J|A|C|S
COMMUNICATIONS

Published on Web 04/09/2003

Alternating DNA and π-Conjugated Sequences. Thermophilic Foldable Polymers

Wei Wang, Wei Wan, Hong-Hui Zhou, Shuqiang Niu, and Alexander D. Q. Li*

Department of Chemistry and Center for Materials Research, Washington State University, Pullman, Washington 99164

Received January 16, 2003; E-mail: dequan@wsu.edu

It is widely accepted that properly folded biopolymers will lose their functions due to unfolding at high temperatures. However, proteins from thermophilic and hyperthermophilic microorganisms have shown extraordinary thermal stability by properly optimizing the delicate balance of weak molecular attractions. The critical issue, which of these interactions establishes the thermostability of proteins, is still not clear.[1] Among the electrostatic interactions, hydrogen bonding, and hydrophobic effects, there appears to be no common determinant of thermostability.[2] We report here that hydrophobic effects in the folding of alternating hydrophobic π-conjugated chromophores and hydrophilic single-strand deoxyribonucleic acid (ssDNA) strengthen as the temperature increases, similar to the inverse temperature transitions observed in elastin.[3] Thus, better folding, albeit counterintuitively, was observed at high temperatures.

DNA has been attached to nanoparticles,[4] proteins,[5] and fluorescent dyes[6] as probes and nanowires. Building on these successes, we have developed methods for efficient incorporation ($\sim$100% yield) of multiple optically active chromophores into the backbone of DNA for folding studies. The general strategy is to insert π-conjugated structures into DNA using asymmetric building blocks with one end activated and the other end protected. Our coupling chemistry is compatible with automated DNA syntheses.[7] Using this strategy, we have constructed foldable sequences of 5′-DNA-(-TEG-Chr-TEG-ssDNA)$_n$-TEG-Chr-TEG-DNA-3′, where TEG is tetraethylene glycol and Chr is the perylene tetracarboxylic diimide chromophore. The ssDNA are either NF-κB binding site (5′-ATC-CGG-AGT-CAG-CCG-GAT-3′), mutated NF-κB binding site (5′-A-GTT-GAG-**T**G**T**-**T**CT-TTC−CCA-GGC-3′; Figure 1A for n = 2), or modified AP1 binding site (5′-ATC-CGG-AGT-CAG-CCG-GAT-3′; Figure 1B and C). Using gel electrophoresis, we have purified these hybrid sequences into single molecular weight polymers corresponding to chromophoric dimers, trimers, tetramers, and pentamers linked via ssDNAs.

Folding of perylene units is signaled by a diagnostic optical absorption change because both covalently bound perylene cyclophanes[8] and folded dimers[9] have an *inverse* intensity distribution among their vibronic states, $A^{0\rightarrow0}/A^{0\rightarrow1} = 0.7$, whereas free perylene molecules have *normal* Franck−Condon progressions with $A^{0\rightarrow0}/A^{0\rightarrow1} \approx 1.6$.[8,10] Hence, the absorption ratio of the $0 \rightarrow 0$ to $0 \rightarrow 1$ transition can be used to quantitate the degree of folding in the DNA-perylene polymers.

At room temperature, both NF-κB and mutated NF-κB polymers form only loosely folded structures due to steric limitations imposed by their bulky DNA bases, as indicated by the diagnostic optical absorption ratio of $A^{0\rightarrow0}/A^{0\rightarrow1} = 1.10-1.15$ for the perylene dimer, trimer, tetramer, and pentamer (Figure 2A). This intensity ratio is intermediate between free monomers and closely packed perylene dimers. Upon heating, however, all of the foldable polymers become more ordered (Figure 1A), as indicated by the change in absorption

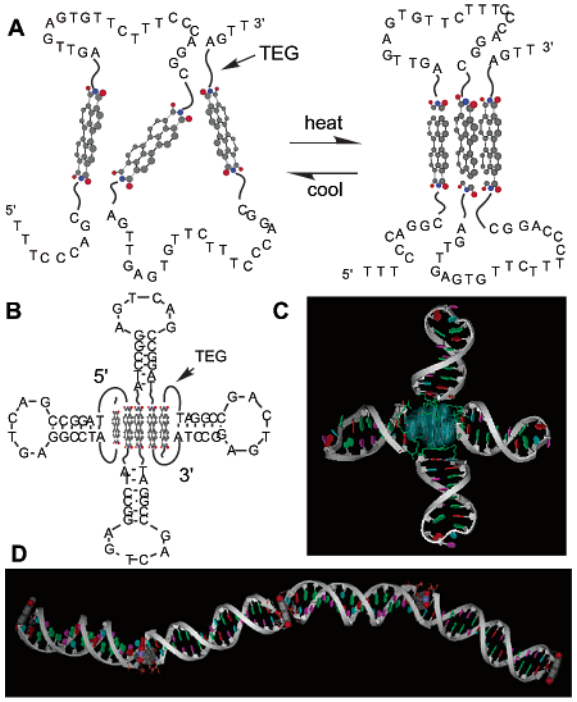

Figure 1. Thermophilic property of chromophoric trimer linked by a mutated NF-κB site with no hybridization in the DNA loops (A) and chromophoric pentamer surrounded by DNA hairpin structures (B and C) that can be unfolded through binding to the complementary DNA (D).

band intensity ratio, $A^{0\rightarrow0}/A^{0\rightarrow1} = 0.77-0.87$ (Figure 2A). This abnormal behavior contrasts with thermal denaturation in most proteins, but it is supported by theoretical calculations[3a] and experimental observations[3b,c] in the hydrophobic collapse of elastin. Moreover, this inverse temperature behavior of folding is largely reversible as the samples were cycled between 20 and 90 °C up to 10 heating−cooling iterations (Figure 2B). The DNA-perylene polymers clearly show that, unlike most other molecular attractions, the strength of hydrophobic forces is more pronounced at high temperatures.

The origin of the inverse temperature behavior comes from an *endothermic* enthalpy $\Delta H°$ (Figure 2C) of 2.72 ± 0.09, 4.44 ± 0.09, 4.78 ± 0.09, and 6.88 ± 0.22 kcal/mol for the folding of two, three, four, and five perylene units, respectively. For an *endothermic* interaction, the stability (K_{fold}) of the folded polymers improves as the temperature increases. The interactions between perylene π-planes mostly come from two contributions: $\pi-\pi$ molecular orbital overlap and hydrophobic forces. To determine which of these two gives rise to the inverse-temperature folding, we replaced the DNA sequences with a phosphotriester to gain solubility in organic solvents. In an organic solvent ($Cl_2CHCHCl_2$), hydrophobic effects are eliminated. Consequently, we observed

10.1021/ja0341900 CCC: $25.00

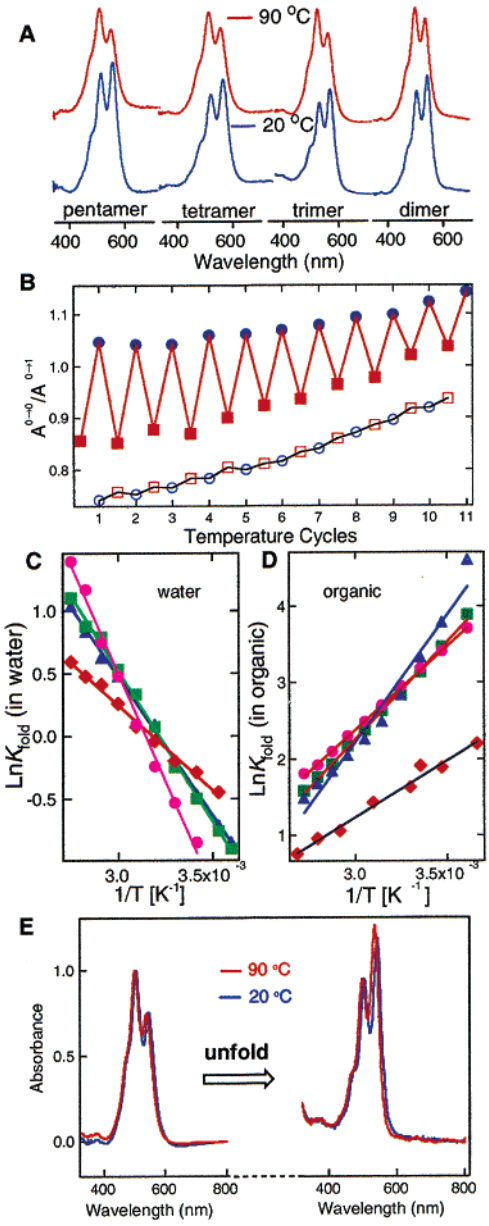

Figure 2. (A) Optical absorption of chromophoric dimer, trimer, tetramer, and pentamer at 20 °C (blue) and 90 °C (red). (B) The 20 °C (blue circles) and 90 °C (red squares) cycles of chromophoric pentamer with no hybridization in either the DNA loops (solid) or the DNA hairpin structures (open). Plots of the equilibrium folding constants, K_{fold}, against inverse temperature yield endothermic processes *in water* (C) and exothermic processes *in organics* (D) for folding of all oligomers. The straight lines were obtained from fitting the van't Hoff equation to experimental data for the dimer (diamonds), trimer (triangles), tetramer (squares), and pentamer (circles). (E) Binding to complementary DNA unfolds chromophoric pentamer with the hairpin structures.

exothermic processes (Figure 2D), with $\Delta H° = -3.01 \pm 0.06$, -6.62 ± 0.43, -5.09 ± 0.09, -4.35 ± 0.04 kcal/mol for the folding of two, three, four, and five perylene units, respectively. Most intermolecular interactions, including hydrogen bonds between DNA bases, are exothermic, thereby generating instability at high temperatures. Consequently, DNA loops cannot contribute to the inverse temperature behavior.

Using hydrophobic effects and six base-pair hairpin stems, we constructed a thermophilic foldable polymer, which can maintain its structural integrity at both low and high temperatures (Figure 1B, C). At high temperatures, hydrophobic attractions derived from perylene play a dominant role in stabilizing the folded structure. At low temperatures, hydrogen bonding between self-complementary hairpin structures plays a major role in perylene folding. The hairpin structure is based on the modification of the AP1 binding site (*vide supra*). The polymer obtained was almost completely devoid of temperature-dependent structural variations (Figure 2B). Because of the cooperative effects between folding of DNA loops and the chromophore core, the overall stability of the folded structure improves, as evidenced by the decrease in $A^{0\rightarrow0}/A^{0\rightarrow1}$ by an additional 10−30% throughout the heating−cooling cycles.

Upon addition of equimolar amounts of the complementary strand (3′-TAG-GCC-TCA-GTA-GGC-CTA-5′) in an annealing buffer (10 mM Tris·HCl, 0.1 M NaCl, 1 mM EDTA) to the thermophilic hybrid polymer, the perylene π-stacking was disrupted, as indicated by the reversal of $A^{0\rightarrow0}/A^{0\rightarrow1}$ from 0.76 to 1.4 (Figures 1D and 2E).

In summary, the integration of synthetic and natural oligomeric sequences into a single macromolecule creates folded nanostructures that possess unusual hyperthermophilic properties. Although temperature is not efficient at unfolding the thermophilic structure, it can be unfolded through molecular recognition-induced nanoactuation events such as DNA hybridization, from which DNA biosensors based on color change can be built.

Acknowledgment. We acknowledge the support of the Chemistry Department, the CMR, and College of Sciences at WSU. This paper is dedicated to A.D.Q.L.'s father, Yong-Zhi Li.

Supporting Information Available: Synthesis and methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Argos, P.; Rossmann, M. G.; Grau, U. M.; Zuber, H.; Frank, G.; Tratschin, J. D. *Biochemistry* **1979**, *18*, 5698. (b) Wallon, G.; Kryger, G.; Lovett, S. T.; Oshima, T.; Ringe, D.; Petsko, G. A. *J. Mol. Biol.* **1997**, *266*, 1016. (c) Rehaber, V.; Jaenicke, R. *J. Biol. Chem.* **1992**, *267*, 10999.

(2) (a) Karshikoff, A.; Ladenstein, R. *Trends Biochem. Sci.* **2001**, *26*, 550. (b) Sanchez-Ruiz, J. M.; Makhatadze, G. I. *Trends Biotechnol.* **2001**, *19*, 132. (c) Kumar, S.; Tsai, C. J.; Nussinov, R. *Protein Eng.* **2000**, *13*, 179. (d) Höcker, B.; Jürgens, C.; Wilmanns, M.; Sterner, R. *Curr. Opin. Biotechnol.* **2001**, *12*, 376. (e) Zartler, E. R.; Jenney, F. E.; Terrell, M.; Eidsness, M. K.; Adams, M. W. W.; Prestegard, J. H. *Biochemistry* **2001**, *40*, 7279.

(3) (a) Li, B.; Alonso, D. O. V.; Daggett, V. *J. Mol. Biol.* **2001**, *305*, 581. (b) Reiersen, H.; Clarke, A. R.; Rees, A. R. *J. Mol. Biol.* **1998**, *283*, 255. (c) Urry, D. W. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 819.

(4) (a) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078. (b) Mitchell, G. P.; Mirkin, C. A.; Letsinger, R. L. *J. Am. Chem. Soc.* **1999**, *121*, 8122. (c) Maxwell, D. J.; Taylor, J. R.; Nie, S. M. *J. Am. Chem. Soc.* **2002**, *124*, 9606.

(5) Saghatelian, A.; Guckian, K. M.; Thayer, D. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 344.

(6) (a) Lewis, F. D.; Letsinger, R. L.; Wasielewski, M. R. *Acc. Chem. Res.* **2001**, *34*, 159. (b) German, I.; Buchanan, D. D.; Kennedy, R. T. *Anal. Chem.* **1998**, *70*, 4540. (c) Waybright, S. M.; Singleton, C. P.; Wachter, K.; Murphy, C. J.; Bunz, U. H. F. *J. Am. Chem. Soc.* **2001**, *123*, 1828. (d) Ren, R. X. F.; Chaudhuri, N. C.; Paris, P. L.; Rumney, S.; Kool, E. T. *J. Am. Chem. Soc.* **1996**, *118*, 7671. (e) Berlin, K.; Jain, R. K.; Simon, M. D.; Richert, C. A. *J. Org. Chem.* **1998**, *63*, 1527. (f) Pirrung, M. C.; Fallon, L.; McGall, G. *J. Org. Chem.* **1998**, *63*, 241. (g) Hakala, H.; Heinonen, P.; Iitia, A.; Lonnberg, H. *Bioconjugate Chem.* **1997**, *8*, 378. (h) Bevers, S.; O'Dea, T. P.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1998**, *120*, 11004.

(7) Gait, M. J. *Oligonucleotide Synthesis: A Practical Approach*; IRL Press: Washington, DC, 1984.

(8) Langhals, H.; Ismael, R. *Eur. J. Org. Chem.* **1998**, *9*, 1915.

(9) Wang, W.; Li, L.-S.; Helms, G.; Zhou, H.-H.; Li, A. D. Q. *J. Am. Chem Soc.* **2003**, *125*, 1120.

(10) Ford, W. E. *J. Photochem.* **1987**, *37*, 189.

JA0341900