ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 106 (1997) 141–144

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY A: CHEMISTRY

Design of photochemical DNA-cleaving molecules via electron transfer

Isao Saito *, Masami Takayama, Hiroshi Sugiyama, Takashi Nakamura

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto, 606-01, Japan

Abstract

Several examples of photochemical DNA-cleaving molecules have been described. Photocleaving amino acid (PCA **1**) has been shown to cut DNA via an electron transfer mechanism. The most readily oxidizable sites in the photochemical one-electron oxidation of duplex DNA are shown to be 5′-guanine of –GG– steps. An important and general rule, referred to as the 'guanine–guanine stacking rule', has been proposed for one-electron transfer from B form DNA. This rule can predict the most electron-donating sites in B form DNA and is important for understanding the HOMO–LUMO interaction of B DNA with electron-accepting molecules. © 1997 Elsevier Science S.A.

Keywords: Electron transfer from DNA; Photochemical DNA cleaver

1. Introduction

There has been much current interest in the design of artificial DNA-cleaving molecules which are chemically stable and activatable by photoirradiation, since such photoactivatable DNA-cleaving molecules can be used to probe the nucleic structure, as designed 'photonucleases', as photofootprinting agents, and as a potent photodrug for photochemotherapy. Most non-photochemical DNA-cleaving molecules, including natural antitumor antibiotics, may often require a co-reagent, a condition hardly compatible with in vivo applications, whereas an advantage might be found in photochemical DNA-cleaving molecules which are non-toxic in the dark, but activatable by a pulse of laser light. Within the last decade our laboratory has focused on exploitation of new methodologies for selective DNA cleavage by photoirradiation [1]. Minimum requirements for designing practically useful photochemical DNA cleavers are (i) high absorptivity in the UVA region (320–400 nm) and (ii) efficient generation of reactive species capable of reacting with nucleobases or DNA sugar backbone. We have demonstrated several different types of selective and non-selective photochemical DNA cleavers as exemplified in Fig. 1 [2–5].

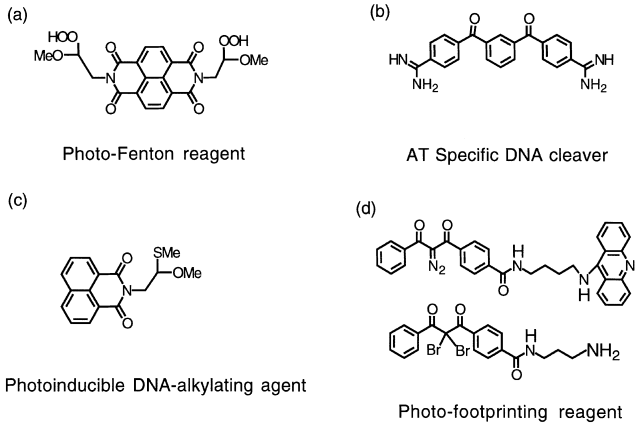


Fig. 1. Photochemical DNA-cleaving agents: (a) photo-Fenton reagent, [2], (b) adenine–thymine (AT) sequence specific DNA cleaver [3], (c) photoinducible DNA-alkylating agent [4], and (d) photo-footprinting agents [5].

2. Results and discussion

We are particularly interested in the design of photocleaving amino acids (PCA) which can be used as a DNA-cleaving unit of DNA-cleaving peptides or hybrid molecules and have prepared PCA **1** and **2**. PCA **1** induced specific DNA cleavage at the 5′-guanine (G) of 5′-GG-3′ steps of duplex DNA by piperidine treatment of the photoirradiated mixture, whereas nitro derivative **2** cleaved the double-stranded DNA preferentially at thymine (T) residues after piperidine treatment [6]. Thus, the sequence selectivity of the DNA cleavage by PCA is highly dependent on the substituent on the aromatic ring. It was also revealed that the first step of T-specific cleavage by **2** is the oxidation of the T methyl group to formyl group initiated by H abstraction by triplet nitro group. Heating 5-formyluracil sites in DNA with piperidine resulted in a

* Corresponding author.

PII S1010-6030(97)00051-8

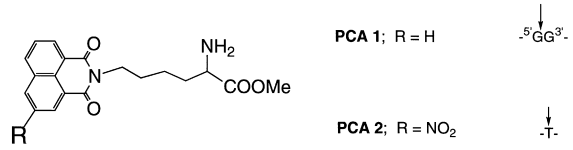
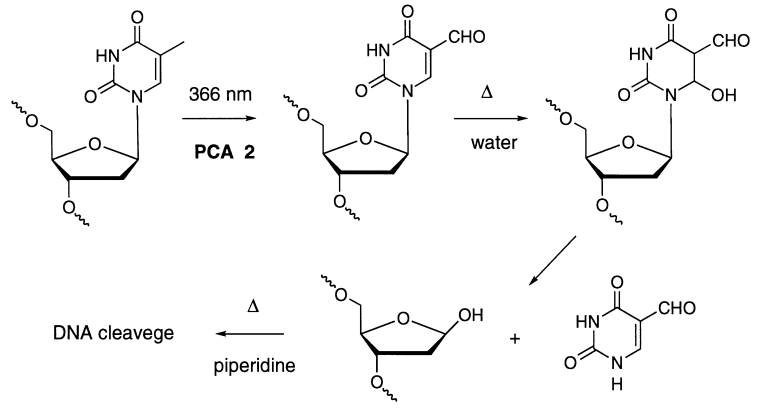


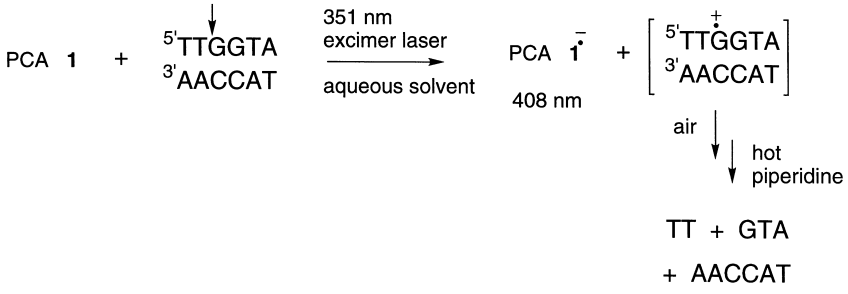
Fig. 2. Photochemical DNA-cleaving amino acids (photocleaving amino acids, PCA).

strand cleavage via the mechanism shown below (Scheme 1, Fig. 2).

The GG specific cleavage by PCA **1** was assumed to proceed via one-electron transfer from electron-rich –GG– steps to photoexcited **1**. Actually, we recently obtained the first direct evidence for one-electron transfer from the –GG– site of duplex hexanucleotide TTGGTA/TACCAA to triplet **1** by detecting $\mathbf{1}^{\bullet-}$ by means of excimer laser flash photolysis (Scheme 2) [7]. Irradiation of **1** with duplex hexamer in sodium cacodylate buffer at 366 nm followed by piperidine treatment and enzymatic dephosphorylation produced TT and GTA as major products, while TACCAA remained unchanged. It should be noted here that photoirradiation of **1** with TTGGTA alone resulted in a non-selective cleavage at G_3 and G_4 in a ratio of 1:1. Such GG-selective photodamage was already observed with other photosensitizers such as ribofla-



Scheme 1.



Scheme 2.

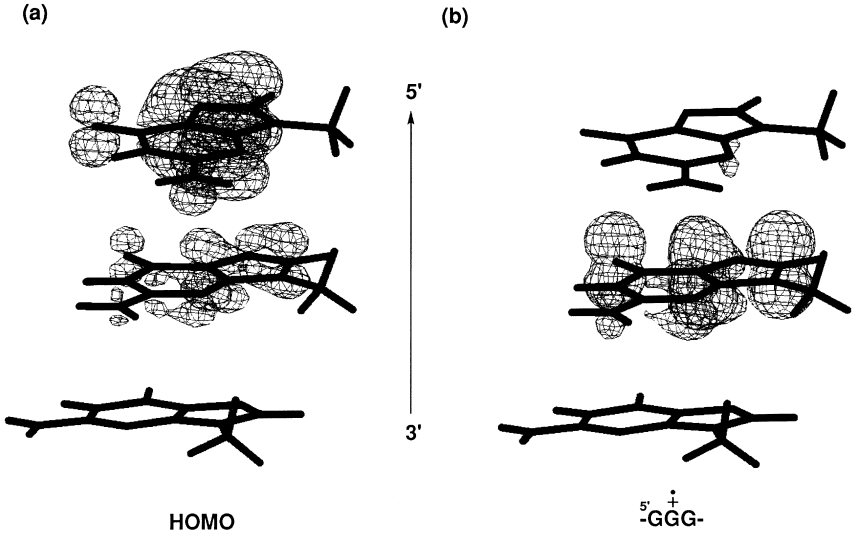


Fig. 3. (a) The HOMO and (b) the most stable cation radical of 5′-GGG-3′ calculated by ab initio 6-31G*.

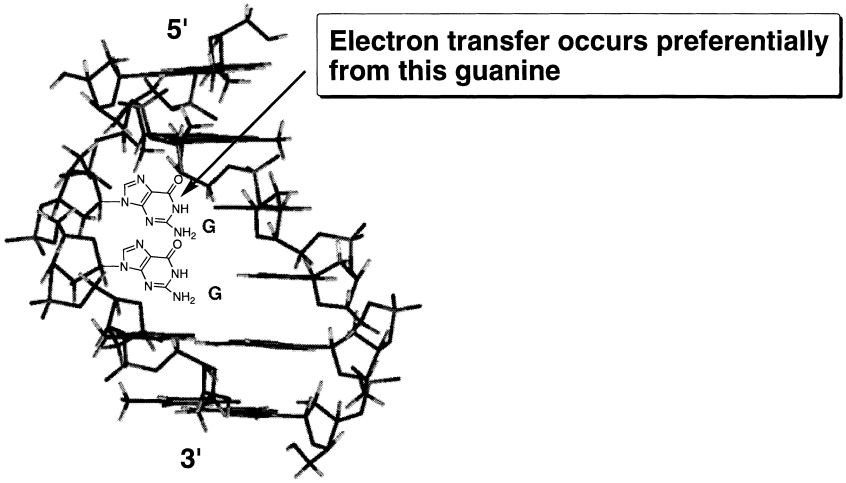


Fig. 4. Schematic drawing of stacked 5′-GG-3′ site in B form DNA. Electron transfer occurs preferentially at 5′-side G.

vin [8], naphthaldiimides [2] and anthraquinone derivatives [9].

There has long been great interest in one-electron oxidations of DNA in connection with DNA damage caused by ionizing radiation, oxidizing agents and photo-oxidation with endogenous photosensitizers. Since guanine is known as the most readily oxidizable base among DNA nucleobases, it has been suggested that the electron loss center created in DNA ultimately ends up at guanine residues via hole migration through the duplex. However, our results indicated that the most readily oxidizable sites in B form DNA toward one-electron oxidation are 5′-G of 5′-GG-3′ steps due the π-stacking interaction of the two guanine bases. This implies that 5′-G of 5′-GG-3′ is a sink in hole migration through DNA, i.e. an electron-loss center created in B form DNA would end up predominantly on 5′-G of GG steps [7].

We examined the photoinduced DNA cleavage of ^{32}P-end labeled DNA fragments in the presence of different electron-accepting sensitizers. Careful examination of the DNA cleavage data revealed a following general trend for the susceptibility of G-containing sites to one-electron oxidation, regardless of photosensitizers used. (i) The DNA cleavage occurred most efficiently at 5′-G of 5′-GG-3′ steps; (ii) DNA cleavage occurred more intensively at the following sequences, –GGGG–>–GGG–>–GG–≫–AG–∼–GA–>–G–. We have carried out ab initio calculations of the lowest ionization potential (IP) of stacked nucleobase models in a B form DNA geometry at 6-31G* level (Fig. 3) [7,10]. The calculated IPs are in the following order: –GGGG–(6.98 eV) <–GGG–(7.07 eV) <–GG–(7.28 eV) <–GA–(7.51 eV) ∼–AG–(7.51 eV) ≪–G–(unstacked, 7.75 eV). This order is in good agreement with the experimental data. Of special importance is that the HOMO of stacked –GG– or –GGG– is always localized predominantly on the 5′-side G, which is compatible with the observed 5′-G selectivity for DNA cleavage (Fig. 1). The susceptibility of each G of 5′-$G_1G_2G_3$-3′ sequence to one-electron oxidation increased in the order $G_2 > G_1 \gg G_3$ with a G_2:G_1 ratio of 58:42. This is not in complete agreement with the calculated HOMO. However, the ab initio calculations indicated that the most stable cation radical is $–G_1–G_2^+ \cdot –G_3–$ owing to the stacking stabilization by the two G bases.

On the basis of experimental data and ab initio calculations [10], we propose a very important and general rule for predicting most electron-donating sites, i.e. the most readily oxidizable sites in B form DNA [3,11]. This rule, referred to as the ‘guanine–guanine (G–G) stacking rule’, shows the DNA sites which are most susceptible to one-electron oxidations such as those in ionizing radiation or numerous chemical and photochemical oxidations.

Guanine-Guanine Stacking Rule

1. Electron-donating ability of G-containing sequences increases in the following order.

5'-GGGG- > 5'-GGG- > 5'-GG- >> 5'-AG- ~ 5'-GA- > -G-

2. The largest HOMO of $(G)_n$ sequences is always localized on the G at 5' side.

5'↓↓↓-GGGG- 5'↓↓-GGG- 5'↓-GG-

3. Electron-transfer from the G at 3' side of $(G)_n$ sequences is least likely to occur.

4. The most readily oxidizable sites of $(G)_n$ sequences are the G being stacked with two G from both sides

5'↓↓↓↓-GGGG- 5'↓↓↓-GGG- 5'↓↓-GG-

Recent results of GG-selective DNA damage [12] induced by direct photoionization using powerful 193 nm excimer laser can also be explained by this GG-stacking rule. Furthermore, this rule is very important in understanding HOMO–LUMO interaction of B form DNA. In such a HOMO–LUMO or a charge-transfer interaction with electron-accepting molecules, the 5′ side G of contiguous ($–G_n–$) is the most electron-releasing and therefore most strongly interacting site (Fig. 4) [13].

Acknowledgements

This work was supported by a Grant-in-Aid for Priority Research from Ministry of Education and in part by CREST of Japan Science and Technology Corporation (JST).

References

[1] I. Saito, *Pure Appl. Chem*., *64* (1992) 1305; I. Saito and K. Nakatani, *Bull. Chem. Soc. Jpn*., *69* (1996) 3007.
[2] I. Saito, M. Takayama, T. Matsuura, S. Matsugo and S. Kawanishi, *J. Am. Chem. Soc*., *112* (1990) 883; S. Matsugo, S. Kawanishi, K. Yamamoto, H. Sugiyama and I. Saito, *Angew. Chem. Int. Ed. Engl*., *30* (1991) 1351.
[3] I. Saito, M. Takayama, T. Nakamura, H. Sugiyama, Y. Komeda and M. Iwasaki, *Nucleic Acids Res. Symp. Ser*., *34* (1995) 191.
[4] I. Saito, M. Takayama and T. Sakurai, *J. Am. Chem. Soc*., *116* (1994) 2653.
[5] I. Saito, T. Sakurai, T. Kurimoto and M. Takayama, *Tetrahedron Lett*., *353* (1994) 4797.
[6] I. Saito, M. Takayama and S. Kawanishi, *J. Am. Chem. Soc*., *117* (1995) 5590.
[7] I. Saito, M. Takayama, H. Sugiyama, K. Nakatani, A. Tsuchida and M. Yamamoto, *J. Am. Chem. Soc*., *117* (1995) 6406.
[8] K. Ito, S. Inoue, K. Yamamoto and S. Kawanishi, *J. Biol. Chem*., *268* (1993) 13221.
[9] D. Breslia and G.B. Schuster, *J. Am. Chem. Soc*., *118* (1996) 2313.
[10] H. Sugiyama and I. Saito, *J. Am. Chem. Soc*., *118* (1996) 7063.
[11] I. Saito, M. Takayama, H. Sugiyama and T. Nakamura, in B. Munier (ed.), *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*, Kluwer, 1996, pp. 163–176.
[12] T. Melvin, M.A. Plumb, S.W. Botchway, P. O'Neill and A.W. Parker, *Photochem. Photobiol*., *61* (1995) 584.
[13] After submitting this manuscript, the following papers have been published for oxidation of guanine in DNA by electron transfer. D.B. Hall, R.E. Holmlin and J.K. Barton, Nature, 382 (1996) 731. E.D.A. Stemp, M.R. Arkin and J.K. Barton, J. Am. Chem. Soc., 119 (1997) 2921.