are independent of temperature (in the range of 5°C to 75°C). By contrast, backbone groups exposed to (and hydrogen-bonded with) solvent water show a temperature dependence similar to that observed in solvated model compounds (2). When this approach is combined with specific isotope-labeling (3), site-specific information about solvent accessibility can be obtained from the variable-temperature IR spectra.

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A Hinge Region Cis-Proline in Bovine Pancreatic RNase a Acts as a Conformational Gatekeeper for C-terminal Domain Swapping

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Structural studies have implicated proline as an important residue for domain swapping and oligomerization due to its increased frequency in hinge regions preceding swapped arms. Proline is unique in its ability to populate both cis and trans peptide bond conformations; we hypothesized that this property may allow proline to act as a conformational gatekeeper, regulating interconversion between monomer and domain-swapped dimer. The hinge region of RNase A contains a proline at residue 114 that adopts a cis conformation in the monomer and extends to a trans conformation in the C-terminal domain-swapped dimer. We find that substitution of P114 with residues that strongly prefer a trans peptide bond (Ala, Gly) results in significant population (~20%) of the C-terminal domain-swapped dimer under near-physiological conditions (pH 8.0, 37°C). This is in stark contrast to dimerization of wild-type RNase A, which requires incubation under extreme conditions such as lyophilization from acetic acid or elevated temperature. Our results suggest isomerization at P114 may facilitate population of a partially unfolded intermediate along the reaction trajectory of RNase A domain swapping, and provide support for a more general role for proline isomerization as a conformational gatekeeper in domain swapping and oligomerization.

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The Importance of Protein-Protein Interactions on the pH-Induced Conformational Changes of Bovine Serum Albumin: A Small Angle X-Ray Scattering Study

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The combined effects of concentration and pH on the conformational states of Bovine Serum Albumin, BSA, are investigated by Small Angle X-Ray Scattering. Serum Albumins, at physiological conditions, are found at concentrations around 35-45 mg/ml (42 mg/ml in the case of humans). In the current work, BSA at 10, 25 and 50 mg/ml, and pH values ranging from 2.0 to 9.0 have been studied. Data were analyzed by means of the Global Fitting procedure, being the protein form factor calculated from Human Serum Albumin, HSA crystallographic structure and the interference function described considering repulsive and attractive interaction potentials within a Random Phase Approximation. SAXS data show that BSA maintains its native state from pH 4.0 up to 9.0 at all investigated concentrations. A pH-dependence of the absolute net protein charge is demonstrated and the charge number per BSA is quantified to 10(2), 8(1), 13(2), 20(2), 26(2) for pHs 4.0, 5.4, 7.0, 8.0 and 9.0, respectively. The attractive potential diminishes as BSA concentration increases. The coexistence of monomers and dimers is observed at 50 mg/ml and pH 5.4, near the BSA isoelectric point. Samples at pH 2.0 show a different behavior, as BSA overall shape changes as a function of concentration. At 10 mg/ml, BSA is partially unfolded and a strong repulsive protein-protein interaction occurs due to the high amount of exposed charge. At 25 and 50 mg/ml, BSA has some refolding and a molten-globule state probably takes place. As a conclusion, the present work confirms that the protein concentration plays an important role on the pH-unfolded BSA state, due to a delicate compromise between interaction forces and crowding effects. This work was recently accepted for publication in the Biophysical Journal.

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The Mechanical Properties of PCNA: Implications for the Loading and Function of Sliding Clamps

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Sliding clamps are toroidal proteins that encircle DNA and act as mobile platforms on which components of the DNA replication and repair machinery bind. While trimeric sliding clamps assemble as stable planar rings, they must be splayed open at one of the subunit-subunit interfaces in order to thread duplex DNA into their central pore. The opening process is driven by the ATP-dependent clamp loader, RFC, whose clamp-interacting sites form a right-handed spiral. Previous molecular dynamics (MD) studies suggested that when PCNA opens, it preferentially adopts a right-handed spiral to match the spiral of the clamp loader. We present evidence from considerably longer MD simulations that PCNA does not have a preference for forming a spiral structure with a particular handedness, although conformations that match the helical pitch of RFC were observed. The strong correspondence between all-atom simulations of PCNA and a coarse-grained elastic model suggest the behavior of the open clamp is primarily due to elastic deformation governed by the topology of the clamp domains. The simple elastic model further allows us to quantitatively describe the energetic cost of deforming PCNA to allow mating with the clamp loader or strand passage once an interface has been disrupted. A picture of PCNA emerges of a protein of considerable flexibility once opened, which is mechanically compliant in the clamp opening process.

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Coordination between N- and C-terminal Kinetics of Hsp90 Investigated by SmFRET

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Hsp90 is a molecular chaperone required for the activation of a large amount of client proteins and survival of the cell during heat shock. It consists of two monomeric chains with dimerization interfaces at the C and N-terminal end[1]. Its chaperone function is dependent on ATP binding and hydrolysis as well as N- terminal and C-terminal dimerization[2].
Up to now mainly the N-terminal dimerization kinetics of Hsp90 has been investigated[3], while the C-terminal interface was assumed to be closed for many minutes, because of its low equilibrium binding constant. We developed a fluorescent based single molecule assay, which allows to investigate C-terminal dimerization independent of N-terminal kinetics. Surprisingly, we find C-terminal dissociation / association kinetics on the timescale of seconds. In addition, this kinetics is nucleotide dependent although the nucleotide binding pocket is far away in the N-terminal domain. Therefore, we conclude that there is coordination through the complete Hsp90 monomer. These findings are confirmed by well defined N-terminal mutations[4].

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