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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 ΔGo = −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.

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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