β-lactoglobulin at pH = 6.7 are biphasic and they show a clear dependence with protein concentration. While the transition that appears at higher temperature is insensible to changes in protein concentration the first transition is stabilized when protein concentration is increased. This result allowed proposing a mechanism that involves dissociation of the dimer in the first instance and subsequent denaturing of the monomer. According to the thermodynamic model of a dimer dissociation presented in a previous work (1), it was determined through calorimetric profiles simulations, that only a dimer with negative dimerization enthalpy shows the dependence on protein concentration observed in these experiments. We also performed isothermal titration calorimetric studies and the association parameters of β-lactoglobulin were obtained at pH = 6.7. The dimerization process is exothermic in accordance with the model. Furthermore, temperature induced β-lactoglobulin dissociation at pH = 6.7 was monitored with infrared absorbance spectroscopy and we can state that there are structural changes related to the dissociation of the dimer.

(1) J. Phys. Chem. B. 112 (45): 14325-14333, 2008.

153-Pos

Structure and Folding Thermodynamics of MfpA, a Pentapeptide Repeat Protein From *mycobacterium Tuberculosis*

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The *Mycobacterium tuberculosis* protein MfpA confers bacterial resistance to the antibiotic fluoroquinolone. MfpA is a dimer in solution and in the crystal. The C-terminal α helices of two monomers form the dimer interface. The shape and distribution of the negative charge on the surface of MfpA mimics those of DNA (1). We quantitatively explored the unfolding, refolding and aggregation of MfpA as a function of temperature, urea concentration and the anionic surfactant SDS by circular dichroism (CD) and intrinsic fluorescence. These analyses reveal a structural transition followed by aggregation of intermediate conformers; the intramolecular and intermolecular interactions occur almost simultaneously. Stacking of phenylalanine side chains stabilize the N-terminal portion of MfpA's pentapeptide thus expanding on the motif of DNA mimicry. The high Arrhenius activation energy of aggregate formation rationalizes the nature of the kinetic trap shown earlier (2) that facilitates aggregate formation. Although secondary structure contents can not be calculated accurately for α/β proteins from their CD spectra (3), the increased α-helical content and a long-wavelength shift of the fluorescence emission maximum show intramolecular secondary and tertiary structure changes along the structural transition of MfpA. Overall, the unfolding and refolding of MfpA in solution is described by the 'double funnel' energy landscape where the 'native' and 'aggregation' funnels are separated by the high kinetic energy barrier that is not overcome during *in vitro* refolding.

[1] S. Hegde, M. Vetting, S. Roderick, L. Mitchenall, A. Maxwell, H. Takiff and J. Blanchard, *Science 308*, 1480-1483 (2005).
[2] S. Khrapunov, H. Cheng, S. Hegde, J. Blanchard, and M. Brenowitz, *J. Biol. Chem. 283*, 36290-36299 (2008).
[3] S. Khrapunov, *Anal. Biochem. 389*, 174-176 (2009)

154-Pos

Concentration and Ion Induced Effects on Nucleotide Binding, Aggregation and Thermal Unfolding Transitions of Reca

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The *Escherichia coli* protein, RecA, catalyzes the DNA pairing and strand exchange reactions that are utilized in DNA recombination and repair. Buffer and salt conditions are known to influence the activity, aggregation state and thermal unfolding of RecA. We have used circular dichroism (CD), fluorescence, infrared and dynamic light scattering studies (DLS) to better understand the salt-induced effects on RecA structure, substrate binding and unfolding. CD and infrared studies were performed in order to monitor the thermally induced unfolding of RecA in the presence of a variety of salts and/or nucleotide and DNA substrates. Previous studies in our laboratory have shown that the concentration and identity of the salt ions resulted in unique influences on RecA unfolding transitions and stability. Unfolding studies performed under salt conditions known to activate RecA's ATPase activity in the absence of DNA showed thermally stable RecA structures. Additional characterization of these stable structures using DLS and fluorescence experiments shows unique aggregation states and nucleotide binding for some of the thermally stable RecA structures. A comparison of the influences of different ions on RecA unfolding, aggregation and nucleotide binding will be presented. These studies may help to elucidate how different ions influence RecA activity, structure, aggregation, and stability.

155-Pos

Influence of Matrix Metalloprotease on the Flexibility of Type I Collagen Fibrils Studied By Atomic Force Microscopy

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Collagen forms the main connective tissue in the body. Collagen turnover is intimately linked with healing of wounds, embryo development and tissue regeneration. Furthermore, the breakdown of collagen in various pathologies such as inflammatory arthritis and cancer is linked to disease progression and is accompanied by profound changes in its structure and mechanical response. Therefore, there has been increased interest in the study of mechanical properties of single collagen fibrils in the past decade. Nevertheless, the influence of the metalloproteases, which degrade collagen fibrils both in healthy tissue and a number of disease conditions, on mechanical properties of collagen has never been studied. In this work we present an investigation of the influence of MMP1 on the bending of type I collagen fibrils. Angular distribution of the segments of single collagen fibrils can be used to characterize the flexibility of collagen fibrils. High resolution images of the type I collagen fibrils were acquired by atomic force microscope (AFM) under ambient conditions in tapping mode. Angular distributions of segments of each collagen fibril were evaluated by an image recognition program from the resulting images. These distributions were compared and analyzed for native collagen type 1 fibrils and after treatment with matrix metalloprotease 1 (MMP1).

156-Pos

Population Analysis of Folding Intermediates From Time-Resolved and Spectral Fluorescence of Single-Tryptophan Apoflavodoxin

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The fluorescence of native apoflavodoxin (i.e. flavodoxin without cofactor FMN) from Azotobacter vinelandii has been extensively used to investigate thermal and denaturant-induced protein (un)folding. The protein populates an off-pathway molten globule-like intermediate during its equilibrium (un)folding. Fluorescence of apoflavodoxin arises mainly from its three tryptophans (Trp74, Trp128 and Trp167). With time-resolved fluorescence anisotropy of wild-type apoflavodoxin, tryptophan-tryptophan energy migration has been quantitatively measured to follow distance variations between two tryptophan pairs during apoflavodoxin (un)folding (N.V. Visser et al. (2008) Biophys. J. 95, 2462-2469). In this study we have followed a more general approach to analyze the time-resolved and steady-state fluorescence results of the single Trp74-mutant apoflavodoxin (Trp128 and Trp167 are replaced by phenylalanine), when it is gradually unfolded by addition of increasing amounts of guanidine hydrochloride. Singular value decomposition (SVD) of data matrices has been used to determine the number of species. In both types of experiments SVD shows the presence of three significant independent components. Therefore we can conclude that the three-state model (native, intermediate and unfolded state of the protein) should be used for further analysis. Each set of experimental data was globally analyzed using the three-state model finally yielding the relative concentrations of all species in the denaturation trajectory. The equilibrium thermodynamic properties were then determined from simultaneous and separate fits of the concentrations obtained from time-resolved and steady-state fluorescence data.

157-Pos

Photon-By-Photon Analysis of Single Molecule Fluorescence Trajectories of a Fast Folding Protein

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Förster resonance energy transfer (FRET) experiments of single protein molecules can be used to measure the structural and dynamical properties of sub-populations, as well as the kinetics of transitions between sub-populations. However, the timescale for study of both the dynamics and kinetics has been limited. To obtain folding and unfolding rate coefficients from FRET trajectories, for example, the bin size must be sufficiently long to calculate an accurate FRET efficiency, but also much shorter than the waiting times in the folded and unfolded states. In cases where the photon count rate is too low for these conditions to hold, an alternative approach is to analyze the photon-by-photon trajectories using maximum likelihood methods. Unlike ensemble methods, where

only the sum of the rate coefficients can be measured for a two state system, maximum likelihood methods allow the determination of the individual rate coefficients, and therefore also the equilibrium constant. Here we apply a maximum likelihood method recently developed by Gopich and Szabo (*J.Phys.Chem.*113pp10965-10973(2009)) to the photon-by-photon trajectories of a two-state designed protein, α_3D, that folds in ~ 1ms, too fast to measure accurately from waiting time distributions in FRET trajectories. Experiments were carried out on both freely-diffusing and immobilized molecules. The FRET efficiency distributions in the free diffusion experiment are broadened by the folding/unfolding transitions occurring within the time bin, a phenomenon similar to line broadening in NMR experiments. Mean FRET efficiencies and rate coefficients extracted using the Gopich-Szabo method were found to be reliable by comparing the sum of the rates with the relaxation rates obtained from the donor-acceptor cross correlation function. Finally, photon trajectories can be divided into folded and unfolded segments at a single photon level using the hidden Markov model (Viterbi algorithm) with extracted parameters.

158-Pos

Conformations and Dynamics of Polypeptide Chains Revealed By Tryptophan-Cysteine Contact Formation Kinetics

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Spectroscopic probes sensitive to intra-chain contact formation events in polypeptides are increasingly used to study the conformational and dynamical properties of different amino acid sequences. Quenching of the triplet state of tryptophan by close contact with cysteine enables the measure of contact formation rates without the need of extrinsic probes, thus being suitable for the study of natural proteins and peptides. We illustrate the use of this method to investigate the unfolded state of small proteins in conditions close to native and the kinetics of weakly structured protein fragments. The coexistence of different conformational states can be revealed from the non-exponential relaxation of the excited triplet, enabling the characterization of both the chain dynamics for each state and the transition kinetics. Moreover, the rate of contact formation measured for the least structured states is compared with those observed for model disordered peptides, allowing to estimate the strength of electrostatic and hydrophobic interactions between residues other than the probes. We test this approach with the widely studied GB1 15-residue C-terminal, which folds into a beta-hairpin structure. The kinetics of elementary conformational steps leading to the folded state is outlined, revealing the presence of misfolded states as proposed in recent computational works.

159-Pos

Electrostatic Interactions Affect the Mechanical Stability of Elastomeric Proteins

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It was predicted that the mechanical stability of elastomeric proteins can be affected by electrostatic interactions (Craig et al, Structure, 12, 2004, 21). To directly test this prediction, we engineered a bi-histidine mutant of a small protein GB1 (biHis-GB1). The two histidines were engineered across two force-bearing beta strands. Histidine residues can exist as protonated or deprotonated states depending on pH, thus we can adjust the pH value of the solution to modulate the electrostatic interactions between the two engineered histidine residues. We used single molecule atomic force microscopy to directly measure the effect of electrostatic interactions on the mechanical stability of biHis-GB1. We found that the unfolding force of biHis-GB1 gradually decreases as the electrostatic repulsion increases due to the lowering of pH value from 8.5 to 4. This result suggested that electrostatic interactions can indeed affect the mechanical resistance of biHis-GB1. We anticipate that this effect can be utilized as an effective method to tune the mechanical stability of elastomeric proteins at the single molecule level.

160-Pos

Molecular Mechanism of Urea-Induced Protein Denaturation

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For more than half a century, urea has been used as a strong denaturant in protein folding/unfolding studies. However, the molecular mechanisms of urea-induced protein unfolding still remain unclear. This lack of understanding is to some extent reflects the scarcity of direct thermodynamic information that can be used to characterize interactions of urea with amino acid side chains and the peptide group. We recently demonstrated that volumetric measurements combined with statistical thermodynamic approach may represent a novel and effective way to tackle this problem [Lee, S. & Chalikian, T. V. (2009) J. Phys. Chem. B. 113, 2443-2450]. In this work, we employ high precision acoustic and densimetric techniques to quantify the solvation properties of solutes in the presence of urea. Specifically, we report the partial molar volumes, V°, and adiabatic compressibilities, K_S°, of *N*-acetyl amino acid amides containing all 20 naturally existing amino acid side chains and oligoglycines, $(Gly)_{1\text{-}5}$, at urea concentrations ranging from 0 to 8 M. Using our developed statistical thermodynamic approach, that links volumetric observables of a solute with solute-solvent and solute-cosolvent interactions in binary solvents, we evaluate the binding constants, k, and elementary changes in volume, ΔV, and compressibility, ΔK_S, accompanying the replacement of water in the vicinity of the solutes with a urea molecule. While the binding constants are essentially similar for all protein groups, the magnitude and the sign of the determined values of ΔV and ΔK_S vary markedly. The latter values reflect the nature of urea interactions with specific functional groups and the concomitant changes in hydration. In general, our results are consistent with a picture in which urea interacts with polar, non-polar and charged groups with comparable affinities, although the underlying forces stabilizing each type of interaction depend on the chemical nature of the interacting group.

161-Pos

Confined Dynamics of a Ribosome-Bound Nascent Globin: Cone Angle Analysis of Fluorescence Depolarization Decays in the Presence of Two Local Motions

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We still know very little about how proteins achieve their native three-dimensional structure in vitro and in the cell. Folding studies as proteins emerge from the mega Dalton-sized ribosome pose special challenges due to the large size and complicated nature of the ribosome-nascent chain complex. This work introduces a combination of three-component analysis of fluorescence depolarization decays (including the presence of two local motions) and in-cone analysis of diffusive local dynamics to investigate the spatial constraints experienced by a protein emerging from the ribosomal tunnel. We focus on E. coli ribosomes and an all-alpha-helical nascent globin in the presence and absence of the chaperones DnaK and trigger factor. The data provide insights on the dynamic nature and structural plasticity of ribosome-nascent chain complexes. We find that the sub-ns motions of the N-terminal fluorophore, reporting on the globin dynamics close to the N terminus, are highly constrained both inside and outside the ribosomal tunnel, resulting in high-order parameters ($>$0.85) and small cone semiangles ($<30^\circ$). The shorter globin chains buried inside the tunnel are less spatially constrained than those of a reference sequence from a natively unfolded protein, suggesting either that the two nascent chain sequences have a different secondary structure and therefore sample different regions of the tunnel or that the tunnel undergoes local structural adjustments to accommodate the globin sequence. Longer globins emerging out of the ribosomal tunnel are also found to have highly spatially constrained slow (ns) motions. There are no observable spectroscopic changes in the absence of bound chaperones. The data presented here show that the ribosome plays an active role in cotranslational folding and it influences the dynamics and conformation, of nascent polypeptides and proteins.

162-Pos

Unraveling the Possible Mechanism Behind Leptomeningeal Amyloidosis Using as Model a Highly Unstable Transthyretin Tetramer

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Transthyretin (TTR) is a 127-residue -βsheet-rich protein homotetrameric that transports thyroxine in the blood and cerebrospinal fluid (CSF). Among all TTR variants, A25T is the most unstable tetramer. Its great instability induces TTR degradation in the endoplasmic reticulum of the hepatocytes, while thyroxine (T4, a natural ligand of TTR) leads to A25T secretion in the CSF by the choroid plexus. In the present study we aimed to determine the structure of A25T by X-ray crystallography in the apo form and in complex with T4. Also, by using high hydrostatic pressure, we have showed that the tetramers of A25T were less stable than the wt and L55P (the most aggressive variant of TTR). Besides, A25T showed to be the most amyloidogenic variant thus far investigated, aggregating in conditions where wt and L55P remain mostly soluble. Using HPLC and native PAGE, we monitored acrylodan-labeled TTR aggregation in the human plasma. The aggregates formed displayed the typical amyloid structure. In the presence of monomers of T119M, a non-amyloidogenic variant, aggregation of A25T was remarkably reduced, pointing to the use of T119M monomers as a strategy to avoid TTR aggregation. The crystal structure of A25T, when compared to that of the wt protein, shaded light into the