Inorg. Chem. **2004**, *43*, 408–410

Probing the Solvent Accessibility and Electron Density of Adenine: Oxidation of 7-Deazaadenine in Bent DNA and Purine Doublets

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Received August 20, 2003

The effect of DNA bending on nucleobase electron transfer was investigated by studying the oxidation of double-stranded sequences containing seven repeats of the known bent sequence d(GGCA$_1$A$_2$A$_3$A$_4$A$_5$A$_6$C) where 7-deazaadenine (zA) was substituted at the A$_3$ position. Native gel electrophoresis was used to show that the sequence remained bent upon substitution of zA, which provides for oxidation of the sequence by Ru(bpy)$_3^{3+}$ (bpy = 2,2′-bipyridine). The Ru(III) oxidant was generated by photolysis of Ru(bpy)$_3^{2+}$ in the presence of ferricyanide, and the oxidation was visualized by high-resolution gel electrophoresis of the radiolabeled DNA sequence following base treatment. Cleavage of the DNA strand at the guanine residues and at the zA residues was observed. Comparison of the oxidation of zA in bent DNA versus the normal B form showed that hybridization of the B form sequence to its Watson–Crick complement produced a reduction in cleavage by a factor of 5.19 ± 0.46 while hybridization of the bent sequence only reduced cleavage by a factor of 1.58 ± 0.23. This result implies that the zA in the double-stranded, bent sequence is much more solvent-exposed than in normal B-form DNA. When the zA occurred in a B-form 5′-zA-G doublet, the reactivity was 6.63 ± 0.10 times higher for the zA compared to the G. This implies an even greater effect of a 3′-guanine on the oxidation potential of zA than in the well-known 5′-GG doublet.

Deviation of DNA structure from the canonical B helix affects the rates and energetics of nucleobase electron transfer.[1−7] Such effects have been studied for both long-range and short-range oxidations of guanine and related bases included in or adjacent to single-stranded regions,[3,5,8] triplexes,[4] quadruplexes,[6,7] and DNA:RNA hybrids,[2,9] so these oxidations provide useful probes of DNA structure. Further, guanine oxidation in the cell is known to play an important role in aging and mutagenesis,[10,11] and novel DNA structures could therefore regulate the extent of gene damage in vivo by altering the rates of base oxidation.[6,12] Bent DNA plays numerous roles in biology,[13−16] but to date, the effects of DNA bending on nucleobase oxidation have not been studied. A difficulty in exploring such effects is the fact that DNA bending is generally obtained when numerous adjacent adenines occur in the sequence.[14] Guanine is the most readily oxidizable nucleobase, and adenine is significantly more difficult to oxidize,[17,18] so it is difficult to study the oxidation chemistry of adenine in sequences that contain guanine. We report here on the use of 7-deazaadenine (zA) as a probe of electron transfer in bent DNA sequences.

An 80-mer oligonucleotide (**1**, Table 1) was synthesized that was composed of seven repeats of the sequence d(GGCA$_1$A$_2$A$_3$A$_4$A$_5$A$_6$C), which is known to produce bent DNA.[19,20] In addition to these seven repeats, a mixed-sequence segment of 10 nucleotides was appended to the 3′ end to ensure complete hybridization. The 10-mer repeating sequence was previously shown to remain bent upon incorporation of zA at the A$_3$ location.[19] In our case, we substituted four zA's into the overall sequence, as shown in

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10.1021/ic034989c CCC: $27.50
Published on Web 12/18/2003

Table 1. Oligonucleotide Sequences

	sequence (5→3′)
1	GGC AAA AAA CGG CAA zAAA ACG GCA AAA AAC GGC AAzA$_{36}$ AAA CGG CAA AAA ACG GCA AzA$_{56}$A AAC GGC AAA AAA CGC zAGT ACT CG[a]
2[b]	CGA GTA CTG CGT TTT TTG CCG TTT TTT GCC GTT TTT TGC CGT TTT TTG CCG TTT TTT GCC GTT TTT TGC CGT TTT TTG CC
3	CAT G$_4$zA$_5$T TAT CzA$_{11}$G$_{12}$ ACT CTzA$_{18}$ TAC TCG CzAG TAC[a]
4[c]	GTA CTG CGA GTA TAG AGT CTG ATA ATC ATG
5[d]	CAT G$_4$G$_5$T TAT CG$_{11}$G$_{12}$ ACT CTG TAC TCG CGG TAC
6[e]	GTA CCG CGA GTA CAG AGT CCG ATA ACC ATG
7[f]	CAG CTA TGA CCA TGA TTA CGC CAA GCT TGC ATG CCT GCA GGT CGA CTC TAG AGG ATC CCC GGG TAC CGA GCT CGA ATT CA

[a] zA = 7-deazaadenine. [b] Complement to **1**. [c] Complement to **3**. [d] Same sequence as **3** with G's replacing zA's. [e] Complement to **5**. [f] Synthesized by PCR as the double-strand.

Table 1. To verify that **1** exhibited the appropriate degree of bending, native gel electrophoresis was performed at varying polyacrylamide concentrations, and the migration of **1** was compared to the migration of a sequence lacking A-tracts (**7**).[19] This experiment showed that **1** was bent as expected (see Supporting Information).

We have reported that the oxidation potential of zA is around 1.05 V,[18,21] similar to that of native guanine (all potentials versus Ag/AgCl). We have also shown previously that the oxidation of guanine by Ru(III) produces an enhancement in the oxidative current of the cyclic voltammogram of $Ru(bpy)_3^{2+}$ in the presence of DNA according to[5]

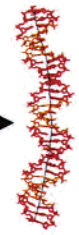
$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^- \quad (1)$$

$$Ru(bpy)_3^{3+} + DNA \rightarrow Ru(bpy)_3^{2+} + DNA_{ox} \quad (2)$$

where "DNA_{ox}" represents DNA where an electron has been removed from guanine. In addition, guanine in DNA oligonucleotides can be oxidized by the "flash-quench" method, where $Ru(bpy)_3^{2+}$ is photolyzed in the presence of ferricyanide.[6,7,22,23] This reaction produces $Ru(bpy)_3^{3+}$, which extracts an electron from guanine to produce a base-labile lesion that can be visualized by high-resolution sequencing on radiolabeled oligonucleotides.

Oligonucleotide **3** was 5′-labeled and photolyzed in the presence of $Ru(bpy)_3^{2+}$ and ferricyanide. As expected, cleavage of the oligonucleotide at the guanine residues was observed following piperidine treatment. In addition, however, piperidine-labile cleavage was observed at the zA residues, which is consistent with the ability of $Ru(bpy)_3^{3+}$ to abstract an electron from zA (gel given in Supporting Information). The extent of reaction of the zA residues was quantitated by phosphorimagery in both the single-strand and duplex forms. As we have described elsewhere,[6,23] larger extents of reaction are observed in single strands when the oxidized base is more exposed to the solvent-bound

Table 2. Comparison of Reactivities of 7-deazaadenine in Bent versus Straight DNA

Sequence #	Representation	Reactivity of zA (SS/DS)
1		1.58[a] ± 0.23[b]
3		5.19[c] ± 0.46[b]

[a] Calculated for **1** as the average of reactivities at single-stranded (SS) zA$_{56}$ and zA$_{36}$ divided by the double-stranded (DS) reactivities at the corresponding $Ru(bpy)_3^{2+}$ concentrations. [b] Standard deviation. [c] Calculated for **3** as the average of reactivities at SS zA$_{18}$ divided by the DS reactivities at the corresponding $Ru(bpy)_3^{2+}$ concentrations. Structures (kindly provided by Dr. Z. Shakked) are for illustration purposes only and are not of the precise sequences used here.

$Ru(bpy)_3^{3+}$. Accordingly, the decrease in reaction upon hybridization for the normal duplex (**3**) was greater than 5-fold (Table 2). In contrast, the difference in reaction for the zA residues in the A-tract regions of the bent oligonucleotide **1** was only a factor of 1.6. Thus, it appears that the bases in the bent A-tract are much more solvent-exposed than the same bases in unbent duplex DNA. The difference between bent and B-form DNA could also be attributable in part to a change in the ease of oxidation of zA in the two structures. Also, $Ru(bpy)_3^{2+}$ and related complexes are known to interact with DNA in the minor groove,[5] so changes in hydration or ion condensation could also contribute to the observed effect.

In addition to the effects of structure and the attendant solvent accessibility on nucleobase reactivity, the enhanced reactivity of guanines situated on the 5′-side of another adjacent guanine has been studied carefully.[24−29] This effect has been attributed to the favorable alignment of the six-membered ring of the 5′-guanine with the N7 of the guanine on the 3′-side (Figure 1);[30] the extent of the enhancement of

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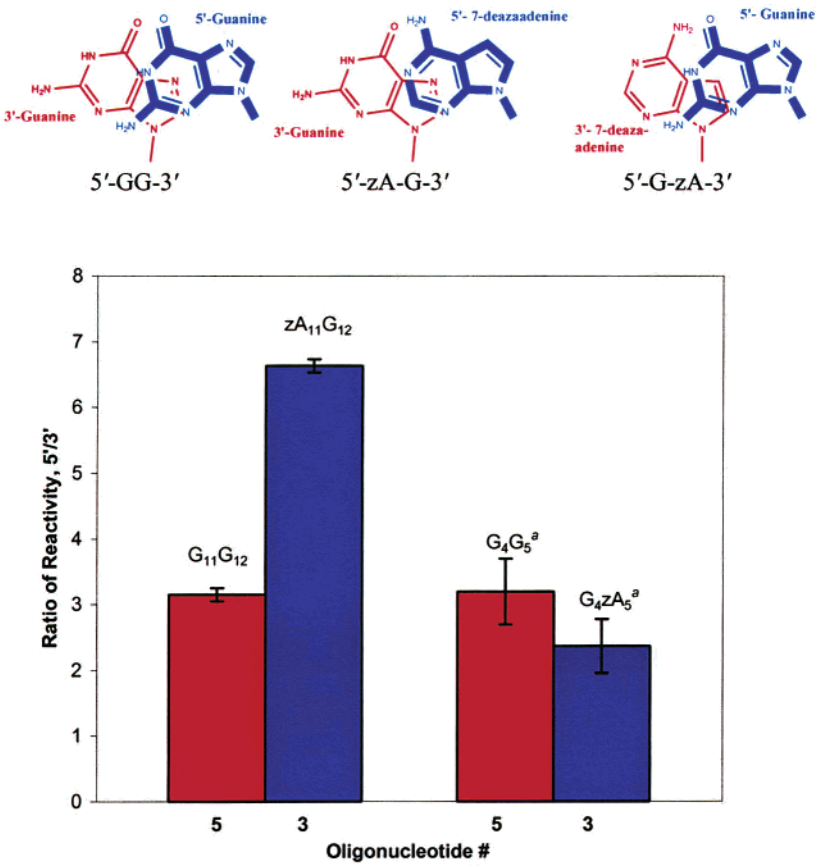

Figure 1. Purine doublet effect. The superscript *a*'s designate larger errors due to bases running near the bottom of the gel. Error bars indicate standard deviation.

reactivity for the 5′-guanine varies somewhat with the precise sequence.[24−29] Given the similar potentials for guanine and zA,[18] we sought to determine whether a similar stacking effect was observed for zA on the 5′-side of a guanine. Oxidation of sequences where zA was substituted for guanine to create a 5′-zA-G doublet showed that in fact an even greater enhancement was observed for zA compared to guanine (Figure 1). The reversed 5′-G-zA doublet showed a diminished effect when compared to that of the corresponding GG doublet, further implicating the guanine N7 on the 3′-base as the primary determinant of the effect. A similar trend has been observed with 7-deazaguanine;[28] however, that experiment is complicated by the fact that 7-deazaguanine is even more readily oxidized than guanine. The result shown in Table 2 with zA provides evidence supporting the role of N7 using a substituted nucleobase *that exhibits a potential similar to that of guanine*.

In summary, we have developed the oxidation chemistry of zA as a readily oxidizable alternative to adenine, which will enable numerous studies where the sequence and structural context of adenine can be evaluated by electron transfer, as has been done for some time now with guanine bases.[1−7] The zA base is available both as a phosphoramidite and as a nucleotide triphosphate,[21] so examination of zA electron transfer in diverse DNA structures is feasible. Here, we apply the concept to the evaluation of the solvent accessibility of bases in bent, A-tract DNA; these experiments show that bases in bent DNA are considerably more accessible than in the normal B form. In addition, the effect of stacking of an oxidizable purine on the 5′-side of a guanine produces a greater enhancement in reactivity for zA than for guanine, while a diminished effect is observed in the reversed 5′-G-zA doublet. This experiment provides further evidence for the importance of guanine N7 in the effect.[30]

Acknowledgment. This research was supported by the North Carolina Biotechnology Center. We would like to thank Dr. Z. Shakked for the structure of bent DNA.

Supporting Information Available: A detailed Experimental Section, plot of distance migrated versus percentage polyacrylamide for native-gel electrophoresis of **1**, and phosphorimages of denaturing polyacrylamide gels of **1**, **3**, and **5** after oxidation with $Ru(bpy)_3^{3+}$. This material is available free of charge via the Internet at http://pubs.acs.org.

IC034989C