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Photoreactivity of 5-Iodouracil-Containing DNA-Sso7d Complex in Solution: The Protein-Induced DNA Kink Causes Intrastrand Hydrogen Abstraction from the 5′ Side of Thymine at the 5′ Side

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Resolution of the crystal structures of numerous DNA−protein complexes over the past decade has facilitated tremendous progress in our understanding of DNA−protein interactions.[1] In some cases, the binding of a protein induces significant DNA conformational changes which are thought to play important biological roles.[2] To address the question of how accurately a crystal structure reflects the structure of the complex, either in solution or in the living cell, a variety of methods have been employed, including footprinting, cross-linking, electrophoretic mobility assays, and mutational analysis.[1] These methods provide important structural information on the complexes for which the crystal structures are not yet available.

For several years, we have been studying the photoreaction of 5-halouracil-containing DNA duplexes[3−5] and have demonstrated that hydrogen (H) abstraction of the deoxyribose moiety from the 5′ side by deoxyuridin-5-yl is largely conformation-dependent. Competitive C1′ and C2′β H abstractions are observed in B-DNA,[3] whereas predominant C1′ H abstraction occurs in DNA−RNA hybrids.[4] Moreover, stereospecific C2′α-hydroxylation occurs efficiently in Z-DNA.[5] Here, we examine the photoreaction of the complex of 5-halouracil-containing DNA with Sso7d protein, which is known to cause a significant kink in DNA in the crystal structure.[6]

Sso7d is a small chromosomal protein from the hyperthermophilic archaeabacterium *Sulfolobus solfataricus*, with high thermal, acid, and chemical stability. The crystal structure of the complex of Sso7d and d(GTAATTAC)$_2$ has been clarified at high resolution.[6] The protein binds in the minor groove, causing a sharp kink (60°) at the TpT step compared with B-form DNA. This kink results from the intercalation of the hydrophobic side chains of Val26 and Met29.

The octanucleotide d(GTAAT$^{\rm I}$UAC)$_2$, which binds Sso7d in a similar manner and appears as a typical B-form DNA in solution, was used for the present study.[7] In accordance with previous observations in B-form DNA, photoirradiation of d(GTAAT$^{\rm I}$UAC)$_2$ produced C1′ and C2′ oxidation products (1′-ox (19%) and 2′-ox (2.9%)) in the absence of Sso7d.[3,4,8] In the presence of Sso7d, the formation of 1′-ox was suppressed, whereas the formation of 2′-ox was enhanced.[9] In addition to these products, the formation of four new products (**1**−**4**) was observed (Figure 1). Under O_2 limiting conditions, reduction of the formations of 2′-ox, **2**, and **3** were observed.

The enzymatic digestion of product **1** revealed that one T of d(GTAATUAC) was oxidized to hydroxymethyluracil. Electrospray mass spectra (ESMS, 2410.2) confirmed that product **1** is a hydroxymethyluracil-containing octamer.[10] Similar enzymatic digestion of product **2** (ESMS, 2408.2) indicated that it contains formyluracil.[11] The fact that $NaBH_4$ reduction of product **2** quantitatively converted it to product **1** confirmed that product **2** is a formyluracil-containing octamer as described previously.[11] These products resulted from the abstraction of H from T5-Me by the adjacent deoxyuridin-5-yl radical. To the best of our knowledge, this is the first example of intrastrand H abstraction from T5-Me by a deoxyuridin-5-yl radical.[12] Product **3** was a diastereomeric mixture of 2′-hydroxylated products, which resulted from abstraction of the C2′ H from the T_5 residue (Figure 1S, Supporting Information). Product **4** was d(GTAATUAC), which suggested that considerable H abstraction occurs from Sso7d. The yields of the products are summarized in Scheme 1.[8] An inspection of the X-ray structure indicates that the T5-Me and T_5−C2′βH are in close proximity to the deoxyuridin-5-yl radical, whereas the T_5-C1′H and T_5-C2′αH are far from the adjacent deoxyuridin-5-yl radical. We conclude that the unusual intrastrand H abstraction from T5-Me by the deoxyuridin-5-yl radical occurred efficiently at the observed bending site in the crystal structure.[6]

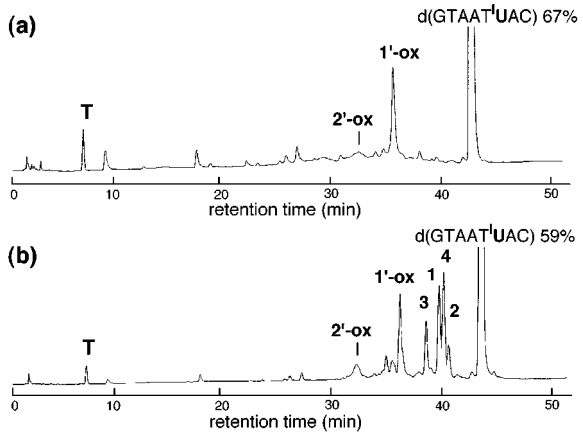

Figure 1. HPLC profiles of UV (302 nm) irradiated (a) d(GTAAT$^{\rm I}$UAC)$_2$ and (b) d(GTAAT$^{\rm I}$UAC)$_2$−Sso7d. The reaction mixture (total volume, 100 μL) contained d(GTAAT$^{\rm I}$UAC) (84 μM strand concentration), Sso7d (42 μM), and 50 mM sodium cacodylate (pH 7.0). Irradiation was performed with a transilluminator (302 nm) for 15 min at 0 °C. The reaction mixtures (20 μL) were analyzed by HPLC on a Chemcobond 5-ODS-H column (4.6 × 150 mm) and detected at 254 nm; elution was performed with 0.05 M ammonium formate (pH 6.5) containing 0−10% acetonitrile over a linear gradient for 50 min at a flow rate of 1.0 mL/min, at 40 °C. Percent yield indicates the unreacted d(GTAAT$^{\rm I}$UAC).

HPLC analysis of the photoirradiated d(GTAAT$^{\rm I}$UAC)$_2$−Sso7d complex indicated that the oxidation product of Sso7d (Sso7d$^{\rm OH}$) (ESMS, 7161) was produced as a major product from Sso7d (ESMS, 7145) as shown in Figure 5S. This product occurred during photoirradiation only in the presence of d(GTAAT$^{\rm I}$UAC)$_2$. In the absence of d(GTAAT$^{\rm I}$UAC)$_2$ or in the presence of d(GTAATTAC)$_2$, photoirradiation of Sso7d did not produce Sso7d$^{\rm OH}$. These results indicate that the deoxyuridin-5-yl radical in the complex efficiently

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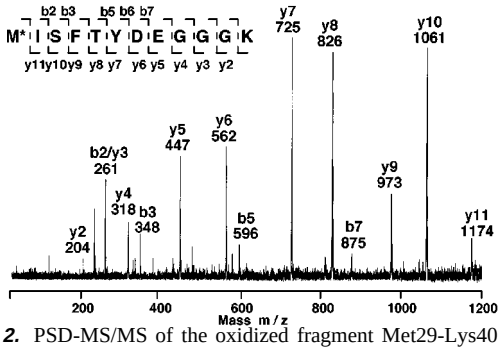


Figure 2. PSD-MS/MS of the oxidized fragment Met29-Lys40 isolated by HPLC. Oxidized fragment 29−40 produced peak at 261 (b2 fragment) instead of 245, whereas a peak at 1174 (y11 fragment) stayed constant in both the oxidized and unmodified 29−40 fragments.

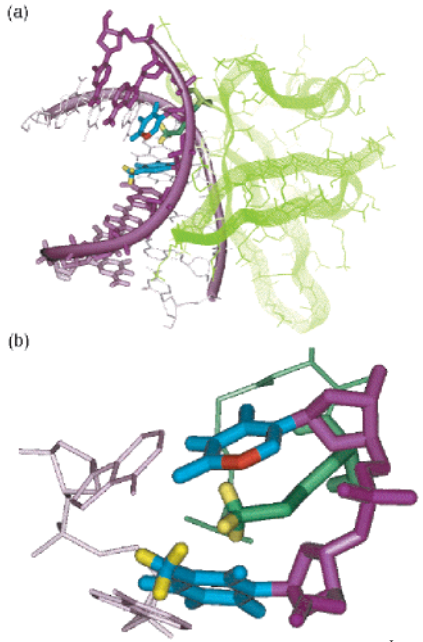
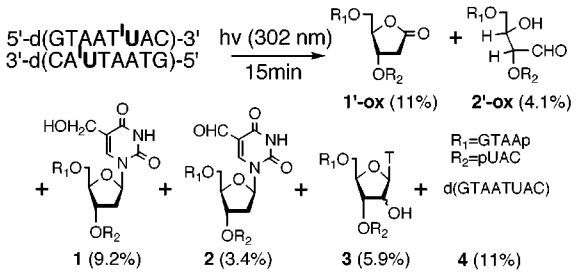


Figure 3. Structure of photoirradiated-Sso7d−d(GTAAT^{I}UAC)$_2$ complex based on the X-ray crystal structure (a) and a close-up view of the reacting region (b). In part b, U_6 and T_5 of d(GTAATUAC)$_2$ are drawn in blue, C5 of U_6 is in red, and putative abstracted Hs by deoxyuridin-5-yl radical is in yellow. In part a, the Sso7d ribbon presentation is drawn in green and Val26 and Met29 are in green.

Scheme 1



oxidizes Sso7d to produce Sso7d^{OH}. HPLC profile of lysyl endopeptidase-treated Sso7d^{OH} and intact Sso7d showed that the oxidized peptide fragment from residues 29 to 40 was formed as a major product (78%) as shown in Figure 2S.

To elucidate the site of the oxidized amino acid residue in fragment 29−40, PSD-MS/MS was performed (Figure 2). The peak at 261 (b2 fragment) was found in oxidized fragment 29−40 instead of at 245 in the unmodified fragment, whereas the peak at 1174 (y11 fragment) stayed constant in both cases. These results indicate that specific photooxidation at Met29 occurred during photoirradiation of the d(GTAAT^{I}UAC)$_2$−Sso7d complex. Further elucidation of the structure of oxidized Met29 was unsuccessful due to the limited amount of the oxidation fragment. Inspection of the X-ray structure suggests that residue Met29 is in close proximity to the deoxyuridin-5-yl radical, which is intercalated from the major groove at the bending site in the crystal structure.

In the present study, we have demonstrated an unprecedented intrastrand H abstraction at the methyl group of T_5, generating products **1** and **2**, together with selective photooxidation at Met29. The reactivity of the deoxyuridin-5-yl radical can be well explained by the crystal structure of the d(GTAATTAC)$_2$−Sso7d complex (Figure 3). These results suggest that the interaction of DNA−Sso7d in solution is substantially similar to its crystal structure. Although several novel DNA structures have been resolved by X-ray crystallography and nuclear magnetic resonance (NMR), the precise biological functions of these structures are not fully understood. This is presumably because of the lack of an appropriate in vivo detection method. Since 5-halouracil-substituted DNA is known to be functional in a living cell system such as in *E. coli*, the photochemical reactions of 5-halouracil-containing DNA provide a powerful tool that can directly probe local DNA conformations and DNA−protein interactions not only in vitro but also in vivo.[13]

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Supporting Information Available: HPLC profile of the enzymatic digest of the photoproduct **1**−**3** (Figure 1S), HPLC profile of UV (302 nm) irradiated d(GTAAT^{I}UAC)$_2$−Sso7d, and HPLC analysis of enzymatic digested Sso7d and Sso7d^{OH} (Figure 2S) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) We examined photoreaction of Sso7d with other ^{I}U-containing hexamers, such as d(G^{I}UAATTAC)$_2$ and d(GTAA^{I}UTAC)$_2$. Even though we observed retarded thymine dimer formation at the TT site or the ^{I}UT site, these complexes did not show any different reactivity of deoxy-uridin-5-yl compared with that in the absence of Sso7d.
(8) Yields were based on the consumed d(GTAAT^{I}UAC).
(9) Partial suppression of the formation of 1′-ox is presumably because, according to X-ray structural analysis, only one of the T^{I}U sites is involved in the sharp kink, and the other site remains in the B-form.
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