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Thermal Desorption Behavior and Binding Properties of DNA Bases and Nucleosides on Gold

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DNA monolayers on gold thin films and electrodes, as well as DNA-protected gold nanoparticles, are the basis for an increasing number of diagnostic applications that involve the use of surface-enhanced Raman spectroscopy (SERS), surface plasmon resonance spectroscopy (SPRS), and electrochemical, scanometric, and colorimetric DNA detection strategies.[1−5] Thus, the nature and strength of interactions of DNA with gold surfaces for both planar films and charged particles are subjects of great interest to researchers in the disciplines of biotechnology and nanotechnology. Indeed, a number of studies aimed at elucidating the binding modes and conformation of DNA and its components (bases and nucleosides) on gold surfaces suggest that the DNA−gold interaction is complex and highly sequence-dependent.[4,6−8] Herein, we use temperature-programmed desorption (TPD) and reflection absorption FT infrared (RAIR) spectroscopy to directly examine the energetics of the DNA base−gold and DNA nucleoside−gold interactions. To the best of our knowledge this is the first study to quantify and compare the energetics of these important interactions between the fundamental chemical components of DNA and gold.

Monolayer films[10] of DNA bases and nucleosides on gold were prepared by incubating Au films in 50 μM aqueous solutions of the adsorbates for 12 h, followed by thorough rinsing with water and drying under a stream of N_2 (see Supporting Information). TPD experiments consisted of simultaneous measurement of mass traces (<300 amu) of the desorbing material and RAIR spectra of the surface and were carried out under UHV conditions (pressure not exceeding 10^{-8} Torr during temperature ramp) and as previously reported for other molecular systems using a heating rate of 0.25 K s^{-1}.[11] Multilayer adsorbate films were prepared by allowing a 20-μL droplet of 1 mM solution of adsorbate to dry on a horizontal Au surface in air without rinsing (see Supporting Information). The multilayer experiments are important for comparison purposes and to differentiate interactions between stacked adsorbates from direct interaction of adsorbates with the gold substrate.[12] Thermal desorption traces for each DNA base in the study showed distinct peaks for *m*/*e* values corresponding to the full molecular mass for the bases, Table 1. RAIR spectra acquired at regular intervals during the temperature ramp were used to monitor the surface structure and the relative surface coverage of the adsorbate during the desorption process. Note that integration of peak areas does not take into account conformational changes that may take place for the adsorbate during the desorption process. Peak areas corresponding to the strongest vibrations in the RAIR spectra of the adsorbates decrease with increasing temperature. Significantly, in all cases the area−temperature profiles indicate a 50% loss of material from the surfaces occurs at temperatures within <10° of the peak maxima in the mass traces, Figure 1. The slight lag between mass trace maxima and IR peak area profile at 50% (dotted guidelines, inset Figure 1) is expected due to limited pumping capacities in the analytical chamber. However, the close correlations between IR and mass traces verify desorption of intact bases from the gold surfaces.

Table 1. Heats of Desorption (ΔH_{des} in kJ mol^{-1}) of DNA Bases and 2′Deoxyribonucleosides from Gold Thin Films

	ΔH_{des}(kJ mol^{-1}) nucleobases		ΔH_{des} (kJ mol^{-1}) 2′deoxyribonucleosides
	TPD mass trace[a]	IR[b]	IR
thymine	111 ± 2[c]	110 ± 2	109 ± 3
cytosine	128 ± 4	130 ± 5	114 ± 2
adenine	131 ± 3	129 ± 4	112 ± 4
guanine	146 ± 2	144 ± 2	120 ± 2

[a] ΔH_{des} values calculated using Redhead's equation for T (K) at mass spectrum peak maxima.[9] [b] ΔH_{des} values calculated from normalized peak area profile from RAIR spectra using T at 50% loss of material and Redhead's equation. [c] Number of samples was at least 3.

Redhead's analysis was used to calculate ΔH_{des} for the bases from the temperature of the mass trace peak maxima and 50% area in IR profiles, Table 1.[9] ΔH_{des} for nucleosides was calculated from the 50% area in the IR profiles (full molecular masses of the nucleosides were not detected in the mass spectra.) There are several striking trends that appear in the data. First, thymine interacts much more weakly with the gold surface than the other nucleobases studied. In general, the pyrimidines, thymine, and cytosine desorb at lower temperatures than the purines, adenine, and guanine in the order of T < C < A < G, Figure 1.

Similar trends have been observed for nucleobase adsorption on graphite surfaces and rationalized on the basis of the different solubilities of the bases in water.[13] On gold the difference in strength of interaction is likely due to the varying ability of the bases to coordinate to the substrate as a result of the different types of possible surface binding moieties (e.g., carbonyls and amides; mono- versus polydentate). Second, in general nucleosides desorb from the gold thin films at a lower temperature than the corresponding nucleobases, Table 1. In a separate TPD experiment, 2′deoxyribose, the sugar that comprises DNA, exhibited only very weak binding to gold surfaces when deposited from a 1 mM aqueous solution (ΔH_{des} < 100 kJ mol^{-1}). Therefore, the ribose moiety likely sterically inhibits the bases from adopting their most strongly bound orientation, resulting in various degrees of destabilization (a slight decrease of 1 kJ mol^{-1} for thymine/thymidine and a decrease of 26 kJ mol^{-1} for guanine/guanosine). For example, in the case of adenine/adenosine this hypothesis is supported by differences in the frequency and relative intensity of the peaks

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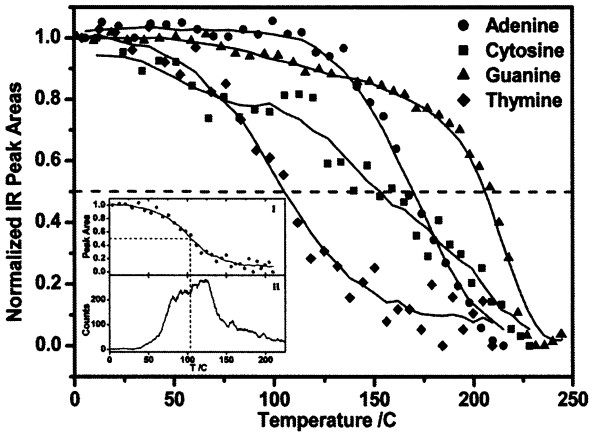

Figure 1. TPD profiles of monolayers of nucleobases adsorbed on Au thin films: thymine (1690−1560 cm^{-1}), cytosine (1750−1550 cm^{-1}), adenine (1750−1550 cm^{-1}), and guanine (1760−1640 cm^{-1}). Note: solid lines for IR area traces are based on adjacent averaging of five points and are meant as a guide to the eye. (Inset) Typical desorption experiment for thymine monolayer: (I) normalized peak area decrease in RAIR spectrum with temperature for 1690−1560 cm^{-1}, (II) mass count trace for full thymine mass (m/e = 127).

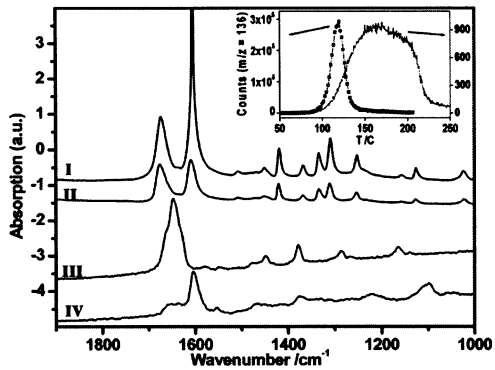

Figure 2. (I) Transmission IR spectrum of isotropic adenine in bulk as KBr pellet. RAIR spectra of (II) multilayer film of adenine on gold prior to desorption, (III) monolayer of adenine on gold (× 100), and (IV) of adenosine on gold (× 100). (Inset) Desorption of adenine layers on gold thin films. Low-coverage layer (solid line), thick multilayer film (filled squares).

associated with the NH_2 scissoring and the in-plane ring C−N stretch, Figure 2. The transmission-mode IR (I) and multilayer (II) spectra of isotropic adenine show peaks for both modes (NH_2 scissoring at 1673 cm^{-1} and in-plane ring C−N stretch 1604 cm^{-1}).[14] For the free adenine molecule, the NH_2 scissoring mode has exclusively an in-plane transition dipole moment. A recent study shows, however, that the ($C-NH_2$)-part of the adenine molecule changes configuration from planar (sp^2) to nonplanar (sp^3) upon coordination to metals.[15] This implies that the NH_2 scissoring mode should display both in-plane as well as out-of-plane components with respect to the ring in the coordinated state, an issue that must be taken into account in orientation analysis. The NH_2 scissoring shifts to 1647 cm^{-1} upon coordination to the gold surface and this is the only peak seen near 1600 cm^{-1} in RAIR spectrum of adenine monolayers on gold (III), Figure 2, suggesting a coplanar orientation at the surfaces. The RAIR spectrum of adenosine (IV), on the other hand, contains peaks for both modes (1647, 1604 cm^{-1}), and the in-plane vibration is the stronger on of the two, indicating that the adenine moiety of the adenosine molecules is oriented at an angle with respect to the surface.

Third, TPD measurements on films of various coverages (monolayer to multilayer) indicate that binding of DNA bases to the gold surface is stronger than interactions between bases in multilayer films. For instance, in the case of adenine, the desorption peak for the multilayer (coverage of ∼150 times the monolayer) is centered at 119 °C, while the desorption peak for the monolayer is centered at 168 °C, Figure 2, inset. Note that multilayer films were distinguishable from low-coverage adsorbate layers by RAIR spectra of the adsorbate layers prior to desorption. The RAIR spectra of the monolayer films prior to heating show peak shifts and intensity changes consistent with surface selection rules for molecules with fixed orientations on a surface, while multilayer adsorbate films have features which are similar to the transmission mode IR spectra of the isotropic nucleobases in KBr pellet form, Figure 2. Fourth, the base components of DNA desorb from Au thin films at lower temperatures than alkanethiol molecules in a self-assembled monolayer of hexanethiol (290 °C compared to ∼220 °C for guanine). This corresponds to ΔH_{des} for alkanethiols of about 167 kJ mol^{-1}.[16] The binding strength per mole of individual bases to gold, regardless of base identity, is weaker than that of an alkanethiol linkage. Thus, although the interaction between alkanethiol-capped oligonucleotides and gold is a cumulative one involving bases and the thiol-tethering groups, the primary interaction would appear to be between the thiol and gold rather than one of the bases and gold. This is important because many researchers have now recognized that there are complex sequence-specific interactions between such oligonucleotides and gold surfaces,[4,6] and this work provides the first quantitative insight into the relative contributions of some of the basic building blocks that are, in part, responsible for the fundamental interactions between oligonucleotides and gold surfaces. Finally, it is noteworthy that the trends observed here reflect the general trends reported by our group for alkanethiol-modified oligonucleotides adsorbed on gold thin film and nanoparticle surfaces in biological buffers.[6] Specifically, it is known that polyT adsorbs much more weakly than polyA DNA strands on both Au thin film and nanoparticle substrates. This observation has been used empirically in the design of oligonucleotide tethers and recognition elements for many detection processes that either use gold electrodes or nanoparticle probes.[17−19]

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Supporting Information Available: AFM images and detailed experimental section of mono- and multilayers of DNA bases on Au-(111)/mica (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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