The C-terminal (331–376) Sequence of Escherichia coli DnaJ Is Essential for Dimerization and Chaperone Activity

A SMALL ANGLE X-RAY SCATTERING STUDY IN SOLUTION

Received for publication, April 4, 2005, and in revised form, April 21, 2005
Published, JBC Papers in Press, April 21, 2005, DOI 10.1074/jbc.M503643200

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DnaJ, an Escherichia coli Hsp40 protein composed of 376 amino acid residues, is a chaperone with thiol-disulfide oxidoreductase activity. We present here for the first time a small angle x-ray scattering study of intact DnaJ and a truncated version, DnaJ (1–330), in solution. The molecular weight of DnaJ and DnaJ (1–330) determined by both small angle x-ray scattering and size-exclusion chromatography provide direct evidence that DnaJ is a homodimer and DnaJ (1–330) is a monomer. The restored models show that DnaJ is a distorted o-shaped dimeric molecule with the C terminus of each subunit forming the central part of the o, whereas DnaJ (1–330) exists as a monomer. This indicates that the deletion of the C-terminal 46 residues of DnaJ impairs the association sites, although it does not cause significant conformational changes. Biochemical studies reveal that DnaJ (1–330), while fully retaining its thiol-disulfide oxidoreductase activity, is structurally less stable, and its peptide binding capacity is severely impaired relative to that of the intact molecule. Together, our results reveal that the C-terminal (331–376) residues are directly involved in dimerization, and the dimeric structure of DnaJ is necessary for its chaperone activity but not required for the thiol-disulfide oxidoreductase activity.

Hsp40 proteins collaborate specifically with Hsp70 proteins and some other factors to participate in a wide range of cellular processes essential to cell survival, such as assisting the folding, assembly, disassembly, and translocation of newly synthesized proteins, by suppressing aggregation or mediating degradation of misfolded proteins (1, 2). The Hsp40 family proteins are divided into three subtypes (3) (see Scheme 1). They all contain a J domain responsible for binding to the ATPase domain and stimulating the ATPase activity of Hsp70. Type I Hsp40 proteins, such as Escherichia coli DnaJ, yeast Ydj1, and human Hdj-2, have a Gly/Phe-rich region, which has been suggested to assist J domain interactions with Hsp70 (4). The Gly/Phe-rich region is followed by a conserved cysteine-rich region forming a zinc finger domain and a poorly conserved C-terminal domain. The peptide binding site(s) has been found to locate within these two domains (5–7). Type II Hsp40 proteins, such as yeast Sis1 and mammalian Hdj-I, also contain the J, a Gly/Phe-rich region, and C-terminal domains but without the zinc finger domain (8, 9). Both type I and type II Hsp40 proteins act as molecular chaperones to bind and deliver non-native proteins to Hsp70 (7, 8, 10), but they are not functionally equivalent (11). Type III Hsp40 proteins, such as yeast YJL162c and virus T antigen, contain only the J domain, and they do not function as molecular chaperones (3).

In addition to the DnaK-dependent co-chaperone activity described above, DnaJ also exerts autonomous DnaK-independent chaperone activity (9, 12, 13), which is characterized by its ability to bind to unfolded proteins and prevent them from irreversible aggregation (10, 11). Moreover, DnaJ has been found to have thiol-disulfide oxidoreductase activity but little, if any, isomerase activity (14).

Although a large number of Hsp40 proteins have been discovered in prokaryotic and eukaryotic cells, only a few structures have been solved. These include the J domains of DnaJ (15) and Hdj1 (16), and the zinc finger domain of DnaJ (17) solved by NMR methods, and the peptide-binding fragments of yeast Sis1 (18) and Ydj1 (19) solved by x-ray crystallographic techniques. To date, no structure has been reported for the entire Hsp40 molecule. DnaJ has been shown to exist as a homodimer of two 41 kDa-subunits, each composed of 376 amino acid residues, by size exclusion chromatography. Nevertheless, the structural elements responsible for dimerization have not been yet elucidated. Yeast type II Hsp40 protein, Sis1, has been shown to dimerize through a short C-terminal stretch of 15 amino acid residues, residues 338–352 (18). Considering that the C-terminal domain II (residues 260–337) of Sis1 is conserved between type I and II Hsp40 proteins, and the sequence (338–352) of Sis1 is highly homologous to residues 331–345 of DnaJ (8), we prepared a truncated DnaJ by removing the C-terminal residues 331–376. Using this truncated DnaJ (1–330) and full-length DnaJ, we investigated the structural and functional roles of residues 331–376 in maintaining the biological activities of DnaJ.

Small angle x-ray scattering (SAXS) has recently been proven to be a powerful tool for investigating the solution structure of biomacromolecules. This, in part, is because of a
significant improvement in the ab initio methods for restoring the three-dimensional shape of a molecule from the observed one-dimensional scattering profile in a model-independent manner (20–23), i.e. no detailed structural data, such as crystal structure coordinates, are required.

In this communication we report, for the first time, determination of the low-resolution structure of full-length DnaJ and DnaJ (1–330) in solution using the SAXS technique with two independent ab initio shape-determination programs, DAMMIN (21) and GASBOR (22). The results clearly indicate that the DnaJ molecule exists as a distorted o-shaped homodimer with the C terminus of each subunit associated to form the central part of the ω, whereas the truncated DnaJ (1–330) fails to dimerize. Further, biochemical studies reveal that monomeric DnaJ (1–330) is severely deficient in peptide binding capacity and therefore lacks the autonomous chaperone activity. However, it retains the full thiol-disulfide oxidoreductase activity of the intact DnaJ homodimer.

**EXPERIMENTAL PROCEDURES**

**Proteins Expression and Purification**—The expression plasmid pUHE21-2 containing the full-length E. coli DnaJ gene was generously donated by Dr. J. Buchner, Institut für Organische Chemie und Biochemie, TU München, Germany. DnaJ protein was expressed and purified according to Zylicz et al. (24) and Tang and Wang (14).

The plasmid carrying the coding sequence for the M 1-P330 fragment of DnaJ was constructed by cloning a DNA fragment with BamHI and PstI sites, amplified from the plasmid pUHE21-2, into the pBluscript II/SK(+) vector, and then subcloning into pUHE21-2 via the same restriction sites. Transformed XL1 blue cells were grown in 2YT medium (1.6% tryptone, 1% yeast extract, 0.5% NaCl) containing 100 μg/ml ampicillin at 30 °C, and the overnight culture was diluted 100-fold and incubated for 6 h followed by induction for 4 h with 0.1 mM isopropyl-β-D-thiogalactoside. The cell pellet was suspended in buffer A (Tris buffer containing 50 mM NaCl and sonicated. If not specified, 50 mM Tris-HCl buffer (pH 8.0) was used in all experiments and is referred to as Tris buffer. The supernatant was loaded onto a DEAE-Sepharose fast flow column equilibrated with buffer A. The flow-through was then applied onto a Bio-Scale S column (Bio-Rad) equilibrated with buffer A and eluted with 10 mM and then 15 ml of Tris buffer containing 150 and 200 mM NaCl, respectively. Examination by SDS-PAGE showed that the fractions eluted with 200 mM NaCl were DnaJ (1–330) with a molecular mass of 36 kDa. The purified DnaJ and DnaJ (1–330) were stored in elution buffer at -80 °C.

**Glyceroldehyde-3-phosphate dehydrogenase (GPDH)** from rabbit muscle (25) and E. coli DebC, a periplasmic thiol-disulfide oxidoreductase (26), were kindly provided by G. P. Ren and Z. Zhao, respectively, of this group. Reduced and denatured RNase A was prepared essentially according to Pigiet and Schuster (27).

**Determination of Concentrations of DnaJ and DnaJ (1–330)** were determined by the Bradford method with bovine serum albumin (BSA) as a standard (28). Concentrations of BSA, GPDH, DebC, and native RNase A were determined spectrophotometrically at 280 nm with the absorption coefficients (A\text{280 cm}^{-1} μg^{-1}) of 0.66, 0.98, 0.7, and 0.695, respectively, and at 275 nm with ε_{275} = 9200 M^{-1} cm^{-1} for fully reduced RNase A (27). Homotetrameric GPDH and homodimeric DnaJ were considered as protomers in the calculation of molar ratios. Intrinsic and ANS fluorescence spectra were measured in a Shimadzu RF-5301 PC spectrophotometer at 25 °C with 280 nm and 380 nm for excitation, respectively. CD spectra in the far-ultraviolet region (200–250 nm) were determined using a Jasco 500 spectropolarimeter at 25 °C.

**SAXS Measurements**—Synchrotron SAXS measurements of DnaJ and DnaJ (1–330) were carried out at the beamline BL-10C of Photon Factory, Tsukuba (29). The scattered signals were detected using a one-dimensional position-sensitive proportional counter with 512 channels.

Samples were encapsulated inside a cell sandwiched by two thin parallel quartz windows 1 mm apart with a volume of 70 μl. All of the SAXS experiments were carried out in a sample holder maintained at 25.0 ± 0.1 °C. SAXS data of buffer and samples were collected alternatively in the frames of 60–300 s to avoid radiation-induced protein damage. Concentrations from 2.0 to 20.0 mg/ml for DnaJ and from 3.0 to 15.0 mg/ml for DnaJ (1–330) in Tris buffer containing 200 mM NaCl were used for the measurements of interparticle interactions.

The data reduction includes normalization of the one-dimensional scattered data to the intensity of the transmitted beam and subtraction of the background scattering of the buffer. All of the scattering curves were then standardized to that of a protein concentration of 1 mg/ml. The low angle data were extrapolated to infinite dilution and merged with the high angle data measured at high protein concentrations to yield final scattering curves.

**Scattering Data Analysis**—The scattering intensity I(q,c), expressed by the Guinier Equation (30),

\[ I(q,c) = I(0,c) \exp \left( -\frac{R_g^2 q^2}{3} \right) \]  

(1)

is a function of scattering vector q and protein concentration c. Here I(0,c) is forward scattering intensity, and R_{g}(c) is apparent radius of gyration at finite concentration.

At low protein concentrations, I(0,c) may be written by,

\[ Kc/I(0,c) = 1/M_w + 2A_g c + ... \]  

(2)

where K is a constant determined by using a series of concentrations of BSA as a reference protein with the known molecular mass of 67 kDa (31). M_w is the relative molecular weight of the protein and can be obtained by extrapolating K/I(0,c) to infinite dilution. A_g is the second virial coefficient resulting from interparticle interference effects and can discriminate between attractive and repulsive interactions. Repulsive interactions lead to positive values of A_g, and attractive interactions lead to negative values.

At the dilute limit, R_{g}(c) is given by,

\[ R_{g}(c)^2 = R_0^2 - B_g c + ... \]  

(3)

where R_0 is the radius of gyration at infinite dilution, and B_g is a parameter reflecting interosleate force potential (32). The sign of B_g means the same as that of A_g (30). The three parameters A_g, R_0, and B_g were calculated using Equations 2 and 3.

The pair-distance distribution function, P(r), given by,

\[ P(r) = \frac{1}{2\pi} \int I(q,r) \sin(qr) dq \]  

(4)
is a measure of the frequency of interatomic vector lengths within a protein molecule and can provide further information about the shape of the scattering particle.

Ab Initio Molecular Shape Determination—The overall shapes of the proteins were restored from the experimental data by two independent programs, DAMMIN (21) and GASBOR (22). Starting from a sphere with a diameter equal to the maximum particle size filled by ~1000 densely packed small beads (dummy atoms), DAMMIN searches for the best dummy atom model through minimizing the discrepancy function, \( f(X) = \chi^2 + aP(X) \), between the calculated and experimental curves using the simulated annealing method. \( aP(X) \) is a loosened penalty with positive weight for \( a > 0 \), and \( \chi \) is the discrepancy between the experimental \( I_{\exp}(q) \) and the calculated \( I_{calc}(q) \) curves

\[
\chi^2 = \frac{1}{N-1} \sum_j \left( \frac{I_{\exp}(q_j) - I_{calc}(q_j)}{\sigma(q_j)} \right)^2
\]

(Eq. 5)

where \( N \) is the number of experimental points, \( \sigma(q) \) is the experimental error at the momentum transfer \( q \). The method is to modify the coordinates of beads randomly, while always approaching the configurations that decrease the energy \( f(X) \). Starting from a random string, simulated annealing was employed to find a compact configuration of beads minimizing the discrepancy. In this method, a constant was subtracted from the experimental data to ensure that the intensity decay follows Porod’s law for homogeneous particles.

The x-ray scattering curves at high angles (\( q > 0.25 \) Å\(^{-1} \)) contain a significant contribution from the internal particle structure. GASBOR is a more versatile ab initio approach, especially for high scattering vectors. A dummy residues model was generated by a random walk Ca chain and was folded to minimize the discrepancy between the calculated and the experimental scattering data. GASBOR uses a simulated annealing approach to find a chain-compatible spatial distribution of an exact number of dummy residues, which correspond to the Ca atoms of amino acid residues. The dummy residues method permits fitting data up to a resolution of 5 Å, if the number of amino acid residues of the protein is specified.

Activity Assay—Thiol-disulfide reductase activity was assayed by measuring the turbidity increase at 650 nm because of insulin reduction (33) and was expressed as a ratio of the slope of the linear part of the turbidity curve to the lag time (34). Oxidase activity was assayed by the oxidative reactivation of reduced and denatured RNase A (27). Aggregation during the refolding of fully denatured GAPDH upon dilution in the presence of DnaJ or DnaJ (1–330) was followed by light scattering according to Tang and Wang (14) to measure the chaperone activity of the proteins.

RESULTS

SAXS Parameters—As shown in Fig. 1 the Guinier plots of experimental SAXS data at all concentrations used were linear in the low \( q \) region, indicating that the proteins do not undergo aggregation.

The function of \( K_r/I(0,c) \) evaluated from the intercepts of the Guinier plots is linear over the entire concentration range (Fig. 2A), and the slope represents the value of \( A_2 \). The square of apparent radius of gyration \( R_g^2(c) \) calculated from the slopes of the Guinier plots, as a function of protein concentration, also showed a linear relation (Fig. 2B), and the slope represents the value of \( B_2 \) (30). The SAXS parameters are summarized in Table I. The molecular mass of DnaJ, 94.7 kDa, is about twice the value 45.4 kDa for DnaJ (1–330). The values of \( A_2 \) and \( B_2 \) of DnaJ and DnaJ (1–330) are all negative, and the negative value of DnaJ (1–330) is ~2–3-fold larger than that for DnaJ, indicating even stronger attractive interactions between the DnaJ (1–330) molecules. The radius of gyration of DnaJ, 62.1 ± 0.3 Å, is greater than the value 39.2 ± 0.3 Å for DnaJ (1–330).

The pair-distance distribution function \( P(r) \) (Fig. 3) is obtained from the entire scattering curve \( I(q) \) in the range of \( q \), from zero to \( q_{max} \), by direct Fourier transformation (Equation 4). In the low angle region \( q < 0.05 \) Å\(^{-1} \), \( I(q) \) was extended to zero by using the radius of gyration at infinite dilution, i.e. \( R_g \) (0) (Table I). In the high angle region \( q > 0.05 \) Å\(^{-1} \), concentrated solutions were used to improve the statistics, as the scattering intensity is not affected by interparticle interactions.

FIG. 1. Dependence of Guinier plots on concentrations of DnaJ (A) and DnaJ (1–330) (B). The protein concentrations were as indicated. The straight lines were fitted with determined data in a region of 4.0 × 10\(^{-1} \)–1.5 × 10\(^{-2} \) (Å\(^{-2} \)) by the least squares method. For clarity, the plots have been arbitrarily shifted along the y axis.

FIG. 2. Protein concentration dependence of \( K_r/I(0,c) \) (A) and \( R_g^2(c) \) (B) of DnaJ (■) and DnaJ (1–330) (○). The values of \( K_r/I(0,c) \) and \( R_g^2(c) \) were calculated as described under "Experimental Procedures."

The maximum linear dimension, \( D_{max} \), determined from \( P(r) \) (Fig. 3) is 170 ± 5 Å for DnaJ and 120 ± 5 Å for DnaJ (1–330). The radius of gyration of DnaJ and DnaJ (1–330), estimated from the \( P(r) \) function by applying GNOM to the entire scattering profile in the range of 0.01 Å\(^{-1} \) to \( q_{max} \) is 61.5 ± 0.3 Å and 40.3 ± 0.3 Å, respectively, which is consistent with the corresponding value estimated from the extrapolated Guinier plots.

The \( P(r) \) functions calculated from the three-dimensional coordinates (PDB code 1BQ0) of the DnaJ sequence (1–77) (the J domain) and (PDB code 1EXK) of the sequence (131–
FIG. 3. Pair-distance distribution function $P(r)$. The $P(r)$ values of DnaJ (curve 1) and DnaJ (1–330) (curve 2) were calculated from the experimental scattering curves using Equation 4 under "Experimental Procedures." $P(r)$ of the sequence 1–77 (curve 3) and 131–209 (curve 4) of DnaJ were computed from the atomic models of the corresponding NMR structures by CRYSOL and GNOM.

209) (the zinc finger domain) using CRYSOL and GNOM were used to compare with those of DnaJ and DnaJ (1–330) (Fig. 3). The main peak at 47 Å and the $D_{\text{max}}$ of 120 Å for DnaJ (1–330) are both much larger than the corresponding values of 15 Å and 42 Å for the sequence 1–77 and 14 Å and 51 Å for the 131–209, respectively. The sum of the $D_{\text{max}}$ values for the sequences of 1–77 and 131–209, 93 Å, is smaller than the value of 120 Å obtained for DnaJ (1–330). This suggests that the two sequences and the fragment (210–330) are arranged to form an elongated DnaJ (1–330) molecule. DnaJ showed a spread $P(r)$ profile with the main peak at 75 Å and a $D_{\text{max}}$ of 170 Å, which is, however, much smaller than twice the $D_{\text{max}}$ of DnaJ (1–330), indicating that the two DnaJ (1–330) subunits are not arranged linearly.

**Ab Initio Molecular Shape**—Dummy atom modeling was performed from the scattering pattern up to $q_{\text{max}} = 0.25 \, \text{Å}^{-1}$ using DAMMIN within a spherical search diameter of $D_{\text{max}} = 120 \, \text{Å}$ for DnaJ (1–330) and 170 Å for DnaJ without symmetrical constraint. The uniqueness and the stability of the restored shapes were checked by repeating modeling. A dozen independent models were then aligned and averaged using programs SUPCOMB (35) and DAMAVER (36), which superimposed all models and determined the common envelope containing all models. In the next round, this common envelope was used as an initial search volume for all new models. Twelve runs of *ab initio* shape determination produced consistent results, as all output models yielded nearly identical scattering patterns (Fig. 4, dashed curves) with a discrepancy of $\chi = 1.3$ for DnaJ (1–330) and $\chi = 1.1$ for DnaJ to the respective corrected experimental data obtained by subtracting a constant in the scattering range up to 0.25 Å$^{-1}$. These final models were again aligned and averaged using SUPCOMB (35) and DAMAVER (36). The averaged dummy atom model of DnaJ at three orthogonal orientations (Fig. 5, left column) shows a molecule basically with a 2-fold symmetry along the middle line of the front view.

The restored dummy residues models were obtained by performing 12 runs with the GASBOR program using 752 dummy residues assuming no molecular symmetry and fitting the scattering profiles up to $q_{\text{max}} = 0.35 \, \text{Å}^{-1}$, i.e. with a resolution of $2\pi q_{\text{max}} = 18 \, \text{Å}$. The results are similar to those obtained by DAMMIN (data not shown). The models restored by both DAMMIN and GASBOR exhibit 2-fold symmetry.

A 2-fold ($P_2$) symmetrical constraint in GASBOR was employed using all 376 residues for one DnaJ monomer to obtain more reliable models. The final models fit the experimental data very well with a discrepancy of $\chi = 1.3$ in the entire scattering range as indicated by the solid curves in Fig. 4. DAMSEL in the package of DAMAVER was employed to align all of the possible pairs of models and to identify the most probable model, which should yield the smallest average discrepancy (Fig. 5, right column). The results suggest that DnaJ exhibits an $\omega$-shape.

Using the same approach described above, DnaJ (1–330) was restored to appear as an elongated roughly S-shaped molecule composed of three regions (Fig. 6, left column). The NMR structures of fragments 1–77 and 131–209 were accommodated well
within the envelope of the restored model of DnaJ (1–330). The NMR structure of the zinc finger domain (fragment 131–209) was superimposed with the restored DnaJ (1–330) model next to the J domain. The zinc finger domain is composed of two zinc fingers in an abnormal topology (17). It has been reported that zinc finger 1 is necessary for the autonomous chaperone activity in cooperation with the C-terminal domain (5, 7, 13), and zinc finger 2 together with the J domain are absolutely essential for the interaction with DnaK (13). Thus, we localized the zinc finger domain in such a way to make zinc finger 1 toward C-terminal domain and the zinc finger 2 close to the J domain, for fitting the different functions of the two zinc fingers.

Superposition of a half of the DnaJ model with one DnaJ (1–330) model obtained by GASBOR reproduced most of the intact DnaJ model, except the C-terminal part, which is absent in DnaJ (1–330) (Fig. 6, right column). The DnaJ exhibits as a distorted \( \omega \)-shaped dimer having the approximate dimensions of 150 \( \times \) 120 \( \times \) 110 Å. From the side view, the N termini of each subunit are swung slightly away from each other at the open end of the \( \omega \). The two C-terminal stretches from each subunit form the central part of the \( \omega \). There seems to be a cavity between the two monomers.

Size Exclusion Chromatography of DnaJ and DnaJ (1–330)—As shown in Fig. 7 DnaJ and DnaJ (1–330) both eluted as a single peak at positions corresponding to apparent molecular mass of 90 and 30 kDa, respectively. Relative to the calculated molecular mass of 41 and 36 kDa for the full-length fragment 1–376 and the sequence 1–330, respectively, it is concluded that DnaJ is a dimer, whereas DnaJ (1–330) is a monomer.

Conformation of DnaJ (1–330)—As shown in Fig. 8A the intrinsic fluorescence spectrum of DnaJ (1–330) showed the same emission maximum of 310 nm, but with 50% decreased intensity compared with that of DnaJ, although DnaJ (1–330) retains all of the fluorophores of the intact molecule. The ANS fluorescence spectrum of DnaJ (1–330) showed an emission maximum red-shifted to 509 from 497 nm for DnaJ and a decrease in intensity (Fig. 8B). The CD spectrum of DnaJ (1–330) was similar to that of DnaJ in both the shape and the
ellipticities at 208 and 222 nm (Fig. 8C).

Sensitivity of DnaJ (1–330) to Hydrolysis by Proteinase K—Limited hydrolysis by proteinase K was performed to compare the structural stability of DnaJ (1–330) with that of the intact molecule. As shown in Fig. 9, digestion of DnaJ by proteinase K for 20 min resulted in three main fragments of H11011 35, 22, and 14 kDa in addition to 20% of the intact protein remaining. When the truncated DnaJ was treated under similar conditions, it was digested almost completely to produce at least four main fragments of H11011 29, 20, 17, and 14 kDa with 25% of the total protein digested to pieces too small to be retained in the SDS gel. When the digestion was extended to 60 min, the DnaJ (1–330) was completely digested with 60% of the total protein running out of the gel, whereas only 10% of the protein ran out of the gel in the case of DnaJ digestion. The results indicate a higher sensitivity of the truncated DnaJ (1–330) to proteolysis by proteinase K compared with the intact molecule.

DnaJ (1–330) Is Severely Deficient in Chaperone Activity—As shown in Fig. 10, the presence of DnaJ efficiently decreased the denatured GAPDH aggregation to less than 5% during refolding when the concentration of DnaJ was increased to a 2-fold molar excess over GAPDH. However, DnaJ (1–330) failed to prevent the aggregation of denatured GAPDH even with its concentration at a 5-fold molar excess over GAPDH. These results reveal the importance of the C-terminal 46 residues in maintaining the peptide binding ability and chaperone activity of the DnaJ molecule.

DnaJ (1–330) Has Full Thiol-Disulfide Oxidoreductase Activities—DnaJ (1–330) at 5 μM showed reductase activity of 2.12 ± 0.14 × 10⁻⁴ ΔA₆₅₀ nm·min⁻¹, which is 1.6-fold higher than the activity of DnaJ (1–330) at 0.12 × 10⁻⁴ ΔA₆₅₀ nm·min⁻¹ observed for DnaJ (activity values are the average of three separate determinations, and Fig. 11A shows one experimental determination). Furthermore, similar to full-length DnaJ, DnaJ (1–330) also showed a marked ability to stimulate the oxidative refolding of denatured and reduced RNase A (Fig. 11B).

DISCUSSION

DnaJ Exists as an ω-Shaped Dimer—The low-resolution structure of the entire DnaJ molecule in solution has been determined for the first time by SAXS using two independent ab initio methods, which showed highly consistent results. The
restored DnaJ model shows an ω-shaped molecule with 2-fold symmetry along the middle line of the front view (Fig. 6A), whereas it appears as a distorted ω assessed from the side view (Fig. 6B) with the two ends swung away from each other at the open end. The superposition of one DnaJ (1–330) model with a half of the ω produced most part of the latter, indicating that the ω is a dimer. The apparent molecular mass of 94.7 ± 3.0 kDa determined from the scattering curves and 90 kDa by gel filtration both provide further evidence that DnaJ is a dimeric molecule in solution in accord with the monomeric molecular mass of 41 kDa.

**DnaJ (1–330) Is an S-shaped Monomer**—Our SAXS analysis revealed that DnaJ (1–330) is an elongated S-shaped molecule composed of three regions with a molecular mass of 45.4 ± 1.8 kDa, which is about half of the 94.7 ± 3.0 kDa determined for DnaJ. This observation is consistent with DnaJ (1–330) being a monomer. The NMR structure of the fragments of 1–77 and 131–209 of DnaJ can be accommodated well within the restored model (Fig. 6). DnaJ (1–330) displayed normal size exclusion chromatography behavior and retained full reductase and oxidase activities (see detailed discussion below). The intrinsic fluorescence spectrum and the CD spectrum of DnaJ (1–330) showed no significant change compared with that of DnaJ. In addition, the limited proteolysis pattern of DnaJ (1–330) by proteinase K was also similar to that of DnaJ under the same conditions although with higher sensitivity (see detailed discussion below). All of the above suggest that the deletion of the C-terminal 46 residues of DnaJ impairs DnaJ association so that the truncated DnaJ (1–330) exists as a monomer, even though it does not cause significant conformational change of the shortened molecule. We believe that the C-terminal (331–376) sequence is directly involved in the dimerization of DnaJ subunits. In addition, the truncated DnaJ (1–330) was expressed in *E. coli* as a soluble protein, unlike the intact DnaJ, which exists as a membrane-bound protein (data not shown). These observations suggest that the C-terminal sequence 331–376 may also be involved in interactions with the membrane. In this respect the transmembrane domain of DjlA, a DnaJ-like protein in *E. coli*, has been reported to be a dimerization domain (37).

**Dimerization Site**—The superposition of two DnaJ (1–330) on the dimeric DnaJ model does reproduce most of the intact DnaJ model, with the remaining being the C-terminal 331–376. It is therefore highly likely that two DnaJ subunits dimerize through the C-terminal 331–376 stretch, as in the case of type II Sis1 (18) and type I Ydj1 (19) of yeast Hsp40 proteins. Removal of the 331–376 sequence impairs the interactions between the two subunits and thus prevents the dimerization of DnaJ. However, the present restored model is not yet able to provide enough information to identify the dimerization site unambiguously. There are two possibilities for the mode of interaction between the two subunits: 1) the 331–376 stretch of residues from each of the subunits may interact with each other to contribute to the dimerization, and 2) this stretch on each subunit may interact with another part of the other subunit, as in the cases of Sis1 (18) and Ydj1 (19).

**Peptide Binding Site and Chaperone Activity**—DnaJ (1–330) shows no ability to prevent the aggregation of denatured GAPDH during refolding, and the lack of chaperone activity is not because of misfolding of this truncated protein. There is no significant conformational change, as discussed above, and full reductase and oxidase activities are retained. Therefore the lack of chaperone activity can only be ascribed to the lack of the C-terminal 331–376 sequence, which is directly involved in dimerization of DnaJ subunits.

Based on the present model we suggest that the central cavity between the two subunits of the dimeric DnaJ molecule is likely to be the site responsible for holding unfolded peptides or proteins in a by simultaneously binding with two binding sites. Removing the C-terminal residues 331–376 prevents the dimerization of DnaJ and thus fails to generate the required cavity for tight binding with the non-native peptide. In other words, the dimeric structure is necessary for the chaperone activity of DnaJ. Consistent with this, it was found that linking the monomeric thioredoxin, which has no chaperone activity, to the association domain of DsbC converts the mutant to a dimeric hybrid, and the dimerization bestows new chaperone activity (38).

Different from the double doughnut structure of chaperones, which contain a somehow isolated cavity for peptide binding, the V- or U- or ω-shaped structure of Sis1 (18), Ydj1 (19), DsbC (39) and DnaJ, provides a sufficiently large and flexible cavity between the two subunits for non-native peptide binding and contributes to the chaperone activity. Such a structure should facilitate the interactions between Hsp40 and Hsp70 proteins. A recent study (40) proposed that the chaperone action of DnaJ (binding to substrate) and its co-chaperone action (stimulating the ATPase activity of DnaK) cooperate in ternary (ATP-DnaK)ₙ-substrate-DnaJₙ complexes.
Enzymatic Activity of DnaJ (1–330)—Monomeric DnaJ (1–330), although with severely deficient in chaperone activity, exhibited slightly higher oxidoreductase activity compared with that of the dimeric DnaJ. This suggests that the enzymatic activity site (CXYC motif) (14) in the monomeric molecule is fully functional, and the dimeric structure is not required for this enzymatic activity. In fact only the CXYC motif in zinc finger 2 serve as the catalytic sites (41), and zinc finger 1 is not required for the activity (14, 41). Similarly, the reductase activity of DsbC also needs only one active site (42).

As mentioned above, the DnaJ (1–330) molecule shows no significant change in conformation and is slightly less stable than the intact molecule. The decreased stability may thus provide higher conformational flexibility necessary for the catalytic activity (43). Similarly, monomeric Sis1 (1–337) exhibited severe deficiency in chaperone activity but could fully stimulate ATPase activity of Ssa1 (18).

**Acknowledgments**—We sincerely thank Drs. J. Buchner and R. Glockshuber for the generous gift of pUHE21-2 and pDsbC, respectively. We are also grateful to Professor C. L. Tsou for his continuous encouragement in this work. The assistance of Dr. K. Katsumi and Professor Y. Izumi in the SAXS experiments is greatly appreciated. We thank Dr. D. Svergun greatly for the solution scattering data of BSA.

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