Iron-Sulfur Cluster Biosynthesis

BIOCHEMICAL CHARACTERIZATION OF THE CONFORMATIONAL DYNAMICS OF THERMOTOGA MARITIMA IscU AND THE RELEVANCE FOR CELLULAR CLUSTER ASSEMBLY*[

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Important for the understanding of the functional properties of the iron-sulfur scaffold IscU is knowledge of the structure and dynamics of this protein class. Structural characterization of Thermotoga maritima IscU by CD (Mansy, S. S., Wu, G., Surerus, K. K., and Cowan, J. A. (2002) J. Biol. Chem. 277, 21397–21404) and high resolution NMR (Bertini, I., Cowan, J. A., Del Bianco, C., Luchinat, C., and Mansy, S. S. (2003) J. Mol. Biol. 331, 907–924) yielded data indicating a high degree of secondary structure. However, the latter also revealed IscU to exist in a dynamic equilibrium between two or more distinct conformations, possibly existing in a molten globule state. Herein, we further characterize the molten globule characteristics of T. maritima IscU by near-ultraviolet circular dichroism, 1-anilino-8-naphthalenesulfonic acid binding, free energy of unfolding, hydrodynamic radius measurements, and limited tryptic digestion. The data suggest unusual dynamic behavior that is not fully consistent with typical protein states such as fully folded, fully unfolded, or molten globule. For instance, the existence of a stable tertiary fold is supported by near-UV CD spectra and hydrodynamic radius measurements, whereas other data are less clearly interpretable and may be viewed as consistent with either a molten globule or fully folded state. However, all of the data are consistent with our previous hypothesis of a protein sampling multiple discrete tertiary conformations in which these structural transitions occur on a “slow” time scale. To describe such proteins, we introduce the term multiple discrete conformers.

Isc proteins are integral components of the iron-sulfur cluster biosynthetic pathway for organisms as diverse as bacteria, Archaea, and eukaryotes (1, 2). By use of these pathways, cells are able to assemble protein-bound Fe-S clusters that are used for redox reactions, enzymatic catalysis, gene regulation, and structural stabilization. The cellular machinery for Fe-S cluster biosynthesis is necessary not only to provide appropriate proteins with their necessary metallocofactors, but, more importantly, to do so without suffering the toxic effects of free iron and sulfide. A main component of the Fe-S cluster biogenesis pathway is the protein IscU. IscU is believed to function as an Fe-S cluster scaffold in which a nascent Fe-S cluster is synthesized and subsequently delivered to target Fe-S approteins (3, 4). Coordination of the [2Fe-2S]1+ cluster to IscU is stabilized by substitution of a highly conserved Asp with Ala at position 40 (Thermotoga maritima numbering) (1, 3, 5, 6). Other characterized proteins within this system include IscS (which delivers sulfur to IscU) (1), IscA (7–10), a [2Fe-2S] ferredoxin (11, 12), IscR (13), and chaperones Hsc66 and Hsc20 (14).

In recent reports, we characterized the factors influencing the stability of IscU-bound clusters (12) and the reactions of IscU in cluster transfer to a target protein, apoferredoxin (11). We also addressed the mechanism of assembly of IscU-bound clusters (15) and identified a natural iron delivery protein (16). Elucidation of the structure of IscU would provide critical insight to our understanding of IscU chemistry; however, the structural characterization of IscU has proven to be challenging. Our structural characterization efforts have thus far been focused on a homolog from the hyperthermophile T. maritima (17). Initial structural characterization by circular dichroism correctly predicted a high degree of secondary structure (3). Other spectroscopic and functional evidence seemed consistent with a high degree of secondary structure and the presence of a stable tertiary fold. The strongest evidence for such was the high degree of chemical shift dispersion observed by 1H-15N heteronuclear single quantum coherence NMR experiments. However, subsequent efforts to further define the three-dimensional structure of T. maritima (Tm) IscU by NMR failed to identify a unique tertiary fold (17). Although the vast majority of the NMR data were consistent with a stable and rigid protein fold, the number of detectable long-range nuclear Overhauser effects was significantly below that which is normally observed for a protein the size of Tm IscU and provided too few distance constraints for satisfactory structural calculations of the tertiary fold. Much of the NMR data were, however, similar to those previously characterized for molten globule states of proteins. The term molten globule is generally used to describe a state in which a protein possesses a high degree of secondary structure without a stable tertiary fold (18–20). Molten globules often show large degrees of chemical shift dispersion, but lack long-range nuclear Overhauser effects (21, 22). The main difference in the results of NMR studies on Tm IscU relative to those reported previously for molten globule proteins is the dynamic data. Molten globules usually show motion on a nanosecond-picosecond time scale detectable by 15N relaxation measurements (23). Tm IscU did not appear to have significant nanosecond-picosecond motion. In addition to the dynamic data, several lines of evidence such as resonance splitting and hy-

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The abbreviations used are: Tm, Thermotoga maritima; ANS, 1-anilino-8-naphthalenesulfonic acid; GdnHCl, guanidine hydrochloride; WT, wild-type.
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drogen exchange suggested that flexibility existed on a millisecond time scale. Thus, it is presently unclear what tertiary state Tm IscU is in and whether that state reflects what is typically described as a molten globule. Therefore, we sought to characterize Tm apo-IscU by methods typically employed to identify molten globules. These methods include near-UV CD, 1-anilino-8-naphthalenesulfonic acid (ANS) binding, free energy hydrodynamic, hydrodynamic mean radius, and limited tryptic digestion. Apo- and holoproteins behave similarly by NMR criteria. However, due to complications arising from the instability of the bound Fe-S cluster of Tm holo-IscU, the majority of the structural data were acquired on Tm apo-IscU. The data suggest unusual dynamic behavior that is not fully consistent with typical protein states such as fully folded, fully unfolded, or molten globule. For instance, the existence of a stable tertiary fold is supported by near-UV CD spectra and the hydrodynamic radius, whereas other data are less clearly interpretable and may be viewed as consistent with either a molten globule or fully folded state. However, all of the data are consistent with our previous hypothesis of a protein sampling multiple discrete tertiary conformations in which these structural transitions occur on a “slow” (microsecond-millisecond) time scale. The significance of such dynamics and how they may be affected by partner protein interactions are discussed.

EXPERIMENTAL PROCEDURES

General Chemicals—ANS was obtained from Molecular Probes, Inc. (Eugene, OR); the low molecular mass protein ladder was from Invitrogen; and all other chemicals were from Sigma. Protein samples were expressed and purified as described previously (3). All protein concentrations were calculated based on monomer equivalents.

ANS Binding—ANS concentration was determined by absorbance using the previously determined extinction coefficient at 350 nm (24). The change in fluorescence emission was monitored by use of a PerkinElmer Life Sciences LS50B luminescence spectrometer with an excitation wavelength of 371 nm and a slit width of 5 nm, whereas emission was monitored at 482 nm with a slit width of 10 nm at 25°C. The buffer used was 100 mM Tris-HCl (pH 7.4). NaCl was varied between 0 and 200 mM. Data were corrected for the inner filter effect when necessary (25).

Near-UV CD—CD spectra were measured on an Aviv Model 202 circular dichroism spectrometer using a 1-cm path length cuvette. The protein concentration was 0.08 mM in 100 mM Tris-HCl (pH 7.4) at 25°C. Spectra were recorded in 0, 50, and 200 mM NaCl. Buffer spectra were subtracted. Spectra are averages of three measurements, except for those in guanidine hydrochloride (GdnHCl).

Dynamic Light Scattering—Light scattering was recorded on a DynaPro-801 (Protein Solutions, Charlottesville, VA) with a temperature-controlled microsample. The laser wavelength and scattering angle were 8294 Å and 90°, respectively. The instrument software (Dynamics Version 3.27) was used to calculate the hydrodynamic radius using the measured transversal diffusion coefficient and the Stokes-Einstein equation (Equation 1),

$$D_T = (kT/6\pi \eta R)$$

(Eq. 1)

where $D_T$ is the translational diffusion coefficient, $k$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the frictional coefficient, $\eta$ is the viscosity of the solution, and $R$ is the hydrodynamic radius. Solution conditions were 50 μM protein in 100 mM Tris-HCl (pH 7.4) at 22.7°C with varying NaCl concentrations of 0, 50, 100, 200, 300, and 450 mM. Samples were filtered through 0.2-μm filters (Whatman AnaTop 10) immediately prior to measurement.

Unfolding—GdnHCl-induced unfolding was monitored by CD. Measurements were carried out on an Aviv Model 202 circular dichroism spectrometer using a 0.1-mm path length cuvette, repeated three times, and averaged. Protein (0.08 mM) was incubated in 100 mM Tris-HCl (pH 7.4), 200 mM NaCl, and 0, 0.5, 1, 1.5, 2.5, 3.0, 3.2, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.7, 5.0, 5.5, 6.0, 7.0, and 7.3 mM GdnHCl overnight at room temperature. The GdnHCl concentrations were prepared by mixing stocks of 100 mM Tris-HCl (pH 7.4) and 200 mM NaCl and an identical solution with 0.1 M GdnHCl in which the pH was adjusted after dissolving all of the components. Unfolding was followed by monitoring the ellipticity at 222 nm as a function of GdnHCl concentration and analyzed by the linear extrapolation method (26), which assumes a two-state unfolding mechanism. Equations 2–4 were used to fit the data,

$$\Delta G_0 = -RT \ln K$$

(Eq. 2)

$$\Delta G_0 = C_m (H_2O) - m[GdnHCl]$$

(Eq. 3)

where $K$ is the equilibrium constant for unfolding, $f_0$ is the fraction of denatured protein, $\Delta G_0$ is the free energy of unfolding, $R$ is the gas constant, $T$ is the temperature in Kelvin, $\Delta G_0 (H_2O)$ is the free energy of unfolding extrapolated to zero denaturant, and the $m$ value is proportional to the amount of polypeptide exposed to solvent upon unfolding.

RESULTS

ANS Binding—ANS is a structural probe whose fluorescence is greatly dependent on its polar environment. For example, ANS fluorescence is negligible in aqueous solution, whereas in an apolar environment, its fluorescence is greatly increased and blue-shifted. ANS addition to native or fully unfolded protein solutions usually does not result in significant fluorescence. However, in the presence of partially folded or molten globule proteins, ANS exhibits a large degree of fluorescence, presumably by penetrating the protein’s hydrophobic core (27).

Under the current solution conditions and in the absence of protein, ANS showed weak fluorescence, with a maximum emission at 516 nm. Upon Tm IscU addition (either WT or D40A), the fluorescence increased ~16-fold and was blue-shifted to 478 nm (Fig. 1). Increasing sodium chloride concentrations resulted in decreased ANS fluorescence in the presence of protein. For example, in 200 mM NaCl, the ANS fluorescence decreased 2-fold. To ensure that a heating step in the purification of Tm IscU (3) was not responsible for the ANS binding properties of the protein, a His-tagged construct that was not subjected to this heat step was tested and found to have similar ANS binding characteristics. The presence of di-thiothreitol did not inhibit fluorescence. As an additional control, human apo- and holoferreredoxins were examined for ANS binding. Human ferreredoxin is regarded as a typical low molecular mass [2Fe-2S] protein that is not considered a molten globule in the apo or holo state. Under identical solution conditions used for the Tm IscU experiments, ANS fluorescence increased $<$2-fold in the presence of either human apo- or holoferreredoxin and slightly increased with increasing NaCl...
concentrations. As is commonly observed (28, 29), Scatchard plots of ANS-IscU titrations were not linear, indicating multiple ANS-binding sites, with the affinity of the first binding site being $\sim 1 \mu M$.

Near-UV CD—Near-UV CD spectra are sensitive to the protein’s tertiary structure surrounding its aromatic residues. Only fully folded proteins show significant signals within this region, whereas fully unfolded and molten globules do not (30). Both WT and D40A Tm IscU showed near-UV CD spectra indicative of a stable tertiary fold with minima at 297 and 299 nm and a maximum near 292 nm. These signals were lost in the presence of high concentrations of GdnHCl (Fig. 2). Sodium chloride did not significantly affect the near-UV CD spectra.

Dynamic Light Scattering—The measured translational diffusion coefficient $(D_T)$ was $8.83 \times 10^{-7}$ and $8.54 \times 10^{-7}$ cm$^2$/s for WT and D40A Tm IscU, respectively. Using the Stokes-Einstein equation, the hydrodynamic radius was calculated to be $2.4 \pm 0.1$ nm for WT Tm IscU and $2.5 \pm 0.1$ nm for D40A Tm IscU, which is normal for a dimeric protein of the size of Tm IscU and is in agreement with $^{15}$N NMR relaxation measurements (17). For comparison, the expected hydrodynamic radii of monomeric and dimeric Tm IscU are 2.0 and 2.5 nm, respectively. Sodium chloride did not have a significant effect on the measured $D_T$ and consequently did not influence the hydrodynamic radius. The translational diffusion coefficient and the hydrodynamic radius are related to the frictional coefficient of the molecule, which is indicative of the overall shape of the protein in solution. In particular, the ratio of the measured frictional coefficient $(f)$ to that calculated for the smallest rigid sphere capable of accommodating the protein $(f_{\text{sphere}})$ is typically between 1.2 and 1.3 for globular proteins. Values larger than this are observed for non-globular proteins with elongated tertiary folds (31, 32). The $f/f_{\text{sphere}}$ for both WT and D40A Tm IscU was 1.2.

Free Energy of Unfolding—The unfolding of Tm IscU was achieved by incubation with increasing concentrations of GdnHCl and was monitored by CD (Fig. 3). Assuming a two-state mechanism for unfolding $(\Delta G_f(H_2O))$ were 3.9 and 5.8 kcal/mol for WT and D40A Tm IscU, respectively. The dependence of the free energy on GdnHCl concentration is often represented by the $m$ value and reflects the amount of polypeptide that is exposed to solvent upon unfolding (33, 34). The $m$ values for WT and D40A Tm IscU were 0.9 and 1.4, respectively. A comparison of unfolding data between rigid protein folds and molten globules from both mesophilic and thermophilic organisms is reported in Table I.

Tryptic Digestion—Tryptic digestion under nondenaturing conditions of WT and D40A Tm IscU was monitored by mass spectrometry and SDS-PAGE (see Supplemental Fig. S1). Both proteins were relatively resistant to protease digestion, with nearly full-length protein fragments persisting for $> 19$ h. After 5 min, the predominant species present was the full-length protein. However, after 15 min, a slightly truncated species appeared that predominated for the duration of the measurements. This fragment spanned Met$^9$ to Pro$^{142}$, i.e. the full-length protein minus the first four residues. The limited proteolytic digestion of WT and D40A Tm IscU was not identical. For instance, after 30 min, several additional large peptides between 6 and 14 kDa were observed for D40A Tm IscU, but not for WT Tm IscU. Additionally, after 19 h, $<30\%$ of WT Tm IscU remained undigested, whereas $>40\%$ D40A Tm IscU sur-

![Fig. 1. ANS binding to Tm IscU. From top to bottom, the lines represent WT Tm IscU, D40A Tm IscU, and ANS alone without protein, respectively. In all samples were identical (50 $\mu M$ protein and $10 \mu M$ ANS). Further details are provided under “Experimental Procedures.”](image1)

![Fig. 2. Near-UV CD spectra of WT (solid line) and D40A (dashed line) Tm IscU in 100 mM Tris-HCl (pH 7.4) and 200 mM NaCl, 7 A u GdnHCl (dotted lines), deg, degrees.](image2)

![Fig. 3. GdnHCl denaturation of WT (open circles) and D40A (closed circles) Tm IscU. Solution conditions were 100 mM Tris-HCl (pH 7.4) and 200 mM NaCl with varying concentrations of GdnHCl.](image3)
DISCUSSION

Molten globule proteins display a variety of biochemical characteristics that are now often used to identify new members of this protein class. These characteristics include ANS binding (27, 35), far-UV CD reflective of a significant degree of secondary structure without near-UV CD signals (36), an expanded hydrodynamic radius that is nevertheless compact in comparison with fully unfolded proteins (37), decreased stability as reflected by $\Delta G_{m}$(H$_2$O) measurements (38), and increased susceptibility to protease digestion (39, 40). However, comparison of these data between different molten globules is complicated by the fact that there is wide variation among literature examples of what is meant by the term molten globule, covering the full range of possibilities between fully folded and fully unfolded proteins, i.e. those that are more similar to a rigid protein fold and those that lie nearer to an unfolded state. Additional complications are related to the method used to induce the molten globule state because most characterized molten globules do not exist under physiological conditions.

The data described here for Tm IscU are not fully consistent with a rigid protein fold, a molten globule state, or a completely unstructured fold. Indeed, none of the data suggest an unstructured conformation, so the structure of Tm IscU must either be a unique molten globule or possess a more typical fold with atypical dynamics. The present data that most clearly indicate a molten globule state because most characterized molten globules do not exist under physiological conditions.

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phobic interactions resulting in forced ANS binding. However, ANS binding to Tm IscU was found to decrease with increasing salt, thus eliminating forced binding as a possibility. Increased structural rigidity of the protein under high salt conditions is an unlikely cause for the decreased ANS fluorescence because the hydrodynamic radius and near-UV CD of Tm IscU are unaffected by NaCl. A more reasonable explanation is interference with electrostatic interactions between the negatively charged sulfonyl group of ANS (ionized between pH 1.5 and 12) (42) and Tm IscU. Although ANS is routinely used to probe for hydrophobic patches, it is known that both the non-polar amilinonaphthalene and the charged sulfonyl group can significantly contribute to protein binding (43). It is also possible that high salt concentrations preferentially stabilize one of the IscU conformations present in solution that does not possess high ANS affinity.

Much of the remaining data are indicative of a rigid protein fold. In particular, Tm IscU exhibits near-UV CD signals that are not observed for unfolded proteins. These bands reflect the asymmetric environment around the protein’s aromatic residues and suggest the formation of a stable hydrophobic core. Although some characterized molten globules show near-UV CD signals (44), they are largely due to the absorption of a coordinated chromophore and not to a well defined hydrophobic core. The hydrodynamic radius of Tm IscU is also normal for a globular protein fold. The measured translational diffusion coefficient can be used to calculate the protein’s frictional coefficient, which for Tm IscU is also typical for a globular fold. Even if this method of frictional coefficient analysis introduces some degree of error, it is clear that the hydrodynamic radius of Tm IscU is far from that of an unfolded protein. Using the method of Tanford et al. (45, 46), the hydrodynamic radius of fully unfolded Tm IscU is expected to be ~26 nm for a monomer and 40 nm for a dimer. For comparison, the hydrodynamic radius of a 85-kDa partner protein, Tm NifS, is 3.5 nm as calculated from crystallographic data (47) using the program HYDROPRO (48).

The free energy of denaturation and the tryptic digestion data are less clearly interpretable. Both techniques are extremely dependent on the characteristics of the individual protein, making comparisons between different proteins a difficult task. For example, values of the free energy of unfolding vary widely among a variety of rigid proteins relative to protein states described as molten globule (Table I). Therefore, even though molten globules show decreased stability in comparison to their associated native state, such differences are only apparent for induced molten globules. For proteins that exist only in a molten globule state under physiological conditions, there is no associated rigid state for comparison. Nevertheless, it is apparent that the ΔG°(H₂O) for Tm IscU is normal in as much as there are proteins with structurally rigid folds that have lower and higher free energies of unfolding. Indeed, nearly 6 x GdnHCl is required to fully unfold both WT and D40A Tm IscU. The results do tend to suggest that D40A Tm IscU is more stable than WT Tm IscU, with ΔG°(H₂O) = 1.9 kcal/mol. Interestingly, this value is similar to the difference in activation energies for Fe-S cluster transfer from D40A and WT Tm IscU to apoferredoxin, with ΔG° = 15.0 and 13.7 kcal/mol, respectively, and ΔG° = 1.3 kcal/mol (12). We reported previously that both the stability of the cluster and the rate constant defining cluster transfer to a target apoprotein are strongly influenced by the solvation state of the cluster pocket (12). It is therefore not unreasonable to expect that the difference in activation energies and cluster stability would dominate any difference in the denaturation free energy of WT versus D40A derivative protein. Even though the free energy of unfolding of Tm IscU appears to be normal, the m values are lower than typically observed for rigid protein folds. Such values can result from a molten globule state (49) or the presence of disulfide bonds (50). Because the dynamic properties of apo- and holoproteins are similar, as judged by NMR (17), it does not seem likely that disulfide bonds are present in IscU. IscU proteins contain three conserved Cys residues that bind the iron-sulfur cluster in the holo state (5, 51), thus preventing disulfide formation. Therefore, even though the free energy of unfolding calculated from denaturation experiments is not instructive with respect to the motional characteristics of the protein, the m value does appear to provide some measure of the rigidity of the protein’s tertiary state. In particular, all entries in Table I that are associated with “flexible” proteins show m values that are ~2.2. However, the m value cannot distinguish between molten globule proteins and proteins that possess motion on a distinct time scale (see definition below of a multiple discrete conformer class of protein).

Analysis of limited proteolytic digestion data suffers from the same complications as that of the free energy of unfolding, i.e. both techniques are more easily understood when comparing native and molten globule states of the same protein. Nevertheless, it is evident that Tm IscU is significantly more compact than an unfolded protein, with the nearly full-length protein being the predominant species present for ~2 h and persisting for ~19 h. This ability of Tm IscU to withstand large amounts of trypsin for long periods of time indicates that many of the tryptic sites of Tm IscU are buried for a significant period of time and thus are not solvent- or protease-accessible during these periods. Similar behavior for a dynamic protein experiencing motion on a microsecond-millisecond time scale has been observed (52). Of the 22 possible tryptic sites (not including Arg141, which is followed by Pro), nine were cleaved within 30 min, suggesting that the regions surrounding these sites are the most solvent-accessible or least structured segments of Tm IscU.

Comparison of the identified trypsin cleavage sites with previous structural data (17) is revealing. Between α1 and β2, four of the seven possible tryptic sites were cleaved (Fig. 4), and from the C terminus of α4 to the end of the molecule, five of the seven possible tryptic sites were cleaved. In fact, the least structured part of Tm IscU lies between α4 and α5, encompassing Cys124, as determined by NMR data. Of the two possible tryptic sites within this region, both were cleaved, further confirming that this region of the protein is unstructured. It is also interesting to note that a putative protein-protein interaction domain within α5 of IscU contains several Lys residues believed to mediate binding with target proteins (7, 12, 17). Of the four Lys residues within α5 of Tm IscU, three were cleaved by trypsin, consistent with these Lys residues being solvent-accessible and thus available for protein-protein interactions (Fig. 5). By limited tryptic digestion criteria, the most stable part of Tm IscU appears to lie in the region from β3 to α4, with none of the seven possible tryptic sites cleaved. However, it
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should be noted that the large peptides produced by limited trypsin digestion at these sites were observed only for D40A-Tm IscU, and not for WT Tm IscU. Also, these large peptides were subsequently rapidly degraded, resulting in large populations of nearly full-length protein and fully digested peptide fragments without significant populations of intermediate species. Such behavior suggests that Tm IscU does not consist of independently folded units and thus requires the whole molecule to fold properly. Therefore, the large fragments resulting from initial cleavage are not structurally stable and thus are rapidly degraded. It appears that D40A-Tm IscU is slightly more stable than WT Tm IscU.

Although the various studies that we have performed to investigate the solution structure of Tm IscU appear to indicate contradictory conformational states, they can be reconciled by a model in which Tm IscU alternates between different conformations on a millisecond time scale, as previously proposed (17). Those techniques that are not influenced by conformational flexibility such as near-UV CD and dynamic light scattering show characteristics indicative of a well folded protein. Such a result is expected if each conformational state that the molecule experiences is well defined relative to the time scales of the measurements. However, those techniques that are influenced by dynamic processes may result in data that resemble less folded structures. For instance, ANS binding and the value from denaturation experiments are consistent with a molten globule state, whereas the methods commonly used to identify molten globules yield unusual and surprising results. In particular, ANS binding and the m value from denaturation experiments are consistent with a molten globule state, whereas the results from other non-NMR experiments support a non-molten globule state. Future characterization of other members of this distinct family of molten globule-like proteins should therefore focus on comparing the results of ANS binding experiments and m values with data from near-UV CD and dynamic light scattering measurements. If trends are found that resemble the results described herein, then the possibility of slow motion conformational exchange should be further confirmed by NMR criteria. We suggest the term “multiple discrete conformers” to define these proteins with unusually slow conformational dynamics and to distinguish this class of protein from that which is nominally categorized as molten globule. Tm IscU therefore represents a class of protein that exists in two or more discrete conformational states that are interchangeable on a microsecond-millisecond time scale.

We have proposed earlier that structural flexibility is necessary for the function of IscU proteins in vivo (17). This function requires holo-IscU to dock with an apoprotein target and then to transfer an intact [2Fe-2S] cluster via a sequence of bond cleavage and bond formation events in which the ligand set from IscU is replaced with that from the target protein. Such a complex reaction presumably demands flexibility on the part of IscU as it prepares the cluster for transfer and then proceeds through a series of intermediate and transition states before cluster transfer can be achieved. Given the fact that IscU can transfer [2Fe-2S] clusters to a number of distinct protein targets, the need for plasticity in protein structure is readily understood. Other than recognition of diverse apo targets for cluster transfer, IscU needs to be capable of interacting with a variety of proteins with different folds to execute its function. Such proteins include sulfur donors, iron donors, holoferrredoxins, and chaperones. It is therefore plausible that, upon interaction with one of these partner proteins, a specific IscU conformation may be selected. Indeed, possible changes in the oligomeric state of IscU are accommodated by such a model. IscU may fluctuate between monomeric and dimeric states on a slow time scale and may form heterodimeric structures when complexed with partner proteins.

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