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Intrastrand 2'β Hydrogen Abstraction of 5'-Adjacent Deoxyguanosine by Deoxyuridin-5-yl in Z-form DNA

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Abstract: In order to identify the site of H abstraction by the deoxyuridin-5-yl in Z-form DNA, we examined the photoreaction of d(CGCG(2'β-D)^{I}UGCG) **2-d**/ d(Cm^{8}GCACm^{8}GCG) **3** whose G$_4$ residue was deuterated with the diastereoselectivity of 2'*R*: 2'*S*=91: 9. Electrospray MS analysis of ribonuclease T1 digested fragments of the 2'α-hydroxylation product **4** demonstrated that 2'α-hydroxylation results from the abstraction of the 2'β-H atom by the deoxyuridin-5-yl in Z-form DNA. © 1999 Elsevier Science Ltd. All rights reserved.

DNA local conformations such as A form, Z form, triplex and DNA-RNA hybrid have been suggested to play an important role in biological processes.[1] However, the biological relevance of DNA local conformations is still not well understood due to the lack of a reliable detection method which is applicable to a living cell. Since the DNA local structures are assumed to appear in a very short period of time, utilization of a photoreaction as a DNA conformational probe would provide important structural information on a short-lived DNA local structure. Recently, we found that the 2'-deoxyuridin-5-yl generated in the fixed geometry of a DNA duplex undergoes H abstraction in a highly DNA conformation-dependent manner. For example, the competitive 1'- and 2'α-H abstractions occurred in the B-form DNA,[2] whereas the predominant 1'-H abstraction occurred in a DNA-RNA hybrid.[3] More recently, we found that preferential 2'α-hydroxylation occurred efficiently in Z-form DNA.[4] Inspection of the X-ray structure of the purine-pyrimidine step in Z-DNA indicated that the 2'β H of purine nucleoside is close to the C5 position of the pyrimidine nucleoside, suggesting a selective abstraction of 2'β-H by the deoxyuridin-5-yl (Figure 1).[5] In this study, we examined the photoreaction of d(CGCG(2'β-D)^{I}UGCG) **2-d**/ d(Cm^{8}GCACm^{8}GCG) **3** in which 2'β-H of the G$_4$ residue was stereoselectively deuterated, and demonstrated that the 2'α-hydroxylation results from the

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abstraction of the 2'β-H by the deoxyuridin-5-yl.

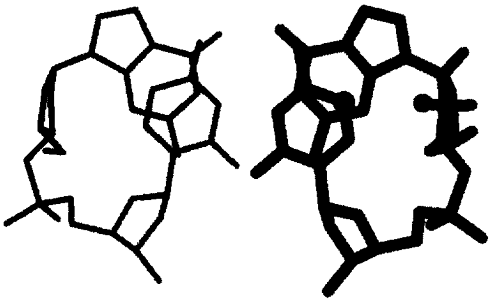
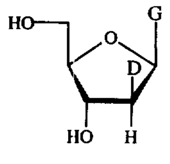


Figure 1. Purine-pyrimidine step of Z-DNA. C2'β hydrogen and C5 carbon are represented by balls. The complementary strand is shown as a thin line.



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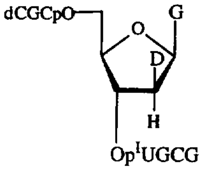
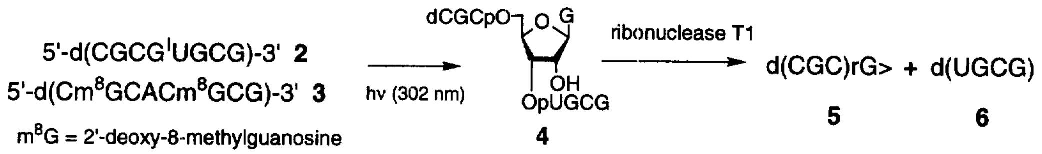


d(CGCG$_{(2'\beta\text{-}D)}$^{I}UGCG)

2-d

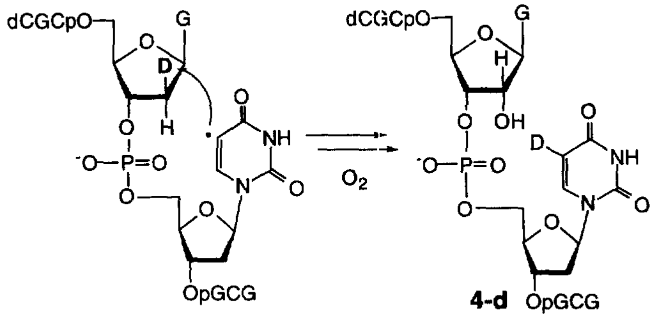
The stereoselectively deuterated nucleoside, (2'*S*)-[2'-^{2}H]-2'-deoxyguanosine **1** with the diastereoselectivity of 2'*R*: 2'*S*= 91: 9, was synthesized according to the previous method[6] and incorporated into the oligonucleotide via the standard phosphoramidite method. The octanucleotide d(Cm^{8}GCACm^{8}GCG) **3** as a complement for d(CGCG^{I}UGCG) **2** and d(CGCG(2'β-D)^{I}UGCG) **2-d** was prepared as previously reported.[4,7] The structure of the octamers was confirmed by enzymatic digestion and by electrospray MS (ESMS). The 5-iodouracil-containing Z-form duplex **2-d/3** was irradiated at 302 nm with a transilluminator. The 2'α-hydroxylated product **4** produced was isolated and treated with ribonuclease T1. As previously reported,[4] the 2'α-hydroxylated product **4** was quantitatively hydrolyzed to d(CGC)rG> (> : cyclic phosphate) **5** and d(UGCG) **6** (Scheme 1).

Scheme 1



In order to identify the position of deuterium (D) after the 2'α-hydroxylation reaction, the hydrolyzed fragments **5** and **6** were subjected to ESMS. Figure 2 shows the ESMS of **5** and **6** obtained from the photoreaction of **2-d/3** and the unlabeled octamers (control), indicating that 2'β-D is specifically abstracted and incorporated into **6**. These results clearly indicate that 2'β-H of G_4 was abstracted by the 5'-adjacent deoxyuridin-5-yl radical as an initiation step for the formation of the 2'α-hydroxylated product **4** (Scheme 2). The intramolecular kinetic isotope effects (KIE) [(k_H/k_D)=(%**4**/%**4-d**)] obtained for the 2'α-hydroxylation by photoirradiation of the ^{I}U-containing Z-form oligonucleotide was found to be 1.2±0.1. The magnitude of KIE obtained in the present study was in the range of 1.0 to 5.8 which were previously reported for 1', 4', and 5' H abstraction by several antitumor antibiotics.[8] However, the KIE value of 1.2±0.1 is substantially lower than the value 7.2 which was observed for the 2'α-H abstraction in B-form DNA.[2c]

Scheme 2



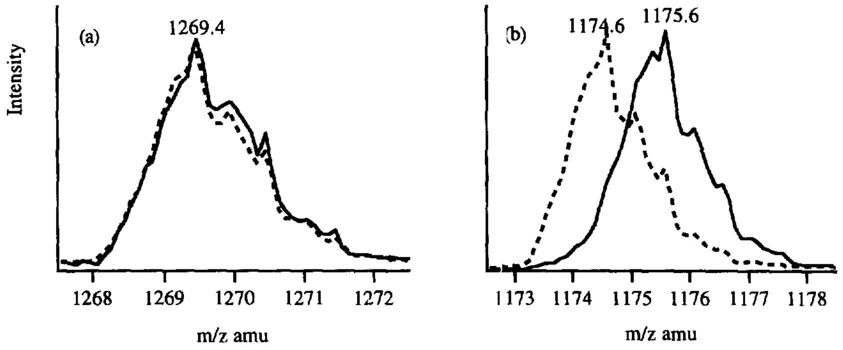


Figure 2. ESMS of (a) d(CGC)rG> **5** and (b) d(UGCG) **6**. MS of the products obtained from the deuterated and unlabeled octamers are indicated by the solid and dashed lines, respectively.

We previously developed a simple method to evaluate the conformational energy needed to achieve the transition state for H abstraction in DNA.[3] The calculation provided a qualitative explanation for the competitive 1'- and 2'α-H abstractions and the selective 1'-H abstraction by the deoxyuridin-5-yl in DNA duplex and DNA-RNA hybrid, respectively. Based on this method, the transition states for the 1'-, 2'α- and 2'β-H abstractions in Z-form DNA were calculated and are summarized in Table 1. These results suggest that the AMBER energy of the 2'β H-abstraction in Z-form DNA is significantly low compared to the other transition states. The smaller KIE observed in Z-DNA would be a consequence of the lower energy for 2'β-H abstraction in Z-form DNA. In summary, using stereoselectively deuterated octanucleotide, we demonstrated that the C2' α-hydroxylation was a consequence of the reaction of O_2 with the C2' radical which was resulted from 2'β-H abstraction by the deoxyuridin-5-yl formed in Z-form DNA.

Table 1. Minimized AMBER Total Energies (kcal/mol) of Octamer Containing Transition States for Deoxyribose Hydrogen Abstraction by Deoxyuridin-5-yl[a]

	Transition state			
Conformation	C1'	C2'α	C2'β	Unmodified
Z-form	-91.1 (14.6)[b]	-90.7 (15.0)[b]	-95.2 (10.5)[b]	-105.7

[a] Starting structures were generated from standard Z DNA in a builder module of Insight II (MSI, San Diego, CA). The models were energy minimized by conjugate gradient (0.001 kcal/molÅ). To prevent unusual distortion of the helix, the terminal residues were tethered to the initial structures (100 kcal/molÅ). Total energies of the molecules were evaluated using AMBER force field and distance-dependent dielectric of 4r. [b] The value in parentheses is the difference in energy from the corresponding unmodified octamers d(CGCGUGCG)/d(CGCACGCG).

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