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Suppression of DNA-Mediated Charge Transport by BamHI Binding

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Summary

A guanine radical cation produced by one-electron DNA oxidation migrates over long distances through the DNA π-stack. Fundamental questions regarding the likelihood of charge transport in genomic DNA, the effects of protein binding, and its biological consequences arise as the next issues of study. Electronic effects of protein binding on the efficiency of charge transport were investigated for the endonuclease BamHI-DNA complex. Direct contact of a positively charged guanidium group of BamHI to guanines in the recognition sequence 5′-GGATCC-3′ completely suppressed one-electron oxidation of the guanine in the protein binding site and dramatically lowered the charge transport efficiency through the sequence. Electronically insulated guanines, by the hydrogen bonding contact of a guanidium group in BamHI, no longer function as a stepping stone in the charge transport through the DNA π-stack.

Introduction

Oxidative DNA damage plays an important role in the processes of aging, mutagenesis, and carcinogenesis. Damage induced by one-electron oxidation occurs selectively at a guanine base because this is the most easily oxidized of the nucleotide bases [1]. Recent studies of DNA oxidation culminated in the findings that a guanine radical cation produced by one-electron oxidation may not localize at the initially oxidized site but may migrate over long distances through the π-stacked array of base pairs [2–11]. Subsequent studies revealed that DNA-mediated transport of the guanine radical cation (charge transport) proceeds via a process of successive "hopping" between neighboring guanines [3–13]. As the number of observations of DNA-mediated charge transport using oligomer duplexes increases, fundamental questions regarding the likelihood of charge transport in genomic DNA, and its biological consequences, arise as the next issues to be studied. Chromosomal DNA in eukaryotes is stored in the nucleus as a form of chromatin, with DNA bound to positively charged histone octamers [14]. The X-ray structure of the nucleosome core particle shows that the structure of the DNA bound to histone deviated from the ideal superhelix geometry [15]. Distortion of the DNA structure changes the degree of base stacking. It has been shown that DNA π-stack disruption induced by protein binding decreased the charge transport efficiency [16–19]. Besides the structural alteration, the protein binding has significant effects on the electronic state of DNA. In particular, the distribution of the electron density on nucleotide bases is modified by the hydrogen bonding of charged groups to nucleotide bases. However, the electronic effects of protein binding on the efficiency of charge transport through a DNA π-stack has remained to be clarified. We describe here, for the first time, how binding of endonuclease BamHI to its recognition sequence 5′-GGATCC-3′, involving hydrogen bonding of a positively charged guanidium group to guanine, effectively suppressed the oxidation of the sequence and the charge transport through the binding site.

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Results and Discussion

Effects of BamHI Binding on the Electronic States of Guanines

BamHI is a restriction endonuclease that binds as a dimer to the palindromic sequence 5′-GGATCC-3′ and hydrolyzes the phosphodiester linkage between the two guanines in the presence of Mg^{2+} [20]. The X-ray structure of the BamHI-DNA complex shows that direct hydrogen bonding involved in the protein-DNA contacts is condensed in a major-groove face of two G-C base pairs (Figure 1A). The protein bound DNA retains a standard B form-like conformation without significant bends and distortions of the base stack. In contrast, the electronic state of the protein bound DNA seems to differ significantly from the free state. Direct hydrogen bonding of a positively charged guanidium group of Arg155 of BamHI to both N7 and O6 of the 5′ guanine of the 5′-GGATCC-3′ binding motif should make the electron density of the guanine in the complex lower than in the free-state DNA. The electrostatic potential mapped on the surface of total electron density in fact shows that guanine in the complex is much more electron deficient than free guanine (Figure 1B). Negative electrostatic potential appeared at the region of N7, and O6 of the free guanine (left) disappeared in the guanine-guanidium-carboxylate triad (right). We expected that, when compared with normal guanine, such guanines with the decreased electron density would become less easily oxidized because of the increase of the ionization potential and would no longer function as a stepping stone in the charge transport via guanine hopping.

Design of Oligomers Containing an Electron-Accepting Nucleotide Base and Hole-Trapping Sites

To address the electronic effects of protein binding on the efficiency of charge transport through the DNA π-stack, we have used oligomer duplexes **1/2** and **3/4**, which both include a BamHI binding site in the middle of the sequence (Figure 2). Oligomers **1** and **3** contain

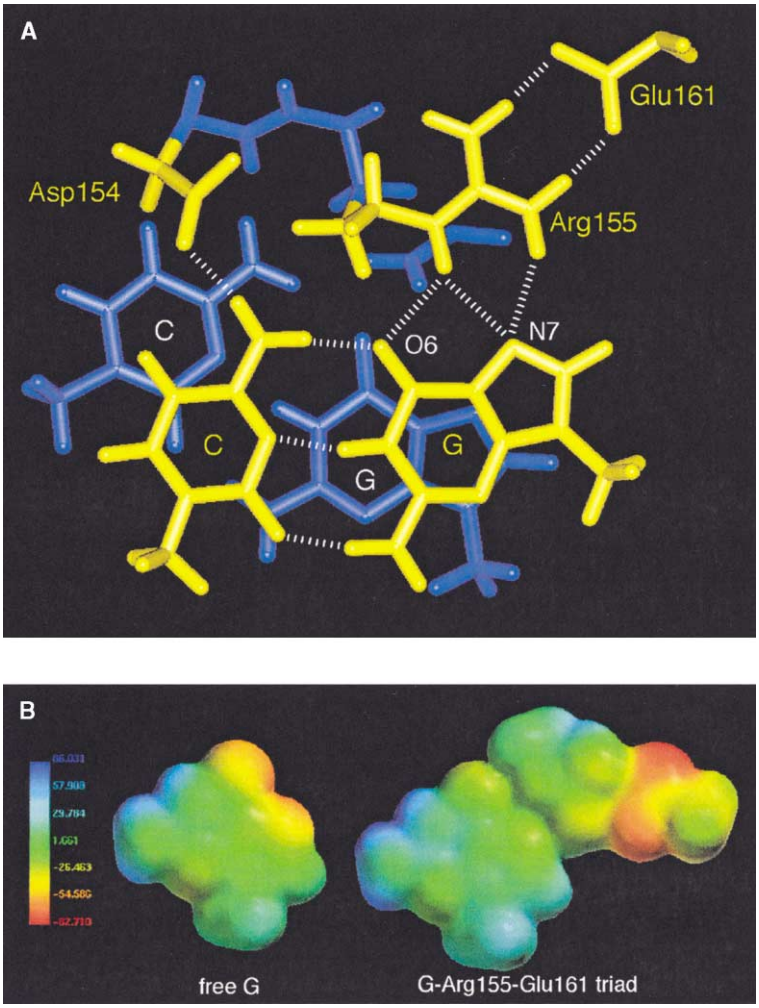

Figure 1. Hydrogen Bonding Contacts between BamHI and G-C Base Pairs in the Recognition Sequence of 5′-GGATCC-3′ and the Effect of Hydrogen Bonding on the Electrostatic Potential

Atomic coordinates were taken from the X-ray structure of the BamHI-DNA complex reported by Newman et al. [20].

(A) The protein-DNA interactions involving 5′- and 3′-side G-C base pairs are colored in yellow and blue, respectively. The N7 and O6 atoms of 5′-side G are directly hydrogen bonded to an Arg155 guanidium group that is also bound to Glu161. The O6 of 3′-side G bound to Asn116. For clarity, a hydrogen bonding network involving 5′ side G-C base pair is shown with a dotted line.

(B) The electrostatic potential mapped on the surface of a total electron density (0.002 electrons/au^3) for free guanine (left) and the guanine-guanidium (Arg155)-carboxylate (Glu161) triad (right). The electron-rich sites are shown in orange, whereas electron-deficient sites are shown in blue.

p-cyanobenzophenone-substituted uridine (^{CNBP}U) [21] as an electron-accepting nucleotide base that initiates one-electron oxidation of a neighboring guanine (i.e., G_8 in oligodeoxynucleotides [ODNs] **1** and **3**) upon irradiation at 312 nm [8–11]. Stacked guanine sites of GG and GGG have lower oxidation potential compared than does a single guanine [22–24] and are frequently used as internal hole traps [2–11]. Thus, a radical cation produced at G_8 can migrate through the π-stack down to $G_{16}G_{17}$ in ODN **1** and to G_8G_9 in ODN **2**, which both directly contact a guanidium group in Arg155 of the BamHI-DNA complex.

Analysis of Guanine Oxidation in the BamHI-DNA Complex

Gel Shift Analysis for the Binding of BamHI to the Duplex and Oxidation of the Complex

We first examined the binding of BamHI to duplex **1/2** and its effect on the oxidation of $G_{16}G_{17}$ at the binding site. Optimum conditions for the complete complex for-

1: 5'-ATT TAT A**G**T AGT GAT **GGA TCC** ATA TTA T-3'
2: 3'-TAA ATA **X**CA TCA CTA **CCT AGG** TAT AAT A-5'
3: 5'-ATT TAT A**G**T AGT GAT **GGA TCC** AT**G** **GG**A TTA T-3'
4: 3'-TAA ATA **X**CA TCA CTA **CCT AGG** TAC CCT AAT A-5'

X = ^{CNBP}U

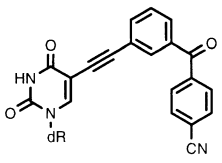

Figure 2. Sequences of Oligomer Duplexes **1/2** and **3/4** Both Containing a BamHI Recognition Sequence, 5′-GGATCC-3′

The modified base (X) denotes *p*-cyanobenzophenone-substituted 2′-deoxyuridine (^{CNBP}U). G_8 and a hole-trapping G triplet ($G_{24}G_{25}G_{26}$) shown in ODN **3** are located on opposite sides of the BamHI binding site. The BamHI binding site, a modified base, G_8, and a G triplet are shown in bold face.

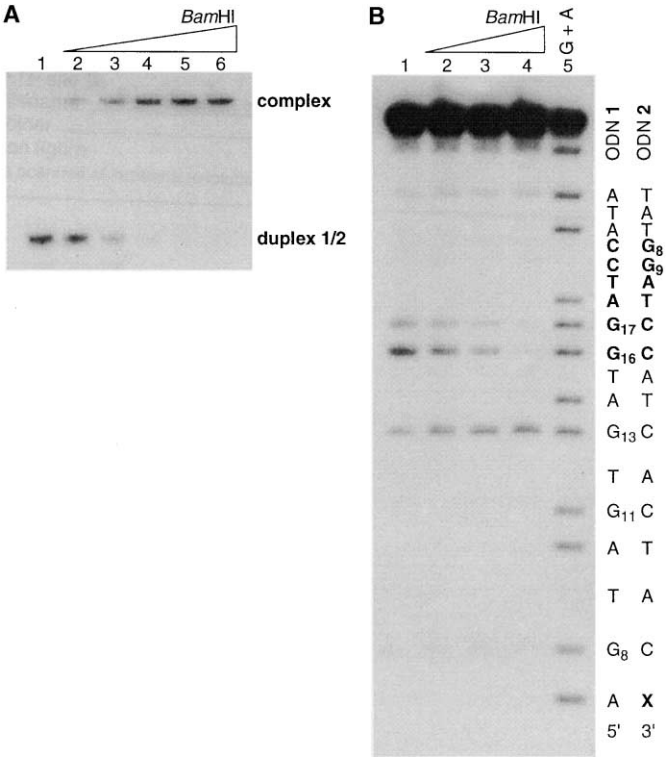

Figure 3. Binding of BamHI to ODN **1** and Its Effect on the One-Electron Oxidation of G's

(A) An electrophoretic mobility shift assay to detect a BamHI-DNA complex formation [25, 26]. Lane 1, DNA only; lane 2, BamHI, 0.15 U/μl; lane 3, 0.3 U/μl; lane 4, 0.6 U/μl; lane 5, 1.2 U/μl; lane 6, 2.4 U/μl.

(B) An autoradiogram of a denaturing polyacrylamide gel for BamHI concentration-dependent photooxidation of duplex **1/2**. Lane 1, DNA only; lane 2, 0.15 U/μl; lane 3, 0.3 U/μl, lane 4, 1.2 U/μl; lane 5, Maxam-Gilbert G + A sequencing reactions. The BamHI site and CNBPU (X) are shown in bold face.

mation between BamHI and duplex **1/2** were determined by an electrophoretic mobility shift assay (Figure 3A). As clearly seen from the figure, the BamHI-DNA complex increased as the concentration of the protein increased. At a BamHI concentration of 1.2 U/μl, duplex **1/2** (<2 nM) was completely transformed into the complex. These conditions (i.e., BamHI, 1.2 U/μl; duplex, <2 nM) that ensure a complete complex formation were used for all the BamHI binding experiments described below. The effect of BamHI binding on the oxidation of the 5′ GG in the recognition sequence was examined as a function of BamHI concentration (Figure 3B). In the absence of the protein (lane 1), distinct and strong cleavage was observed at G_{16}, with additional weak cleavages at G_{13} and G_{17}. It is well established in one-electron oxidation of DNA that (1) a GG site is more strongly oxidized than a GA site and (2) oxidation of a GG site is selective for the 5′ guanine. These observations ensure that G_{16} cleavage of ODN **1** proceeds by one-electron oxidation. With an increase in the concentration of BamHI, the band intensity at G_{16} decreased and eventually faded away in the presence of 1.2 U/μl of the protein (lane 4). Intensity of the cleavage band at G_{16} decreased as the fraction of BamHI-**1/2** complex increased. Complete disappearance of the G_{16} band demonstrated that $G_{16}G_{17}$ was insulated from one-electron oxidation upon complex formation. In marked contrast to G_{16}, at G_{13} in a GA site the band intensity increased with protein concentration (c.f., lanes 1 and 4). These opposing results regarding the efficiency for the oxidation of G_{16} and G_{13} suggest that the G_{13}A site of duplex **1/2** becomes the most easily oxidized site in the BamHI bound duplex because of the insulation of $G_{16}G_{17}$ by the guanidium group.

Suppression of Guanine Oxidation in Both Strands at the BamHI Recognition Site of the BamHI-DNA Complex

Because BamHI binds as a dimer to the palindromic sequence of 5′-GGATCC-3′, two GG sites in the sequence should be equally insulated from one-electron oxidation. In the absence of the protein, both $G_{16}G_{17}$ in ODN **1** (Figure 4A, lane 2) and G_8G_9 in ODN **2** (Figure 4B, lane 2) showed similar oxidization patterns under the irradiation conditions. In contrast, cleavage bands at both GG sites completely disappeared in the presence of BamHI (1.2 U/μl) (lane 3 in Figures 4A and 4B). Simultaneous suppression of oxidation at both GG sites shows that insulation of both GG sites from one-electron oxidation is due to the binding of BamHI to the recognition sequence.

Analysis of Charge Transport through the BamHI-DNA Complex

Because we had established that BamHI binding suppressed one-electron oxidation of GG in the recognition sequence, we then investigated charge transport through the site of BamHI binding. Since a hole injecting G_8 and a hole-trapping $G_{24}G_{25}G_{26}$ in duplex **3/4** are located on opposite sides of the BamHI binding site, hole migration from G_8 to the G triplet must proceed through the site of BamHI binding. In the absence of the protein, the guanine radical cation produced at G_8 in the duplex **3/4** migrates down to $G_{24}G_{25}G_{26}$ through the $G_{16}G_{17}$ATCC sequence (Figure 5, lane 4). Strong cleavage bands are observed at G_{24} and G_{25} in the G triplet, in addition to the cleavage at G_{16} of the GG site. Only a faint band was observed at G_{13} in the GA site. In the presence of BamHI, band intensity at the G triplet decreased with the con-

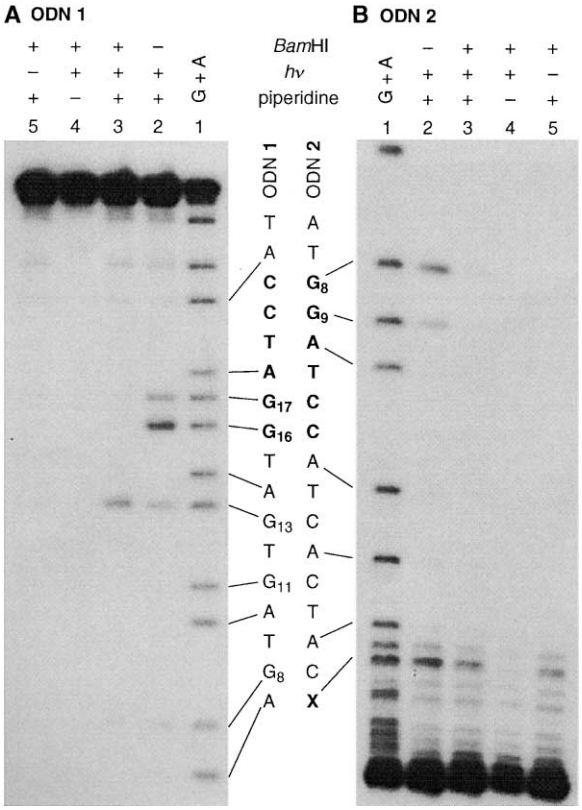

Figure 4. Autoradiograms of a Denaturing Polyacrylamide Gel for Photooxidation of Duplex **1/2** in the Presence of BamHI

ODNs **1** (A) and **2** (B) were separately 5′-32P-end labeled and hybridized to a nonlabeled complementary strand. Lane 1, Maxam-Gilbert G + A sequencing reactions; lane 2, in the absence of BamHI; lanes 3–5, BamHI. ODNs in lanes 2–4 were irradiated at 312 nm. All samples except that in lane 2 were heated with piperidine. The BamHI site and ^{CNBP}U (X) are shown in bold face. For clarity, the autoradiogram for ODN **2** was shown upsidedown.

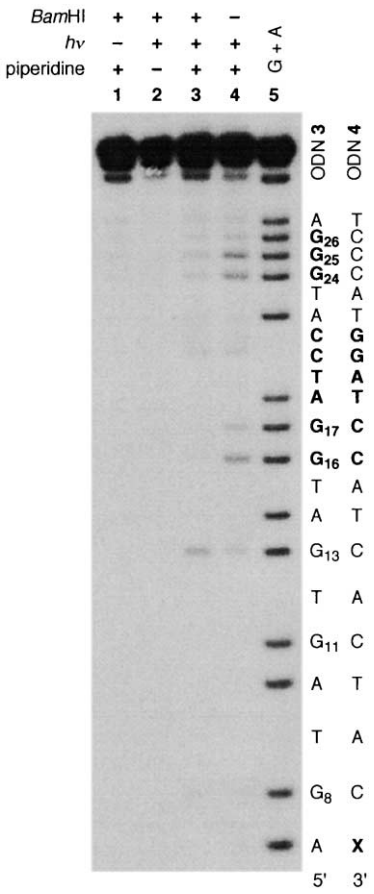

Figure 5. An Autoradiogram of a Denaturing Polyacrylamide Gel for Photooxidation of Duplex **3/4** in the Presence of BamHI

Lanes 1–3, in the presence of BamHI; lane 4, in the absence of BamHI; lane 5, Maxam-Gilbert G + A sequencing reactions. ODNs in lanes 2–4 were irradiated. All samples except that in lane 2 were heated with piperidine.

comitant disappearance of the band at G_{16} (lane 3). Densitometric analysis of the bands in lanes 3 and 4 showed that oxidation of the G triplet in the protein bound duplex was suppressed more than 3.7-fold (Figure 6). However, accurate analysis was not feasible because of very weak cleavages at the G triplet in the complex. In spite of the presence of the hole-trapping G triplet, the predominant site for one-electron oxidation in the protein bound duplex is the G_{13}A site, suggesting a considerable decrease in the efficiency of the charge transport from G_{13} to G_{24}. These results clearly show that BamHI binding to DNA not only suppressed the one-electron oxidation of GG in the recognition sequence but also lowered the charge transport through the site of the protein binding.

The electrostatic contacts between positively charged amino acid residues and negatively charged DNA are extremely important for protein-DNA interactions. Nonspecific electrostatic contacts of proteins are mostly to the phosphate anion of the DNA backbone, but sequence-specific protein bindings involve direct or water-mediated hydrogen bonding of charged groups to nucleotide bases. Contact via hydrogen bonding of a guanidium group in arginine to guanine is one of the most commonly observed protein-DNA interactions and is indispensable for the sequence recognition. Our results described here show that direct contact of a guanidium group to guanine dramatically decreased the susceptibility of the guanine for one-electron oxidation and lowered the efficiency for charge transport through the guanine.

Significance

The guanine radical cation produced by one-electron oxidation of DNA is not localized at the initially oxidized guanine site but migrates over long distances through the π-stacked array of base pairs. This quite striking property of DNA has been thoroughly studied through the use of oligomer duplexes containing various types of electron acceptors, showing that DNA mediates charge transport in a highly sequence-dependent manner. With these studies, fundamental questions regarding the likelihood of charge transport in genomic DNA, where most DNA was bound to proteins, arise as the next issues to be studied. Besides the structural alteration, the protein binding has significant effects on the electronic state of DNA. We found for the first time that binding of the endonuclease

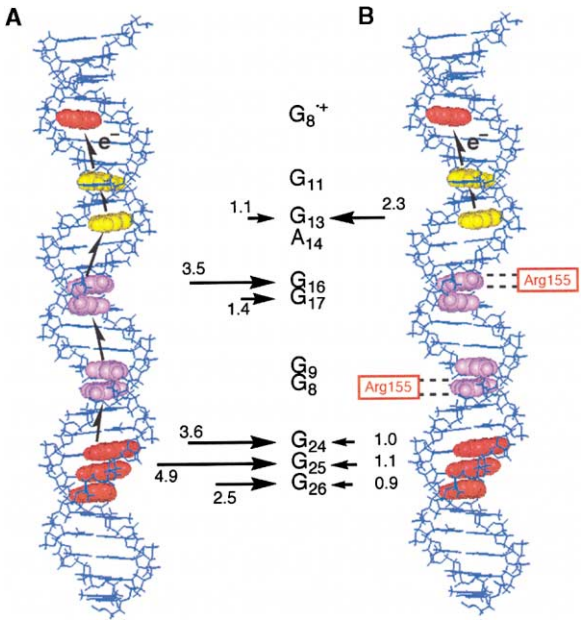

Figure 6. Illustrations of the Charge Transport in Duplex **3/4** in the Absence and Presence of BamHI

(A) In the absense of BamHI.
(B) In the presence of BamHI.
Horizontal arrows and the numbers shown on the site of guanine oxidation indicate the band intensity relative to that of G_{24} in the protein bound duplex. Band intensities are determined by densitometry of the lanes 3 (Figure 5A) and 4 (Figure 5B) in Figure 5.

BamHI to its recognition sequence 5′-GGATCC-3′ completely suppressed one-electron oxidation of the guanine in the protein binding site and dramatically lowered the charge transport efficiency through the sequence. This remarkable effect of BamHI binding on DNA-mediated charge transport is most ascribable to electronic insulation of the guanine in the binding site by direct contact of the positively charged guanidium group of the protein via hydrogen bonding. Contact via hydrogen bonding of a guanidium group in arginine to guanine is one of the most commonly observed protein-DNA interactions and is indispensable for sequence recognition. Our results described here show that direct contact of a guanidium group to guanine dramatically decreased the susceptibility of the guanine for one-electron oxidation and lowered the efficiency for charge transport through the guanine.

Experimental Procedures

Gel Mobility Shift Assay for the Binding of BamHI to Duplex DNA

5′-^{32}P-end-labeled ODN **1** (<2 nM) was hybridized with ODN **2** and incubated with various concentrations of BamHI (0.15–2.4 U/μl) in 100 mM NaCl, 1 mM EDTA·Na, and 10 mM Na cacodylate (pH 7.0). After incubation at 30°C for 20 min, an amount of glycerol (50%) equal to 10% of the reaction volume was added, and the resulting sample was loaded onto 12% polyacrylamide gel at 200 V for 2 hr.

Gel Analysis for the Guanine Oxidation of the BamHI-DNA Complex

5′-^{32}P-end-labeled ODN (<2 nM) was hybridized to its complementary strand in 100 mM NaCl, 1 mM EDTA·Na, and 10 mM Na cacodylate (pH 7.0). Duplex was incubated with BamHI (0.15, 0.3, and 1.2 U/μl) at 30°C for 20 min. The BamHI-DNA complex was irradiated at 312 nm at 30°C for 1 hr. After piperidine treatment (90°C, 20 min) of the irradiated duplex, the samples were suspended in denaturing loading buffer and electrophoresed through a denaturing 15% polyacrylamide/7 M urea gel at 1900 V for 100 min. Cleavage of the labeled strand was quantified by densitometry with Molecular Analyst software (BIORAD).

Synthesis and Purification of DNA Oligomer Containing ^{CNBP}U

Automated DNA synthesis was carried out by a standard β-(cyanoethyl)phosphoramidite method with Applied Biosystems 392 DNA synthesizer. The coupling of d^{CNBP}U phosphoramidite was conducted as usual except for the coupling time of 15 min. Synthesized oligomers were deprotected and removed from the solid support by treatment with concentrated ammonia at 37°C for 24 hr. Purification of the oligomers was performed on a CHEMCOBOND 5-ODS-H HPLC column with a linear gradient of 5%–20% acetonitrile in 100 mM triethylammonium acetate for 30 min at a flow rate of 3.0 ml/min. The purity and concentration of the synthesized oligodeoxynucleotides was determined by a complete digestion of the oligomer with snake venom phospodiesterase (0.15 U/μl), alkaline phosphatase (50 U/μl), and nuclease P1 (50 U/μl) at 37°C for 2 hr to a mixture of 2′-deoxynucleosides. Oligodeoxynucleotides containing ^{CNBP}U were further identified by MALDI-TOF mass measurements (**1**: observed, 8754.5; calculated, 8753.9. **2**: observed, 9624.9; calculated 9621.4).

Calculations of Electrostatic Potentials

Electrostatic potentials of free guanine and the guanine-Arg155-Glu161 triad in the BamHI-DNA complex were calculated at the B3LYP/6-31G(d) level with quantum chemical software SPARTAN (version 5.1). Geometries of the molecules were obtained from the coordinates of the X-ray structure of the BamHI-DNA complex deposited in the protein data bank and used for the calculations without any change. The electrostatic potentials were mapped on the surface of a total electron density (0.002 electrons/au^3) of each molecule.

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