides, APA09r (Fig. 1A) and APA14r (Fig. 1B), indicate there is little or no regular helical structure. The secondary structure calculated for each peptide in either the oxidized or reduced form is shown in Table I. The fluorescein-labeled peptides APA08 and APA09 were not soluble at concentrations required for NMR. However, analysis of oxidized APA14c by \(1^H\) NMR spectroscopy identified intense \(H^N-H^N\) sequential NOEs and medium range NOEs characteristic of a helix between residues 9 and 16 (Fig. 2); the presence of a helical conformation is confirmed by the small values of \(^3J_{HN-Ha}\) for these residues. The data for residues 17–19 are not indicative of a well formed helix, suggesting that they form a frayed terminus to the helix or adopt a turn conformation. These findings are in line with the structure modeled for APA14 and are consistent with the three-dimensional structures determined for other apamin peptides (35). Many of the chemical shifts of oxidized APA14c are found to be very different from random coil values, suggesting that the peptide does adopt a well defined structure. The addition of dithiothreitol to the peptide resulted in a loss of the characteristic helical NOEs, an increase in the \(^3J_{HN-Ha}\), scalar coupling constants (Fig. 2, lower panel), and movement of the resonances to approximately random coil positions. Helical interactions were also absent from rotating frame Overhauser spectroscopy spectra, indicating that the loss of NOE intensity was not the result of a change in rotational correlation time. Thus, the disulfide bonds are required to maintain

\footnote{R. S. McDowell, unpublished data.}
Amphipathic α-Helical Peptides Bind to cpn60

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A

![Graph A](image)

B

![Graph B](image)

**Fig. 4. Fluorescence of bisANS at increasing concentrations of oxidized APA14.** A, spectra. Oxidized APA14 was titrated into a cuvette containing 10 μM bisANS. The spectra from bottom to top represent oxidized APA14 concentrations of 0, 0.5, 0.8, 2.0, 3.9, 5.7, and 7.4 μM, respectively. Excitation of bisANS was performed at 397 nm, and emission was detected at 500 nm at 20 °C. The bisANS fluorescence spectra blue shift as the concentration of oxidized APA14 increases. B, fluorescence intensity of bisANS versus [APA14c]. Maximum fluorescence intensities of spectra shown in A are plotted as a function of the APA14c peptide concentration.

APA14 in a helical conformation. The predicted structure of these peptides is shown in Fig. 3, and the surface hydrophobicity of each peptide is also presented.

**Low Peptide Concentrations Are Critical to Avoid Aggregation—**Oxidized APA09c and APA14c are amphipathic helical peptides with exposed hydrophobic faces. Thus, the question of solubility and formation of aggregates was addressed. BisANS fluorescence was used to detect aggregation of oxidized APA14c (Fig. 4) and reduced APA14r (data not shown). A cuvette containing 10 μM bisANS was titrated with increasing amounts of either oxidized APA14c or reduced APA14r. There was relatively little increase in bisANS fluorescence in the range of peptide concentrations from 0 to 2.0 μM, but there was a substantial increase that occurred above 2 μM APA14. As shown in Fig. 4B, the fluorescence response is reminiscent of similar titrations that are used to define the critical micelle concentrations of amphiphilic detergents (36). Thus, significant self-association of the peptide occurs when its concentration is higher than 2–3 μM. To avoid artifacts and interpretive complications from aggregation, all experiments with APA08, APA09 (the fluorescein-labeled form of APA14), and APA14 were done at a concentration of 1 μM.

**Binding of Oxidized and Reduced APA09 to cpn60 Can Be Measured by Fluorescence Anisotropy—**Fluorescence anisotropy binding studies were done using the fluorescein-labeled APA09 peptides (same sequence as APA14). Initial experiments were done to determine any differences in the binding affinity of oxidized APA09c and reduced APA09r.

The anisotropy of 1 μM APA09c free in solution had a value of 0.061 ± 0.004. The anisotropy of 1 μM APA09c in the presence of 10 μM cpn60 was 0.084 ± 0.003. On the other hand, there was virtually no change in the anisotropy of free APA09r (0.055 ± 0.008) as compared with APA09r in the presence of cpn60 (0.050 ± 0.006). The dissociation constant for oxidized APA09c was determined as described under “Experimental Procedures.” Cp60 was titrated into a solution of 1 μM oxidized APA09c over a concentration range of 1–65 μM cpn60. The inverse of the change in anisotropy was plotted as a function of the inverse of the cpn60 monomer concentration, and the data were fit with a straight line (Fig. 5B). The anisotropy of bound peptide was determined from the y-intercept of the straight line as explained under “Experimental Procedures.” The concentration of cpn60/APA09c complex formed was then plotted against the concentration of free cpn60 multiplied by the concentration of free APA09c (Fig. 5). The data were fit to a straight line, and by taking the inverse of the slope, a K_d was estimated to be approximately 106 (± 6) μM. Independent of the assumptions used for the analysis of these data, it is clear that the oxidized peptide shows considerable binding, whereas the reduced version of the same peptide shows no detectable interaction. The oxidized APA08c peptide was subjected to the same fluorescence anisotropy experimental conditions, and no change in anisotropy was detected. Thus, the oxidized APA08c hydrophilic peptide did not bind to cpn60 as observed by fluorescence anisotropy measurements.

**Oxidized APA14 Induces Hydrophobic Exposure on cpn60—**Changes in bisANS fluorescence were used to assess changes in hydrophobic exposure in solutions containing cpn60 together with APA14c. When 10 μM bisANS was mixed with 1 μM cpn60, the maximum fluorescence intensity was observed at 502 nm. When 1 μM oxidized APA14c was added, the maximum fluorescence intensity increased by a factor of 1.7 with no change in the wavelength maximum. Fig. 6 shows the time course of the bisANS fluorescence intensity after the addition of 2 mM DTT to the APA14c-cpn60 mixture. The decrease in bisANS fluorescence that followed the reduction of the peptide could be modeled by a single exponential with a decay rate constant = 0.065/min (Fig. 6). A control sample showed that the fluorescence intensity of 10 μM bisANS did not change upon the addition of 2 mM DTT. These results indicate that the complex of cpn60 with APA14c displays considerably more hydrophobic exposure than the sum of the individual components, and the result requires the presence of the oxidized and not the reduced peptide.

**DISCUSSION**

Cpn60 can bind a wide variety of proteins in non-native states, implying that the specific primary sequence does not supply the information required for recognition (9, 11, 14, 37). Because cpn60 does not bind proteins in their native folded state, the recognition does not require formation of native tertiary structure (14). The binding of various proteins must occur by a more general mechanism of molecular recognition. One common feature of partially folded proteins is the solvent exposure of hydrophobic residues that would be buried in the native state. As proteins fold, local regions can form secondary structures such as α helices that can align residues to produce hydrophobic surfaces. These surfaces could provide the necessary binding motif for recognition by the cpn60 hydrophobic binding sites.

The oxidized peptides APA09c, APA14c, and APA08c used in this study address the issue of secondary structural require-
mments for binding to cpn60 and suggest a possible mechanism of cpn60 binding to unfolded proteins via induced hydrophobic exposure on cpn60 as a result of an initial amphipathic peptide binding.

The small size of APA09 compared with that of the cpn60 tetradecamer (798 kDa) allowed the binding of the peptide to cpn60 to be followed by fluorescence anisotropy. Oxidized APA09c was determined to bind weakly to cpn60 with a $K_d = 10^6$ M, assuming one binding site on cpn60. No binding of reduced APA09r was detected under the same experimental conditions. This result shows the effect of organizing the hydrophobic surface onto a helical motif. Although the binding interactions of these small peptides are weak, they should be viewed as a part of the many weak interactions that would occur with a partially folded protein containing numerous elements of local secondary structure. This implied multiple binding of a protein to the oligomeric cpn60 is supported by the previous observation that cpn60 quaternary structure is stabilized by interactions with partially folded proteins (38). That the hydrophilic peptide, APA08, shows no binding to cpn60 supports the suggestion that hydrophobic interactions are important in forming the cpn60-protein complex. These results are complementary to the data from Fenton et al. (13), where polypeptide binding studies were performed on various mutants of cpn60 to demonstrate the requirement for hydrophobic residues on cpn60 to bind partially folded proteins. The present studies suggest that initial binding of amphipathic elements to cpn60 can induce the formation of increased hydrophobic exposure. When the interactions with the peptides are eliminated by reduction, the induced hydrophobic surfaces are lost. This may be related to the effects seen with proteins whose exposed, interactive surfaces are buried as they fold with two consequences: (a) they are bound less tightly to cpn60, and (b) the interactive surfaces on cpn60 are hidden and buried.

![Figure 5](image.png)

**Figure 5.** Determination of the dissociation constant for oxidized APA09. A, as described under “Experimental Procedures,” Equations 1–5 were used to determine the $K_d$ of APA09c. The concentration of fluorophore, APA09c, was held constant at 1 μM, whereas cpn60 was titrated into the cuvette containing APA09c. A plot of $[C_F]$ versus $[C][F]$ was fit to a straight line according to Equation 8, with the inverse of the slope being equal to the $K_d$. A straight line was fitted to the data points, and from the slope of this line the $K_d$ was determined. Panel B shows the data used to determine the anisotropy of bound APA09c as described in the text.
protected against nonproductive interactions.

The 2.8 Å crystal structure of cpn60 (12) as well as mutational analysis (13) have suggested that the polypeptide binding site on cpn60 resides in hydrophobic sequence within the apical domain of each monomer. The crystal structure in this region has poor resolution, presumably due to flexibility or malleability in this area of the apical domain (12). It is tempting to speculate that interactions with amphipathic structural elements could modify the exposure in this part of the structure and interactions with nucleotides or the co-chaperonins could modulate the interactions. Thus, organized amphipathic structures on unfolded proteins may not only be a part of the general recognition motif for cpn60 binding, but they may also serve as a means of amplifying the hydrophobic surface exposed by cpn60. Finally, the observed strong net binding to cpn60 of partially folded proteins may result from multiple weak interactions stemming from the symmetrical association of many subunits. This would allow some flexibility of the bound protein by dissociation at some of the sites without permitting its release from the complex.

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