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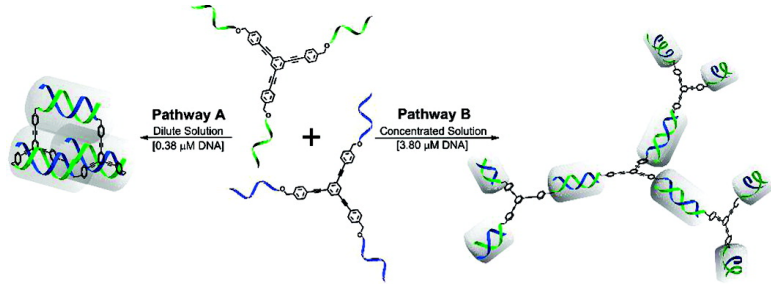

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Cooperative Melting in Caged Dimers of Rigid Small Molecule-DNA Hybrids

Brian R. Stepp, Julianne M. Gibbs-Davis, Dorothea L. F. Koh, and SonBinh T. Nguyen*

Department of Chemistry and International Institute for Nanotechnology, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113

Received March 2, 2008; E-mail: stn@northwestern.edu

Over the past two decades, DNA hybrid materials have become the materials of choice in the development of new strategies for genetic detection,[1] templated synthesis,[2] programmed self-assembly,[3] and molecular computing.[4] Hybridized mixtures of materials containing multiple DNA strands, such as DNA-functionalized comb polymers[5] and DNA-functionalized gold nanoparticles (GNPs),[6] have notably sharpened dehybridization, or melting, profiles compared to unmodified DNA:DNA duplexes. This enhanced melting property can be used to improve the selectivity for single-nucleotide polymorphisms (SNPs) and has been attributed in one model to cooperative melting of neighboring duplexes.[7] This "neighboring-duplex" model proposes that hybridization of complementary multi-DNA materials leads to a network of interlinked DNA duplexes where the condensed ion cloud around each DNA duplex can appreciably overlap with those of its neighbors. Under these conditions, the coalesced ion cloud stabilizes the duplexes, causing them to melt as a *cooperative* unit in a switchlike fashion.[8] Such cooperative melting is predicted to be observable with as few as 2−3 cooperative duplexes,[9,10] beyond the limit of alternative theories that ascribe sharp melting behavior to macroscopic phase transition (see Supporting Information (SI) for details).[11]

To date, switch-like melting transitions have only been observed for large polymer-DNA[5,8] and GNP-DNA aggregates[6] linked through numerous DNA duplexes. Herein, we report the first observation of cooperative melting in discrete DNA-hybrid structures where caged dimers are formed from small molecule-DNA hybrids (SMDHs) possessing *only* three DNA strands around a rigid (r) small-molecule core (Figure 1, pathway a). This result demonstrates the important roles that local geometry and ion concentration play in DNA hybridization/dehybridization processes and points the way for designing new DNA-based materials with enhanced recognition properties.

Our rSMDHs were designed with a rigid tris(phenylacetylene) core that allows for the placement of exactly three DNA strands at 120° intervals with a fixed distance from the central benzene. While these rSMDH cage dimers possess fewer DNA strands per structure than the polymer-DNA and GNP-DNA hybrids, they exhibit similarly sharpened melting transitions. When compared to unmodified DNA:DNA duplexes, their melting temperatures (T_m) increased by >10 °C and their transitions narrowed from >20 to 3 °C.

Previous theoretical[12] and experimental[13] studies on the aggregation behavior of complementary structures with three or more "arms" surrounding a central hub found that A:B dimers tend to form when the corresponding partners are combined in dilute solutions. Accordingly, we expect that a dilute 1:1 mixture of rSMDH molecules possessing complementary DNA strands, attached through the 5′ terminus, would result in caged dimers (Figure 1, pathway a). By design, the rigid small-molecule core and short spacer sequences consisting of six thymidine units (T_6) would allow these dimers to have all three duplexes approximately parallel to each other with 20−40 Å duplex center−duplex center distances, within the theoretical distance necessary for ion-cloud overlap.[9] In contrast, concentrated samples are not expected to melt cooperatively as they should form large aggregates (Figure 1, pathway b) with the ion clouds not in parallel alignment.

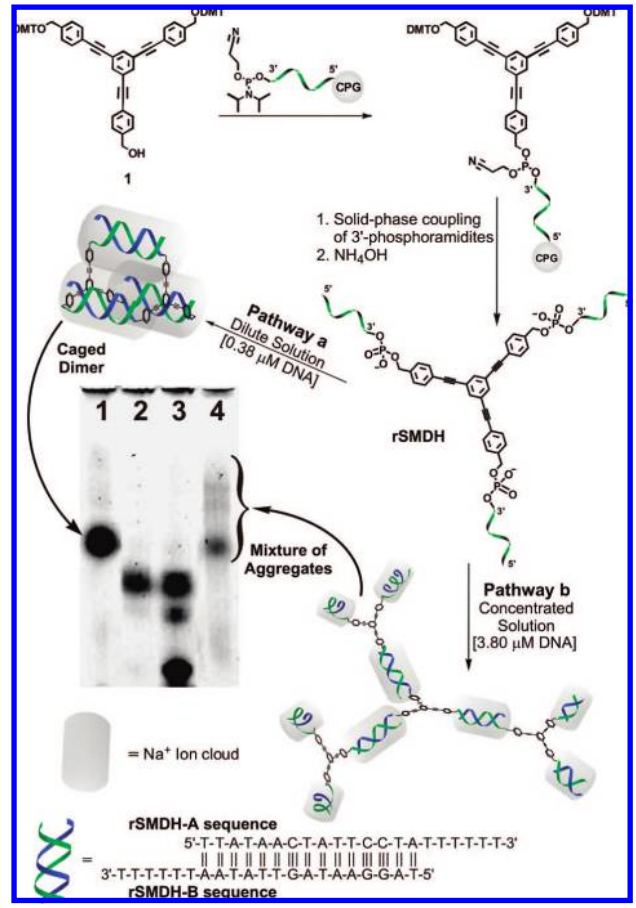

Figure 1. Synthetic scheme for rSMDHs and possible hybridized structures between rSMDH-A and rSMDH-B, with a digital image of a native PAGE gel containing rSMDH materials: lane 1, A:B rSMDH caged dimers formed in dilute solution; lane 2, purified nonhybridized rSMDH-A; lane 3, crude sample of nonhybridized rSMHD-A showing a large percentage of cores with incomplete DNA conjugation. Both the 1-arm and 2-arm functionalized materials can be seen eluting faster than the fully conjugated rSMDH-A. Lane 4, A:B rSMDH aggregates formed in concentrated solution. While the concentrated sample may have up to 50% of the dimer present (based on the gel data, see also Figure SI-2), the observed melting is noncooperative.

Symmetric rSMDH materials were prepared by synthesizing the initial DNA arm from the surface of a controlled porosity glass bead (CPG), followed by addition of the small-molecule core **1** and coupling of the two remaining DNA arms (Figure 1).[13] Following removal from the solid support, the crude rSMDH products were purified by size-selective dialysis to give the desired triply functionalized rSMDHs.

Hybridized rSMDHs were formed by combining equimolar amounts of two complementarily functionalized rSMDHs in phosphate buffer at 50 °C, annealing the mixture at 50 °C for 5 min, and allowing the mixture to cool to room temperature over 8 h. The melting profile of the hybridized mixture was ascertained by heating the samples from

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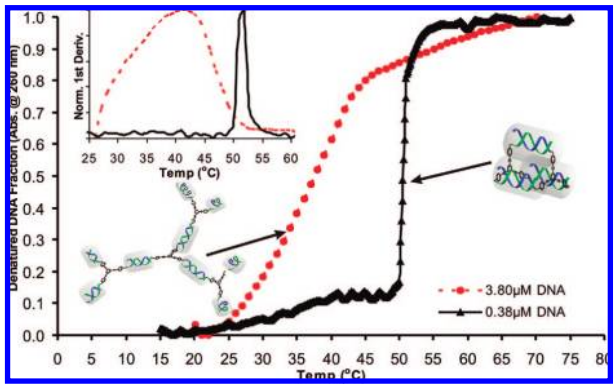

Figure 2. Melting curves for rSMDH:rSMDH hybridization mixtures at high (3.80 μM) and low (0.38 μM) concentrations in saline buffer (10 mM PBS, pH = 7.0, 150 mM NaCl). (Inset) First derivatives of the melting curves.

Table 1. Melting Data for Unmodified DNA (DNA:DNA) and rSMDH (rSMDH:rSMDH) Hybrids

entry	hybridization mixture	[DNA] (μM)	[NaCl] (mM)[a]	T_m (°C)	fwhm (°C)
1	DNA:DNA	0.38	150	39.2	20.0
2	DNA:DNA	3.80	150	44.1	23.0
3	rSMDH:rSMDH	3.80	75	37.5	20.0
4	rSMDH:rSMDH	3.80	150	41.5	17.5
5	rSMDH:rSMDH	3.80	300	49.0	22.0
6	rSMDH:rSMDH	0.38	75	43.2	3.2
7	rSMDH:rSMDH	0.38	150	51.5	2.0
8	rSMDH:rSMDH	0.38	300	56.0	3.7

[a] NaCl concentration in a 10-mM PBS buffer solution at 7.0 pH.

15 to 70 °C at a rate of 1 °C per minute while monitoring the increase in UV absorbance at 260 nm at 0.1 °C intervals.

As predicted by the neighboring-duplex model, the T_m and the overall melting range of rSMDH mixtures differ significantly depending on the concentration (Figure 2). Most notably, the dilute sample (0.38 μM [DNA]) has a T_m of 52 °C and a fwhm (full width at half-max of the derivative curve) of 2.5 °C, 12 °C higher and considerably sharper than a DNA:DNA duplex of the same sequence (Table 1, entries 1 and 2). Conversely, the concentrated sample (3.80 μM [DNA]) has a T_m of 41.5 °C and a fwhm of 17.5 °C—nearly identical to that of unmodified DNA duplexes.

Using the neighboring-duplex theory, the number of duplexes interacting cooperatively can be determined from the salt concentration dependence of the melting temperature for the rSMDH hybridization mixtures (Table 1, entries 3−8).[7,8] The average number of cooperative duplexes is calculated to be 2.97 for the caged dimers in the dilute samples and 0.91 for the concentrated samples where larger aggregates are favored (see SI for details). This "speciation" is further confirmed by the nondenaturing PAGE gel shown in Figure 1: the dilute sample (lane 1) eluted quickly as a well-defined spot, signifying a single entity with low charge/volume ratio, consistent with the compactness of the caged dimer. In contrast, the concentrated sample (lane 4) eluted slowly as a long band with many species. The species in both lanes 1 and 4 eluted slower than the free rSMDH A (lane 3), consistent with their hybridized states.

Shchepinov et al. reported that DNA hybrids with a flexible dendrimer core can form 1:1 dimer structures upon annealing.[13] While these flexible dimers also exhibited increased melting temperatures, their dehybridization profiles are broad like that of unmodified DNA duplexes. Together with our data, this observation suggests that while multivalent interactions are responsible for enhanced melting temperature, the rigid core of our rSMDH system is vital in forcing the connected DNA duplexes to remain in the parallel geometry that is necessary for cooperative interactions via shared ion clouds.[9] It is noteworthy that mixtures containing a high concentration of rSMDHs did not exhibit an enhanced melting temperature (Table 1, cf. entries 2 and 4 vs 7), presumably due to the lack of multivalent interactions between neighboring rSMDHs: on average each rSMDH is only linked to another rSMDH through a single DNA duplex (see SI for details).

In conclusion, well-defined rSMDHs possessing three DNA strands have been synthesized and used to form caged dimers in dilute solutions. These caged dimers comprise the first discrete small molecule-DNA hybrids that exhibit both switchlike dehybridization properties and enhanced melting temperatures similar to aggregates of DNA-modified gold nanoparticles[6] and polymer-DNA hybrids.[5] These results provide conclusive evidence to support the neighboring-duplex model as a critical tool for understanding DNA hybridization/dehybridization processes at the discrete molecular/supramolecular level where phase-transition theory relying on aggregate formation/dissolution cannot be applied. It may also allow researchers to push the limits in the design of the next generation of DNA-based materials to maximize DNA's natural recognition ability via precisely spaced and finely tuned duplex interactions.

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Supporting Information Available: Synthetic procedures and characterization data for rigid small molecule-DNA hybrids; methods and data for hybridization experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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