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Photochemistry of highly organized biomolecules: Sequence-selective photoreaction of DNA

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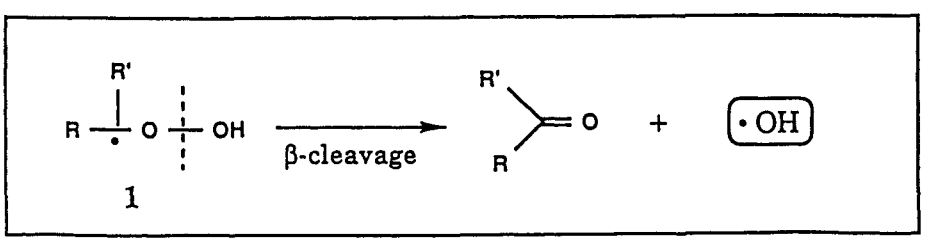
Abstract. Two types of highly sequence-selective photoreactions of DNA are described. One example includes a specific photocleavage of DNA duplex at 5' side of 5'-GG-3' sequence by a "photo-Fenton reagent", whereas the other is the sequence-selective photoreaction of 5'-A^{Br}U- site of DNA duplex which results in formation of a 2-deoxyribonolactone residue with release of free adenine via selective hydrogen abstraction from C2' of deoxyribose residue.

1. INTRODUCTION

Stereo- and sequence-specific photoreactions occur in highly organized biomolecules such as DNA duplex and are the subject of much current mechanistic and practical interest.[1] Disclosed are two types of highly sequence-selective photoreactions of DNA recently discovered in our laboratory. Our goal in this area is to devise a new, efficient photochemical method for cutting DNA at specific sites. One of our approaches is based on the design of an organic molecule that binds to specific site of DNA and generates free hydroxyl radicals (· OH) by photoirradiation with long-wavelength light.[2] Another example is a sequence-selective photoreaction of 5-halouracil-containing DNA initiated by electron transfer followed by hydrogen abstraction from C1' of DNA deoxyribose to result in a cleavage of a DNA strand.[3]

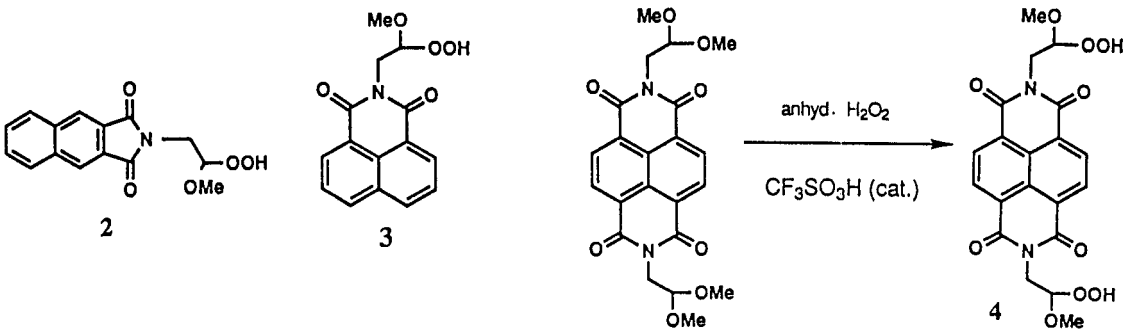
2. DESIGN OF 'PHOTO-FENTON REAGENT' AND -GG- SPECIFIC PHOTOCLEAVAGE OF DNA

In view of the high level of interest in the hydroxyl radical (· OH) in biological and other systems, approaches toward the development of efficient methods for · OH generation without using transition metal ions and hydrogen peroxide have been investigated.[4] Our objective is to design an efficient organic precursor that generates · OH by low-energy irradiation, such as long-wavelenght UV light (> 350 nm) or more preferably by visible light irradiation. Such molecules, referred to as "photo-Fenton reagents", are particularly attractive as a controllable and mechanistically less complicated · OH source for applications in a number of biologically important reactions such as cross-linking of biopolymers[5] and cleavage of DNA[6] or proteins.[7] The design criteria include the ease of synthesis, stability at ambient temperature, solubility in aqueous solvent and ability to produce · OH by irradiation with long-wavelength light. Our strategy for the generation of · OH is based on the well known photochemical g-hydrogen abstraction of phthalimide carbonyl[8] and the earlier finding that hydroperoxyalkyl radicals such as **1** undergo extremely facile b-cleavage of the labile O-O bond giving · OH.[9]



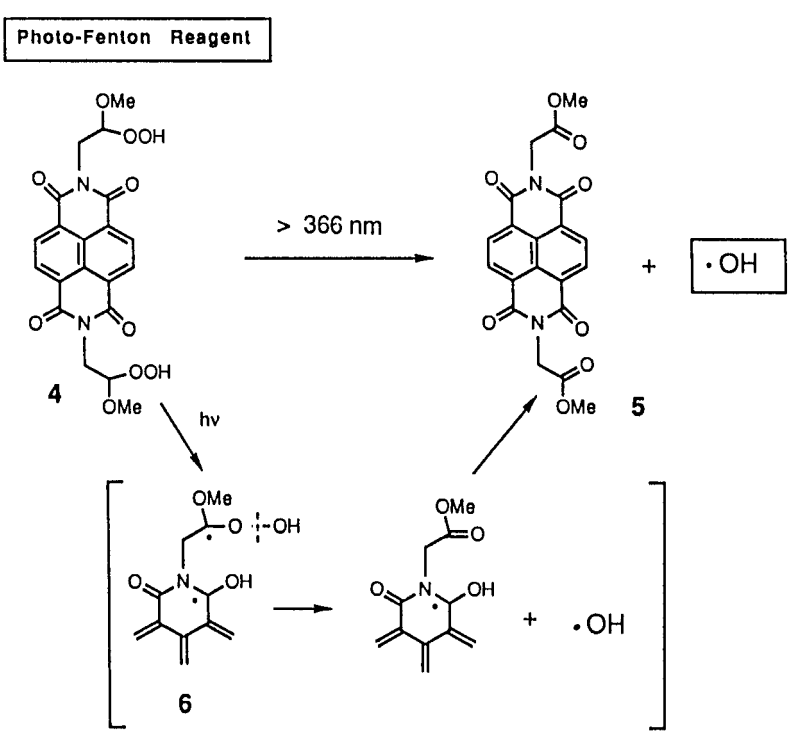
1

We have prepared the following hydroperoxides **2**, **3** and **4** from the corresponding dimethyl ketals by treatment with ethereal hydrogen peroxide in the presence of triflic acid in dichloromethane.[10] Particularly, bis(hydroperoxy)-naphthaldiimide **4** was designed in order to improve the efficiency of · OH generation per molecule and to enhance the absorption at longer wavelength.[2] The thermally stable hydroperoxide **4** was soluble in aqueous organic solvent (up to 2.5 mM in acetonitrile-water 8: 92) and has a very strong absorption at 377 nm (log ε = 4.45).



Photolysis of **4** in acetonitrile at 366 nm proceeded rapidly to give ester **5** quantitatively with a quantum yield of ϕ = 0.18 (Scheme 1).[2] In the presence of adamantane, 1-adamantanol, 2-adamantanol and adamantanone were obtained, and their formation was inhibited by addition of · OH scavenger such as dimethyl sulfoxide. Generation of · OH was also confirmed by an ESR spin trapping method using DMPO as a spin trapping reagent. Upon brief exposure of a solution of **4** and DMPO in sodium cacodylate buffer (pH 7.0) to 366 nm light, intense ESR signals characteristic of a · OH-DMPO adduct were detected.[10] Exclusive formation of ester **5** accompanied by · OH generation is consistent with a mechanism involving γ-hydrogen abstraction by the naphthaldiimide carbonyl followed by cleavage of the labile O-O bond of the resulting biradical **6**.[11]

Scheme 1



Next, we have examined the DNA-cleaving activity of **4** upon exposure to 366 nm light by using supercoiled circular ϕX174 RFI DNA (form I). Single-strand breaks and a small amount of double-strand breaks at higher conversion were observed, as evidenced by the production of form II and form III DNA, respectively, by means of gel electrophoresis.

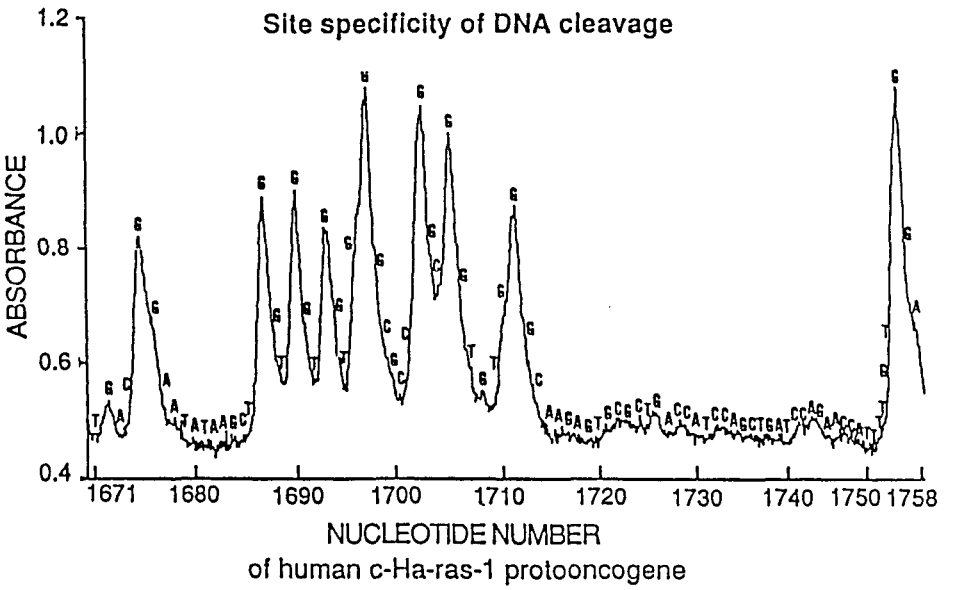


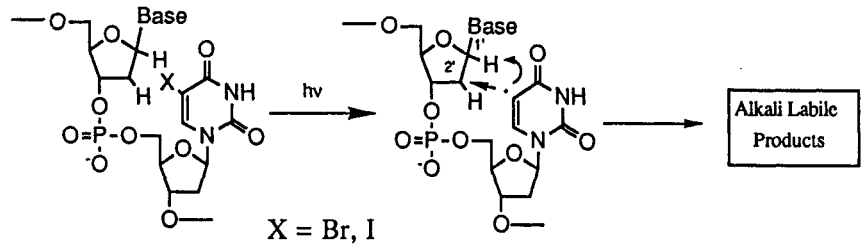
Fig. 1. Site specificity of the DNA cleavage in the photoirradiaiton of **4**. A solution containing the ^{32}P-5'-end-labeled 261-bp fragment of human c-Ha-*ras*-1 protooncogene and 10 μM of **4** in 50 mM sodium cacodylate buffer (pH 7.0) was irradiated for 20 min. After piperidine treatment (1 M, 90 °C, 20 min) the DNA fragments were separated by electrophoresis. The relative amounts of oligonucleotides produced by the photoreaction were measured by a laser densitometer. The horizontal axis shows the nucleotide number of the DNA fragment (from left to right, 5' - 3').

Addition of sodium benzoate as · OH scavenger to the reaction system inhibited the DNA cleavage. The base and sequence specificity of DNA cleavage was analyzed by using ^{32}P-end-labeled DNA fragments. As shown in Fig. 1, **4** induced DNA strand cleavage preferentially at the 5' site of 5'-GG-3' sequences after piperidine treatment. No cleavage was observed at other sites, including single G residues. This is in marked contrast to the photocleavage of DNA mediated by singlet oxygen, where the cleavage occurs equally at each G residue after piperidine treatment.[12] The DNA cleavage was enhanced approximately 10-fold upon treatment with piperidine at 90 °C, suggesting that · OH reacts preferentially with the DNA base, particularly with the guanine base rather than the sugar backbone. The cleavage of double-stranded DNA by · OH with piperidine treatment, usually occurs at every nucleotide position with some preference for G and T.[13] Therefore, we believe that the observed specific cleavage is a consequence of the selective binding of **4** to 5'-GG-3' sequence. In fact, the binding constant of **4** to calf thymus DNA as measured by the equilibrium dialysis method was 1.50 x 10^4. This method of · OH generation is very efficient and could be quite attractive for the use in applications requiring · OH in a number of other systems. Recently, **4** adsorbed on silica gel was also used for photocrosslinking of eye lens proteins.

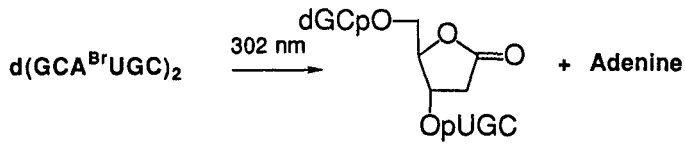
3. SEQUENCE- AND SITE-SELECTIVE PHOTOREACTION OF 5-HALOURACIL-CONTAINING DNA

For many years replacement of thymine in DNA by 5-bromouracil (BrU) has been known to enhance photosensitivity with respect to DNA-protein photocrosslinking, single strand break and creation of alkali labile site.[14] However, the detailed chemistry associated with the radical-induced degradation of BrU-incorporated DNA has not been well understood. We have examined the photoreaction of a number of BrU or 5-iodouracil (IU)-containing deoxyoligonucleotides of defined sequence in order to look into the detailed chemistry of light-induced degradation of 5-halouracil-containing DNA initiated by intramolecular hydrogen abstraction by uracilyl-5-yl radical in the same strand (Scheme 2).

Scheme 2



We found that deoxyoligonucleotides containing a 5'-$A^{Br}U$- site in the middle of the duplex structure undergo an extremely facile photoreaction to produce a deoxyribonolactone residue 7 with release of free adenine as exemplified by photolysis of self-complementary hexanucleotide $d(GCA^{Br}UGC)_2$ as illustrated below. This type of photoreaction occurs most effectively with duplex hexamers containing a 5'-$A^{Br}U$ site in the middle; no such reaction being observed with single stranded hexanucleotides such as $d(GCA^{Br}UCG)$, *i. e.*, duplex structure and the 5'-$A^{Br}U$ sequence are essential for the efficient formation of the 2-deoxyribonolactone residue and free base release.

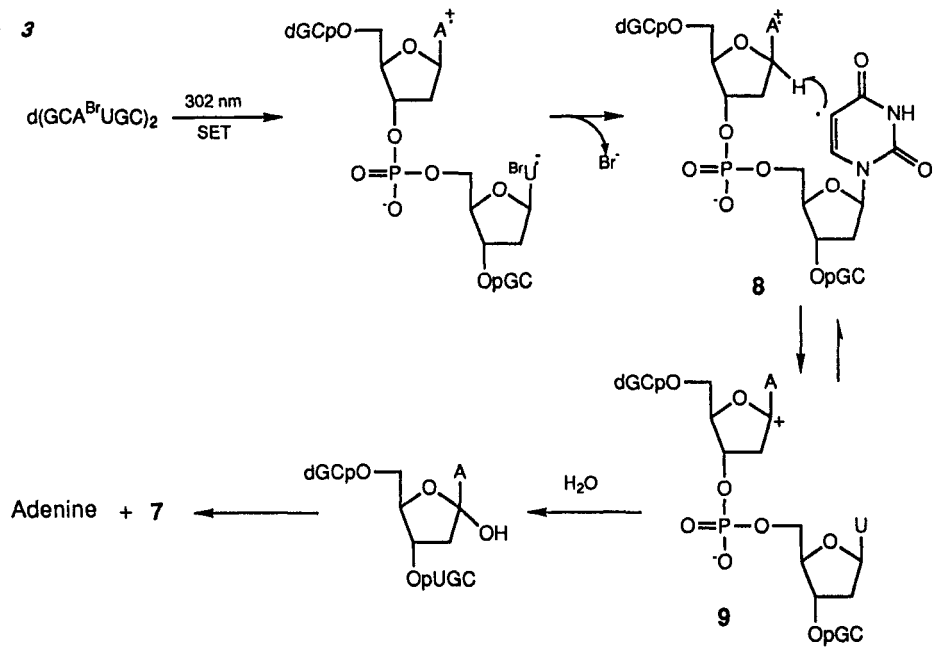


302 nm, 0°C, 30 min
oligomer 1 mM (base conc.)
NaCl 1 M
Na cacodylate (pH 7) 50 mM

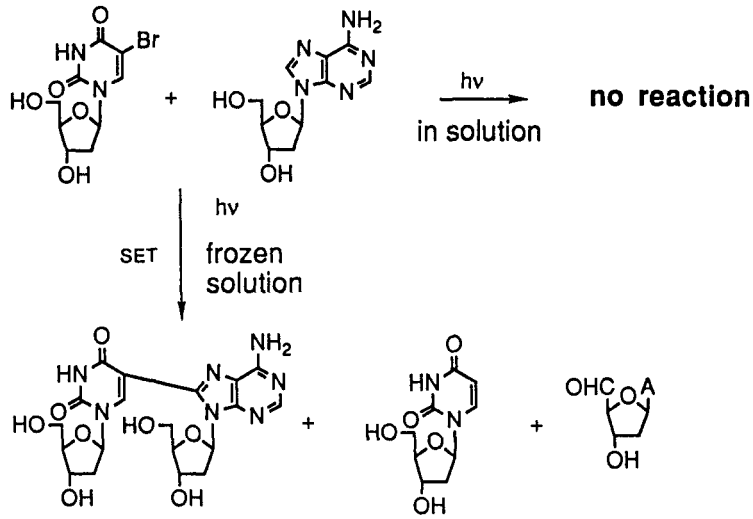
7

The quantum yield (ϕ = 1.4 x 10^{-2} at 0 °C) for the formation of 2-deoxyribonolactone-containing oligomer 7 from duplex $d(GCA^{Br}UGC)_2$ is remarkably, higher than that for the photoreduction of monomeric BrU in water containing 2-propanol (ϕ = 1.8 x 10^{-4}).[15] While the reason for the specific and higher quantum yield photoreaction of the 5'-$A^{Br}U$ sequence is unclear, an attractive mechanism appears to involve an intramolecular electron transfer from an adenine chromophore to an adjacent BrU in a specially oriented complex in the highly organized duplex (Scheme 3). The resulting BrU anion radical would

Scheme 3



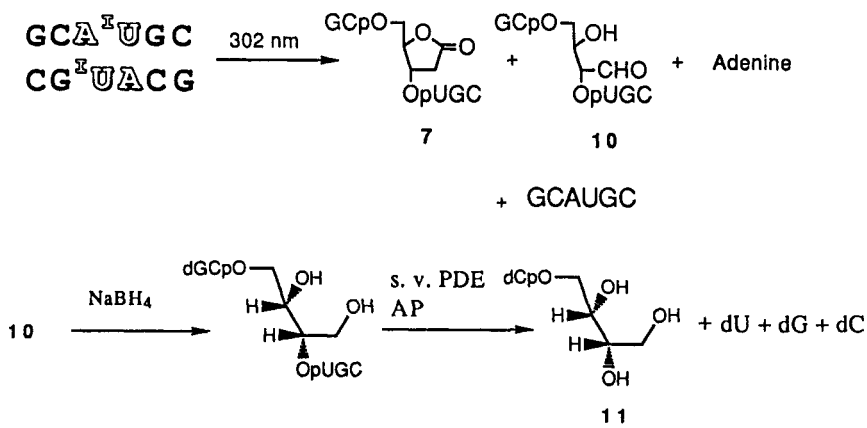
Scheme 4



release Br anion to produce uracilyl-5-yl radical **8**, which can immediately abstract neighboring C1' hydrogen of the adenosine radical cation to give cationic species **9**. Hydrolytic cleavage of the N-glycosidic bond would provide **7** with release of free adenine.

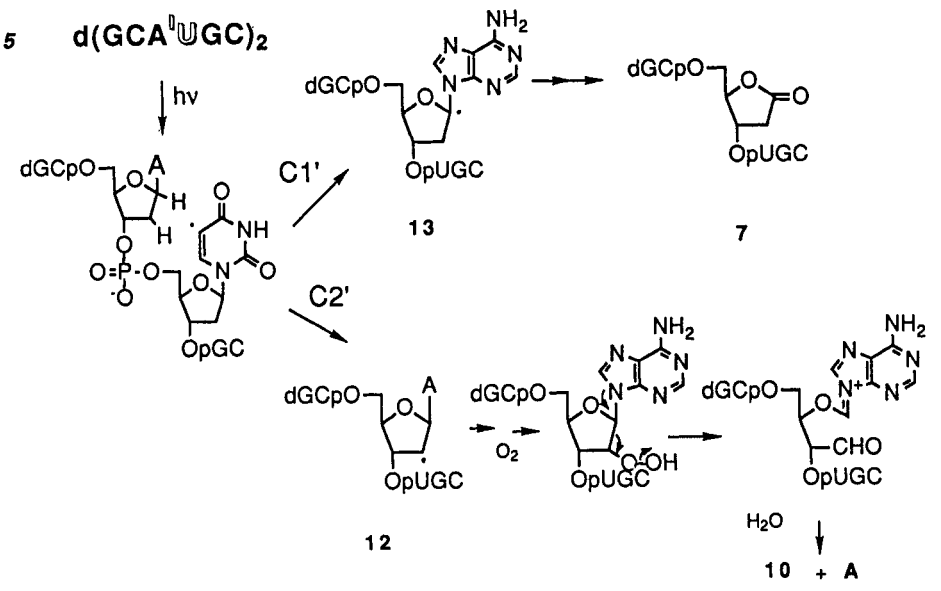
In support of this hypothesis for the existence of such a specially oriented complex between adenosine and BrU, we found that irradiation of a frozen aqueous solution containing equimolar amounts of both 5-bromouridine and deoxyadenosine results in an efficient formation of the photoproducts shown in Scheme 4, in marked contrast to the fact that no photoreaction occurs at all in a solution phase under similar conditions.

In contrast, photoreaction of 5-iodouracil (IU)-containing hexanucleotides proceeded less selectively to produce a different type of photoproduct together with those resulting from deoxyribose C1' hydrogen abstraction. For example, irradiation of self-complementary duplex $d(GCA^{I}UGC)_2$ provided 2-deoxyribonolactone-containing oligomer **7**, dehalogenated product d(GCAUGC) and a new product **10** resulting from C2' hydrogen abstraction, together with release of free adenine as illustrated below. The structure of **10** was confirmed by following chemical degradation followed by independent synthesis of the resulting **11**.



In order to look into the general picture for the photoreaction of IU-containing DNA, photoreaction of various types of IU-containing hexanucleotides was examined. In many cases examined, hydrogen abstraction by uracilyl-5-yl radical occurs competitively at C1' and C2' positions of adjacent deoxyribose moiety. Such a competitive hydrogen abstraction from C1' and C2' also occurs even in a single-stranded IU-containing hexanucleotide. This is in marked contrast to the case of BrU-containing hexanucleotides. These results suggest that photoreaction of IU-containing DNA proceeds via a different mechanism from that observed for BrU-containing DNA. While there are ample examples for electron-transfer type photochemistry of BrU,[16] photoreaction of IU is generally believed to proceed via a homolytic cleavage of C-I bond. Thus, the resulting uracilyl-5-yl radical abstracts C2' and

Scheme 5



C1' hydrogens of the deoxyribose moiety at 5' side to produce **12** and **13**, respectively. In fact, inspection of the B DNA model indicates that both C2' and C1' hydrogens are very close to C5 of the IU residue. One of the possible mechanisms for the formation of **10** is shown in Scheme 5.

These observations are the first demonstration for the direct observation of C2' oxidation product from oligonucleotides of defined sequence. The chemistry observed here in the photoreaction of 5-iodouracil-containing hexanucleotides is very important for understanding the molecular mechanism of DNA degradation resulting from deoxyribose C2' hydrogen abstraction induced by DNA-damaging agents such as natural antitumor antibiotics and footprinting agents.

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