sulfate proteoglycans) supported dimerization or polymerization of the FGFRs are thought to be required to activate the signaling pathway. The D2 domain is suggested to bind with both HSPGs and FGFs to form a ternary complex. Xray and NMR solution structures of the D2 domain have been analyzed using the CAPTURE cation-pi program. The CAPTURE program indicates cation-pi interactions between residues Y155:R152(Xray), W191:R203 (NMR) and possibly F237:K151 (Xray). Biophysical characterization of the mutants at each cation and pi pair, identified by CAPTURE, shows a significant destabilization resulting from the Y155A, W191A and R203E mutations. Results from differential scanning calorimetry show a reduction in melting temperature by 10-14 °C for Y155A, W191 and R203 mutants of D2. The reduction in the stability of the D2 domain is corroborated by results of ANS binding, thermal denaturation and a limited trypsin digestion experiments. The HSQC of D2 Y155A shows limited chemical shift perturbation of residues in the vicinity of the mutation site. The W191A and R203E mutations show significant 1H-15N chemical shift perturbations in their HSQC spectra. The results obtained in this study show that cation-pi interactions contribute significantly to the thermodynamic stability of proteins. In addition, our results indicate that cation-pi predictions made on the solution NMR structures are more reliable than those predicted based on crystal structures.

2317-Pos

Equilibrium Population of the Folding Intermediate of RNase H and its Importance in the Folding Trajectory

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Proteins can sample a variety of partially folded conformations during the transition between the unfolded and native states. Some proteins never significantly populate these high-energy states and fold by an apparently two-state process. What factors govern which conformations are accessible to a protein as it folds? We have attempted to re-route the folding of ribonuclease H from E. coli by manipulating its regional stability. Using phi-value analysis, we compare the structures of the transition states for folding of RNases H that fold with and without a detectable partially folded intermediate and find that both versions of RNase H fold through a similar trajectory with similar high energy conformations. In light of the general importance of this species on the folding pathway, we attempted to populate the intermediate at equilibrium by destabilizing the region of the protein that is unfolded in this form. Surprisingly, a single change at Ile 25 (I25A) resulted in the equilibrium population of the intermediate under near-native conditions. The intermediate was undetectable in a series of HSQC's, revealing the dynamic nature of this partially unfolded form on the timescale of NMR detection. The dynamic nature of the RNase H intermediate may be important for its role as an on-pathway, productive species that promotes efficient folding.

2318-Pos

Solvation of Hydrophobic Amino Acid Side Chains and Peptide Backbone in Aqueous Glycine Betaine and Trimethylamine N-oxide Solutions

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There is a large class of small, water-soluble molecules broadly referred to as osmolytes that may stabilize or destabilize biomacromolecular structures. For example, trimethylamine N-oxide (TMAO) and glycine betaine are prominent stabilizers of proteins, while urea is a strong denaturant. Although these osmolytes have been employed in protein studies for more than 70 years, the mechanisms of their action are still largely unknown. One reason for this deficiency is the lack of direct thermodynamic data that can be used to quantify solute-solvent interactions versus solute-osmolyte interactions. In this work, we use high precision densimetry and ultrasonic velocimetry to examine the solvation properties of amino acid side chains and the peptide group in binary mixtures of water and TMAO or glycine betaine. Specifically, we report the partial molar volume, V°, and adiabatic compressibility, Ks°, of N-acetyl amino acid amides (alanine, valine, leucine, isoleucine, phenylalanine) and oligoglycines (Gly)1-5 in binary mixtures containing 0 to 4 M TMAO or glycine betaine. We use our volumetric results to evaluate the osmolyte-dependent group contributions of amino acid side chains and the peptide group. We analyze these osmolyte-dependent group numbers to evaluate the binding constants and elementary changes in volume and compressibility accompanying the replacement of water of hydration in the vicinity of the solutes with a TMAO or glycine betaine molecule. We compare these data with similar results previously obtained in our laboratory for the interactions of urea with protein groups. In general, we discuss the implications of our results for elucidating the mechanism of stabilization/destabilization of protein structures by osmolytes.

2319-Pos

Site-Specific Determination of Conformational Flexibility from a Side Chain Perspective: Native State Thiol Exchange of E. coli Ribonuclease H

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Tools such as crystallography and hydrogen exchange (HX) have revealed a wealth of knowledge about protein function, folding, and dynamics. Here we explore the use of thiol exchange (SX) to gain further insight into the energy landscape of E. coli ribonuclease H (RNase H). Similar to HX, SX investigates the solvent accessibility and conformational fluctuations of specific positions in a protein, but while HX measures exchange of the backbone amide proton, SX takes advantage of cysteine's unique reactivity to measure solvent accessibility of the side chain. Native state SX results for a hyperstabilized mutant of E. coli RNase H reveal a partially unfolded form (PUF) at equilibrium with the native state, as is seen by HX. The structured regions of the PUFs measured by the two techniques agree overall, with some slight differences due to probing the side chain rather than the backbone. Moreover, while for some positions the SX experiments revealed this equilibrium information, the same experiments yielded direct kinetic information about protein opening events. Thus, in one set of experiments we have measured both kinetic and equilibrium parameters describing the folding of E. coli RNase H.

Enzymes

2320-Pos

Functional Effects of N-Metal Binding Domain Deletion and Specific Mutations on the ATP7B (Wilson Disease) Copper ATPase

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We obtain high yield heterologous expression of ATP7B (Wilson disease) protein in COS1 cells infected with adenovirus vector. The recombinant protein recovered with the microsomal fraction of the infected cells undergoes high levels of phosphorylation with ATP through the minute time scale, most of which involves serine residues (Ser^{478}, Ser^{481}, Ser^{1121} and Ser^{1453}), as demonstrated by proteolysis and mass spectrometry (J Biol Chem. 2009; 284:21307-16). We now find that incubation within the second time scale yields mostly alkali labile phosphorylation which we attribute to formation of phosphoenzyme catalytic intermediate (EP). In fact, this rapid phosphorylation does not occur following $D^{1027}N$ (conserved catalytic aspartate in P-ATPases) or C983A/C985A (transmembrane copper binding domain site) mutations. The WT phosphoenzyme intermediate reaches steady state levels within 2 seconds, and undergoes 3 sec^{-1} turnover at 30 °C. We also find that the H1069Q mutant (nucleotide binding domain mutation found in Wilson disease) does not form the catalytic phosphoenzyme intermediate within the second time scale, and reduces phosphorylation of serine residues as well. Finally, we find that an extensive deletion eliminating the first five out of six copper sites of the N-metal binding domain (NMBD, a unique feature of ATP7A and B which is not present in other P-type ATPase) does not interfere with formation and rapid turnover of phosphorylated enzyme intermediate. It is noteworthy that, as in previous work with the bacterial copper ATPase CopA (J Biol Chem. 2008; 283: 22541-9), mutation of the NMBD copper site close to the A domain sequence slows substrate utilization kinetics, indicating interference with A and N domains movements. (Supported by 5 R01 HL069830-08).

2321-Pos

Characterization of Membrane Bound Phospholipase-Lipid Complex

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Phospholipases are interfacial enzymes that catalyze hydrolysis of lipids in membranes. Their activity is significantly higher at the surface of lipid aggregates than on monomeric substrates. Enzymatic activity occurs in three sequential steps of 1. enzyme-interface binding; 2. bound enzyme-lipid binding at the active site; 3. lipid hydrolysis. The interface binding in step 1 puts the enzyme in an "open" conformation. In recent work we formulated a specific role for the complex formed in step 2 between the membrane-bound enzyme and phospholipid in the rate of hydrolytic cleavage of the lipid, which involves the thermodynamic properties of the complex. In this work we present results of thermodynamic characterization of the enzyme-lipid complex in vesicles. Existence of an energy barrier for the complex formation is postulated. The heat capacity of the formation of the complex in vesicles was measured by Differential Scanning Calorimetry. The DSC thermograms indicate the existence of a peak in