The co-chaperone GrpE is essential for the activities of the Hsp70 system, which assists protein folding. GrpE is present in several organisms, and characterization of homologous GrpEs is important for developing structure-function relationships. Cloning, producing, and conformational studies of the recombinant human mitochondrial GrpE are reported here. Circular dichroism measurements demonstrate that the purified protein is folded. Thermal unfolding of human GrpE measured both by circular dichroism and differential scanning calorimetry differs from that of prokaryotic GrpE. Analytical ultracentrifugation data indicate that human GrpE is a dimer, and the sedimentation coefficient agrees with an elongated shape model. Small angle x-ray scattering analysis shows that the protein possesses an elongated shape in solution and demonstrates that its envelope, determined by an ab initio method, is similar to the high resolution envelope of *Escherichia coli* GrpE bound to DnaK obtained from single crystal x-ray diffraction. However, in these conditions, the *E. coli* GrpE dimer is asymmetric because the monomer that binds DnaK adopts an open conformation. It is of considerable importance for structural GrpE research to answer the question of whether the GrpE dimer is only asymmetric while bound to DnaK or also as a free dimer in solution. The low resolution structure of human GrpE presented here suggests that GrpE is a symmetric dimer when not bound to DnaK. This information is important for understanding the conformational changes GrpE undergoes on binding to DnaK.

Nascent proteins in the cell sometimes require the assistance of one or more protein complexes named molecular chaperones to fold correctly (1, 2). An important chaperone complex is composed of the molecular chaperones Hsp70, 1 Hsp40 (or DnaK and DnaJ, respectively) and GrpE, which are highly expressed and important for several cell processes (3–7). The Hsp70 affinity for unfolded proteins is regulated by nucleotide binding to its nucleotide binding domain (NBD), which has a molecular mass of about 45 kDa (5, 8). The Hsp70 C terminus forms the substrate binding domain (SBD), which is capable of binding hydrophobic amino acid residues and has a molecular mass of about 20 kDa (8, 9). Co-chaperones Hsp40 and GrpE interact both in vivo and in vitro with DnaK (6, 10, 11), stimulating its ATPase activity (12) and regulating the ability of DnaK to bind and stabilize unfolded proteins (13). The importance of GrpE in the Hsp70 chaperone machinery is shown by the following: it is essential for bacterial viability at all temperatures (14), GrpE acts as an exchange factor that releases nucleotides bound to DnaK (12), and it is important for DnaK recycling (11). The first indication that GrpE was a homodimer came from cross-linking studies with glutaraldehyde (15). Subsequently, analytical ultracentrifugation experiments (16) showed that GrpE has a dimeric structure with an elongated shape that binds DnaK with 2:1 stoichiometry.

The crystallographic high resolution structure of residues 34–197 of *Escherichia coli* GrpE (EcGrpE1–197), complexed with the *E. coli* DnaK-NBD (EcDnaK1–383) corroborates that GrpE forms a dimer and shows that only one of the subunits, known as the proximal monomer, binds to Hsp70 (17). In this structure, the GrpE dimer is asymmetric because the proximal monomer adopts a more open conformation than the distal monomer. Knowing whether this asymmetric conformation remains while GrpE is free in solution is necessary for understanding this protein structure-function relationship in the cell. The GrpE C-terminal domain, EcGrpE141–197, is β-struc-
tured and binds to the DnaK-NBD causing the release of ADP from the NBD (17). The function of the GrpE N terminus remains to be understood fully. The GrpE40–86 domain forms a long coiled-coiled α-helical structure (17) and may function as a thermosensor because it appears to be responsible for the GrpE thermal transition at physiological temperatures (18). GrpE appears to be the only component of the Hsp70 chaperone machinery which undergoes a thermal transition at a physiologically relevant temperature (19). The GrpE99–137 domain forms a four-helix bundle (17) and likely acts as the stabilization center for dimerization (18). GrpE is present in eukaryotes (20–22), and it is generally assumed that they share high structural similarity with their prokaryote homologs. Thus, it...
is important to characterize the structure and function of GrpE from diverse organisms to test this hypothesis. The sequence of human mitochondrial GrpE (23) is represented in Fig. 1 along with the E. coli GrpE sequence showing that they share about 30% identity. The mitochondrial form of human GrpE is expressed as an immature protein that matures when its mitochondrial signaling peptide is lost.

Human GrpE was cloned and expressed in E. coli and purified by ion exchange chromatography and preparative molecular exchange chromatography. The secondary structure characterized by circular dichroism (CD) showed that the protein is folded. Temperature-induced unfolding followed either by CD or by differential scanning calorimetry (DSC) showed that human GrpE unfolding is only partially reversible, whereas E. coli GrpE exhibits reversible unfolding up to 60 °C (19). Analytical ultracentrifugation data indicate that the protein has the molecular mass of a dimer, and the calculated sedimentation coefficient agrees with the value expected for a protein with an elongated shape. The elongated shape was also derived independently from small angle x-ray scattering analysis (SAXS). The hydrodynamic parameters derived from SAXS data agree with those determined by analytical ultracentrifugation. Our envelope models are similar to the envelope of the crystallographic structure of E. coli GrpE bound to the DnaK-NBD determined by Harrison et al. (17). The low resolution structure generated from our SAXS data in solution suggests that GrpE is a symmetric dimer when not bound to DnaK.

The implications of our conclusions concerning human GrpE conformational changes in structure-function relationships of this co-chaperone are discussed.

EXPERIMENTAL PROCEDURES

Cloning, Expression, and Purification—The human cDNA IMAGE clone (GenBank accession number BE614754) was used for cloning the mitochondrial GrpE (Mt-GrpE#1, GenBank accession number Q9HA7V). Two primers were used to amplify the cDNA by PCR and create the restriction enzyme sites NdeI and XhoI for cloning into pET23a vector (Novagen). The 5'-primer (5’-TCTCCCCGCGATATGTTGCACAAG-3’) containing the NdeI restriction site was designed to anneal downstream to the mitochondrial peptide signal, eliminating this sequence in the recombinant protein. The correct cloning was confirmed by DNA sequencing using an ABI 377 Prism system (PerkinElmer Life Sciences). These procedures created the pET23aHMGrpE#1 vector, which was transformed in E. coli strain BL21(DE3) for protein expression by adding 0.4 mmol/liter isopropyl thio-

Circular Dichroism (CD) Spectroscopy—CD measurements were recorded on a Jasco J-810 spectropolarimeter with temperature controlled by Polycold Type Controlled System PFD 465S. The degree of purifica-

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Analytical Ultracentrifugation—Ultracentrifugation equilibrium experiments were performed with a Beckman Optima XL-A analytical ultracentrifuge. The protein was tested in concentrations of 50, 100, and 200 μg/ml in 25 mmol/liter Tris-HCl buffer at pH 7.5, with 150 mmol/liter NaCl and 0.5 mmol/liter DTT, with no apparent aggregation. The sedimentation velocity experiments were carried out at 20 °C, 40,000 rpm (AN-60T rotor), and the scan data acquisition was taken at 230 nm. The sedimentation equilibrium experiments were made at 20 °C at speeds of 7,000, 9,000, and 11,000 rpm using the AN-60T rotor. Scan data acquisition was done at 230 nm. The analysis involved fitting a model of absorbance versus cell radius data by nonlinear regression. All fits were done with the ORIGIN software package (MicroCal Software) supplied with the instrument. The van Holde-Weischedel (27) (sediment coefficient plot), Second Moments (28), and the Sedimentation Time Derivative (g(t) integral distribution) (29) methods were used to analyze the sedimentation velocity experiments. The methods used for analyzing both velocity and equilibrium experiments allow the calculation of the apparent sedimentation coefficient s, the diffusion coefficient D, and the molecular weight M. The ratio of the sedimentation to diffusion coefficient gives the molecular weight;
evaluated from the corrected and normalized SAXS curves by the indirect Fourier transform program GNOM (32, 33). A constant was subtracted from the experimental data to ensure that the intensity at higher angles decayed as $q^{-4}$ following Porod’s law for a two-electron density model (34). The value of the constant was derived automatically from the outer part of the curve using a linear fit to $q^4I(q)$ versus $q^4$ plots by the shape determination program DAMMIN (35), which will be described later. This procedure reduces the contribution from scattering because of the short range fluctuations of the internal protein structure and yields an approximation of the “shape scattering curve” (i.e. the scattering intensity produced by the excluded volume of a particle with a spatially constant density).

The low resolution shape of GrpE was obtained from the experimental data using the ab initio method implemented in DAMMIN (34). A sphere of diameter $D_{\text{max}}$ was filled by a regular grid of points corresponding to a dense hexagonal packing of small spheres (dummy atoms) of radius $r_0 < D_{\text{max}}$. The structure of the dummy atom model (DAM) is defined by a configuration $X$, assigning an index to each atom corresponding to solute (0) or solute particle (1). The computed scattering intensity curve of the DAM is compared with the intensity curve determined experimentally and the model is progressively modified by successive minimization trials of a function $f(X)$ defined as follows,

$$f(X) = x - aP(X)$$  \hspace{1cm} (Eq. 3)

where $x$ is the discrepancy between the experimental and modeled SAXS intensity functions given by the following,

$$x = \frac{1}{N} \sum_{j=1}^{N} \left( \frac{I(q_j) - I_{\text{exp}}(q_j)}{\sigma(q_j)} \right)^2$$  \hspace{1cm} (Eq. 4)

where $N$ is the number of experimental points, $I_{\text{exp}}(q_j)$ is the experimental intensity, and $\sigma(q_j)$ is the standard deviation in the $j$th point. In Equation 3, $\alpha$ is a positive constant, and $P(X)$ is an added penalty function that avoids solutions with loose bonds or disconnected structures.

The DAMMIN program searches for a compact interconnected configuration $X$, minimizing the function $f(X)$ defined by Equation 3. Starting from the initial configuration corresponding to a sphere with a radius $r = D_{\text{max}}/2$, where $D_{\text{max}}$ is the maximum diameter determined by the GNOM program, filled with small spheres (dummy atoms) with $r_0 < R$, a simulated annealing algorithm is employed for the minimization procedure (35). DAMMIN finally generates the best structure model containing a fraction of the initial small dummy atoms and also yields the SAXS intensity of the resulting model.

The coordinate set of positions for E. coli GrpE was obtained from Protein Data Bank (1DKG) (17). Relative positions of the domains were found with an automated procedure that iteratively rotates their envelope functions to minimize the discrepancy with the ab initio low resolution structure. The models were displayed using the program MASSHA (36). $R_g$, maximum intraparticle distances ($D_{\text{max}}$), envelope functions, and scattering curves were calculated from these atomic coordinates by the CRYSIOL program taking into account the influence of the hydration shell (37). SUPCOMB (38) was used to superimpose ab initio low resolution models onto crystallographic structures.

The HydroPro program (39) was used to estimate the translational diffusion coefficient $D_t$, the $R_g$, the sedimentation coefficient $s$, and tip-to-tip distance from the ab initio model generated by SAXS data at 20 °C. The HydroPro software was set up with radius of the atomic elements of 3.5 from ab initio development, sigma factors from 5 to 8 (as indicate by supplier) and minibeads radius (SIGMIN and SIGMAX) elements of 3.5 (from 20/H9268). The amino acid sequences of mitochondrial human GrpE (Mt-GrpE#1, GenBank accession no. Q9HAV7) and E. coli GrpE (GenBank accession no. NP311503) are represented by the one-letter codon, and similar residues are shown inside boxes. The thin black line below the first residues in the human GrpE sequence indicates the mitochondrial peptide signal, and the arrow represents the amino acid Leu-27 that was mutated to Met (the recombinant human GrpE produced here has this Met as the first codon; for details see “Experimental Procedures”). The thick boxes underlining the E. coli GrpE represent the main regions in this protein as seen in its crystallographic structure: white, EcGrpE/0–85 region formed by long coiled-coil $\alpha$-helix; gray, four-helix bundle (EcGrpE/95–137); and black, $\beta$-sheet domain (EcGrpE/137–191).

**RESULTS**

**Cloning, Expression, and Purification—Human and E. coli GrpE amino acid sequences are shown in Fig. 1 for comparison, the two proteins have about 53% similarity and 30% identity.** During the cloning of the human GrpE cDNA two sequence changes were made to allow recombinant expression of the functional protein in strain BL21(DE3). First, the cDNA 5′-sequence that codes for the mitochondrial peptide signal sequence was deleted. In human cells this peptide is removed from the protein when it reaches the mitochondria, but the bacterial host does not have the machinery to make this deletion. Without this modification, our expressed recombinant GrpE would have an N-terminal portion that is not present in the mitochondrial GrpE. This change causes the first residue in the mature protein to be leucine, which is not recognized as a start codon by strain BL21(DE3), thus the second change replaced this codon for a methionine codon. DNA sequencing confirmed the correct cloning and sequence of this mutated cDNA (data not shown). Human GrpE was expressed in a soluble state and in large amount, and the purification procedure resulted in a single protein band with the expected molecular mass (21.5 kDa) in SDS-PAGE (Fig. 2). Purified human GrpE shows no sign of impurities or degradation, is soluble (even in water), and folded (see below).

**Secondary Structure Analysis by CD and Thermal Analysis—**CD spectropolarimetry was used to assess the protein secondary structure content as a function of temperature (Fig. 3, a and b). Analysis of the global shape of the far UV CD spectra indicates that human GrpE is folded and soluble, allowing measurements down to near 180 nm. The far UV CD spectrum shows characteristics of a highly $\alpha$-helical protein, and analysis by the CDNN Deconvolution software indicates that about 55% of the protein is in this form (Fig. 3a). The CDNN software used the whole spectrum from 260 to 180 nm for secondary structure analysis, thus the calculation benefits from the good CD signal at low wavelengths. Increasing temperature causes the protein to unfold; there is a slight change...
in the CD signal at 222 nm from 4 to 50 °C and a more accentuated change above 50 °C (Fig. 3b). This temperature dependence is verified by DSC, the protein started to unfold at about 50 °C (Fig. 4). The unfolding of human GrpE is not completely reversible even if the protein is heated to only 50 °C as shown by both CD and DSC experiments (Figs. 3b and 4). The unfolding measured by DSC shows at least two unfolding events, one with a midpoint at 57 °C and the other, probably aggregation, occurs above about 62 °C (Fig. 4).

**Hydrodynamic Analysis by Analytical Ultracentrifugation**—The hydrodynamic properties of human GrpE were analyzed by both sedimentation velocity (Fig. 5a) and sedimentation equilibrium (Fig. 5b). In all of the experiments performed, GrpE behaved as a monodisperse protein system and did not show any sign of aggregation at concentrations from 50 up to 200 μg/ml. Sedimentation velocity analysis shows that human GrpE has an apparent sedimentation coefficient of 2.25 ± 0.05 s and a predicted molecular mass of 42 ± 1 kDa (Table I). The results of sedimentation equilibrium experiments were consistent with a dimeric protein state and a molecular mass of 43 ± 1 kDa at all velocities and concentrations tested; the analysis of residuals evidenced the high quality of the fit. The data generated by analytical ultracentrifugation are thus consistent with a dimeric model for GrpE with an elongated shape and a molecular mass of 42.5 ± 1.5 kDa (Table I).

**SAXS Measurements**—The SAXS curves at 5.0 and 19.4 mg/ml exhibit a similar q dependence, and therefore the protein is in the same configuration in both solutions, and the solutions are both in the dilute state so that the basic assumption of isolated proteins producing independent scattering amplitudes is obeyed. The experimental SAXS curves after normalization and subtraction of parasitic scattering are displayed in Fig. 6, a and b, together with the computed curve from a DAM model as described under “Experimental Procedures.” In Fig. 6a the SAXS intensity is plotted on a logarithmic scale to show the goodness of fit of the modeled function within the high q range. In Fig. 6b the SAXS intensity is given on a linear scale together with a Guinier plot (log I(q) versus q², inset) (45), which allows determination of the Rg of the protein from the slope of the linear portion within the low q limit of the curve. The distance distribution function associated with the scattering object, p(r), was evaluated with the GNOM package, and Rg was determined from the Guinier plot, also by applying the GNOM program. The molecular mass was obtained from SAXS results by comparing the intensity extrapolated to q = 0 with the corresponding data on bovine serum albumin and determined to be 38 ± 2 kDa. This value indicates that the GrpE protein is in a dimeric state, both at low and high concentrations (5.0 and 19.4 mg/ml, respectively).

The experimental values of the maximum diameter of the protein, Dmax = 155 Å obtained from the curve plotted in Fig. 7 and the Rg = 45.2 Å, obtained from the Guinier plot, and calculated by GNOM program, suggest that the protein is very elongated. This is also evidenced by the shape of the distance distribution function p(r) plotted in Fig. 7, which exhibits the typical asymmetry corresponding to very elongated scattering particles. This elongated shape of human GrpE caused the protein to elute from a gel filtration column earlier than expected for a typical globular protein with similar molecular mass (data not shown).

**Ab Initio Shape Determination of Human GrpE by DAMMIN**—The human GrpE shape was determined by an *ab initio* procedure using the DAMMIN program (35). In this method, a sphere of diameter Dmax is filled with densely packed small spheres (dummy atoms) with radius r0 ≪ Dmax. This method uses a simulated annealing algorithm and searches to minimize a function that accounts for the differences between ex-
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Fig. 4. DSC. Human GrpE temperature-induced unfolding was investigated by DSC with protein concentrations of 7, 45, and 130 μmol/liter and in two sets of buffers: 25 mmol/liter Hepes pH 7.6, 50 mmol/liter KCl, and 5 mmol/liter MgCl₂, and 25 mmol/liter Tris-HCl, 150–500 mmol/liter NaCl, and 1 mmol/liter β-mercaptoethanol. The scan rate varied from 0.5 to 1.5 °C/min, and the temperature measurement range was from 10 to 100 °C. Curves are presented for the first (solid line) and second (dotted line) temperature-induced unfolding of GrpE, showing that the unfolding is irreversible (even if the first scan was up to only 57 °C). The protein started to unfold at about 50 °C. There are at least two unfolding events, one has a midpoint at 57 °C, and the other is probably aggregation that occurs above about 62 °C. The human GrpE unfolding profile shown here is independent of salt concentration from 50 to 500 mM, of concentration from 7 to 20 μmol/liter, and of scan rate.

Fig. 5. Analytical ultracentrifugation experiments. a, sedimentation velocity. Sedimentation velocity experiments were carried out at 20 °C, 40,000 rpm (AN-60Ti rotor), and with scan data acquisition at 230 nm. The figure shows the experiment with 200 μg/ml GrpE in 25 mmol/liter Tris-HCl buffer, pH 7.5, 150 mmol/liter NaCl, and 0.5 mmol/liter DTT. The g(ś*) distribution was fitted using the ORIGIN (MicroCal Software) with a Gaussian giving a sedimentation coefficient of about 2.25 Svedberg (s_20,W = 2.34). The plot also shows that GrpE is homogenous. b, sedimentation equilibrium. Sedimentation equilibrium experiments were made at 7,000, 9,000, and 11,000 rpm (AN-60Ti rotor) with scan data acquisition at 230 nm. The best fit of experimental data for 100 μg/ml GrpE at 7,000, 9,000 and 11,000 rpm at 20 °C is shown (for details, see “Experimental Procedures”). The random distribution of the residuals (bottom panel) is a good indication that the fit is correct. The fit agrees with a dimer structure of human GrpE (43 ± 1 kDa).

The crystal structure of E. coli GrpE (17) was used for modeling the human GrpE with the SWISS-MODEL, PredictProtein, and HyperChem programs (see “Experimental Procedures”). These programs yielded the estimates of the global structure of human GrpE shown in Fig. 8, b–e. Fig. 8b shows the human GrpE dimer structure modeled from the proximal monomer of the E. coli GrpE crystallographic structure (17) fitted into the DAM model. The fit of the β-sheet domains is not satisfactory. In the GrpE dimer, the proximal monomer is bound to DnaK-NBD, whereas the distal monomer is not (17). Analyses of Fig. 8, c–e, where the human GrpE dimer structure modeled from the distal monomer of E. coli GrpE is fitted into...
TABLE I
Mathematical, structural and dynamic parameters of human GrpE in solution obtained from different experimental procedures and modeling methods

| Mathematical, structural, and dynamic parameters | SAXS\(^a\) | DAM\(^\dagger\) | AUC\(^\ddagger\) | HydroPro |
|-----------------------------------------------|-------------|-------------|-------------|-----------|
| Free parameters                               | 20\(^*\)    | 2098        |             |           |
| Discrepancy X                                 | 1.4         | 1.8         |             |           |
| Molecular mass (kDa)                          | 38.0        | 42.5        |             |           |
| \(R_g\) (Å)                                   | 45          | 46          | 45          |           |
| \(D_{\text{max}}\) (Å)                       | 155         | 160         | 156         |           |
| \(D\) \((\times 10^{-7} \text{ cm/s})\)      | 4.7\(^\dagger\) | 4.8\(^\dagger\) |             |           |
| \(s^*\) (Svedberg)                           | 2.25        | 2.20        |             |           |
| \(P_0/f_0\)                                  | 1.7         |             |             |           |

\(^a\) Calculated from experimental data.

\(^\dagger\) DAM parameters of the dummy atoms model averaged over 20 models.

\(^\ddagger\) Equilibration sedimentation and sedimentation velocity experiments.

\(^\dagger\) Number of Shannon channels given by \(D_{\text{max}} - q_{\text{max}}/TT\).

\(^\dagger\) Calculated by the second moment method.

the DAM model, indicate that the \(\beta\)-domains and the \(\alpha\)-helical tail fit well. The comparison of Fig. 8, \(b\) and \(c\), suggests that, in solution, human GrpE is in the distal monomer conformation. Fig. 8\(f\) shows the superposition of the \(E.\ coli\) GrpE dimer structure obtained by x-ray diffraction (17) onto the low resolution DAM model for the human GrpE. The proximal monomer (right), which binds to DnaK-NBD, does not exhibit a satisfactory fit, whereas the distal monomer (left) yields a good fit. Our model of the region corresponding to the first 33 residues of GrpE, deleted in the crystal structure (17), took into account the \(\alpha\)-helical secondary structure prediction and the best fit in the envelope (Fig. 8, \(c\) and \(d\)). To evaluate the reliability of the structure obtained from the SAXS results, the HydroPro program was used to calculate the hydrodynamic parameters of the DAM model, yielding values very similar to those determined experimentally (Table I). Such results provide additional support for the low resolution structure proposed here for human GrpE in solution ensuring that it represents a good approximation of the real conformation of the protein.

**DISCUSSION**

GrpE plays an important function in protein folding. It forms a stable complex with DnaK (Hsp70) which dissociates in the presence of ATP (46), helping to fold nascent proteins (13). GrpE is essential for bacterial viability (14), lowers the amount of DnaK required for function (47), and acts as the nucleotide exchange factor for the Hsp70 DnaK subfamily (12). Human mitochondrial GrpE was cloned in its mature form, without the mitochondrial peptide signal. This recombinant protein was produced in large scale and was purified as a soluble molecule. The purified protein is folded and presents a high amount of \(\alpha\)-helices as a component of its secondary structure. The shape of the human GrpE CD spectra and the calculated secondary structure are similar to \(E.\ coli\) GrpE (17). Although no detailed information about the tertiary structure is given by far UV CD spectra analysis, the secondary structure information itself is an indication that human and \(E.\ coli\) GrpE share at least some structural homology. However, the thermal unfolding profile of human GrpE measured either by CD or DSC differs from prokaryotic GrpE (18, 19), showing that these proteins differ in stability. The midpoint of the first thermal transition of human GrpE is at about 57 °C and is only partially reversible even if it is heated only to 50 °C. The first thermal transition of \(E.\ coli\) GrpE is at about 50 °C and is reversible if the protein is heated only up to the end of the transition at 60 °C (19). The first thermal transition in \(E.\ coli\) GrpE has been suggested to be the unfolding of the N terminus (18), a region with lower similarity to human GrpE than the C terminus (Fig. 1). Several works suggest that the N and the C termini of GrpE have different functions. The N terminus apparently acts as a temperature sensor for DnaK-DnaJ-GrpE function (18, 19, 48). We believe that the N-terminal region in human GrpE is probably more stable than in \(E.\ coli\) GrpE, thus shifting the thermal transition of this region to a higher temperature. This causes the N- and C-terminal regions to unfold simultaneously and thus not be able to refold. \(E.\ coli\) GrpE thermal unfolding is reversible.

**FIG. 6.** Experimental SAXS curves of human GrpE in solution. **a**, plot of log I versus q (logarithmic scale) focusing on the features of SAXS curves at high q, b, plot of I versus q (linear scale) focusing on the features at small q. **Inset** Guinier plot (log I versus q\(^2\)) within the small q range. 1, experimental curve; 2, experimental curve after subtraction of a constant value as described under “Results”; 3, scattering intensity computed from the DAM model (DAMMIN). Curves 1 and 2 are indistinguishable.
when it is heated above the first thermal transition, where only the N-terminal domain is unfolded (18). Unfortunately, the thermal induced unfolding of human GrpE also results in aggregation, which prevents a more precise thermodynamic analysis.

The analysis of the quaternary structure of human GrpE strongly indicates that this protein exists as a dimer. Both sedimentation velocity and equilibrium sedimentation measurements of human GrpE in solution point to a protein with a molecular mass of 42–43 kDa at all concentrations tested. This molecular mass is about twice the molecular mass of the human GrpE monomer. This result is in good agreement with the analytical ultracentrifugation data generated for E. coli GrpE (16). The analysis of the crystal structure of E. coli GrpE confirms that this protein is a dimer with an elongated shape. Our hydrodynamic analysis of human GrpE also agrees with an elongated shape for this protein, and our analytical ultracentrifugation analysis leads to a sedimentation coefficient compatible with a nonglobular shape for human GrpE. SAXS data were used to generate an ab initio model (DAM) of human GrpE (see discussion below) which resulted in an elongated dimer model for GrpE. Together, the results obtained with analytical ultracentrifugation and SAXS data demonstrate that human GrpE has a high friction coefficient (f) for a protein of 42–43 kDa, and consequently a high translational friction ratio (P). The hydrodynamic parameters computed from this model are in good agreement with those determined experimentally, which gives confidence in both our results and the structural model presented here. The hydrodynamic analysis of human GrpE indicates that this protein has structural similarity to E. coli GrpE.

The elongated shape of GrpE is the most likely reason for the apparently much too high molecular mass calculated from molecular exclusion chromatography experiments (16). Several works have indicated that the elongated shape of GrpE serves the purpose of allowing its N terminus to also interact with DnaK-SBD. The deletion of the first 33 amino acid residues abolishes the release of substrate by DnaK (17), the mutation of residue Glu-53 results in a temperature-sensitive phenotype for lambda replication (49), and the GrpE\textsubscript{L,89} fragment affects the release of peptide by DnaK but not the exchange rate of nucleotide (48). These results suggest that GrpE reduces DnaK affinity for substrate by interacting with SBD. Although the question of whether the GrpE N terminus binds to DnaK or not is still a matter of discussion, our data indicate a clearly elongated shape of human GrpE, the dimer being large enough and sufficiently flexible to accomplish this function.

The high resolution structure of E. coli GrpE bound to NBD of DnaK shows that this protein is a dimer with a cruciform format (17). The C terminus is composed mainly of β-sheet in a rounded form, and the N terminus is composed of elongated α-helices that almost coil around each other. GrpE interacts with DnaK in 2:1 stoichiometry (16), and the crystallographic data (17) show that this interaction is asymmetric: only one GrpE monomer interacting with NBD of DnaK. The overall structure shown herein for human GrpE in solution also has a cruciform format and fits well with the E. coli GrpE high resolution structure (17). Together, all of the results indicate that the human GrpE structure is quite similar to the E. coli homolog. The fact that human and E. coli GrpE have similar global structures is expected because these proteins share about 53% similarity. Conservation of the amino acid sequence in this family suggests that the structure is also likely to be conserved.

To test our bead model we generated two possible human GrpE structures from each monomer of E. coli GrpE. The monomer of E. coli GrpE that is bound to DnaK has the C-

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**Fig. 7. Distance distribution function.** Pair distance distribution, \( p(r) \), of human GrpE showing the typical asymmetry that is expected for elongated (prolate) scattering objects. The \( D_{\text{max}} = 155 \) Å, is indicated by an arrow.

**Fig. 8. Low resolution DAM models derived from the results of the SAXS experiment and molecular modeling for human GrpE.** a, front view of human GrpE DAM model showing the elongated leaf-like shape. Grid spacing is 20 Å. b, front view of human GrpE dimer structure modeled from proximal monomer of E. coli GrpE crystallographic structure (17) fitted into the DAM model (for details, see “Experimental Procedures”). In the GrpE dimer, the proximal monomer is bound to DnaK-NBD, whereas the distal monomer is not. This figure indicates that the fit of the β-sheet domains is not satisfactory, c, front; d, side; and e, top view of human GrpE dimer structure modeled from the distal monomer of E. coli GrpE fitted into the DAM model (for details, see “Experimental Procedures”). The β-domains and the α-helical tail are well fit by the model. Comparison of (b) and (c) indicates that, in solution, human GrpE is in the distal monomer conformation. These models suggest that the binding of DnaK moves the β-domain away from the helical axis and that GrpE is an asymmetric dimer when not bound to DnaK. f, superposition of the E. coli GrpE dimer structure solved by single crystal x-ray diffraction (17) into the low resolution DAM model for the human GrpE. The proximal monomer (right), which binds to DnaK-NBD (17), does not yield a satisfactory fit, whereas the distal monomer (left) evidences a satisfactory fit. The model was displayed with the programs MASSHA and PYMOL (pymol.sourceforge.net).
terminal domain more “open,” compared with the unbound monomer (Fig. 8f) (17). The human GrpE structure generated using the bound monomer as a template does not fit well to the bead model generated from SAXS results (Fig. 8b), whereas the structure generated using the unbound monomer as a template exhibits a much better fit (compare Fig. 8, b and c). This suggests that in solution, when not bound to DnaK, GrpE is a symmetric dimer with each monomer in a “closed” conformation. The analysis of the E. coli GrpE structure (17) shows that the monomer and the dimer have perpendicular asymmetry, the C terminus has a different shape than the N terminus. The structure of the C terminus has a different shape than the N terminus. The monomer and the dimer have perpendicular asymmetry, the C terminus of one of the monomers bends away from the axis (this monomer is known as the proximal monomer). The binding of DnaK then causes the C-terminal domain of the proximal monomer to open, probably to expose residues that will bind to DnaK. It is interesting to note that Harrison et al. (17) showed that part of the surface between the β-domain and the four-helix bundle domain makes contact with DnaK. This part is more accessible in an open conformation.

The crystal structure resolution of GrpE was done with a protein deleted of the first 33 residues, opening the terminal domain more.

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