structure unfolding, even though protein-urea interactions are considered crucial. Here, we provide nuclear magnetic resonance (NMR) spectroscopy and 3D reconstructions from X-ray scattering to develop the ''push and pull'' hypothesis, which helps to explain the initial mechanism of chemical unfolding of proteins in light of the physical events triggered by high pressure (HP). In studying MpNep2 from Moniliophthora perniciosa, we observed that the effects of pressure and urea on structure are different (but correlated and complementary), and this difference has a major impact on unfolding. Using HP-NMR, we tracked at least two cooperative units of MpNep2 as they moved uphill in the energy landscape; this process contrasts with the overall structure unfolding that occurs upon reaching a threshold concentration of urea. These observations explain the differences between the molecular mechanisms that control the physical and chemical unfolding of proteins, thus opening up new possibilities for the study of protein folding and providing a new interpretation of the nature of the cooperativity in the folding and unfolding process.

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Probe the Heme Iron Ligand and Conformational Change of Misfolded States of Cytochrome C through EPR Spectroscopy

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Cytochrome c is a heme-protein existing in the membrane of mitochrondria which plays a pivotal role in apoptosis. Besides its important biological functions, it is a model system for protein folding and unfolding studies. Recently, Soffer et al. [Soffer et al. Biochemistry 2013, 52, 1397−1408] claimed that a meta-stable misfolded state of horse heart ferricytochreome c could be obtained after exposing the protein to the ferricyanide solution at very alkaline pH for an extended period of time, and it did not undergo any remarkable change in its secondary and tertiary structures upon adjusting the solution to folding promoting conditions at neutral pH. Yet the axial coordination of the heme iron as well as the electronic structures for the misfolded protein remained undetermined. For this purpose, in this work, electron paramagnetic resonance (EPR) spectra of the misfolded protein in the solution at different pH values were recorded, and DFT calculation was conducted to predict the EPR g-tensor at different axial ligand configurations. Our EPR data indicate that the misfolded protein at very alkaline condition exhibits a hexacoordinated low spin state, while at pH 5 the protein adapt the configuration similar to the native-like low-spin ferric heme complex which possesses a histidine/methionine coordination environment. For pH value between 6 and 7, the EPR spectra appear to be the superposition of the two species. With the aid of DFT calculation, also checked by the EPR and other spectroscopic data, the possible hexacoordinated low spin state with a hydroxyl ion as the proximal ligand and the pentacoordinated quantum mixed state of the heme iron for misfolded protein at different folding conditions were discussed. This work was partially supported by Hundred Talents Program of Chinese Academy of Sciences.

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Constant pH Simulations with the Double Reservoir pH Replica Exchange

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We present the double reservoir pH replica exchange (DR-pH-Rex) method, which can improve convergence of constant pH simulations in situations where proper sampling is difficult. This method separates the sampling of protonation states from much of the sampling of protein conformations. The DR-pH-Rex method was inspired by the single reservoir temperature replica exchange method. The DR-pH-Rex method relies on pre-generation of two Boltzmann-weighted structural reservoirs of conformations that correspond to two end states at pH values where titratable groups are fully protonated and fully deprotonated. The end state conformations are coupled to simulations at intermediate pH values through the pH replica exchange (pH-Rex) methodology. We tested this method on three different systems: amino acid model compounds Lys and Glu, a small peptide, and a challenging case of V66K variant of Staphylococcal nuclease (SNase) which exhibits two distinctly different conformations of Lys-66 with slow transitions between the two conformations. We benchmarked the performance of the method through comparison with results of the pH-Rex method, which already provides better sampling than regular constant pH simulations. For the model compounds pH-Rex and DR-pH-Rex methods yield identical results. For the peptide, the pKa values are comparable between the two methods, but the DR-pH-Rex method exhibits faster convergence and less noise. For the V66K variant the pH-Rex method fails to properly sample conformational transitions of Lys-66 and results in two different pKa values when different conformations are used as starting structure. The DR-pH-Rex method, however, results in convergence of pKa values. Additionally we construct a four state model with two protonation states and only two conformations of Lys-66 and show that the pKa values calculated from DR-pH-Rex simulations are in excellent agreement with the pKa values determined from the four state model.

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Determinants of Domain Swapping in Staphylococcal Nuclease

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Previous studies have shown that the deletion of residues 114-119 (comprising Val-114, Tyr-115, Lys-116, Gly-117, Asn-118, and Asn-119) in wild type staphylococcal nuclease (SNase) results in a C-terminal domain swapped dimer. The 114-119 segment constitutes the loop that precedes that C-terminal helix that is swapped. In the crystal structure of the domain swapped state the dimers make a well-packed interface. The domain swapped dimer is identifiable as a dimer crystallographically, by native gel electrophoresis, by light scattering, by analytical ultracentrifugation, and by NMR spectroscopy. Chemical denaturation of the domain swapped dimer leads to a single transition between folded and unfolded states, suggesting that the monomeric form is unstable. This is consistent with the effect of the 114-119 truncation on the midpoints of the thermal and acid unfolding transitions, which show that the domain swapped dimer is considerably less stable than the parent protein. In the highly stable Δ+PHS form of SNase, which is 6.4 kcal/mol more stable than the wild type, the 114-119 truncation also leads to a domain swapped state, suggesting that the tendency to swap is governed more by the local conformational properties of the 114-119 turn than by the propensity of the swappable helix to exist in the ''open'' conformation necessary for swapping. The 114-119 truncation is less destabilizing in Δ+PHS SNase than in the wild type SNase. A series of constructs in which residues 114 to 119 were removed systematically suggest that swapping is promoted by shortening of this segment, regardless of which residues are removed, and that only 2 to 3 residues need to be removed for swapping to be possible. However, so long as flexibility in the loop is retained through the presence of Gly-117, the protein appears to be capable of populating the monomeric state.

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Molecular Basis of Tetramerization and pH-Gating in the KcsA Potassium Channel Cytoplasmic Domain

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The intracellular C-terminal domain (CTD) of KcsA, a bacterial homotetrameric potassium channel, is a 40-residue long segment which natively adopts a helical bundle conformation with four-fold symmetry. A hallmark of KcsA behavior is a pH-induced conformational change which leads to opening of the channel at acidic pH. While crystal structures of full-length KcsA failed to observe a pH-effect upon the CTD, other biophysical methods have presented evidence to the contrary. We approached the question of CTD structure and its pH-dependence by studying the behavior of soluble peptides corresponding to residues 128-160 of the CTD (CTD34). A combination of NMR and sedimentation equilibrium experiments established CTD34 to be a tetramer with a K_D of (2.0 ± 0.5) x 10^{-11} M^3 at neutral pH, and this tetrameric species undergoes pH-dependent dissociation, rendering CTD34 fully monomeric below pH 5.0. The structural basis for this phenomenon is destabilization of the tetrameric CTD34 by protonation of residue H145 in the monomeric form of the peptide.

Molecular factors contributing to CTD tetramerization were investigated by comparing the tetrameric stability of single alanine mutants as determined by NMR, SE and molecular dynamics. Single-residue contributions to tetramer stability were in the 0.5-3.5 kcal/mol range. Hydrophobic interactions between residues lining the tetramer core generally contributed more to formation of tetramer than the inter-subunit salt-bridges between R147 and D149/E152. A third class of residues outside the helical interface influenced tetramer stability via the tetramerization on-rate by changing the inherent helical propensity of CTD34 which promotes tetramer formation. We conclude that the CTD is an independent tetramerization domain modulated by pH, and tetramer formation is controlled by a combination of on- and off-rate effects which conform to current paradigms of protein folding.