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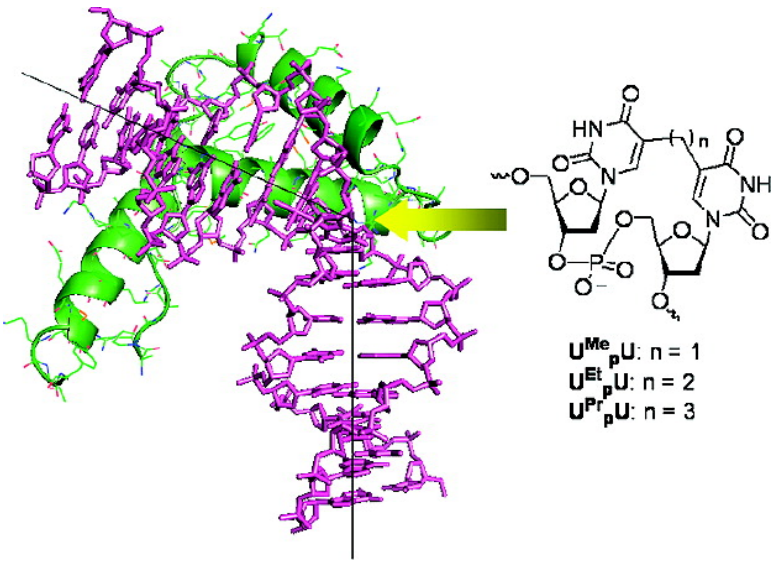
J. Am. Chem. Soc., **2007**, 129 (34), 10300-10301• DOI: 10.1021/ja071335k • Publication Date (Web): 02 August 2007



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Modular Bent DNAs: A New Class of Artificial DNAs with a Protein Binding Ability†

Shunpei Murata, Youko Mizumura, Kaori Hino, Yoshihito Ueno, Satoshi Ichikawa,* and Akira Matsuda*

Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku, Kita-12, Nishi-6, Sapporo 060-0812, Japan

Received March 1, 2007; E-mail: matuda@pharm.hokudai.ac.jp

Intracellular events including transcription, recombination, and replication are governed by DNA-protein interactions, where the binding of transcription factors to local higher-order DNA structures induces further sequential structural alterations and formation of multiprotein-DNA complexes.[1] Specificity determinants for intricate DNA-protein interactions would be categorized as sequence-specific and structure-specific recognition. Although extensive studies on the sequence specific recognition of DNA by proteins have been conducted,[2] the structural basis and characteristic traits of structure-specific DNA recognition, in which higher-order DNA structures are often involved, remain less well researched with limited success in defining these interactions.[3] Therefore, DNAs with altered conformational behavior should be useful in understanding structure-specific DNA recognition in biological systems and in the development of nucleic acid-based therapeutics. One of the best-known systems of structure-specific DNA recognition is an interaction of cisplatin−DNA adducts with high-mobility group box proteins (such as HMGB1).[4] The primary cisplatin−DNA adduct is a 1,2-d(GpG) intrastrand cross-link, which induces a dramatic structural distortions in the DNA helix, including bending and unwinding of the DNA helix.[5−7] This unique bending is believed to serve as a structure-specific recognition signal for these proteins including HMGB1. Of great significance is this single-point modification of DNA, where platinum plays a role as a "clasp" linking a neighboring G−G base sequence, and which results in significant global alteration of the structural features of DNA to acquire new functions. We designed novel oligonucleotides (ODNs) containing a cyclic 2′-deoxyuridylate dimer which possesses an alkylene linkage as a "clasp" with various lengths (Figure 1, $\mathbf{U^{Me}_pU}$, $\mathbf{U^{Et}_pU}$, and $\mathbf{U^{Pr}_pU}$). Uracil base was chosen because of easier synthetic accessibility. Here, we describe the bending property of the newly synthesized ODNs and their HMGB1 A-box protein binding property as an evaluation of the function of artificial bent ODNs.

The corresponding amidite blocks of **1**−**3** were synthesized as shown in Scheme S1−S3 (Supporting Information) and utilized to synthesize ODNs **C1**−**C3**. First, fluorescence resonance energy transfer (FRET) experiments were conducted to verify a degree of global bending of ODN **C1**−**C3** induced by a local chemical modification. 5′-6FAM-d(TCTCCTTCA**XX**ACTTCCTCT)-3′ and its complementary strand 5′-Cy3-d(AGAGGAAGTAATGAAG-GAGA)-3′ were set up as a donor and an acceptor, respectively.[8] The FRET experimental data, including the calculated bending angle of each ODN, are summarized in Table 1. FRET effects were significantly observed for ODN **C1**−**C3**, and the cross-links clearly introduced bending of the ODNs (Supporting Information). The estimated bending angles of each ODN **C1**, **C2**, **C3**, and **Pt** are 94°, 86°, 84°, and 58°,[9] respectively, and the shorter the length of the alkylene clasp, the deeper the bending angle of the ODN. ODNs **C1**−**C3** possess a deeper bending nature than ODN **Pt**. These experimental results unambiguously reveal deep bending of the global ODN structure, which is systematically controlled by local chemical modifications. On the other hand, ODN **C2′**, which contains an newly synthesized acyclic dimer ($U^{Et}U$), has a small bending angle (52°), which clearly shows that the cyclic 2′-deoxyuridylate structure is necessary to induce deep bending. We speculated that the bending position, including the cyclic 2′-deoxyuridylate dimer, must be highly distorted. To better understand the local structure of **C1**−**C3**, the thermal stability of the double stranded ODNs as well as those containing a mismatched base pair was compared by measuring T_m (Table 2). Compared to the control ODN, the T_m of each **C1**, **C2**, and **C3** was decreased by 12.0, 6.0, and 3.0 °C, respectively. The degree of decrease was well correlated to a length of the alkylene clasp, and introduction of the shorter clasp resulted in higher thermal destabilization of the ODNs. Mismatch incorporation at the YY position further decreased the T_m value. This trend was remarkable in the case of **C2** and **C3**, and double mismatched ODNs (YY = CC) were much more destabilized than complementary and single mismatched ones (YY = AC or CA). These results indicate the existence of base−pair interaction, including hydrogen bonding and/or stacking at the local bending site. ODN **C2′** contains $U^{Et}U$, which is an acyclic dimer

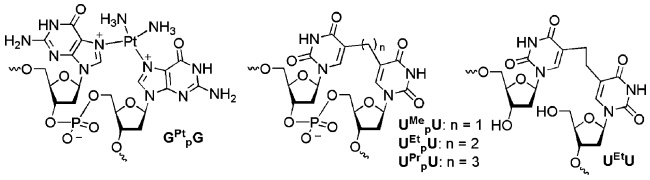

Figure 1. Structure of 1,2-d($\mathbf{G^{Pt}_pG}$) cross-link and designed alkylene cross-linked cyclic 2′-deoxyuridylate dimers

Table 1. FRET Experiments

5′-6FAM-d(TCTCCTTCA**XX**ACTTCCTCT)-3′
3′-d(AGAGGAAGT**YY**TGAAGGAGA)-Cy3-5′

ODN	XX	YY	energy transfer ($F\epsilon$[a])	absolute distance[b] (Å)	bending angle[c] (deg)
TT	TT	AA	0.272	65.0	n.d.[d]
Pt	G^{Pt}_pG	CC	0.477	56.9	58
C1	U^{Me}_pU	AA	0.794	44.7	94
C2	U^{Et}_pU	AA	0.714	48.1	86
C3	U^{Pr}_pU	AA	0.688	49.1	84
C2′	$U^{Et}U$	AA	0.436	58.5	52

[a] F_ϵ = FRET efficiencies, determined by measuring the intensity of the sensitized emission of the donor (F_{da}) normalized to the fluorescence of the donor alone (F_d) and the acceptor alone (F_a) (see Supporting Information). [b] Distances calculated from the energy transfer efficiencies with a Forester distance R_0 of 56 Å. [c] Angles calculated by the way a ODN was deemed to a cylinder molecule. [d] Not determined.

† Part 249 of Nucleosides and Nucleotides. Part 248: see Ohtawa, M.; Ichikawa, S.; Teishikata, Y.; Fujimuro, M.; Yokosawa, H.; Matsuda, A. *J. Med. Chem.* **2007**, *50*, 2007−2010.

10.1021/ja071335k CCC: $37.00

Table 2. Thermal Stability of the Double Stranded ODNs Incorporating Cross-linked 2′-Deoxyuridylate Dimers[a]

XX	YY	T_m (°C)[b]	ΔT_m (°C)	XX	YY	T_m (°C)[b]	ΔT_m (°C)
GG	CC	59.1		$U^{Et}{}_pU$	CC	41.5	−14.9
$G_{Pt}{}^PG$ (ODN **Pt**)	CC	56.2	−2.9[c]	$U^{Et}U$ (ODN **C2′**)	AA	46.5	−9.9[d]
TT	AA	56.4		$U^{Et}U$	CA	43.3	−13.1
$U^{Me}{}_pU$ (ODN **C1**)	AA	46.4	−12.0[d]	$U^{Et}U$	AC	43.8	−12.6
$U^{Me}{}_pU$	CA	42.2	−14.2	$U^{Et}U$	CC	38.9	−17.5
$U^{Me}{}_pU$	AC	43.7	−12.7	$U_p{}^{Pr}U$ (ODN **C3**)	AA	53.4	−3.0[d]
$U^{Me}{}_pU$	CC	39.7	−16.7	$U^{Pr}{}_pU$	CA	47.5	−8.9
$U^{Me}{}_pU$ (ODN **C2**)	AA	50.4	−6.0[d]	$U^{Pr}{}_pU$	AC	47.2	−9.3
$U^{Et}{}_pU$	CA	46.2	−10.2	$U^{Pr}{}_pU$	CC	40.5	−15.9
$U^{Et}{}_pU$	AC	46.2	−10.2				

[a] Sequence of ODN is 5′-d(TCTCCTTCA**XX**ACTTCCTCT)-3′/3′-d(AGAGGAAGT**YY**TGAAGGAGA)-5′. [b] Conditions: 1 μM each ODN, 10 mM sodium cacodylate−HCl (pH 7.0), 100 mM NaCl. Average of three measurements. [c] T_m (modified) − T_m (GG/CC). [d] T_m (modified) − T_m (TT/AA).

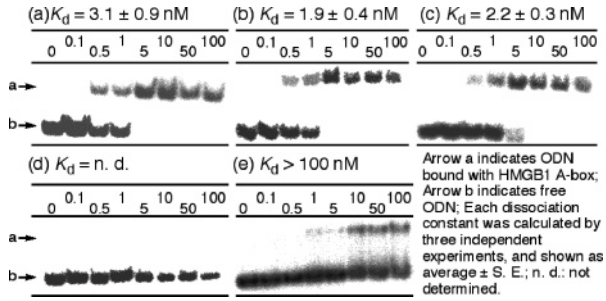

Figure 2. Electromobility shift assay (EMSA) of oligodeoxynucleotide (ODN) with high mobility group protein A-box (HMGB1 A-box): (a) ODN **Pt**, (b) ODN **C1**, (c) ODN **C2**, (d) ODN **C3**, (e) ODN **C2′**

with the phosphate backbone disconnected. Therefore, ODN **C2′** is more thermally destabilized than **C2**.

With sharply bent ODNs in hand, we next set about demonstrating protein binding ability. HMGB1 A-box protein was chosen as a binding partner, and electrophoretic mobility shift assays with these bent ODNs were performed (Figure 2). ^{32}P-labeled ODNs including **Pt** as a control were incubated with the A-box protein at 4 °C for 30 min, and the mixture was analyzed by gel electrophoresis. The results indicated that formation of the protein-ODN complexes had occurred in a dose dependent manner. Apparent dissociation constants (K_d) of ODN **Pt**, **C1**, and **C2** were determined to be 3.1 ± 0.9, 1.9 ± 0.4 and 2.2 ± 0.3 nM, respectively, and both ODNs **C1** and **C2** exhibited slightly higher affinity than ODN **Pt**. Furthermore, formation of the complex of ^{32}P-labaled **Pt** and the A-box protein was competitively inhibited by the addition of ODN **C1** or **C2** (Figure 3). This observation indicated that newly synthesized ODNs **C1** and **C2** were recognized by HMGB1 A-box protein in a manner similar to the cisplatin-crosslinked ODN **Pt**. The ODN **C2′** possesses poor binding ability to the A-box protein compared to ODN **C2**, indicating the importance of having a local bending structure for A-box binding (Supporting Information). Contrary to this successful protein binding for ODNs **C1** and **C2**, ODN **C3**, containing a propylene bridged 2′-deoxyuridylate dimer, failed to form a complex even at high concentration (0.1 μM) of the A-box protein. Of great interest is the observation of different binding natures of synthesized modular ODNs to the protein, although all ODNs **C1**−**C3** exhibit a similar range of bending angles. Selectivity in bent ODN recognitions observed in these results might be explained by a difference in a local structure of chemical modification itself and/or global structural alteration induced by its introduction such as unwinding of the helix. In fact, the CD spectrum of ODN **C3** was different from that of **C1** or **C2**,

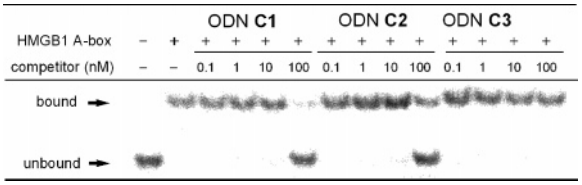

Figure 3. Competition assay with ODN containing cyclic 2′-deoxyuridylate dimer.

which retained B-form structures (Supporting Information), although detailed structural analysis is still necessary.

It is interesting that the ODNs **C1** and **C2** containing uracil bases in the bent units showed the same protein binding affinity as the ODN **Pt**. It is known that HMGB1 also binds to UV-damaged DNA containing a cis-syn cyclobutane pyrimidine dimer with only a moderate affinity (K_d = 1.2 μM).[10] Structural study of a complex of the A-box protein and cisplatin cross-linked DNAs revealed that Phe37 found in the A-box protein is one of the key residues interacting by $\pi-\pi$ stacking with the 3′-guanine base and $\delta-\pi$ stacking with the 5′-guanine residue.[7] The stacking nature of cis-syn cyclobutane thymine dimer is largely decreased because of loss of the 5,6-double bond. From this study, the stacking interaction would largely contribute to the 3 orders of magnitude gain in the binding affinity of **C1** and **C2** to the A-box protein.[11]

In conclusion, we have synthesized modular bent ODNs containing the cyclic 2′-deoxyuridylate dimer and have revealed that these ODNs have deep bending angles and bind tightly to the HMGB1 A-box protein. This strategy could provide ready access to systematic preparations of structurally altered ODNs, which would be a useful system for studying structure-specific DNA recognition.

Acknowledgment. We thank Prof. M. E. Bianchi for the generous gift of HMGB1 A-box plasmid. This work was supported by grants-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Supporting Information Available: Synthetic and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Pabo, C. O.; Sauer, R. T. *Annu. Rev. Biochem*. **1992**, *61*, 1053−1095.

(2) (a) Seeman, N. C.; Rosenberg, J. M.; Rich, A. *Proc. Nat. Acad. Sci. U.S.A.* **1976**, *73*, 804−808. (b) Garvie, C. W.; Wolberger, C. *Mol. Cell* **2001**, *8*, 937−946. (c) Luscombe, N. M.; Thornton, J. M. *J. Mol. Chem*. **2002**, *320*, 991−1009.

(3) (a) Pohler, J. R. G.; Norman, D. G.; Bramham, J.; Bianchi, M. E.; Lilly, D. M. L. *EMBO J.* **1998**, *17*, 817−826. (b) Wolfe, S. A.; Ferentz, A. E.; Grantcharova, V.; Churchill, M. E. A.; Verdine, G. L. *Chem. Biol*. **1995**, *2*, 213−221.

(4) Thomas, J. O.; Travers, A. A. *Trends Biochem. Sci*. **2001**, *26*, 167−174.

(5) Yang, D.; van Boom, S. S. G. E.; Reedijk, J.; van Boom, J. H.; Wang, A. H.-J. *Biochemistry* **1995**, *34*, 12912−12920.

(6) Huang, H.; Zhu, L.; Reid, B. R.; Drobny, G. P.; Hopkins, P. B. *Science* **1995**, *270*, 1842−1845.

(7) Ohndorf, U.-M.; Rould, M. A.; He, Q.; Pabo, C. O.; Lippard, S. *Nature* **1999**, *399*, 708−712.

(8) 6FAM = 6-carboxyfluorescein; Cy3 = indodicarboxycyanine-3. (a) Norman, D. G.; Grainger, R. J.; Uhrin, D.; Lilly, D. M. J. *Biochemistry* **2000**, *39*, 6317−6324. (b) Bassi, G. S.; Murchie, A. I. H.; Walter, F.; Clegg, R. M.; Lilley, D. M. J. *EMBO J*. **1997**, *16*, 7481−7489.

(9) The estimated bending angle of ODN **Pt** obtained in this study was within that reported by several experiments (see ref 5−7).

(10) Pasheva, E. A.; Pashev, I. G.; Favre, A. *J. Biol. Chem*. **1998**, *273*, 24730−24736.

(11) Jones, S.; Daley, D. T. A.; Luscombe, N. M.; Berman, H. M.; Thornton, J. M. *Nucleic Acid Res*. **2001**, *29*, 943−954.

JA071335K