Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Photochemistry and Photobiology B: Biology 82 (2006) 37–44
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

Guanine-specific DNA damage photosensitized by the dihydroxo(tetraphenylporphyrinato)antimony(V) complex

Kazutaka Hirakawa [a,*], Shosuke Kawanishi [b], Jin Matsumoto [c], Tsutomu Shiragami [c], Masahide Yasuda [c]

[a] Division of Applied Science and Basic Engineering, Faculty of Engineering, Shizuoka University, Johoku 3-5-1, Hamamatsu, Shizuoka 432-8561, Japan
[b] Department of Environmental and Molecular Medicine, Mie University School of Medicine, Edobashi 2-174, Tsu, Mie 514-8507, Japan
[c] Department of Applied Chemistry, Faculty of Engineering, Miyazaki University, Gakuen-Kibanadai, Miyazaki 889-2192, Japan

Received 4 March 2005; received in revised form 1 August 2005; accepted 8 August 2005
Available online 17 October 2005

Abstract

The dihydroxo(tetraphenylporphyrinato)antimony(V) complex (SbTPP) demonstrates bactericidal activity under visible-light irradiation. This phototoxic effect could be caused by photodamage to biomolecules, but the mechanism has not been well understood. In this study, to clarify the mechanism of phototoxicity by SbTPP, DNA damage photosensitized by SbTPP was examined using [^{32}P]-5′-end-labeled DNA fragments. SbTPP induced markedly severe photodamage to single-stranded rather than to double-stranded DNA. Photo-irradiated SbTPP frequently caused DNA cleavage at the guanine residue of single-stranded DNA after *Escherichia coli* formamidopyrimidine-DNA glycosylase or piperidine treatment. HPLC measurement confirmed the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an oxidation product of 2′-deoxyguanosine, and showed that the content of 8-oxodG in single-stranded DNA is larger than that in double-stranded DNA. The effects of scavengers of reactive oxygen species on DNA damage suggested the involvement of singlet oxygen. These results have shown that the mechanism via singlet oxygen formation mainly contributes to the phototoxicity of SbTPP. On the other hand, SbTPP induced DNA damage specifically at the underlined G of 5′-GG, 5′-GGG, and 5′-GGGG in double-stranded DNA. The sequence-specificity of DNA damage is quite similar to that induced by the type I photosensitizers, suggesting that photo-induced electron transfer slightly participates in the phototoxicity of SbTPP. In conclusion, SbTPP induces DNA photodamage via singlet oxygen formation and photo-induced electron transfer. A similar mechanism can damage other biomacromolecules, such as protein and the phospholipid membrane. The damage to biomacromolecules via these mechanisms may participate in the phototoxicity of SbTPP.
© 2005 Elsevier B.V. All rights reserved.

Keywords: Dihydroxo(tetraphenylporphyrinato)antimony(V) complex; DNA damage; Singlet oxygen; Electron transfer; Photocatalyst

1. Introduction

Porphyrin chromophores play important roles as photosensitizers operating under visible-light irradiation. Photoexcited high-valent metalloporphyrins have high oxidation ability compared with low-valent metalloporphyrins [1–5]. The photo-oxidation ability of porphyrin complexes can be applicable to the utilization of visible-light photocatalytic systems for sterilization, decomposition of harmful compounds, or both. Dihydroxo(tetraphenylporphyrinato)antimony(V) (SbTPP) bonding on the surface of a silica gel particle demonstrates remarkable bactericidal activity for *Escherichia coli* cells under illumination with fluorescent light (400–700 nm) [6]. Upon exposure to visible light, the SbTPP photocatalyst showed much superior bactericidal activity to the commercially available TiO_2 semiconductor photocatalyst. Moreover, upon exposure to sunlight, the bactericidal activity of SbTPP was observed, and the bactericidal effect was effective for continuous

* Corresponding author. Tel./fax: +81 53 478 1287.
E-mail address: tkhirak@ipc.shizuoka.ac.jp (K. Hirakawa).

1011-1344/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2005.08.008

treatment on a column photoreactor under fluorescent light irradiation [6]. Photodamage to biomolecules by SbTPP should contribute to the phototoxic activity. However, the mechanism underlying biomolecular damage photosensitized by SbTPP is not well understood.

Photo-induced damage to biomacromolecules has been investigated using DNA as one of the target molecules [7–11]. We have previously reported the mechanism of DNA photodamage induced by photosensitizers via the type I mechanism (photo-induced electron transfer) and the type II mechanism (singlet oxygen ($^1O_2$) formation) [12–17]. Consecutive guanine residues, such as underlined G of 5′-<u>G</u>G or 5′-G<u>G</u>G, are selectively damaged through the type I mechanism, leading to the formation of labile sites, whereas the type II mechanism causes the oxidation of every guanine residues. In this study, to gain insights into the mechanism of biomacromolecule damage by photo-irradiated SbTPP, we examined SbTPP-mediated photolesions of ^{32}P-labeled DNA fragments as target molecules. DNA damage was analyzed by an electrophoresis after piperidine or *E. coli* formamidopyrimidine-DNA glycosylase (Fpg) treatment to cleave the labile site formed by SbTPP photosensitization. The formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), an oxidation product of 2′-deoxyguanosine (dG), was also measured using an electrochemical detector coupled to high-performance liquid chromatography (HPLC-ECD).

2. Materials and methods

2.1. Materials

SbTPP bromide was synthesized according to the previous report [18–20]. Restriction enzymes (*Ava*I, *Hind*III, and *Pst*I) and T_4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). Restriction enzymes (*Apa*I, *BssH*II, *Eco*RI, and *Xba*I) and calf intestine phosphatase were from Boehringer Mannheim GmbH (Mannheim, Germany). [γ-^{32}P]-ATP was from New England Nuclear (Boston, MA). Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA) and bathocuproinedisulfonic acid were from Dojin Chemicals Co. (Kumamoto, Japan). Superoxide dismutase (SOD) (3000 units/mg from bovine erythrocytes) and catalase (45,000 units/mg from bovine liver) were from Sigma Chemical Co. (St. Louis, MO). Methional (3-methylthiopropionaldehyde) was from Tokyo Kaksei (Tokyo, Japan). Fpg was from Trevigen Co. (Gaithersburg, MD). Ethanol, D-mannitol, and sodium formate were from Nacalai Tesque Inc. (Kyoto, Japan). Sodium azide and piperidine were from Wako Chemicals Co. (Osaka, Japan).

2.2. Preparation of ^{32}P-5′-end-labeled DNA fragments

DNA fragments were obtained from the human *p*53 [21] and *p*16 [22] tumor suppressor genes and the c-Ha-*ras*-1 protooncogene [23]. The DNA fragment of the *p*53 tumor suppressor gene was prepared from pUC18 plasmid, ligated fragments containing exons of *p*53 gene. A ^{32}P-5′-end-labeled double-stranded 211-bp fragment (*Hind*III* 13972-*Apa*I 14182) was prepared according to the method described previously [24]. Two fragments containing exon 1 or 2 of the human *p*16 tumor suppressor gene [22] were obtained as described previously [25]. The 5′-end-labeled 460-base pair fragment (*Eco*RI* 9481-*Eco*RI* 9940) containing exon 2 was also further digested with *Bss*HII to obtain the 5′-end-labeled 309-base pair fragment (*Eco*RI* 9481–*Bss*HII 9789). The DNA fragment of the c-Ha-*ras*-1 protooncogene was prepared from plasmid pbcNI, which carries a 6.6 kb *Bam*HI chromosomal DNA segment containing the c-Ha-*ras*-1 gene. The 5′-end-labeled 337 bp fragment (*Pst*I 2345–*Ava*I* 2681) and the 261-bp c-Ha-*ras*-1 fragment (*Ava*I* 1645–*Xba*I 1905) were obtained according to a method described previously [26]. Nucleotide numbering starts with the *Bam*HI site [23]. The asterisk indicates the ^{32}P labeling. In the present study, several kinds of DNA fragments were used. These kinds of DNA fragments do not affect the specificity of DNA damage by photosensitized reaction.

2.3. Detection of damage to isolated DNA photosensitized by SbTPP

The standard reaction mixture in a microtube (1.5-mL Eppendorf) contained the ^{32}P-DNA fragment (<1 μM) and 10 μM/base calf thymus DNA, indicated amounts of SbTPP, and 5 μM DTPA in a 10 mM sodium phosphate buffer (pH 7.8). Calf thymus DNA was used to adjust the total concentration of DNA. DTPA was used to remove the contaminated metal ions. The reaction mixtures were exposed to visible-light using the fluorescent lamp REX (λ_{max} = 612 nm, 1.0 mW cm^{-2}) (Raytronics Co., Saitama, Japan). Subsequently, the DNA was treated with 1 M piperidine for 20 min at 90 °C or 10 units of Fpg in the reaction buffer (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 10 mM EDTA, and 0.1 mg mL^{-1} BSA) for 2 h at 37 °C. Fpg can catalyze the excision of piperidine-resistant 8-oxodG and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua). The DNA fragments were subjected to electrophoresis on an 8 M urea/8% polyacrylamide gel. The autoradiogram was obtained by exposing an X-ray film to the gel. The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam–Gilbert procedure [27] using a DNA-sequencing system (LKB 2010 Macrophor, Pharmacia Biotech, Uppsala, Sweden). The relative amount of DNA fragments was measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL, Pharmacia Biotech).

2.4. Measurement of 8-oxodG formation in calf thymus DNA photosensitized by SbTPP

Formation of 8-oxodG was measured by a modification of a reported method [28]. The reaction mixture in a tube

Download English Version:

https://daneshyari.com/en/article/30801

Download Persian Version:

https://daneshyari.com/article/30801

Daneshyari.com