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

places a cationic group in the major groove at the edge of a G-C pair. The low temperature NMR and x-ray crystal structures of some of these DNA appear identical to unmodified DNA; however, the thermodynamic analyses show that these modified bases have a significant impact on the dynamic structure of DNA. In most cases, a reduction in thermodynamic stability driven by enthalpy changes was observed. The only modification that is thermodynamically as, or more, stable than the corresponding unmodified DNA is the 7-aminomethyl-7-deaza-guanine. The thermodynamic effects of the different substitutions are associated with the folding enthalpies and hydration. Interpretation of how these base modifications affect DNA structure and stability will be discussed.

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220-Pos

Melting Behavior of DNA Complexes With Joined Triple and Duplex Motifs

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One focus of our laboratory is to understand how sequence, duplex and triplex stabilities, and solution conditions affect the melting behavior of complex DNA structures. We used a combination of UV and circular dichroism (CD) spectroscopies and differential scanning calorimetry (DSC) techniques to obtain a full thermodynamic description of the melting behavior of seven DNA complexes involving joined triplex and duplex motifs. Six of these complexes are formed intramolecularly while the seventh forms an intermolecular complex.

The circular dichroism spectra at low temperatures indicated that all complexes maintained the ''B'' conformation. UV and DSC melting curves of each complex show biphasic or triphasic transitions. However, the transition temperatures, T_Ms, of the intramolecular complexes remained constant with increasing strand concentration, while the T_M of the intermolecular complex did not. This confirms their molecularity.

Deconvolution of the DSC thermograms allowed us to determine standard thermodynamic profiles for the transitions of each complex. For each transition, the favorable folding free energy terms result from the characteristic compensation of a favorable enthalpy and unfavorable entropy contribution. The magnitude of these thermodynamic parameters (and associated T_Ms) indicate that the overall folding of each complex depends on several factors: a) the extent of the favorable heat contributions (formation of base pair and base triplet stacks that are compensated with both the ordering of the oligonucleotide and the putative uptake of protons and ions; b) inclusion of the more stable C^+GC base triplets; c) stabilizing the duplex stem of the complex; d) complex molecularity; and e) solution conditions, such as pHand salt concentration.

Overall, the melting behavior of each complex corresponds to the initial disruption of the triplex motif (removal of the third strand) followed by the partial or full unfolding of the duplex stem.

221-Pos

Formation and Quantification of Two-Photon Induced DNA Photolesions

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The generation of DNA photolesions with a high degree of spatial confinement presents unique opportunities to study the recruitment of UV damage repair proteins to localized damage sites. Photolesion formation is typically accomplished by exposure to UV light which is difficult to manipulate with conventional optics, thus limiting the spatial control over the site of irradiation. As an alternative, we use two-photon absorption of visible light to mimic UV exposure in a form that can be manipulated by conventional optics. We frequency double the output of a tunable Ti:sapphire laser using a barium borate crystal to generate femtosecond pulses of 340-540 nm light. Sample irradiation is performed on 10-20μL volumes confined in a multiwell plate and scanned by a focused beam in a raster pattern through different axial planes. We have adapted a sensitive PCR-based assay to quantify the amount of two-photon induced damage. The assay is premised on a reduction in DNA transcription efficiency by the presence of bulky photolesions; decreased amplification of a sample relative to a control indicates the amount of damage. The assay and laser irradiation system are being tested on linearized pBR322 plasmid, and validated by comparison to direct UV exposure. Our preliminary results indicate that the degree of lesion formation exhibits a nonlinear dependence on power, which is in keeping with the intensity dependence expected for two-photon absorption. Additionally, maximal two-photon DNA damage occurs at wavelength lower than twice the single photon absorption maximum. We are analyzing our results to obtain quantitative information about the yield of photolesions generated by two-photon absorption.

222-Pos

6MI Enhanced Fluorescence in a Specific DNA Pentamer Sequence

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The development of fluorescent nucleoside analogs, which hydrogen bond in the same fashion as their counterparts and minimally distort the structure of duplex DNA, has greatly improved the amount of information that can be obtained from both steady-state and time-resolved fluorescence experiments. Reduction in quantum yield observed when probes are incorporated into an oligomer or a duplex limits their potential application. 6-methylisoxanthopterin (6-MI) is a fluorescent guanosine analog which H-bonds with cytosine similar to guanosine. Investigating the photophysical properties of the nucleoside analog; we discovered a pentamer DNA sequence (ATFAA; where F=6-MI) that exhibits an enhancement of fluorescence upon formation of duplex DNA. The enhanced 6-MI fluorescence within a duplex broadens the potential applications by allowing binding and other experiments to occur at nanomolar concentrations. Within, the sequence context of ATFAA, time-resolved measurements reveal that the fluorescent populations shift from 0.4 to 7.2 ns upon formation of duplex and the relative quantum yield increases from 0.2 to 0.8. This implied the pentamer ATFAA fluorescence enhancement is due to 6MI adopting a single conformation that is either ''flipped out'' from the duplex or sterically constrained. To further investigate the enhancement of fluorescence upon duplex formation, we characterized oligonucleotides local and global structure. Temperature melt and iodide quenching experiments support a model in which enhancement of fluorescence is due to a solvent inaccessible geometry of 6MI remaining H-bonded to cytosine. An increase in solvent accessibility and reduction in the quantum yield were achieved through the introduction of a 3′ bulge or mismatch in the highly fluorescent duplex; suggesting limited dynamics of the 6MI is due to steric hiderance on the 3′ side. This information can now be used to generate other sequence contexts in which 6-MI will exhibit enhanced fluorescence upon duplex formation.

223-Pos

Alteration of Nucleic Acid Fluorescence by An Extenal Molecule and Its Practical Application in Enzymology

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The low fluorescence yield of nucleic acids makes it necessary either to attach extrinsic fluorophores, or add fluorescent intercalators in the case of dsDNA. We have found that the precence of 3-bromopropan-1-ol enhances the fluorescence yield of adenine, adenosine, 6-methylpurine and 7-methyladenine. In contrast, guanine, hypoxanthine, cytosine and poly-Adenosine did not exhibit this effect. This is due to an apparent shift in pKa of these molecules. In this work, we will focus our attention on adenine. Monitoring fluorescence from adenine as a function of 3-bromopropan-1-ol concentration, we constructed a Benesi-Hildebrandt plot that revealed the formation of a 1:1 complex with an equilibrium constant and Gibbs free energy of K = 1.7E-5 and $\Delta Go = -28.7$ kJ/mol, respectively. We determined the fluorescence yield of adenine to increase about two orders of magnitude once the complex is formed. A second aspect of our work was to explore practical applications of this phenomenon. The observation that hypoxanthine was not similarly fluorescence enhanced allowed us to observe the kinetics deamination of adenine catalyzed by the enzyme adenosine deaminase (ADA). The reaction involves the exchange of an amino group for a hydroxyl group. The standard assay for ADA relies on the difference of absorption measurements. This standard assay is of limited sensitivity, since the absorption spectra of the substrate and product are overlapping, and the magnitude of their extinction coefficients are similar. The method we are developing relies on fluorescence spectroscopy, which proves to be more sensitive and exclusively detects adenine. Via this method we were able to study the kinetics of this reaction and determine the Michaelis constant and Vmax. The production of hypoxanthine was confirmed using HPLC separation techniques.

224-Pos

Cationic Sequence Dependence in Nucleic Acid Structures

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Nucleic acids require cationic shielding to overcome inherent self-repulsive electrostatics. The cations that take this role are collectively referred to as screening ions and exchange with those in the bulk solution. Here molecular dynamics simulations were performed for a large variety of helical stems to investigate the behavior of cations around nucleic acids. We show that cations have specific affinity with high residence times for polypurine stretches. Polypurine tracts are implicated in viral physiology, ribosomal entry points, and as aptamers for divalent cations. Also the examination of HIV-1 TAR RNA core