DNA recombination and DNA repair. Buffer and salt conditions influence the aggregation and activity of RecA. In low salt conditions, RecA is a DNA-dependent ATPase. However, prior research demonstrated that high salt concentrations allow RecA to hydrolyze ATP in the absence of DNA and at levels comparable to those obtained in the presence of DNA [Pugh, B. F. and Cox, M. M. (1988) *Journal of Biological Chemistry 263*, 76-83]. We have used circular dichroism (CD) and fluorescence spectroscopies to better understand the salt-induced effects on RecA structure and function. CD and fluorescence studies were performed in order to monitor the thermally induced unfolding of RecA in the presence of a variety of salts. We found that different salts had unique effects on RecA unfolding transitions and stability. Unfolding studies performed under salt conditions known to activate RecA's ATPase activity showed unique, thermally stable RecA structures. A comparison of the influences of different ions on RecA unfolding will be presented. These studies may help to elucidate how different ions influence RecA activity, structure, aggregation, and stability.

421-Pos Board B300

Folding/Unfolding of Glycolipid Transfer Protein: Molten Globule-Like Intermediates?

Ravi Kanth Kamlekar[1], Roopa Kenoth[1], Helen M. Pike[1], Franklyn G. Prendergast[2], Sergei Yu. Venyaminov[2], **Rhoderick E. Brown[1]**.
[1]University of Minnesota, Austin, MN, USA, [2]Mayo Clinic College of Medicine, Rochester, MN, USA.

Glycolipid transfer proteins (GLTPs) are small, soluble, single-chain proteins (~24 kDa) that selectively accelerate the intermembrane transfer of glycolipids in vitro. The GLTP-fold is unique among lipid-binding proteins. However, little is known about GLTP stability and folding/-unfolding. During isolation of heterologously expressed GLTP, FPLC size exclusion chromatography showed peaks corresponding to monomer, multimer, and a third peak of intermediate elution volume. Unexpectedly, native gel electrophoresis showed that the intermediate protein peak migrated as monomer rather than dimer, raising the possibility of a molten globule-like state. Intrinsic GLTP tryptophan fluorescence showed a blue-shifted (~2nm) emission wavelength maximum (λmax), indicating an altered tryptophan environment compared to monomer. ANS binding resulted in a large blue shift (~20nm) in λmax and dramatically enhanced emission intensity (~120%). Far-UV-CD showed retention of ordered secondary structure (>95%), but substantially reduced cooperativity during thermally-induced melting. Near-UV-CD analysis of induced optical activity of GLTP Trp/Tyr residues was insufficient to establish tertiary folding changes. To further evaluate GLTP unfolding intermediates, the effect of urea was studied. Trp emission changes suggested a two-step unfolding pathway involving intermediate formation at 4M urea and characterized by blue-shifted Trp emission. Additional urea induced further unfolding marked by red-shifted Trp emission. Far-UV-CD analyses of the 4M urea-induced intermediate indicated reduced ordered secondary structure and cooperative melting at lower temperature compared to native GLTP, but the near-UV-CD signal did not provide definitive insights into tertiary folding status. ANS binding showed 1nm blue shift and 60% increase in fluorescence intensity compared to untreated GLTP. Conditions are identified under which GLTP may exist in molten globule-like and other partially unfolded states. Studies on the significance of these intermediates with respect to function are underway. [Support: NIH/NIGMS GM45928 & GM34847, NIH/NCI CA121493, The Hormel & Mayo Foundations]

422-Pos Board B301

Elucidating The Specificity Determinants Responsible For ClpX-Adaptor Interaction

Tahmeena Chowdhury, Peter Chien, Robert T. Sauer, Tania A. Baker.
MIT, Cambridge, MA, USA.

The ClpXP proteolytic machinery in bacteria consists of the AAA+ ATPase protein ClpX and the serine peptidase ClpP. ClpX recognizes, unfolds, and translocates substrates into the protease chamber where hydrolysis of the polypeptide occurs. To control substrate-specificity, the cell uses adaptor proteins, such as SspB, to modulate substrate selection. The N-terminal domain of ClpX is the adaptor-docking site and also directly recognizes some substrates. However, very little is known about the sequence specificity of this important ClpX N-domain protein-binding site.

Recently, a homolog of *Escherichia coli* SspB was identified in *Caulobacter crescentus*. Despite structural and functional homology, there is limited sequence similarity between the two SspB adaptors. To learn more about ClpX-adaptor interaction specificity, we carried out a systemic mutational analysis of *C.crescentus* SspB to identify the residues responsible for its interaction with ClpX. Functional assays monitoring adaptor-stimulated degradation of a model fluorescent substrate (GFP with a degradation signal) were used to assess SspB variants. In addition, monitoring direct-binding between the ClpX N-domain and variant fluoresceinated-SspB peptides by fluorescence anisotropy provided a quantitative assay for the interaction. Results reveal that a minimal C-terminal region (residues: ^{152}KIVSLDQFRKK^{162}) of SspB is responsible for docking with ClpX, and at least 5 specific residues (I^{153}, V^{154}, L^{156}, R^{160}, K^{162}) within this region play key roles in the interaction. Additional residues (F^{159}, K^{161}) may also be contributing albeit more subtly. This binding region is much longer and shares little sequence homology with the *E.coli* SspB ClpX-binding region (^{161}LRVVK^{165}). However, *C.crescentus* SspB binds *E. coli* ClpX and can clearly deliver substrates to it. This cross-species interaction demonstrates the sequence-versatility of the ClpX N-domain interactions in a functionally relevant manner and highlights the challenge in identifying ''consensus sequences'' for AAA+ protease interaction signals.

423-Pos Board B302

The Effect of Salts and Co-Solvents on the Cytochrome c Folding Pathway within a Sol-gel Glass

Eric S. Peterson, Michael A. Erbele, Brock B. Jas.
Concordia College, Moorhead, MN, USA.

The folding reaction of ferric cytochrome c (cyt c) was examined in the presence of several salts and co-solvents in both solution and within porous silica sol-gel glasses to characterize the conformational changes that cyt c can undergo if constrained to remain in a compact state. The sol-gel pores restrict protein motions to a volume slightly larger than the native state. Unfolding was induced by changing pH and addition of guanidine. The populations of four species characterized by their heme ligation were determined using UV/VIS absorption spectroscopy: the native HM state (His18/Met80), the partially folded HW (His18/water) and HH (His18/His33) intermediates, and the 5C (water) unfolded state. In solution, the native HM state unfolds primarily into the HH species, while in the gel the HW species is formed preferentially. This indicates that the steric constraints within the gel pores hinder some backbone motions. We and others have previously shown that the water solvating the protein within the gel pores is more ordered than bulk water, leading to a decreased hydrophobic effect. Here we present the effects of several salts and co-solvents on the folding pathway of cyt c. We find that addition of some, but not all, salts and co-solvents can alter both the folding kinetics and which conformations are accessed by the protein. The results are discussed in the context of molten globule folding models and the Hofmeister ranking of chaotropic agents.

424-Pos Board B303

Prosegment Catalyzes Pepsin Folding to a Kinetically Trapped Native State

Derek R. Dee, Rickey Y. Yada.
University of Guelph, Guelph, ON, Canada.

Pepsin, an archetypical aspartic peptidase, initially contains an N-terminal, 44 residue prosegment (PS) that, upon folding, is removed to yield native pepsin (Np). Np is irreversibly denatured above pH 6, the basis for which was not understood. Our previous studies comparing Np and a denatured and subsequently refolded state (Rp) indicated that Rp was thermodynamically more stable than Np, having a higher T_m and being reversibly unfolded (Dee, D., et al., 2006, *Biochemistry 45*, 13982). Thus, it was suspected that Np is blocked from refolding by a large energy barrier, which is reduced by the action of the PS.

It was found that the exogenous addition of a synthetic 44 residue PS peptide catalyzes folding of Rp to the Np state. The PS displays foldase activity that follows Michaelis-Menten kinetics ($K_m = 3.1 \pm 2.0$ μM, $k_{cat} = 0.011 \pm 0.002$ sec^{-1}), binds with higher affinity to the product (Np, $K_i = 41 \pm 6$ nM) of the folding reaction than to the substrate (Rp, $K_d = 2.0 \pm 0.2$ μM), and increases the rate of folding by a factor of 10^5 compared to uncatalyzed folding. By comparing the rates of Np unfolding ($t_{1/2} = 9 \pm 1$ days) and uncatalyzed refolding of Rp to Np ($t_{1/2} = 64 \pm 6$ days), it is shown that Np is thermodynamically metastable relative to Rp, in agreement with previous calorimetry data (Dee, D. et al.).

The data support a model whereby the PS catalyzes folding before being removed, resulting in a kinetically trapped Np state that is stabilized by a large barrier to unfolding rather than by a lower free energy. Considering the high structural and functional similarities among the aspartic peptidases, PS-catalyzed folding and kinetic stabilization mechanisms may be quite common.

425-Pos Board B304

Osmolytes Control Peptide Folding and Aggregation

Regina Politi, Shahar Sukenik, Daniel Harries.
Hebrew University, Jerusalem, Israel.

Nature has developed many strategies to ensure that protein folding occurs in vivo with efficiency and fidelity. Among the most widely employed