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Thermodynamic Analysis of Nylon Nucleic Acids

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The stability and structure of nylon nucleic acid duplexes with complementary DNA and RNA strands was examined. Thermal denaturing studies of a series of oligonucleotides that contained nylon nucleic acids (1–5 amide linkages) revealed that the amide linkage significantly enhanced the binding affinity of nylon nucleic acids towards both complementary DNA (up to 26 °C increase in the thermal transition temperature (T_m) for five linkages) and RNA (around 15 °C increase in T_m for five linkages) compared with nonamide linked precursor strands. For both DNA and RNA complements, increasing derivatization decreased the melting temperatures of uncoupled molecules relative to unmodified strands; by contrast, increasing lengths of coupled copolymer raised T_m from less to slightly greater than T_m of unmodified strands. Thermodynamic data extracted from melting curves and CD spectra of nylon nucleic acid duplexes were consistent with loss of stability due to incorporation of pendent groups on the 2′-position of ribose and recovery of stability upon linkage of the side chains.

Introduction

Several examples of DNA-templated organic reactions have been reported,[1–3] but far fewer stereo- and regiospecific DNA-mediated polymerization reactions are known.[4] In previous work, we have reported the attachment of dicarboxyl and diamino groups to the 2′-position of uridine nucleoside analogues.[5] These side chains were condensed to form short segments of a nylon-like polymer pendent from the nucleic acid backbone. The long-term goal of this work is to use the topological control afforded by nucleic acids[6] to direct the topology of polymers of industrial importance. Our synthesis of nylon nucleic acid oligomers is markedly more robust than the previous version, so that we are now able to isolate enough material for a study of the effect of structural restrictions from the nylon-like polymer on thermal stability. In the design of the strands reported in our earlier work, the modified nucleosides were flanked on both sides by short stretches of oligo-dT. The disadvantage of an oligonucleotide containing exclusively rU or dT nucleobases is that there is no control over the position or manner in which it binds its oligo-dA complement, including the possibility of triplex formation.[7] Furthermore, the melting temperatures of homopolymer duplexes that contain only A and T are lower than those that also contain G and C, so to carry out thermodynamic studies, higher strand concentrations are required; however, this leads to a more complicated system, which might contain aggregated concatamers that consist of overlapping oligomers.[8]

In this study, the modified nucleosides in the oligonucleotide are flanked by general DNA sequences that contain all four bases, not only oligo-dT. This is one step further toward the goal of controlling the topology of industrial polymers by nucleic acids, as more bases must be used for such a system to be effective. In the work reported here, two to six modified nucleosides are located in the middle region of 18-mer oligonucleotides. After condensation between carboxyl and amino groups, short nylon-like polymers with one to five amide linkages resulted. Thermodynamic properties of nylon nucleic acid strands with their DNA or RNA complements were investigated by thermal denaturing experiments with precursor strands (prior to amide coupling) and unmodified DNA for comparison. Thermodynamic parameters were extracted from melting curves based on van't Hoff analysis. Duplex formation was also detected and analyzed by circular dichroism.

Results and Discussion

Strands were designed to place a contiguous stretch of modified uridine nucleotides in the middle region of a heterobase sequence. The number of modified nucleotides ranged from two to six. The sequences are shown and the strands are named in Table 1. We denote uridine nucleotides that contain single amino or carboxyl modifications as Un or Uc, respectively, and diamino or dicarboxyl modifications are labeled as Unn or Ucc, respectively (Figure 1). The nylon nucleic acids are underlined at the coupled nucleotides (e.g., UnUc). Oligonucleo-

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Table 1. Oligonucleotides (ODNs) used in this study and MALDI-TOF MS analysis. Strand **1** is the unmodified DNA that was used as control; **2a**–**6a** are uncoupled precursor strands with 2, 3, 4, 5, or 6 modified nucleotides incorporated; and **2b**–**6b** contain corresponding nylon nucleic acid sequences.

ODNs	Sequence	Calculated	Found
1	5′-GCATAGTTTTTTGTCTAC	–	–
2a	5′-GCATAGTTUnUcTTGTCTAC	5678.9	5678.8
2b	5′-GCATAGTTUnUcTTGTCTAC	5660.9	5661.3
3a	5′-GCATAGTTUnUccUnTGTCTAC	5840.8	5839.5
3b	5′-GCATAGTTUnUccUnTGTCTAC	5804.8	5803.7
4a	5′-GCATAGTUcUnnUccUnTGTCTAC	6000.4	6000.3
4b	5′-GCATAGTUcUnnUccUnTGTCTAC	5946.4	5947.0
5a	5′-GCATAGTUcUnnUccUnnUcGTCTAC	6161.7	6161.9
5b	5′-GCATAGTUcUnnUccUnnUcGTCTAC	6089.7	6090.9
6a	5′-GCATAGUcUnnUccUnnUccUnGTCTAC	6322.9	6323.0
6b	5′-GCATAGUcUnnUccUnnUccUnGTCTAC	6232.9	6233.9

tide strands with free carboxyl and amino groups were synthesized by automated phosphoramidite synthesis, cleaved from the solid support, and deprotected by using procedures similar to those described previously—although the incubation temperature for deprotection was 50 °C rather than room temperature.[5a] After purification by denaturing gel electrophoresis, all strands were characterized by MALDI-TOF mass spectrometry. A new amide formation procedure was developed in which the precursor strand was hybridized with a complementary hairpin DNA molecule before the coupling reaction.[9] Schematic drawings in Figure 1 illustrate the templated synthesis of nylon nucleic acid molecule **3b**. After annealing with a 46-mer hairpin molecule, a strand that contained pendent groups formed a duplex with complementary DNA. Duplex formation prevents various potential side reactions, such as coupling with amine groups on nucleobases, or reaction with remote pendent groups. Amide formation catalyzed by DMT-MM also followed previous procedures, except that the reaction mixtures were shaken gently during the coupling reaction. The use of a hairpin with a longer sequence facilitated electrophoretic separation of the modified molecule from its complement after coupling (Figure S1 in the Supporting Information).

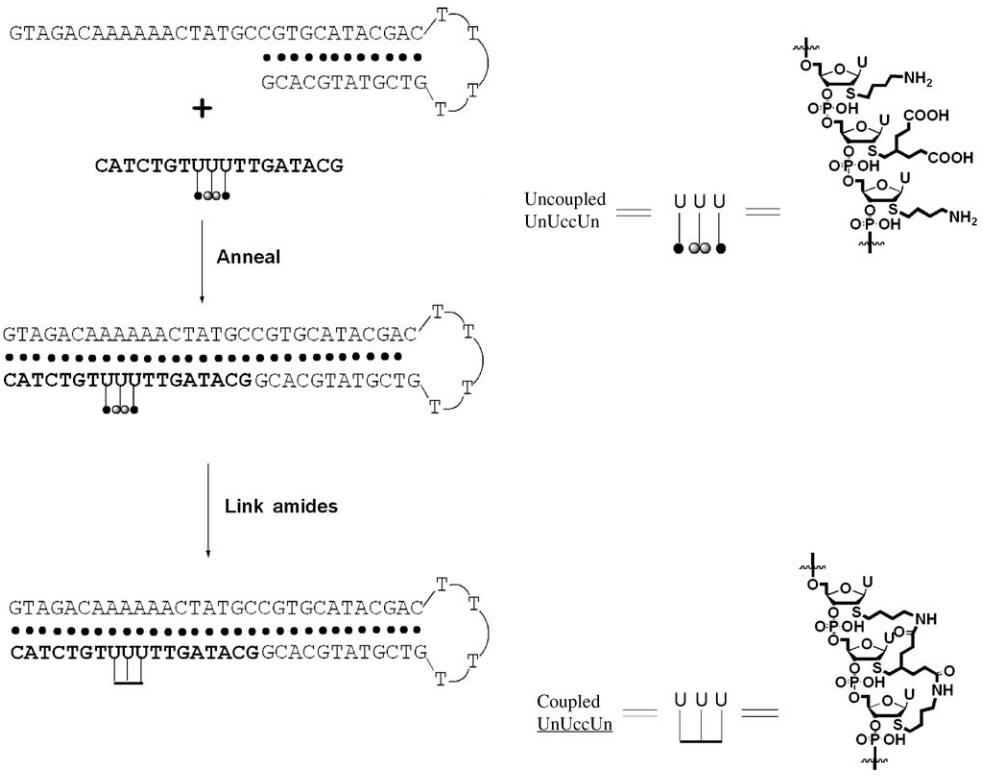

Figure 1. Schematic depiction of the templated synthesis of nylon nucleic acid molecule **3b**. A DNA hairpin template strand with a sequence complementary to strand **3a** forms a stable duplex. Proximal amines and carboxylates are then connected by using chemical ligation. The nylon nucleic acid product is separated from the hairpin template by denaturing gel electrophoresis.

Thermal denaturing studies

The duplex stabilities of nylon nucleic acid-containing strands hybridized with unmodified DNA and RNA complements were determined by thermal denaturing experiments that monitored absorbance at 260 nm. Figure 2 (DNA) and Figure 3 (RNA) show thermal-transition profiles plotted as the change in absorbance $(A_T - A_{T_0})/A_{T_0}$ vs. temperature. In all cases, at least two consecutive heating–annealing cycles were applied. Superimposable heating and annealing profiles indicate that transition processes are kinetically reversible. The effect of extra polyamide linkages was examined by comparing the melting temperatures of **1**–DNA duplex or **1**–RNA duplex (by using the unmodified DNA **1** as control). Melting temperatures (T_m)—defined as the temperature at which half of the double-strand complexes had dissociated[10]—are summarized in Table 2. Uncoupled duplexes showed cooperative melting behavior, but coupled oligomers melted over a relatively broad temperature range. Nevertheless, single transition behavior was assumed for the thermodynamic analysis.

The uncoupled precursor strands formed duplexes with DNA and RNA that were less stable than analogous duplexes of unmodified DNA. Thus, introduction of 2′-pendent thioalkyl diamino or dicarboxyl groups destabilized the duplex. In the case of DNA, the melting temperature decreased as the number of modified nucleotides was increased. This destabilization could be attributed to steric effects exerted by the bulky 2′ moieties. Although 2′-*O*-methyl and 2′-*O*-methoxymethyl substituents are known to stabi-

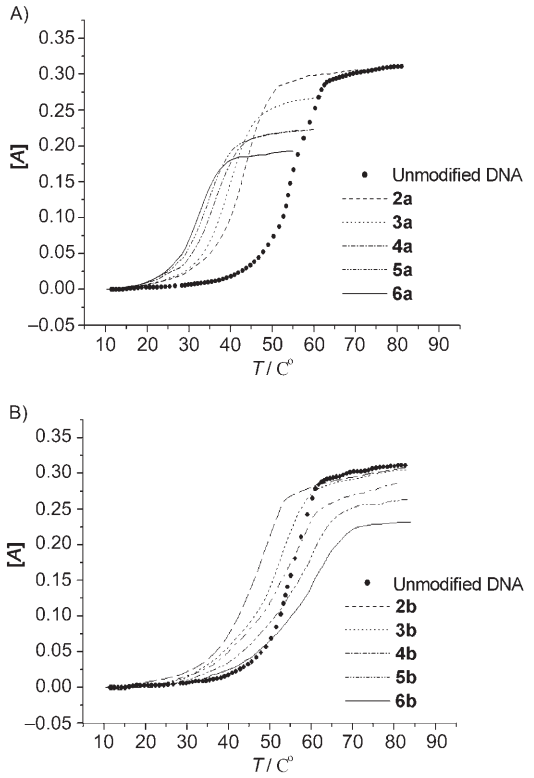

Figure 2. Melting curves for modified oligonucleotides, A) before and B) after coupling with complementary DNA. $[A]=(A_T-A_{T_0})/A_{T_0}$, where A_{T_0} is the absorbance at 10 °C; UV absorption was monitored at 260 nm.

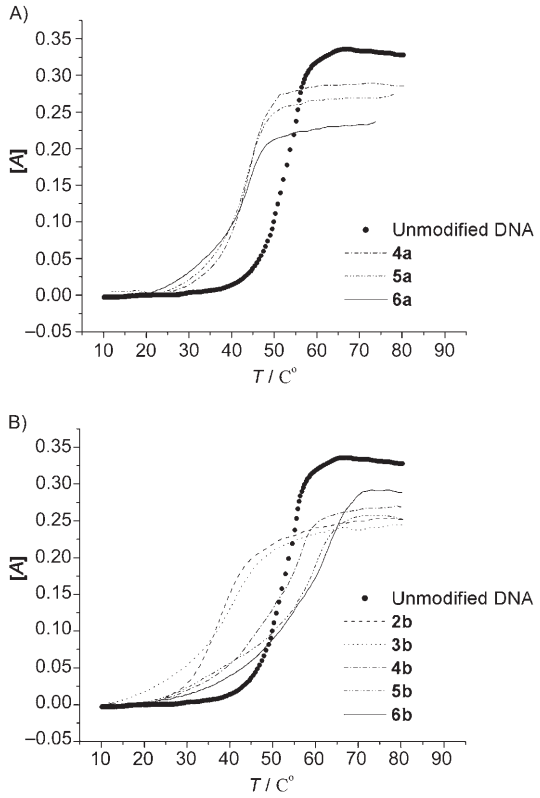

Figure 3. Melting curves for modified oligonucleotides, A) before and B) after coupling with complementary RNA. $[A]=(A_T-A_{T_0})/A_{T_0}$, where A_{T_0} is the absorbance at 10 °C; UV absorption was monitored at 260 nm.

lize duplexes with DNA, 2′-*O*-alkyl modification lowered binding affinity towards complementary DNA strands with increasing size of the alkyl chain.[11] Destabilization might also result from altered sugar puckering of the 2′-deoxy-2′-alkylthio ribose, as the less electronegative[12] 2′-alkylmercapto modification should lead to a decrease in the preponderance of the 3′-endo conformer.[13] The higher ratio of 2′-endo conformer could perturb the backbone, which might lead to further distortions, in addition to the steric hindrance exerted by bulky 2′-S pendent groups. Furthermore, this destabilization effect might be associated with the observation that oligonucleotides that contain dT are more stable than analogous strands with dU.[14] In addition, the charges on the pendent groups could also have a local effect on melting, although the exact nature of the effect is unclear; overall, the number of added positive and negative charges was equal in all molecules. For duplex molecules in which the uncoupled precursor strands were hybridized with RNA destabilization of the duplex was also observed. However, the decrease in melting temperature in response to an increase in the number of modified nucleotides was not as large (Table 2 B, Figure 3 A).

In contrast with the uncoupled strands, coupling of the amino and carboxyl groups led to higher stability in duplex molecules. Coupled strand **2b**, which contained one amide bond, gave a 3.7 °C increase in T_m for the duplex with complementary DNA as compared with the uncoupled precursor **2a**, but was still much lower than control strand **1**. However, duplex stability with natural DNA was further enhanced with the number of amide bonds introduced. For strand **3b** (two amide linkages) T_m increased to 51.2 °C and was 11.7 °C higher than its uncoupled counterpart. This trend continued, so that strands with four or five linkages in fact formed more stable duplexes with their natural DNA complement than the unmodified control. Coupled strand **6b** had a T_m 2.8 °C higher than unmodified DNA and 26 °C higher than its uncoupled analogue. The situation for the RNA duplexes was similar. Higher melting temperatures were obtained with increasing numbers of amide linkages, so that coupled strand **6b** had a T_m 3.6 °C higher than the unmodified DNA–RNA control. This enhanced thermostability can probably be attributed to conformational restriction imposed by the presence of a second polyamide backbone that links adjacent nucleotides in nylon nucleic acids. Conformational restriction has been reported to account for increasing the stability of duplexes for other nucleic acid analogues,[15] including peptide nucleic acid analogues.[16] Most such restrictions have been reported previously within a single nucleotide and have rarely involved links between adjacent nucleotides, as in the present case. Nielsen and colleagues reported covalent links between nucleotides and observed stabilization of nucleic acid three-way junctions.[17]

The enthalpies and entropies of the thermal transition were determined by using van't Hoff analysis (Table 2, Figures 4 and 5).[18] Compared with unmodified DNA–DNA and DNA–RNA duplexes, there is a significant difference in both enthalpy and entropy for all modified strands, including the fully coupled strand **6b**, which showed higher T_m for both types of duplexes. This reflects the broader temperature range of the transition

Table 2. T_m value and thermodynamic data for nylon nucleic acid duplexes with DNA complement and RNA complement.[a,b]

A)

	Nylon nucleic acid precursor strands						Coupled nylon nucleic acids					
	T_m [°C]	ΔH° [kJ mol^{-1}]	ΔS° [J mol^{-1} K^{-1}]	$T\Delta S^\circ$ [kJ mol^{-1}] (298 K)	ΔG [kJ mol^{-1}] (298 K)		T_m [°C]	ΔH° [kJ mol^{-1}]	ΔS° [J mol^{-1} K^{-1}]	$T\Delta S^\circ$ [kJ mol^{-1}] (298 K)	ΔG [kJ mol^{-1}] (298 K)	ΔT_m[c] [°C]
1	55.1	−384	−1050	−312	−71.3	**1**	55.1	−384	−1050	−312	−71.3	–
2a	42.8	−381	−1090	−324	−57.6	**2b**	46.5	−264	−708	−211	−53.5	3.7
3a	39.5	−376	−1080	−323	−53.3	**3b**	51.2	−210	−530	−158	−52.6	11.7
4a	35.8	−366	−1060	−317	−48.7	**4b**	52.9	−209	−523	−156	−52.9	17.1
5a	34.0	−341	−989	−295	−45.9	**5b**	55.3	−197	−481	−143	−53.6	21.3
6a	31.9	−317	−916	−273	−43.6	**6b**	57.9	−193	−464	−138	−54.7	26.0

B)

	Nylon nucleic acid precursor strands						Coupled nylon nucleic acids					
	T_m [°C]	ΔH° [kJ mol^{-1}]	ΔS° [J mol^{-1} K^{-1}]	$T\Delta S^\circ$ [kJ mol^{-1}] (298 K)	ΔG [kJ mol^{-1}] (298 K)		T_m [°C]	ΔH° [kJ mol^{-1}]	ΔS° [J mol^{-1} K^{-1}]	$T\Delta S^\circ$ [kJ mol^{-1}] (298 K)	ΔG [kJ mol^{-1}] (298 K)	ΔT_m[c] [°C]
1	52.9	−467	−1310	−391	−75.6	**1**	52.9	−467	−1310	−391	−75.6	–
2a	–	–	–	–	–	**2b**	37.4	−262	−719	−214	−47.8	–
3a	–	–	–	–	–	**3b**	39.4	−199	−508	−151	−47.4	–
4a	43.1	−377	−1070	−319	−57.8	**4b**	50.5	−196	−495	−148	−48.9	7.4
5a	42.4	−313	−874	−260	−53.0	**5b**	54.8	−186	−448	−134	−52.8	12.4
6a	41.8	−242	−651	−194	−48.3	**6b**	56.5	−177	−416	−124	−53.0	14.7

[a] Complementary DNA sequence: 5′-GTAGACAAAAAACTATGC; complementary RNA sequence: 5′-GUAGACAAAAAACUAUGC. [b] The melting curves (A_{260} vs. temperature) were recorded in medium salt hybridization buffer (40 mM sodium cacodylate, 100 mM sodium chloride, pH 7.3) that contained the two complementary strands (1 μM). [c] ΔT_m is the T_m difference between nylon nucleic acids and the corresponding uncoupled precursor strands.

observed for the duplexes that contained modified strands. Although the latter portions of the T_m curves for strands **5b** and **6b** were observed at clearly higher temperatures than for the controls, initiation of the curve occurred at temperatures well below the control. This broad range suggests lower cooperativity in the melting of duplexes that contained modified strands; this is reflected in the enthalpy and entropy. The less-sharp dependence on temperature could result from distortions in duplex structures that arise from the macrobicyclic rings associated with the nylon nucleic acid ladder polymer structure. Another contribution to this behavior could arise from the heterogeneity of the nylon nucleic acid region of each strand, which is flanked by unmodified DNA sequences.

Within the series of duplexes with DNA (**2**–**6**), the thermodynamic analysis revealed that for the nylon nucleic acids and precursor strands the enthalpy of duplex formation becomes less favorable, but entropy more favorable with increasing numbers of modified nucleotides. However, the values are very different from those from unmodified DNA **1**: the values of enthalpy and entropy for the melting of strand **6b** were only about half those of unmodified DNA **1**. The strongly favorable entropy can probably be attributed to restricted conformation exerted by 2′ amide linkage.[15f] A similar trend was also observed for thermodynamic parameters of nylon nucleic acids that were hybridized with RNA strands (Table 2B). It is likely that nylon nucleic acids impose similar restrictions on the duplex formed with RNA.

Circular dichroism spectroscopy

CD measurements were undertaken to compare the secondary structures of hybridized uncoupled precursor strands and coupled nylon nucleic acids with natural DNA and RNA duplexes.[19] In Figure 6 CD spectra of duplexes that involved nylon nucleic acid **6b** with DNA and RNA complements are shown and compared with the corresponding uncoupled precursor strand **6a** and unmodified DNA **1**. In Figure 6A, the overall CD curves of both uncoupled and coupled strands were similar to DNA control strand **1** and were consistent with the B-form structure. Both uncoupled and coupled strands showed a less intense negative band at around 250 nm and a more intense positive band at around 270–280 nm—the latter was shifted toward shorter wavelengths compared with **1**. Although the maximum number of modified nucleotides accounts only for one third of the strand, it is clear that perturbation by the modified nucleotides is present. In general, it appears that incorporation of the 2′-alkylthio substituent causes a small change in the spectra, and linking the substituents to form nylon nucleic acids brings the spectra back towards that of the unmodified strand **1**. Similar effects were observed for strands with 2–5 modified nucleotides, for which CD data are shown in the Supporting Information. In the series **2a**–**6a**, as more nucleotides with pendent groups were incorporated into the sequence, the uncoupled precursor strands showed increasing differences from the spectra of the control. However, the spectra of all of the duplexes formed between coupled molecules **2b**–**6b** and DNA were nearly superimposable. Therefore, coupling of the amides fixed the conformation of the strands to preclude the continuous deviation from the B-form observed in the uncoupled sequences.

Both uncoupled precursor strand **6a** and nylon nucleic acid **6b** showed the A-form structure in the CD spectra when they were paired with their RNA complement (Figure 6B). Similar to the DNA **1**–RNA control, there is an intense positive band cen-

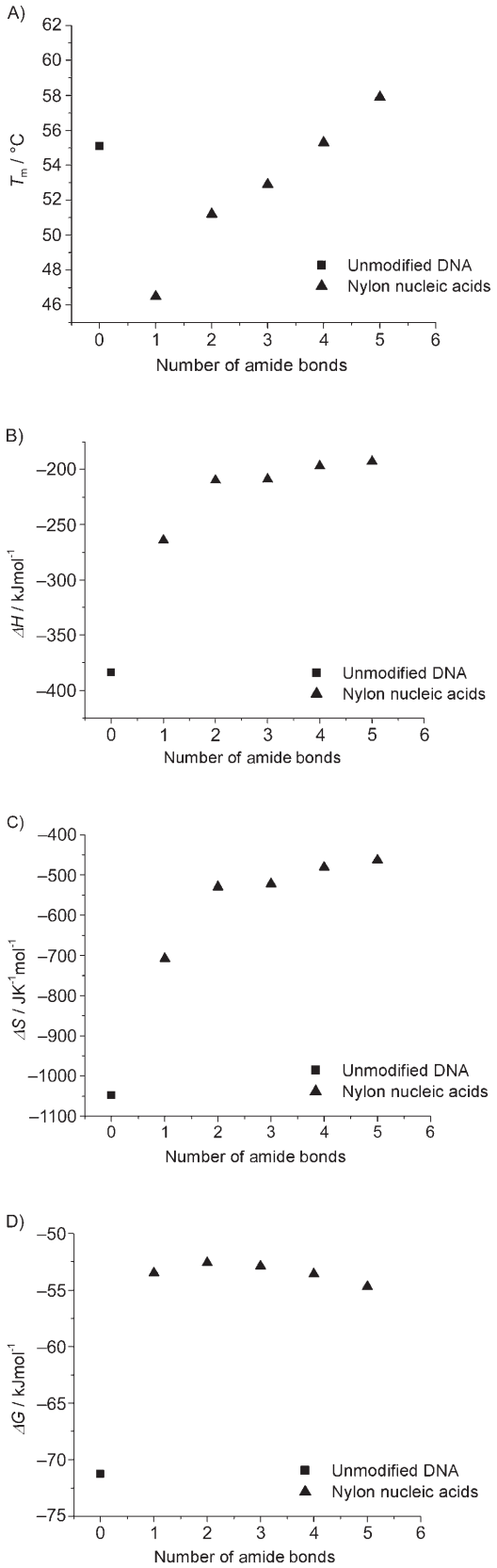

Figure 4. Thermodynamic data plots of nylon nucleic acid–DNA duplexes. A) T_m change as a function of the number of amide bonds formed; B) ΔH as a function of the number of amide bonds formed; C) ΔS as a function of the number of amide bonds formed; D) ΔG (298 K) as a function of the number of amide bonds formed.

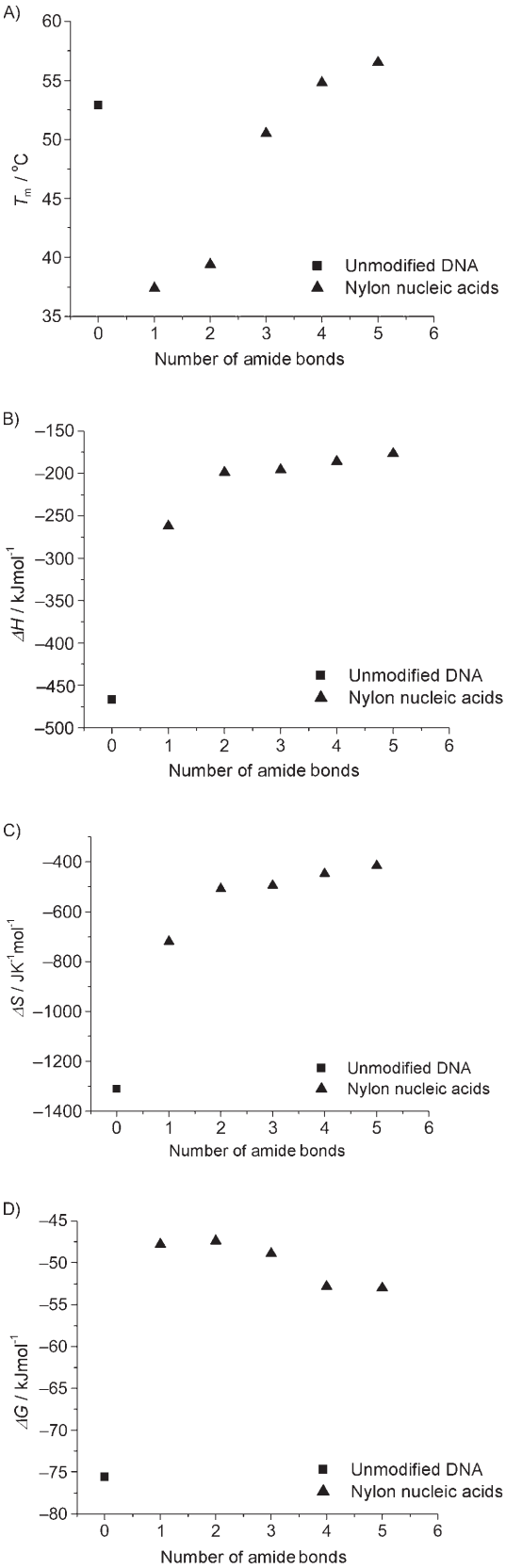

Figure 5. Thermodynamic data plots of nylon nucleic acid–RNA duplexes. A) T_m change as a function of the number of amide bonds formed; B) ΔH as a function of the number of amide bonds formed; C) ΔS as a function of the number of amide bonds formed; D) ΔG (298 K) as a function of the number of amide bonds formed.

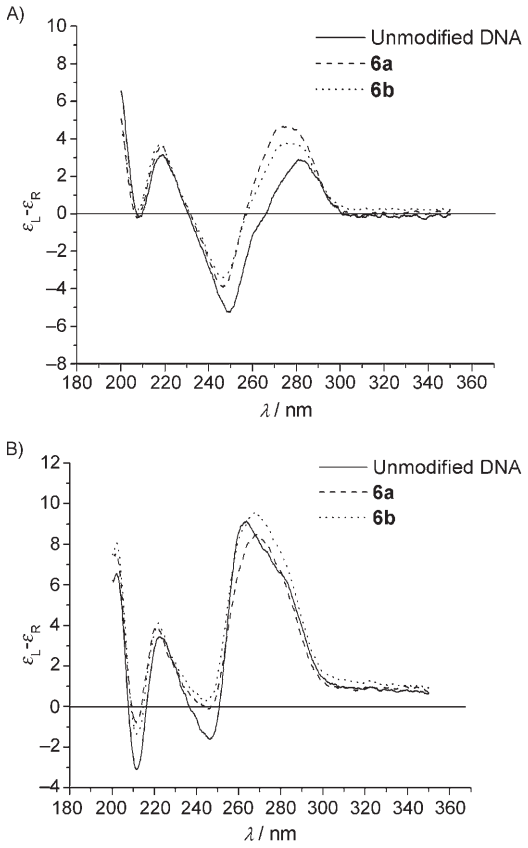

Figure 6. A) CD spectra of duplexes of unmodified DNA strand **1**, uncoupled **6a** and coupled nylon nucleic acid **6b** with DNA complement; B) with RNA complement. Duplex concentration: 4 μM; buffer: sodium phosphate (10 mM), sodium chloride (150 mM), and EDTA (0.1 mM, pH 7.0).

tered at around 265 nm. However, the two negative bands at 210 and 245 nm are less intense than the unmodified reference. In contrast to the control, the CD spectrum of the uncoupled precursor strand–RNA duplex for the series **2a**–**6a** showed a decrease in intensity of the 265 nm band, and this was also accompanied by a small bathochromic shift. For coupled **2b**–**6b**, the changes in CD spectra were much smaller (Figure S3). The polyamide linkage of the nylon nucleic acid perturbed the conformation of duplexes with RNA less than the pendent groups of precursor strands. Thus, there is no compelling evidence from the CD spectroscopy to suggest that a structural perturbation is the reason for the less-sharp dependence on temperature, as seen in the melting data.

Overall, the effect of introducing 2′-alkylthio-substituted nucleotides into DNA sequences brought about a small distortion of the conformation with a significant reduction in the stability of duplexes. However, linking the pendent groups together to form nylon nucleic acids reversed this trend, and even produced an increase in stability in the longer copolymer chains.

Given that many modified oligonucleotides have been studied for biomedical applications,[20] a natural question arises regarding the potential suitability of nylon nucleic acids for such a purpose. The increased stability observed with nylon nucleic acid–DNA duplexes is encouraging in this regard. The increase in the transition temperature per nucleotide for nylon nucleic acid bound to its complementary DNA strand is competitive with many modified oligonucleotides,[21] although not as large as some, such as locked nucleic acids.[15c,d,22] Stabilization of duplexes in our system appears to originate from conformational restriction enforced by the amide links. Prior examples of conformational constraints have involved primarily those within a single nucleotide. A few examples of rigidifying linkers between remote nucleotides have been reported.[23] To our knowledge, rigidification of the oligonucleotide backbone by covalent connections between adjacent nucleotides has not been explored previously. It seems likely that further optimization of nylon nucleic acid linkers could lead to even more stable duplexes. Further work, including examination of enzyme resistance, cell permeability, and other properties, will be needed to properly assess the potential of nylon nucleic acid for in vivo applications.

Conclusions

We have designed and synthesized a series of strands that contain modified uridine nucleotides flanked by heterobase sequences. The thermostability of nylon nucleic acid–DNA/RNA duplexes was studied by monitoring thermal denaturation by absorbance at 260 nm. It was found that fully coupled nylon nucleic acid strands display greater stability of duplex formation, compared with the analogous precursor strands. With an increase in the number of coupled units, nylon nucleic acid strands showed increased binding affinity to complementary DNA or RNA, while the precursor strands showed a decreasing trend. At 1 μM DNA concentration, after four or more amide linkages were formed (strands **5b**, **6b**), the nylon nucleic acid hybridized even more stably with its complement than the unmodified DNA control. These data suggest that the formation of the nylon linkage can stabilize the formation of duplex DNA or RNA by strands modified with nylon precursors. Analysis of thermodynamic data extracted from melting curves suggests that this stability results from a balance between lost enthalpy and lowered negative entropy as a function of modified nucleotide incorporation. Such differences can be attributed to conformational restriction exerted by the nylon linkages. Solution structural analysis by CD spectroscopy suggests that nylon nucleic acids hybridized with DNA have spectra that are more characteristic of B-form than A-form conformation, while nylon nucleic acids hybridized with RNA showed evidence of A-form conformation. However, in both cases the CD spectra of coupled polymers showed less conformational change with increasing numbers of modified nucleotides incorporated than did the precursor strands (i.e., with free pendent carboxylates and amines prior to amide bond formation). This observation is consistent with the conformational restriction hypothesis. These results provide a good model for understanding the factors that affect DNA binding ability.

Experimental Section

Materials: Buffer solutions were prepared from chemicals purchased from Aldrich and doubly distilled water, and then adjusted

to desired pH values. For experiments involving RNA, all laboratory equipments were autoclaved prior to use and sterilized water and buffers were purchased from Ambion (Austin, TX, USA). Preparation of the modified phosphoramidite uridine monomers and synthesis of nylon nucleic acid precursor strands followed the procedures previously reported,[5a] except that strands were cleaved from resin and deprotected at 50 °C instead of room temperature. All commercial nucleic acid strands and nylon nucleic acid uncoupled precursor strands were purified by denaturing gel electrophoresis (20% acrylamide; running buffer: 89 mM Tris·HCl, pH 8.0, 89 mM boric acid, 2 mM EDTA). For purification of uncoupled strands, in order to avoid a side reaction of amino groups with formamide upon heating, care was taken not to dissolve DNA strands in formamide before they were loaded onto gels. Concentrations of oligonucleotides were determined by UV spectroscopy (OD_{260}).

MALDI-TOF MS analysis: MALDI-TOF mass spectra were recorded by using a Bruker OmniFLEX MALDI-TOF spectrometer. A 3-HPA matrix solution was prepared by dissolving 3-HPA (18 mg) in CH_3CN (150 μL) and H_2O (150 μL). An ammonium citrate comatrix solution was prepared by dissolving ammonium citrate (35 mg) in H_2O (1 mL). The working matrix solution was obtained by mixing 3-HPA matrix solution (40 μL) and ammonium citrate comatrix solution (10 μL). An oligonucleotide sample (20~100 μM, 2 μL) was mixed with the working matrix solution (2.5 μL) by using a vortex, and then centrifuged. The mixture was deposited on a target. DNA molecules of known masses were used as either external or internal calibrants in the measurements.

Thermal denaturing studies: Pairs of complementary strands (700 pmol each) were dissolved in buffer (40 mM sodium cacodylate, 100 mM sodium chloride, pH 7.3) to a final volume of 700 μL, and annealed, overnight, over the temperature range 90 °C–room temperature. The samples were transferred to quartz cuvettes with 1 cm path lengths and the same cacodylate buffer was used as the blank. Thermal denaturation was monitored at 260 nm on a Spectronic Genesys 5 spectrophotometer by using a Neslab RTE-111 programmable circulating bath. At least two consecutive heating–cooling cycles were applied with a linear temperature gradient of 0.1 °C min^{-1}. Heating and cooling ramps were superimposable in all cases; this indicates equilibrium conditions. Absorbance vs. temperature curves were converted into θ vs. temperature curves (where θ is the fraction of oligomers in the associated state) by subtracting upper and lower base lines. These upper and lower linear base lines define temperature-dependent extinction coefficients for associated and dissociated states. The T_m was defined as the temperature at which half of the strands are in the associated form and half in the dissociated form, that is, $\theta = 0.5$.[10] Thermodynamic data were generated from van't Hoff plots of the data ($\ln K$ vs. $1/T$).[10]

CD experiments: CD spectra were recorded from 350 to 200 nm on an AVIV Model 202SF circular dichroism spectropolarimeter at room temperature in 0.2 cm cuvettes. Two complementary strands (2.8 nmol each) were dissolved in buffer (10 mM sodium phosphate, 150 mM sodium chloride, 0.1 mm EDTA) to a final volume of 700 μL and annealed as described above. The duplex solution was filtered through a 0.2 μm syringe filter (Millex) before the CD spectra were recorded. At least three scans were collected for each sample and a buffer baseline was subtracted from the average of these scans to yield the CD plots. The single strand CD spectra were recorded under the same conditions except at 15 μM concentration.

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Keywords: circular dichroism · conformational restriction · DNA analogues · template synthesis · thermodynamics

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