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Hydrogen/deuterium exchange studies of a peptide octamer of Aib in a mixed DMSO/MeOD solvent system

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2734-Pos Board #B17

NMR Studies of a model amyloidogenic SH3 Domain Protein

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Protein misfolding and subsequent aggregations are a common feature of amyloid diseases. More than 16 different proteins or polypeptides have been identified to be associated with amyloid diseases, which include prion diseases, type II diabetes, Alzheimer's and Parkinson's diseases. Extensive biochemical studies have shown that soluble, globular proteins are converted to partially unfolded intermediate states, and aggregate into insoluble amyloids. Detailed understanding of the mechanism of the amyloid-forming process at the molecular level will, therefore, be of great importance in developing effective therapic strategies and designing new drugs.

Recently, it was also demonstrated that a β-barrel phosphatidylinositol 3-kinase (PI3K) SH3 domain protein that is not related to amyloid diseases can aggregate into amyloids under partially denaturing conditions. More interestingly, two distinct amyloidogenic conditions for the *cytotoxic* protofibrils (pH of 5.5 with 25% TFE) and *non-toxic* mature fibrils (pH of 2) were reported. Thus, we have been investigating amyloid-forming processes for the two different types of amyloids using NMR spectroscopy. In this meeting, we report NMR studies of intermediate states involved in the amyloid forming-processes for the cytotoxic protofibril and mature fibrils. Our $^{1}H/^{15}N$ HSQC NMR experiments suggest that the two intermediate states adopt quite distinct conformations, presumably leading to the different morphologies of the amyloids. Detailed hydrogen/deuterium exchange NMR experiments of the intermediate states will also be presented.

2735-Pos Board #B18

Hydrogen/deuterium exchange studies of a peptide octamer of Aib in a mixed DMSO/MeOD solvent system

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Hydrogen/Deuterium exchange (HX) is a mature technique that has been used to characterize biomolecular structures in conformational ground states, in the act of folding, and in dynamic fluctuation about conformational ground states. We have performed HX studies to characterize the structure and conformational fluctuations of an octamer of ?-aminoisobutyric acid, 4-fluorobenzoyl-$(Aib)_8$-OtBu. This homooligomer of Aib is expected to adopt a 3_{10} helical structure. Oligomers of Aib exhibit remarkably stable 3_{10} helical structures, even in DMSO at elevated temperatures. In our study, we initiate H/D exchange through the addition of methanol-d_4 to a peptide solvated in DMSO. Although the temperature-dependencies of the eight amide hydrogen ^{1}H NMR resonances in DMSO indicate the presence of 3_{10} helical structure, room-temperature H/D exchange studies reveal greater kinetic lability of amide hydrogens than would be expected from a well-developed 3_{10} helical conformation. Room temperature half-lives for exchange in our system are > 10 hrs, allowing us to probe relatively rare conformational fluctuations. As the temperature is raised, some amide protons become more protected from exchange, suggesting that the 3_{10} helical structure becomes more developed with increasing temperature in this solvent system. Our observations are consistent with previous studies that showed increasing 3_{10} helical content with increasing temperature. We will discuss our findings in terms of rare fluctuations about a ground-state conformation for this octamer of Aib. Supported by Research Corporation CCSA #CC5899.

2736-Pos Board #B19

NMR Measurements of Activation Barriers to Amide Hydrogen-Deuterium Exchange Reveal Sensitivity of Hydrogen-Bond Strengths to Side Chain Steric Effects in 310-Helical Peptides

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A variety of existing experimental evidence suggests that 3_{10}-helices may play a significant role in the dynamic and functional behaviors of proteins. However, the study of helical structures is complicated by the fact that most amino acid sequences do not spontaneously adopt helical conformations. The stericly hindered amino acid Aib (α-methylalanine, B) has been shown to enhance helical stability, and homopolymers of Aib have been shown to form exceedingly stable 3_{10}-helices in organic solvents. In this work, we characterize the temperature-dependent kinetics of amide hydrogen-deuterium exchange for several Aib-rich octapeptides using ^{1}H NMR. Peptides were dissolved in deuteromethanol and the decay of the amide proton signal was modeled using pseudo-first order kinetics. Peptides composed of Aib (B8) or mixtures of Aib and Ala (AAXY, where X and Y indicate the positions of the Ala residues) were examined. The activation barriers to exchange in AA45 are decreased near the two Ala residues, such that one face of the helix center experiences increased solvent exposure. The helix is not broken or frayed, as evidenced by very low exchange rates and high barriers to exchange at the C-terminus. In contrast, exchange rates in B8 are roughly consistent across the helix from residues 3-8, decreasingly slightly at the C-terminus. Preliminary results suggest similar behavior of AA35 to that of B8. Thus, the contiguous positioning of the Ala residues in AA45 creates a site of reduced steric hindrance, causing reduced hydrogen-bond strengths and increased solvent exposure, while separation of the Ala residues by a single Aib restores helical regularity. These results suggest that steric hindrance may play an important role in the dynamics of 3_{10}-helices in natural systems.

2737-Pos Board #B20

Conformational and Thermal Stability Studies of Tryptophan-Zipper Style beta-Hairpin Peptides

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The infrared (IR), electronic (ECD), and vibrational (VCD) circular dichroism spectra of two short tryptophan containing beta-hairpin peptides, with a sequence SWTWEXXKWTWK differing only at XX by the ordering of GN vs. NG residues in the turn sequence, were measured over a range of temperatures to investigate their thermal stability and thermally induced conformational changes. IR spectra lack an isobestic point suggesting a multi-state conformational transition having good reversibility. The ECD spectra reflect the tryptophan interaction and have an isodichroic point but less reversibility and suggest a kinetic trap. The spectral variation with temperature for both FTIR (amide I' frequency) and ECD (intensity) can be fit simultaneously to a two-state thermodynamic model. However with factor analysis, the intensity variation in the IR yields a third component, consistent with a complex process. The process can be represented as involving an ensemble of partially folded states using the simplified statistical mechanical micro-state model of Munoz et al. These conclusions are in some contrast to earlier work by Grubele and co-workers on the NG variant.

2738-Pos Board #B21

Photon echo spectroscopy as a probe of conformational dynamics in folded and unfolded heme proteins

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Three pulse photon echo peak shift (3PEPS) experiments on the Soret band of folded and unfolded Fe(II), Fe(III) and Sn-substituted cytochrome c (cyt c) were used to characterize its conformational dynamics and heterogeneity. The fast decay in the 3PEPS data is caused the convolution of the laser pulse with high frequency heme vibrations and the short B-state lifetime. The time constant for the rise of the peak shift (~450 fs in the Fe(III) samples) is due to the Q-state lifetime. The peak shift rises as the Q-state population decays because excited state absorption from the Q-state contributes a free-induction decay (i.e., a transient compromise of rephasing capability) to the echo signal. The amplitude of this rise is proportional to the ratio of excited state to ground state extinction coefficients. Vibrational relaxation in the ground state does not appear as a separate time constant in these data. However, the relaxation of population into the spectral window of the laser pulses diminishes the magnitude of the echo signal, and thus limits the ability of the 3PEPS experiment to measure dynamics at long times. In fluorescent hemes, the 3PEPS signal is longer-lived, so measurements on the 5-10 ns time scale are feasible. This model is quantitatively consistent with the slower rise time of the peak shift and the Q-state lifetime observed in the Sn-substituted protein, and may be used to fit the data with a set of experimentally determined kinetic parameters and the spectral density characterizing the amplitude and time scales of protein dynamics. The 3PEPS data on acid-unfolded cyt c show pH-dependent inhomogeneous broadening which are explained in terms of the structural events occurring during unfolding.