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Probing dimer interface stabilization within a four-helix bundle of the GrpE protein from *E. coli* via internal deletion mutants: conversion of a dimer to monomer

Andrew F. Mehl^{a,1}, UG Nalin^a, Zohair Ahmed^a, Aaron Wells^a, and Tilemahos D. Spyratos^a

^a Department of Chemistry and the Program in Biochemistry, Knox College, 2 East South St. Galesburg, IL 61401 U.S.A.

Abstract

Insight into protein stability and folding remains an important area for protein research, in particular protein-protein interactions and the self-assembly of homodimers. The GrpE protein from *E. coli* is a homodimer with a four-helix bundle at the dimer interface. Each monomer contributes a helix-loop-helix to the bundle. To probe the interface stabilization requirements, in terms of the amount of buried residues in the bundle necessary for dimer formation, internal deletion mutants (IDMs) were created that sequentially truncate each of the two helices in the helix-loop-helix region. Circular dichroism (CD) spectroscopy showed that all IDM's still contained a significant amount of α -helical secondary structure. IDM's that contained 11 or fewer of 22 residues originally present in the helices, or those that lost at least 50% of residues with less than 20% the solvent accessible surfaces (that is, hydrophobic residues) were unable to form a significant amount of dimer species as shown by chemical cross-linking. Gel filtration studies of IDM3.0 (one that retains 10 residues in each helix) show this variant to be mainly monomeric.

Keywords

Protein stability; dimer interface; homodimer; four-helix bundle; hydrophobicity

1. Introduction

Most proteins in nature exist in higher order oligomeric structures with the most common being the dimeric species. For those dimers where monomer association is coupled with protein folding, the subunit interface is considered a nucleation site where there is a core of residues that are almost entirely buried in the dimer [1]. Additionally, it has been shown that both hydrophilic interactions and the amount of hydrophobic contact area at the interface play an important role in stabilization of the complex [2], [3] and [4]. The extent of reduced solvent-accessible surface area in protein-protein interactions are studied both theoretically and experimentally [5] and [6].

The four-helix bundle is a fairly common structural motif that is often formed at the dimer interface [7]. Furthermore, the role of the macrodipole within an α -helix toward stabilization

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¹Corresponding author. Tel. 1*(309) 341-7270; fax 1*(309) 341-7601; amehl@knox.edu.

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of the four-helix bundle in the anti-parallel arrangement versus the unfavorable parallel situation is also of interest in protein stability [8] and [9].

Here we probe the stabilization of a homodimeric protein that has a four-helix bundle at the subunit interface. The GrpE heat shock protein from *Escherichia coli* contains a square-type arrangement within the four-helix bundle with each monomer providing two adjacent helices that are anti-parallel with an interaxial angle (Ω) of 20° [10]. The GrpE structure also contains an unusual feature at the dimer interface, namely two long helices (88 residues) from the NH₂-terminal end interacting in a parallel fashion. It has been shown previously that this “tail” region is not required for dimer formation [11] and [12]. In this paper a mutagenic approach is utilized where internal deletion mutants (IDM's) have been constructed to sequentially truncate the two α -helices that participate in the formation of the four-helix bundle. Five IDM's with decreasing amount of residues were created to probe the amount of hydrophobic contact area (and subsequent contribution of the macrodipole present) that is required for the retention of the stabilization center within the four-helix bundle. Results indicate that a minimum of 12 amino acids in each helix, and a corresponding total value of 22 hydrophobic residues (defined as those with less than 20% solvent-accessible surface) in the four-helix bundle core, are necessary for the formation of a stable dimer species.

2. Materials and methods

2.1. Reagents and materials

Reagents and materials and their sources were Affi-Gel Blue Gel resin (Bio-Rad); DEAE Cellulose (Sigma); Mono-Q 10/100 GL FPLC column (GE Healthcare); Superdex 200 HR 10/30 FPLC column (GE Healthcare); Bradford Dye reagent (Bio-Rad); EDC and Sulfo-NHS (Pierce Endogen); restriction enzymes and T4 DNA ligase (New England Biolabs); Amplitaq® DNA Polymerase (Applied Biosystems); HEPES (free acid form) and glycerol (Amresco). All other biochemicals were from Sigma-Aldrich.

2.2. Determination of protein concentration

The concentrations of all the purified proteins were determined by the method of Bradford [13], using Bovine γ -globulin as a standard.

2.3. Strains and plasmids

Strain RLM 988 (C600dnaK103) was used for the expression of the full-length GrpE. Either strain RLM 569 (C600, recA, hsdR, tonA, lac⁻, pro⁻, leu⁻, thr⁺, dnaJ⁺) or strain DA262 [14] were used for the expression of all the internal deletion mutants (IDMs). Plasmid pRLM156 was used as the expression vector for all the proteins described in this paper. This plasmid is the same as pRLM76 [15] except for the fact that the polylinker region is more versatile. Plasmid pRLM 159 [16] carrying the *grpE* gene was used for the template in the Polymerase Chain Reaction (PCR) mediated cloning of the IDM's.

All the IDM's were created using the same procedure; to summarize the approach: two segments of DNA retaining the code for the wanted protein regions, each containing the code for a truncated α -helix, along with a common restriction enzyme site (Ava I) were PCR amplified using the *grpE* gene as a template. After subjecting each to the Ava I enzyme the isolated pieces were ligated together. This new piece was then cut with appropriate restriction enzymes (sites were also originally PCR amplified) so that it could be cloned into the pRLM156 expression vector. Since the deleted region of DNA coded for a loop region connecting two α -helices in the protein (see Fig.2A) the primers used in the PCR reaction also provided a sequence that coded for a new but very similar loop sequence (see Fig. 2B).

The details for this cloning procedure will be provided for IDM1.0. To generate the first piece of DNA coding for an α -helix this is truncated on the NH₂-terminal end, a forward primer (F1, see Table 1) contained a *Sall* restriction enzyme site, a consensus ribosome binding site, and the *grpE* coding sequence (underlined) starting with the initial sequence. A reverse primer (R105, see Table 1) contained an *AvaI* restriction enzyme site, a sequence to code for a new loop region, and the *grpE* coding region (underlined) ending with the DNA sequence that codes for residue 105. PCR amplification was performed in a reaction mixture (100 μ l) contained 25 ng of template plasmid DNA, 100 pmol of each primer, 50 μ M of each of the four dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% (w/v) gelatin, and 2.5 units of Amplitaq DNA polymerase. To generate the second piece of DNA coding for an α -helix that is truncated on the COOH-terminal end, a forward primer (F121, see Table 1) containing an *AvaI* restriction enzyme site and the coding sequence (underlined) starting at residue 121 was utilized. A reverse primer (R197, see Table 1) contained a *PstI* restriction enzyme site, two tandem translation stop codons, and the complement *grpE* ending coding sequence (underlined). PCR amplification was carrying out in the same manner as above. After gel purification (1.7% agarose) of the PCR generated pieces of DNA, each was digested to completion with *AvaI* and gel purified, then ligated together. After gel purification (1.7% agarose) of the ligated product, this DNA fragment was digested to completion with *Sall* and *PstI* and then ligated to pRLM 156 which had been similarly digested. DNA from the ligation reaction was transformed into either RLM 569 cells or DA 262 cells, and ampicillin-resistant clones were screened for the ability to overproduce a polypeptide with the appropriate size IDM when grown at 42 °C. Plasmid preparations (Qiagen Midi Kits®, Valencia, CA, USA) were carried out on a number clones that showed significant overexpression, and these were sequenced to verify the correct amino acid sequence (Protein and Nucleic Acid Chemical Laboratory, Washington University School of Medicine, St. Louis, MO, USA). Table 1 provides the appropriate names for the new plasmids and strains for all the IDM's.

2.4. Expression and purification of GrpE and the IDM's of GrpE

The expression and purification of wild-type GrpE protein has been described previously [16]. The expression and purification of IDM1.0 and IDM2.0 utilized the same exact protocol as that of the wild-type GrpE protein, namely a three step purification method involving DEAE cellulose, Affi-gel Blue, and Mono-Q resins was employed. IDM2.5, IDM2.75, and IDM3.0 used the same protocol as that of the wild-type except that during the Affi-Gel Blue chromatography step a salt gradient was needed. During this step a 400-ml linear gradient of 0.0–0.5 M NaCl was utilized for IDM2.5 and the peak of protein elution centered at 0.15 M NaCl. A 400-ml linear gradient of 0.0–1.0 M NaCl was employed for both IDM2.75 and IDM3.0; and the peak of protein elution centered at 0.30 and 0.70 M NaCl respectively for each.

2.5. Circular dichroism spectroscopy and thermal unfolding experiments

Circular dichroism spectra were recorded using an OLIS (Online Instruments Inc., Bogart, GA USA) RSM 1000 spectrometer configured with a CD module for dual beam CD. Initial spectra were obtained used the following conditions: T, 20 °C; pathlength, 0.1 cm; protein concentrations, 0.5–0.7 mg/ml; scanned from 260–190nm (2 nm step size) with a rate dependent on the amount of signal produced at each wavelength. Temperature was controlled using a Peltier-type temperature control system (OLIS and Quantum Northwest, Spokane, WA, USA). Thermal unfolding studies were carried out using the same conditions described above. A full scan was taken at each temperature from 6°C to 94°C (every 2 °C) with an equilibration time of 2 min at each temperature. Depending on the particular IDM being analyzed, the entire data set was fitted to either a two or three species model (see Results and Discussion) using the GlobalWorks™ (OLIS, Bogart, GA, USA) software. The

software allows a singular value decomposition (SVD) factor analysis on the entire data set using all the points from each scan at each temperature (a three dimensional data matrix). The data presented in Table 3, namely the transition temperatures (T_m) and enthalpies of unfolding (ΔH_u) for each transition, were obtained from the fitting results.

2.6. EDC crosslinking reactions

All crosslinking reactions were carried out at 25°C. Samples (50 μ l total volume) contained 25 mM HEPES/KOH (pH 7.6), 0.5 mM EDTA, 33 mM EDC, and 10 mM Sulfo-NHS. Reactions were initiated by the addition of protein (45 μ g) and incubated for 2 hr. Reactions were stopped by the addition of 30 μ l of gel loading buffer and immediate incubation in boiling water for 5–10 min. Samples were analyzed by SDS-PAGE (12.5% resolving and a 4.5% stacking gel) and the crosslinked and uncrosslinked proteins were identified by staining with Coomassie Brilliant Blue R-250.

2.7 Gel filtration

Size exclusion chromatography was carried out at 4° C on a Superdex 200 HR 10/30 column on a FPLC (GE Healthcare). A 100 μ l aliquot containing 10–50 μ g protein in 100 mM sodium phosphate, pH 7.6 and 10 % (v/v) glycerol was loaded onto the column previously equilibrated with the same buffer. Elution of the protein was obtained using the same buffer at a flow rate of 0.4 ml/min.

3. Results and discussion

3.1 Rationale and design of the internal deletion mutants of GrpE

The GrpE protein from *E.coli* contains a four-helix bundle formed by two monomers, each providing two α -helices via a helix-loop-helix arrangement (see Fig. 1). Truncating the α -helices, and thus reducing the dimer interface area in the four-helix bundle region, required removing residues from both helices within each monomer. Our plan was to remove approximately one turn in each helix sequentially by deleting 4 residues at a time (see Fig. 2A, 2B and Table 2). Initially, since IDM2.0 (deletion of ~two turns) retained a dimeric structure and IDM3.0 (deletion of ~three turns) did not (see below), we then created two more IDM's to define further the amount of interface area required for four-helix bundle (dimer) formation, namely IDM2.5 and 2.75. Since we used a PCR based approach to create the mutants we couldn't duplicate the exact sequence in the wild-type loop region because of technical reasons of existing DNA sequence in the plasmid vector and the required restriction enzyme sites used for cloning. The new loop region (see Fig. 2B) contains proline, lysine, and alanine residues similar to that of wild-type; and two glycine residues to allow for flexibility of a loop structure. Also a glutamic acid was engineered into the loop to mimic the presence of an aspartic acid found in the native sequence.

3.2 Expression and purification of the GrpE IDM proteins

All the IDM's were over-expressed and purified in a similar manner as that for the wild-type GrpE protein. Figure 3 shows the final purified proteins used in this study; the level of purity was determined by densitometry to be greater than 94% for each. IDM's 2.5, 2.75, and 3.0 exhibited different behavior with the Affi-Gel Blue chromatography step; they all showed some affinity for the resin and a salt gradient was needed to elute each from the column with the peak of elution occurring at 0.15 M NaCl for IDM 2.5, 0.30 M NaCl for IDM 2.75, and 0.70 M NaCl for IDM 3.0. This behavior is presumably due to the increased net ionic charge of the monomeric form leading to the presence of some affinity for the resin. The Affi-Gel Blue resin has a number of different properties which include ionic bonding. Additionally, it

should also be noted that the peak of elution for these IDM's from the Affi-Gel Blue resin was very broad, especially for IDM 2.5 and IDM 2.75.

3.3 Circular dichroism spectroscopy

In order to determine if proper protein folding of the IDM's occurred, CD spectroscopy was carried out to identify the presence of protein secondary structure, namely that of α -helical content. Fig. 4 shows the far UV CD spectra for all the IDM's and also for the native GrpE protein. All of the mutants show minima in ellipticity at 208 and 222 nm, indicative of significant α -helical content and thus all are presumably folding into a globular structure.

3.4 Probing quaternary state of IDM's

3.4.1 EDC X-linking—The EDC chemical crosslinker has been used previously to probe the oligomeric state of GrpE and other mutants [12]. Here we subjected the IDM's to the EDC reagent for a sufficient length of time to produce a dimeric species. Figure 5 shows the results of the cross-linking experiment for the IDM's and our findings are summarized in Table 2. IDM 1.0 behaves just as wild-type in that all of the protein is converted to the dimeric species in the time frame of the experiment. IDM's 2.0 and 2.5 show significant conversion to dimeric species with some remaining monomer. The results for IDM's 2.75 and 3.0 show a reduction in the conversion to dimer and a high percentage of remaining monomer. Additional incubation time did not produce an increase in dimeric species for IDM 2.75 or 3.0 (results not shown). These results suggest that a minimum of 12 residues in each helix of the helix-loop-helix region of the monomer are required for sufficient dimerization presumably via four-helix bundle formation.

3.4.2 Gel filtration chromatography—To further characterize the differences in oligomeric states between the IDM's, gel filtration chromatography was carried out using a Superdex 200[®] gel filtration column. Figure 6 shows the results of the gel filtration studies. As noted in a prior study [12] this technique cannot be used to accurately determine a molecular mass of GrpE but is presented for comparison purposes between the native species and the IDM's. The results show that all the IDM's except IDM3.0 have an elution time similar to that of the wild-type, namely in the range of 31–32 minutes (with IDM2.75 eluting at a point nearest to 32 min). IDM3.0, however, gives an elution time of closer to 34 minutes. These results support the notion that IDM3.0 is behaving as a monomeric species with a smaller mass.

3.5 Stability studies of IDM's via thermal denaturation

To access the stability of the IDM's in a quantitative manner, thermal denaturation studies were carried out following the protein unfolding via CD. The data for the IDM's and the native GrpE are given in Fig. 7 and the results of the transition temperatures (T_m) and enthalpies of unfolding (ΔH_u) are summarized in Table 3. The data for IDM's 1.0, 2.0, and 2.5 were globally fit (see materials and methods) to a three species model since all three showed the propensity for the dimeric state. Since IDM2.75 and 3.0 showed mostly a monomeric state, the data for these mutants were fit to a simple two state model for the unfolding process. IDM1.0 gave very similar values for T_m 's and ΔH_u 's to that of wild-type except for the higher value for the second transition enthalpy ($\Delta H_{u(2)}$). IDM2.0 gave a reduced temperature for the first transition and a reduced $\Delta H_{u(1)}$; however, the value for the $\Delta H_{u(2)}$ was greatly increased. Since the second transition is thought to be mainly due to the unfolding of the four-helix bundle region, [11] and [17], this suggests that these two mutants may have enhanced stability in the bundle. As can be seen from Fig. 7, IDM2.5 shows more variation in the data and subsequently the fitting process showed more deviation than with IDM1.0 and 2.0. Nevertheless, we decided to utilize the three state model for

IDM2.5 because the chemical crosslinking still revealed a significant amount of dimeric species. However, this mutant shows less of the dimeric state than either IDM1.0 and 2.0 and this may explain the greater variation. Consequently, we placed less emphasis of the results for the thermal denaturation of IDM2.5 and have not drawn any strong conclusions from the data.

IDM2.75 and 3.0 showed denaturation profiles with a single point of inflection and the fitting process gave low standard deviations using a simple two-state model of unfolding. Both mutant proteins showed low transition temperatures and enthalpies of unfolding.

4. Conclusion

The four-helix bundle is a common structural motif found in proteins and in many instances is formed when individual subunits interact to form a higher order oligomeric structure. In this work we have defined a minimum of residue contact at the dimer interface that is necessary for dimer formation of the GrpE protein from *E. coli*. Chemical crosslinking and gel filtration chromatography results with internal deletion mutants of GrpE that reveal that 12 of the original 22 residues of the α -helix that is involved with a four-helix bundle formation at the dimer interface must be present for the dimer to assemble. A survey of the literature reveals that this minimum is consistent with proteins having four-helix bundles at the dimer interface; for example, the structure of the dimer interface for the GADD45g protein shows a small 10 residue α -helix involved in a four-helix bundle [18]; the Hepatitis B Capsid homodimer has a four-helix bundle involving a 24 residue α -helix connected to a 12 residue α -helix via a short loop of 5 amino acids in each monomer [19]; and the stress protein YciF has a short α -helix of 14 residues at the dimer interface of a four-helix bundle [20].

Chemical crosslinking revealed that the amount of dimer species is significantly reduced for IDM2.75 where this mutant is missing 50% of the residues that have <20% solvent-accessible surface. Furthermore, IDM3.0 which contains 10 of the original 22 residues in each α -helix and only retains 44% of the original hydrophobic residues, shows the smallest amount of dimeric species formed with the crosslinking studies. However, CD and gel filtration analysis reveals that this mutant still possesses significant α -helical content and presumably is folding into a monomeric globular structure. Thus the reduction in buried surface area clearly has led to a loss in the protein-protein interaction for both IDM2.75 and IDM3.0 that is required for dimer formation, but does not obliterate the protein folding completely; although these two IDM's have fairly low stability as observed in the thermal denaturation studies. Future work will entail elucidating the structure of the intriguing IDM3.0 GrpE monomeric variant.

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Abbreviations

IDM	internal deletion mutant
PCR	polymerase chain reaction
CD	circular dichroism spectroscopy

EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
sulfo-NHS	N-hydroxysulfosuccinimide
HEPES	N-(2-hydroxyethyl)peperazine-N'(2-ethanesulfonic acid)

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Fig. 1. Ribbon diagram of the GrpE dimer (amino acids 34–197). The structure is based on the X-ray crystallographic data collected for a deletion mutant of GrpE that is missing the first 33 amino acids at the NH₂-terminal end and is in a complex with the ATPase domain of DnaK [10]. The three main regions depicted in the figure are: the NH₂-terminal α -helical “tail” portion composed of residues 34–87 from each monomer, the four-helix bundle composed of residues 88–137 from each monomer, and the COOH-terminal β -sheet domain. The image was produced using MOLMOL (Protein Data Bank code for the GrpE:DnaK complex: 1DKG).

Figure 2A

Figure 2B

Wild-Type	90	100	110	120	130
...	LEKFINELLPV	IDS	LDRALEVADKANP	DMSAMVEG	IELTKSMLDVVRKF...
IDM1.0	90	100	121*	130*	
...	LEKFINELLPV	IDS	LDRAKGAGPEEG	IELTKSMLDVVRKF...	
IDM2.0	90	100	125*	130*	
...	LEKFINELLPV	IDS	SGKAGPELT	KSMLDVVRKF...	
IDM2.5	90	130*			
...	LEKFINELLPV	IGKAGPEL	KSMLDVVRKF...		
IDM2.75	90	130*			
...	LEKFINELLPV	GKAGPE	KSMLDVVRKF...		
IDM3.0	90	130*			
...	LEKFINELLPG	KAGPES	MLDVVRKF...		

Fig. 2.

(a) Monomeric representation for the GrpE protein showing the strategy for design of the internal deletion mutants. Three IDM's are shown: IDM1.0 missing residues 106–120, IDM2.0 missing residues 102–124, and IDM3.0 missing residues 98–128. The image was created using Accelrys DS Visualizer 2.0. (b) The single letter code for the amino acid sequence encompassing the region from position 88–137 that contains the helix-loop-helix segment. The wild-type sequence shows the loop region in bold and the new loop region for the IDM's is also in bold. The residue numbering for the IDM's after the loop region (*) is the same as the wild-type to emphasize which amino acid residues were deleted. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F,

Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

Fig. 3.

Electrophoretic analysis of purified wild-type GrpE and the various internal deletion mutants. A 13% SDS-polyacrylamide gel showing purified wild-type GrpE and the five IDM's used in this study: IDM1.0, IDM2.0, IDM2.5, IDM2.75, and IDM3.0 (as indicated). Polypeptide bands were visualized by staining with Coomassie brilliant blue R-250. Molecular weight markers are (from top to bottom) phosphorylase *b* (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α -lactalbumin (14,400).

Fig. 4.

Circular dichroism spectra of GrpE and the IDM's. Far-UV CD spectra were obtained for wild-type GrpE and the five IDM's used in this study. Conditions are described under Materials and methods. (○) Wild-type GrpE; (□) IDM1.0; (●) IDM2.0; (△) IDM2.5; (■) IDM2.75; (▲) IDM3.0.

Fig. 5.

Electrophoretic analysis of wild-type GrpE and the various IDM's after treatment with the EDC crosslinker (as indicated at the top of the figure). Crosslinked products and uncrosslinked proteins were resolved by electrophoresis using a 15% resolving and 4.5% stacking SDS-polyacrylamide gel and visualized by staining with Coomassie Brilliant Blue R-250. Molecular weight markers are (from top to bottom) phosphorylase *b* (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α -lactalbumin (14,400).

Fig. 6.

Analysis of GrpE and the various IDM's by size exclusion chromatography. Samples were loaded on a Superdex 200 column and eluted as described in Materials and methods. (○) Wild-type GrpE; (△) IDM1.0; (---) IDM2.0; (●) IDM2.5; (◇) IDM2.75; (□) IDM3.0. The molecule mass standards shown at the top are catalase (232 kD), albumin (67 kD), and ovalbumin (43 kD).

Figure 7A

Figure 7B

Figure 7C

Figure 7D

Figure 7E

Figure 7F

Fig. 7.

Thermal unfolding of GrpE and the IDM's followed by CD. Conditions are described under materials and methods. (a) Wild-type GrpE. (b) IDM1.0. (c) IDM2.0. (d) IDM2.5. (e) IDM2.75. (f) IDM3.0.

Table 1

Primer DNA sequences employed for the creation of the IDM's

Mutant	Primer Name	Primer Sequence	Plasmid Name	Strain Name
All	F1	5'-CCACCGTTCGACAAAGAGAGATATATTAATGAGTAGTAAAGAACAC-3'		
	R197	5'-CCACGGCGCCCTGCAGGTATACCTATTAAAGCTTTTGGCTTCGGCTAC-3'		
IDM 1.0	R105	5'-CCACCTAGGACTCGGGACCCGCCCTTCCCGCACGATCCAGGGCTATC-3'	pAFM33	AFM53
	F121	5'-CCACCTAGGAATTCCTAGGATGCCCGGAGGAAAGGCAITGAGCTGAC-3'		
IDM 2.0	R101	5'-CCACCTAGGACTCGGGACCCGCCCTTCCCGCTATCAATCACCGGCA-3'	pAFM34	AFM55
	F125	5'-CCACCTAGGAATTCCTAGGATGCCCGGAGCTGACGCTGAAAGTCGAT-3'		
IDM 2.5	R99	5'-CCACCTAGGACTCGGGACCCGCCCTTCCCAATCACCGGCAGCAATTC-3'	pAFM37	AFM62
	F127	5'-CCACCTAGGAATTCCTAGGATGCCCGAGCTGAAAGTCGATGCTGGA-3'		
IDM 2.75	R98	5'-CCACCTAGGACTCGGGACCCGCCCTTCCCGACCGGCAGCAATTCGTT-3'	pAFM38	AFM63
	F128	5'-CCACCTAGGAATTCCTAGGATGCCCGAGAGTCGATGCTGGATGT-3'		
IDM 3.0	R97	5'-CCACCTAGGACTCGGGACCCGCCCTTCCCGGCAGCAATTCGTTGA-3'	pAFM35	AFM56
	F129	5'-CCACCTAGGAATTCCTAGGATGCCCGATCGATGCTGGATGTTGT-3'		

Table 2

Properties of GrpE and the Internal Deletion Mutants (IDM's) of the GrpE Protein

Name	Total Number of Residues ¹	Deleted Region	Residues Remaining In each Helix	Residues Remaining in the Four-Helix Bundle region with <20% SAS ²	% of residues remaining in the Four-Helix Bundle region with <20% SAS	EDC X-linked Species
WT	197	None	22	36	100	Dimer
IDM1.0	194	106–120	18	32	89	Dimer
IDM2.0	186	102–124	14	24	67	Dimer
IDM2.5	182	100–126	12	22	61	Dimer (major)
IDM2.75	180	99–127	11	18	50	Monomer (major)
IDM3.0	178	98–128	10	16	44	Monomer (major)

¹ Value for the IDM's includes the new 6 residue loop segment (see Fig. 2B).

² Solvent-accessible surfaces (obtained from [10])

Table 3

Transition midpoint temperatures (T_m) and Enthalpies of unfolding (ΔH_u) for GrpE and the IDM's

Protein	T_{m1} (°C)	ΔH_{u1} (kcal mol ⁻¹)	T_{m2} (°C)	ΔH_{u2} (kcal mol ⁻¹)
WT ¹	51.2	50.9	78.5	54.3
IDM1.0	51.5	58.5	73.0	73.0
IDM2.0	45.3	46.2	73.0	96.0
IDM2.5	39.4	36.2	82.0	56.0
IDM2.75	35.1	34.2	na ²	na
IDM3.0	31.7	20.0	na	na

¹ Results represent an average of at least two trials for each protein.

² Not applicable