G6-53 unfolds at ~250 pN. Using their characteristic unfolding forces as a reporter, we were able to directly quantify the partitioning of G6-53 between the apo and Ni2+ bound states at different Ni2+ concentration and measure the binding affinity of Ni2+ to G6-53. The distinct unfolding forces of apo and holo forms of G6-53 also allow us to discriminate different species in the process of folding and Ni2+ binding and measure their kinetic evolution. We unfolded G6-53 by force and waited to allow it to fold and bind with Ni2+. We found that the unfolded G6-53 folds to apo form before incorporating Ni2+. The folding rate of G6-53 is independent of Ni2+ concentration, while the binding rate of Ni2+ to apo form of G6-53 is directly proportional to the Ni2+ concentration. Our kinetic data can be fully described using a ''folding before binding'' model. We anticipate that this novel assay will find unique applications in the study of various protein-ligand interactions.

215-Pos

Binding of Antimicrobial Lactoferricin Peptides to Targets in the Angiogenesis Pathway

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Peptides derived from lactoferricin B (LfB25; F*KC*<u>*RRWQWR*</u>*MKK*LGAPSITCVRRAF; +8), a 25-residue cationic innate immunity peptide released from bovine lactoferrin, exhibit broad spectrum antimicrobial and anti-angiogenic properties. An increase in drug-resistant bacteria and the role of angiogenesis in promoting tumor growth make LfB peptides attractive candidates for future drug development. An important principle for the design of peptide drugs is to reduce the number of amino acids and the sequence complexity, while maintaining maximal activity and minimal toxicity. LfB25 is proposed to inhibit angiogenesis, the formation of new blood vessels, by competing with fibroblast growth factor (FGF) for binding to negatively charged heparin sulfate proteoglycans on endothelial cell surfaces. Previously we used isothermal titration calorimetry (ITC) to characterize the binding of LfB25 and LfB6 (RRWQWR-NH_2; +4; underlined above), the 'antimicrobial core' of LfB25, to heparin and sucrose octasulfate (SOS), a heparin analogue. The binding of LfB25 with SOS and heparin was found to fit a two site model, with Kd values on the order of 10^{-6} and 10^{-7}; whereas the isotherms for LfB6 fit a single binding site model, with Kd values on the order of 10^{-6}.

We now report ITC binding assays for two new LfB peptides, LfB25-$Ala^{3,20}$ and LfB11-$Ala^{2,9}$ (K<u>A</u>RRWQWR<u>A</u>KK-NH_2; +7;sequence *italicized* above). To remove the disulfide bond, the two cysteines were replaced to give LfB25-$Ala^{3,20}$. To reduce sequence length and complexity, in LfB11-$Ala^{2,9}$ a cysteine and a methionine were changed to alanine, and the sequence was reduced to 11 residues. The binding of both LfB25-$Ala^{3,20}$ and LfB11-$Ala^{2,9}$ to SOS and heparin fit a single-site model, with Kd values on the order of 10^{-6}, similar to those for LfB6. Results from antimicrobial and hemolytic assays will be presented.

Physical Chemistry of Proteins & Nucleic Acids

216-Pos

Salt-Dependence of DNA-Protein Binding: A Study of Four DNA-Binding Families

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Long-range salt-mediated electrostatic interactions are crucial for DNA-protein complex formation and stability. The DNA backbone has a strong anionic character, while the DNA-binding proteins here studied display a large positive surface potential patch due to positively charged amino acids facing the DNA-binding site. A linear relationship between ln(Kobs) and ln[M+], where [M+] is the 1:1 salt concentration, is often interpreted as an indication of electrostatic effects and it is named SKobs. This parameter is usually equated to the number of ion pairs found in the complex. We determined the electrostatic binding free energy as a function of 1:1 salt concentration with the non-Linear Poisson-Boltzmann (NLPB) equation to predict SKobs. We investigated four families of DNA-binding proteins: (i) Homeodomains, (ii) High Mobility Group (HMG)-Box proteins, (iii) Interferon Regulatory Factors, and (iv) basic-region Leucine Zippers for which there is experimental binding data from the same laboratory. We correlated structural features to charge distribution, and determined surface accessibility of residues. We found a qualitative relationship between our NLPB predictions of SKobs and the experimental SKobs for homeodomains and for HMG proteins, but not for families in which protein and DNA suffer severe bend and conformational changes. This observation indicates SKobs is sensitive to conformational adaptability and thus this effects have to be accounted in order to improve NLPB predictions of SKobs. We did not find a relationship between SKobs and number of ion pairs, but we found that SKobs is better correlated with the Coulombic interaction energies between molecules of the complex.

217-Pos

Sorption, Intercalation and Cooperativety: the Modes of Interaction of Actinomycin to DNA

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The interaction of Actinomycin-D to DNA has been long investigated given it inhibits the synthesis of ribonucleic acid, inhibits the growth of cancer cells and induces apoptosis. So far, thermodynamic and structural studies have demonstrated that Actinomycin-D intercalates to DNA double helix preferentially to G-C pairs. There is also evidence that binding affinity is modulated by nearest base pairs flanking the intercalation site. However, the mechanism of Actinomycin-D interaction to DNA, and thus its energetic, is still ill understood. While some studies show evidence that ActD intercalation to natural DNA occurs via a mechanism consistent with a model of one independent and equivalent sites, other studies show evidence of the existence of two classes of independent binding sites; other yet show that the binding at low saturation is cooperative. In this work we measured the binding of Actinomycin-D to calf thymus DNA by optical titration and dialysis equilibrium under different solution conditions. Thus, we have found the conditions where the different kinds of binding reported in the literature can be reproduced. Through the analysis of the data correlating experimental design and solutions conditions, we were able to characterize the complexity of ActD interactions with DNA. In this work we show experimental evidences that intercalation of ActD at low drug/DNA ratio is cooperative; that the strong binding site is a consequence of cooperative binding; and that ActD not only intercalates to the DNA double helix but it also binds to the helix surface with a affinity which is in the same order of that measured upon intercalation.

218-Pos

Urea Destabilization of DNA and RNA Double Helices: Preferential Interactions with Nucleobase Conjugated Pi-Pi-Systems

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Thermal denaturation transition temperatures of AT (adenine-thymine)- and AU (adenine-uracil)-rich double helices decrease to a greater extent in aqueous urea solutions than GC (guanine-cytosine)-rich double helices. The work presented here seeks to identify the chemical functional groups urea preferentially interacts with to account for the greater destabilization of AT- and AU-rich double helices. Vapor pressure osmometry was used to determine the preferential interaction coefficients of urea with nucleoside 5′-monophosphates (5′-NMPs) to quantify the accumulation of urea near the 5′-NMP solvent accessible surface areas. Additionally, molecular dynamics (MD) simulations of the 5′-NMPs in explicit water and 1 molal urea predict urea preferential interactions above and below the nucleobase plane through pi-pi interactions. These MD simulation results are supported by the strong correlation between the fraction of accessible surface area devoted to the base conjugated pi-system and the preferential interaction coefficients determined from vapor pressure osmometry. Implications for urea destabilization of DNA and RNA double helices are discussed.

219-Pos

The Effect of Site-Specific Modifications of DNA on Thermodynamic Stability, Ion Binding and Hydration

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Cations, which associate with DNA in both the major and minor grooves, play a significant role in determining DNA conformation. In the major groove, cations are associated with the $N7/O^6$ edge of guanines, while in the minor groove they are found at A-T pairs. Both G-C and A-T have potential cation binding sites that when modified should result in the reorganization of salts and water, which in turn would affect local conformation and stability. We report herein the biophysical characterization of DNA duplexes in which we altered the N-7 position in the major groove of purines (7-deaza-guanine, 7-aminomethyl-7-deazaguanine, 7-hydroxymethyl-7-deazaguanine and 7-deaza-adenine) and at N-3 position of adenine in the minor groove (3-deazaadenine and 3-methyl-3-deazaadenine). These modifications alter the electronic properties of the heterocyclic bases and specifically eliminate DNA cation binding sites in the different grooves, or in the case of 7-aminomethyl-7-deazaguanine