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Journal Item

Formation of oxidative photoproduct of tri-acetyl-modified 6-thioguanosine by UVA irradiation

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Abstract

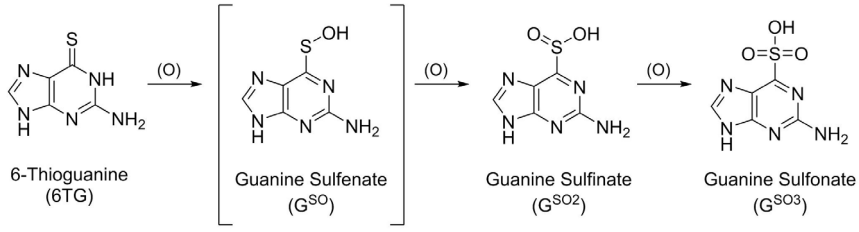
Thio-substituted nucleobases such as 6-thioguanine are known to be photosensitive to UVA light and capable of generating singlet molecular oxygen ($^1O_2$*). 2',3',5'-Tri-*O*-acetyl-6-thioguanosine (ta6TGuo) was prepared and its photochemistry was investigated. ta6TGuo in the aerated acetonitrile solution under UVA irradiation generates $^1O_2$* that can also oxidize ta6TGuo itself and finally convert into tri-acetylated guanosine sulfonate (taGuo^{SO3}). The decay rate constant of ta6TGuo was found to be in good agreement with the formation rate constant of taGuo^{SO3}, revealing that the first step in the reaction of ta6TGuo with $^1O_2$* would be the rate-determining step in forming taGuo^{SO3} as the final product. Thiolated nucleosides such as 6-thioguanosine could be used as a photosensitive agent for light-induced therapies. The key feature in the UVA irradiation of the thionucleosideis to produce $^1O_2$*, but the resultant oxidative products would also contribute to the effect on therapies.

Introduction

The thiopurines such as 6-thioguanine (6TG) are prescribed as cancer therapeutic and immunosuppressive agents [1-12]. They are converted into the active form, thioguanine nucleotide, by metabolic activation in body, and then incorporated into cellular RNA and DNA [13]. 6TG as well as thiolated pyrimidine bases such as 4-thiothymidine has an absorption band in the UVA region (320-400 nm), where the normal DNA/RNA bases do not absorb in the UVA region, instead at 260-300 nm (UVB and UVC regions) [3-20]. 6TG localizing in tumor cell was reported to generate reactive oxygen species with low dose of UVA light, causing cellular apoptosis [6]. Thus, the thiopurines can be used as potential photosensitive agents for light-induced therapies due to their unique properties.

The key characteristic in UVA irradiation of 6TG is to produce singlet molecular oxygen ($^1O_2$*), that oxidizes 6TG to unstable guanine sulfenate (G^{SO}) and guanine sulfinate (G^{SO2}). Both of the intermediates can be further oxidized to form guanine sulfonate (G^{SO3}) as the stable photoproduct (Scheme 1) [9,10]. G^{SO3} was reported not capable of forming stable base pairs with any of the normal DNA bases in duplex oligonucleotides [7]. Crosslinking of proteins or low molecular weight thiol compounds to DNA involving an intermediate derived from oxidized 6TG has been proposed as a previously unrecognized hazard in sunlight-exposed cells of thiopurines-treated patients [9].

6-Thioguanine is hardly soluble in hydrated organic solvents, thus it is difficult to carry out its spectroscopic studies. Recently, we prepared 2',3',5'-tri-*O*-acetyl-6-thioguanosine (ta6TGuo, see structure **1** in Figure 1) for better solubility and easier



Scheme 1. Oxidation mechanism of 6TG by singlet molecular oxygen.

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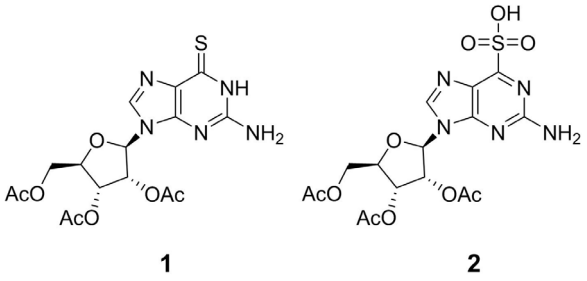

Figure 1. Structure of ta6TGuo(**1**) and taGuo^{SO3} (**2**).

handlings, and reported that ta6TGuo under UVA irradiation generates $^1O_2$* with a high quantum yield [21]. In this article, we report our findings from absorption and emission spectroscopic studies of ta6TGuo in aerated acetonitrile. From our HPLC analysis, the guanosine sulfonate (taG^{SO3}) can be regarded as the major photoproduct by the UVA light. The decay rate constant of ta6TGuo and the formation rate constant of the photoproduct are also presented, and the reaction mechanism is discussed.

Experimental

The preparation of ta6TGuo was carried out as reported in a recent publication [21]. Briefly, guanosine was acetylated by adding acetic anhydride, followed by thiolation with Lawesson's reagent. The isolated ta6TGuo was purified with column chromatography and used for spectroscopic studies.

Absorption spectra were recorded with a spectrophotometer (Jasco U-best V550). Emission and excitation spectra were measured with a spectrofluorometer (Jasco FP6500). A XeCl excimer laser (Lambda Physik COMPex 102; 308 nm) was used as an excitation light source. Incident laser fluence, estimated by a joulemeter (Gentec ED200), was 0.18 mJ cm^{-2} per shot. A Xe lamp (Hamamatsu L2273, 150 W) was used in the continuous UV-light irradiation experiment. All measurements were carried out at room temperature. Photoproducts were analyzed using a HPLC system with a UV-vis detector (Shimadzu, SPD-10A). Samples with the mobile phase consisting of a mixed solvent of acetonitrile/water (1/2v/v) passed through a column (Kanto Chemical; Mightysil, RP-18 GP 150-4.6 5μm) at 40°C and were quantified by the UV detection at 340 nm. The mobile phase was delivered at a flow-rate of 0.3 mLmin^{-1}.

Results and Discussion

ta6TGuo (**1** in Figure 1) has an intense absorption peak at 346 nm ($\varepsilon = 2.82 \times 10^4$ M^{-1}cm^{-1} [21]). When the sample solution was irradiated by a XeCl excimer laser (308 nm) under the aerated condition, the

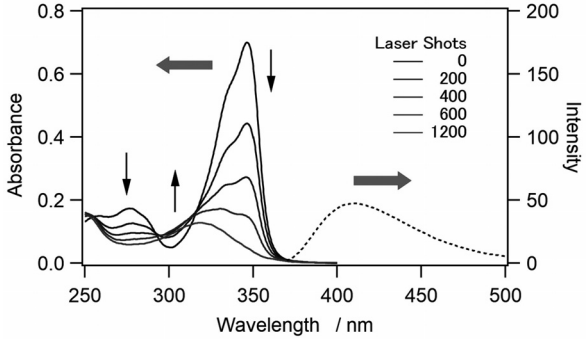

Figure 2. Change of absorption spectra (solid lines) of ta6TGuo in acetonitrile byirradiation of XeCl excimer laser shots and an emission spectrum (broken line) of the photoproduct obtained with the excitation of 340 nm.

intensity of the absorption band decreased gradually as shown in Figure 2. After irradiation of 1200 laser shots, the absorption band for ta6TGuo disappeared almost completely, and the residual absorption derived from a photoproduct appeared at around 320 nm. A new emission band was observed with its maximum at around 410 nm when the photoproduct was excited at 340 nm, as shown in Figure 2 (broken line), while no emission band from ta6TGuo was observed. Thus, ta6TGuo itself was intrinsically non-fluorescent, revealing that ta6TGuo will have a high quantum yield of triplet formation as well as 6-thio-2'-deoxyguanosine [22]. The excitation spectrum obtained by monitoring the emission was identical to the absorption spectrum of the photoproduct. The absorption and emission spectral features of the photoproduct and its intense fluorescent property are similar to those of guanine sulfonate (G^{SO3}) (see Scheme 1) [3,7,9,10,12]. Thus the photoproduct, afforded by the UV irradiation of ta6TGuo, can be assigned to tri-acetylated guanosine sulfonate, taGuo^{SO3} (see structure **2** in Figure 1). In our previous work, the formation quantum yield of singlet molecular oxygen ($^1O_2$*) by ta6TGuo with UV irradiation was determined to be as large as 0.37 ± 0.01 in the oxygen saturated acetonitrile solution [21]. Hence, taGuo^{SO3} is also produced from oxidation of ta6TGuo by $^1O_2$*.

Figure 3 shows the HPLC charts of ta6TGuo in acetonitrile with and without continuous UV-irradiation. The peak at 4.3 min. (a photoproduct) increased, while the peak at 12.0 min. (the starting material ta6TGuo) decreased along with continuous UV irradiations. Based upon the absorption spectral changes of ta6TGuo by the laser irradiation, the photoproduct eluting at 4.3 min. can be assigned to be its fully oxidized product, namely taGuo^{SO3}. Irradiation time dependences of the peak area for ta6TGuo and taGuo^{SO3} are shown in the inset of Figure 3. Here, the peak area was estimated by fitting with the Gaussian function to remove the interference

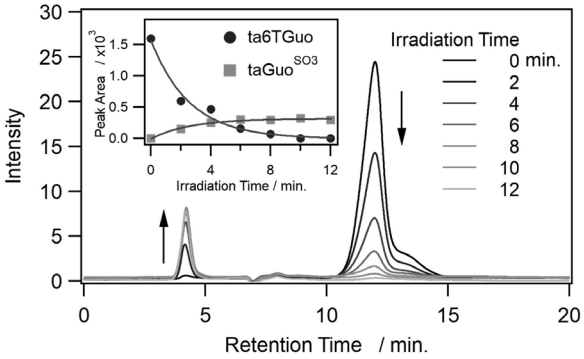

Figure 3. The HPLC charts of ta6TGuo in acetonitrile with and without irradiation. Inset: Irradiation time dependence of the peak area for ta6TGuo (circle) and taGuo^{SO3} (square).

of impurities like the shoulder at 13 min. beside the ta6TGuo signal. The decay rate constant of ta6TGuo (**1**) and the formation rate constant of taGuo^{SO3} (**2**) were determined with a single exponential equation to be 0.39 ±0.04 min^{-1} and 0.38 ± 0.05 min^{-1}, respectively. Ren et al. presented the oxidation mechanism of 6TG to afford G^{SO3} (see Scheme 1) [9]. UVA irradiation of 6TG generates $^1O_2$* that oxidizes 6TG to the unstable guanine sulfenate (G^{SO}). G^{SO} can undergo further oxidation to guanine sulfinate (G^{SO2}) that can be further oxidized to guanine sulfonate (G^{SO3}). In aqueous solution the G^{SO2} peak in the HPLC chart was clearly seenbeside the G^{SO3} peak [9]. However, in this work only the single peak of taGuo^{SO3} was observed in acetonitrile, suggesting that the oxidation of taGuo^{SO} (the sulfenate form) and taGuo^{SO2} (the sulfinate form) to taGuo^{SO3} would be quite fast in acetonitrile. In addition, the decay rate constant of ta6TGuo was found to be in good agreement with the formation rate constant of taGuo^{SO3}. These results indicate that the first step in the reaction of ta6TGuo with $^1O_2$* is likely to be the rate-determining step to yield taGuo^{SO3} as the final product.

In conclusion, we investigated the photochemistry of tri-acetyl-modified 6-thioguanosine (ta6TGuo), which is soluble in organic solvents. ta6TGuo in the aerated acetonitrile solution with UVA irradiation generates $^1O_2$* that oxidizes ta6TGuo to its guanosine sulfonate form (taGuo^{SO3}) ultimately. The decay rate constant of ta6TGuo agrees well with the formation rate constant of taGuo^{SO3}, suggesting that the first step in the reaction of ta6TGuo with $^1O_2$* is likely to be the rate-determining step in formingits corresponding sulfonate (taGuo^{SO3}). Thionucleosides such as 6-thioguanosine can be further developed as a potential photosensitive agent for light-induced therapies. The main characteristic of thioguanosine by UVA irradiation is to produce $^1O_2$*. However, its oxidative products may also contribute to the effect on photo-induced therapies. Further studies of the oxidative products of thio-substituted guanosines are underway.

Notes

The authors declare no competing financial interest.

References

[1] Lapage G. A. Incorporation of 6-thioguanine into nucleic acids. Cancer Res.,1960;20: 403-408.

[2] Lepage G. A., Jones M. Further studies on the mechanism of action of 6-thioguanine. Cancer Res., 1961; 21: 1590-1594.

[3] Rubin Y. V., Blagoi Y. P., Bokovoy V. A. 6-Thioguanine luminescence probe to study DNA and low- molecular-weight systems. J. Fluores., 1995; 5: 263-272.

[4] Qu P., Lu H., Ding X., Tao Y., Lu Z. Influences of urea and guanidine hydrochloride on the interaction of 6-thioguanine with bovine serum albumin. Spectros. Acta A, 2009; 74: 1224-1228.

[5] Massey A., Xu Y.-Z., Karran P. Photoactivation of DNA thiobases as a potential novel therapeutic option. Curr. Biol., 2001; 11: 1142-1146.

[6] O'Donovan P., Perrett C. M., Zhang X. *et al*. Azathioprine and UVA light generate mutagenic oxidative DNA damage. Science, 2005; 309: 1871-1874.

[7] Zhang X., Jeffs G., Ren X. *et al*. Novel DNA lesions generated by the interaction between therapeutic thiopurines and UVA light. DNA Repair, 2007; 6: 344-354.

[8] Brem R., Li F., Karran P. Reactive oxygen species generated by thiopurine/ UVA cause irreparable transcription-blocking DNA lesions. Nucleic Acids Research, 2009; 37: 1951-1961.

[9] Ren X., Xu Y.-Z., Karran P. Photo-oxidation of 6-thioguanine by UVA: The formation of addition products with low molecular weight thiol compounds. Photochem. Photobiol., 2010; 86: 1038-1045.

[10] Ren X., Li F., Jeffs G., Zhang X., Xu Y.-Z., Karran P. Guanine sulphinate is a major stable product of photochemical oxidation of DNA 6-thioguanine by UVA irradiation. Nucleic Acids Research, 2010; 38: 1832-1840.

[11] Daehn I., Brem R., Barkauskaite E., Karran P. 6-Thioguanine damages mitochondrial DNA and causes mitochondrial dysfunction in human cells. FEBS Letters, 2011; 585: 3941-3946.

[12] Zou X., Zhao H., Yu Y., Su H. Formation of guanine-6-sulfonate from 6-thioguanine and singlet oxygen: A combined theoretical and experimental study. J. Am. Chem. Soc., 2013;

135: 4509-4515.
[13] Elion G. B. The purine path to chemotherapy. Science, 1989; 244: 41-47.
[14] Harada Y., Suzuki T., Ichimura T., Xu Y.-Z. Triplet formation of 4-thiothymidine and its photosensitization to oxygen studied by time-resolved thermal lensing technique. J. Phys. Chem. B, 2007; 111: 5518-5524.
[15] Suzuki T. Photodynamics of thio- and aza-substituted DNA/RNA bases and singlet oxygen formation. Photomed. Photobiol., 2008; 30: 31-32.
[16] Harada Y., Okabe C., Kobayashi T. *et al*. Ultrafast intersystem crossing of 4-thiothymidine in aqueous solution. J. Phys. Chem. Lett., 2010; 1: 480-484.
[17] Kuramochi H., Kobayashi T., Suzuki T., Ichimura T. Excited-state dynamics of 6-aza-2-thiothymine and 2-thiothymine: Intersystem crossing and singlet oxygen photosensitization. J. Phys. Chem. B, 2010; 114: 8782-8789.
[18] Isozaki T., Ikemi J., Suzuki T. Multi-photon excitation studies of 6-thioguanosine as a potential agent for photodynamic therapy. Photomed. Photobiol., 2012; 34: 67-68.
[19] Suzuki T., Isozaki T. Excited state characteristics of aza- and thio-substituted nucleic acid bases: an application for photodynamic therapy. Kokagaku (Photochemistry), 2015; 46: 154-160.
[20] Suzuki T., Miyata S., Isozaki T. Absorption spectra of 8-oxoguanosine and 8-thioguanosine. Photomed. Photobiol., 2015; 37: 21-22.
[21] Miyata S., Yamada T., Isozaki T., Sugimura H., Xu Y.-Z., Suzuki T. Absorption characteristics and quantum yields of singlet oxygen generation of thioguanosine derivatives. Photochem. Photobiol., in press.
[22] Pollum M., Ortiz-Rodríguez L. A., Jockusch S., Crespo-Hernández C. E. The triplet state of 6-thio-2'-deoxyguanosine: intrinsic properties and reactivity toward molecular oxygen. Photochem. Photobiol., 2016; 92: 286-292.