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

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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 *Escherichia 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.

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 [2–4]. The extent of reduced solvent-accessible surface area in protein–protein interactions is studied both theoretically and experimentally [5,6].

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

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 NH_2-terminal end interacting in a parallel fashion. It has been shown previously that this "tail" region is not required for dimer formation [11,12]. In this paper a mutagenic approach is utilized where internal deletion mutants (IDMs) 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

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-hydroxyethl)peperazine-N′(2-ethanesulfonic acid).

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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′-CCACCGTCGACAAGAAGGAGATATATTAATGAGTAGTAAAGAACA-3′		
	R197	5′-CCACGGCGCCCTGCAGGTATACCTATTAAGCTTTTGCTTTCGCTAC-3′		
IDM 1.0	R105	5′-CCACCTAGGACTCGGGACCCGCCTTCCCCGCACGATCCAGGCTATC-3′	pAFM33	AFM53
	F121	5′-CCACCTAGGAATTCCTAGGATGCCCGAGGAAGGCATTGAGCTGAC-3′		
IDM 2.0	R101	5′-CCACCTAGGACTCGGGACCCGCCTTCCCGCTATCAATCACCGGCA-3′	pAFM34	AFM55
	F125	5′-CCACCTAGGAATTCCTAGGATGCCCGAGCTGACGCTGAAGTCGAT-3′		
IDM 2.5	R99	5′-CCACCTAGGACTCGGGACCCGCCTTCCCAATCACCGGCAGCAATTC-3′	pAFM37	AFM62
	F127	5′-CCACCTAGGAATTCCTAGGATGCCCGAGCTGAAGTCGATGCTGGA-3′		
IDM 2.75	R98	5′-CCACCTAGGACTCGGGACCCGCCTTCCCCACCGGCAGCAATTCGTT-3′	pAFM38	AFM63
	F128	5′-CCACCTAGGAATTCCTAGGATGCCCGAGAAGTCGATGCTGGATGT-3′		
IDM 3.0	R97	5′-CCACCTAGGACTCGGGACCCGCCTTCCCCGGCAGCAATTCGTTGA-3′	pAFM35	AFM56
	F129	5′-CCACCTAGGAATTCCTAGGATGCCCGAGTCGATGCTGGATGTTGT-3′		

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 (Amreseco). 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 1) were PCR amplified using the *grpe* gene as a template. After subjecting each to the Ava 1 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 that is truncated on the NH_2-terminal end, a forward primer (F1, see Table 1) contained a *SalI* 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 μl) contained 25 ng of template plasmid DNA, 100 pmol of each primer, 50 μM 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 *SalI* 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

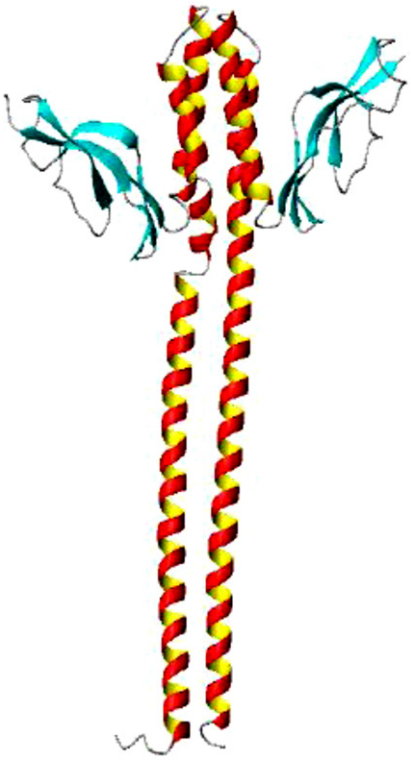

Fig. 1. Ribbon diagram of the GrpE dimer (amino acids 34–197). The structure is based on the X-ray crystallographic data collected for a deletion mutant of GrpE that is missing the first 33 amino acids at the NH_2-terminal end and is in a complex with the ATPase domain of DnaK [10]. The three main regions depicted in the figure are: the NH_2-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).