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.

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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.

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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).

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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.

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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