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Flavin-sensitized photoreduction of thymidine glycol

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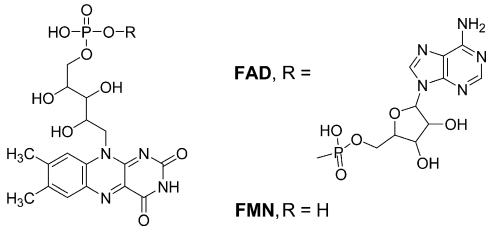
Abstract—Photochemical reactivity of flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) toward thymidine glycol (dTg) has been investigated. Fluorescence intensity of FAD was enhanced as increasing the concentration of dTg, suggesting that adenosine moiety of FAD interacts with dTg. However, photoreduction of dTg using reduced form of FAD gave repaired thymidine in almost the same yield as when reduced FMN was used alternatively, and thus such interaction seems to have no effect on the reduction. Oligodeoxynucleotides containing dTg were also photochemically repaired by reduced form of flavins in different yields depending on the sequence, which could be related to electron affinity of the nucleobases in DNA.

Flavin-binding proteins (flavoproteins) have received extensive interest since this group of enzyme family catalyzes a wide variety of reactions in biological systems.[1–3] Flavins adopt three different redox states and their protonated forms in aqueous media, and each form shows different redox properties. DNA photolyase photochemically catalyzes the repair of UV-induced pyrimidine lesions in DNA via electron transfer between reduced form of flavin adenine dinucleotide ($FADH^-$) cofactor and the lesion.[2] Some previous studies on the electron transfer process suggest the configuration of the flavin may affect the reactivity.[3] It has been predicted that adenine and flavin form a charge transfer complex in the ground state due to the U-shaped configuration of FAD. Meanwhile, we have found that $FADH^-$ photochemically induces electron transfer to thymidine glycol (dTg), a well-known oxidative DNA damage structure, in aqueous solution and generates 5,6-dihydro-6-hydroxythymidin-5-yl radical via relatively slow elimination of hydroxyl ion, which is then further reduced to thymidine (dT) or 6-hydroxy-5,6-dihydrothymidine (HOdT, Scheme 1).[4] In addition, photoreduction of a series of short oligodeoxynucleotides containing thymine glycol (Tg) by $FADH^-$ afforded corresponding repaired oligodeoxynucleotides. On the other hand, photo-reductive repair of Tg was not observed in a Tg-containing DNA tethered with an internal electron donor, probably because back-electron transfer to the electron donor radical cation is much faster than elimination of hydroxyl ion from the radical anion of Tg.[4a] Although no enzyme that catalyzes repair of Tg in DNA by photochemically induced electron transfer has been found so far, details on the reactivity of flavins provide extensive knowledge for an understanding of the roles and reaction mechanisms of flavins in a wide variety of redox enzymes. In this study, we have investigated photoinduced electron transfer between the flavin chromophore and Tg, and the subsequent repair into thymine to understand redox reactivity of Tg in single-stranded DNA. Implications of the formation of Tg in DNA for reductive electron transfer through the sequence are also discussed.



To investigate the photochemical process of flavin-sensitized reaction of dTg, we undertook Stern–Volmer analysis of fluorescence of flavins [FAD and flavin mononucleotide (FMN)] in the presence of dTg[5]. As shown in Figure 1, fluorescence intensity of FMN at 520 nm decreased at higher dTg concentrations, and the relative intensity gave a linear Stern–Volmer plot,

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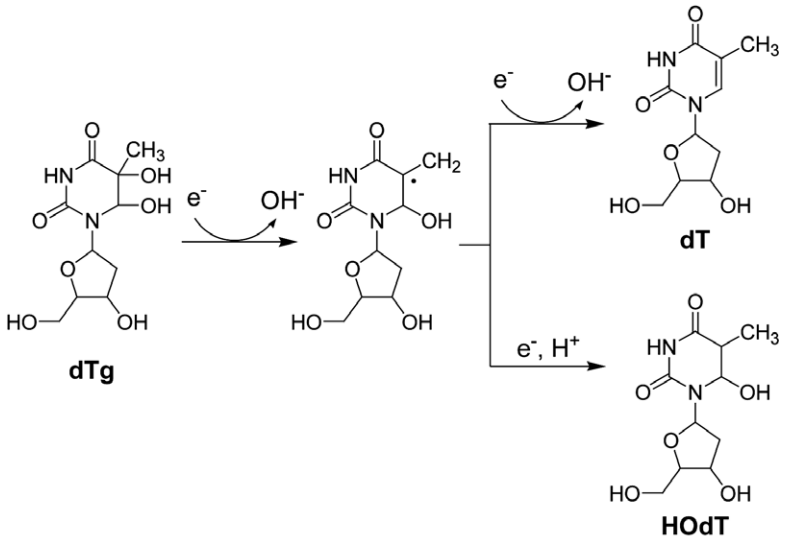


Scheme 1. Reductive repair of thymidine glycol.

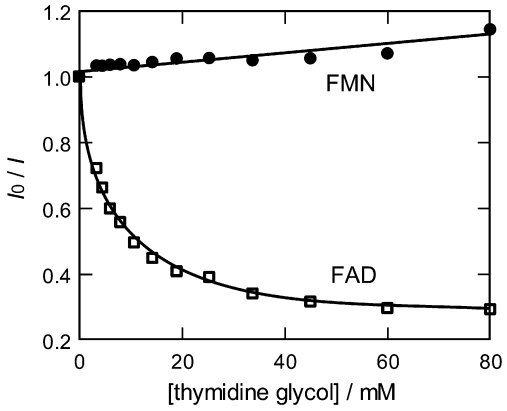


Figure 1. Stern–Volmer plots for fluorescence quenching of (•) FMN and (□) FAD by thymidine glycol. Relative intensity (I_0/I) of the emission at 520 nm was measured in the presence of 0.1 mM flavin in deoxygenated phosphate buffer solution (pH 7.0).

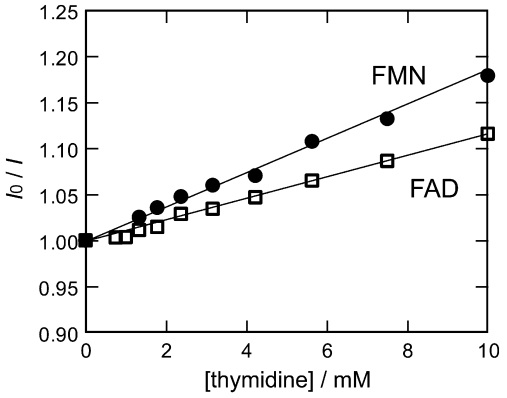


Figure 2. Stern–Volmer plots for fluorescence quenching of (•) FMN and (□) FAD by thymidine. Upon excitation at 370 nm, relative intensity (I_0/I) of the emission at 520 nm was measured in the presence of 0.1 mM flavin in deoxygenated phosphate buffer solution (pH 7.0).

$$I_0/I = 1 + K_{SV}[Q], K_{SV} = k_q\tau_0,$$

where τ_0 is the lifetime of excited state of flavin, I_0 and I are the fluorescence intensity at 520 nm in the absence or presence of quencher, respectively. The linear plot gave a dynamic quenching rate constant [$k_q = (3.5 \pm 0.4) \times 10^8\ M^{-1}\ s^{-1}$] for the FMN-dTg solution.[6] On the other hand, it was interesting that fluorescence intensity of FAD increased as increasing the concentration of dTg (Fig. 1). For a reference, we also examined the analysis for fluorescence quenching of FAD or FMN by thymidine, as shown in Figure 2; however such fluorescence enhancement was not observed [$k_{q,\ FMN} = (3.8 \pm 0.1) \times 10^9\ M^{-1}\ s^{-1}$; $k_{q,\ FAD} = (4.0 \pm 0.1) \times 10^9\ M^{-1}\ s^{-1}$]. Previous studies on photophysical properties of FAD have found that the excited state of flavin is intramolecularly quenched by covalently linked adenosine, suggesting that flavin and adenosine form a stacking conformation.[3] In this context, it is predicted that dTg may weakly interact with adenosine, thereby inhibiting the intramolecular quenching between the flavin and adenosine.

Figure 3 shows the effect of dTg on the fluorescence intensity of FMN (0.1 mM) measured in the presence of adenosine (15 mM). The successive addition of dTg into the solution resulted in a recovery of the FMN fluorescence, which is consistent with the fluorescence behavior of FAD. On the other hand, however, it is known that base pairing interaction between Tg and adenine in DNA is less than that between adenine–thymine pairs in DNA.[7] Specific discrimination of dTg by FAD might be a result of non-Watson–Crick type interaction between adenosine moiety and dTg.

Next, flavin-sensitized photoreduction of dTg into dT was examined. It has been suggested that photoexcitation of the reduced flavin generates its excited singlet state which can induce reductive repair of pyrimidine lesions.[2] We have shown previously that dTg undergoes one-electron reduction by photoexcited aromatic amines or hydrated electrons to afford 6-hydroxy-5,6-dihydrothymin-5-yl radical, which is further reduced to thymine.[4] Here reduced forms of flavins were generated

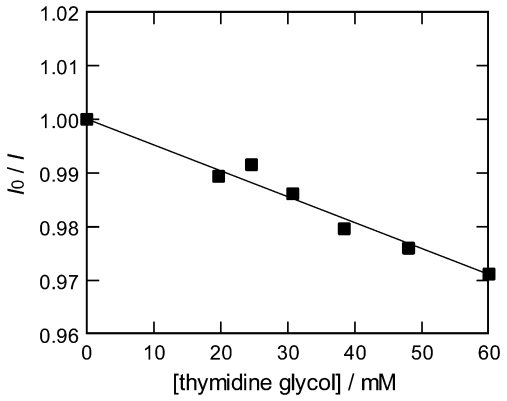
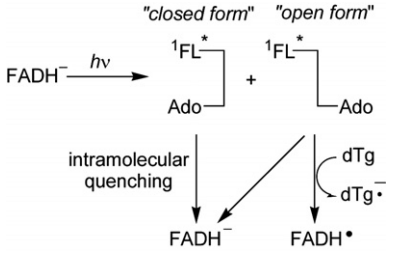


Figure 3. Relative intensity of fluorescence of FMN (0.1 mM) at 520 nm upon excitation at 370 nm in the presence of adenosine (15 mM) and various concentrations of thymidine glycol (0–60 mM) in deoxygenated phosphate buffer (pH 7.0).

in situ by adding ethylenediaminetetraacetic acid (EDTA) in the reaction[8] for investigating photoreduction of dTg. Flavin (FAD or FMN, 0.1 mM), EDTA·2-Na (20 mM), and various concentrations of dTg (0.1–20 mM) were exposed to UV-light ($\lambda > 300$ nm) in Ar-saturated phosphate buffer solution (2.5 mM, pH 7.0), and then restored dT was quantitated by the use of HPLC (Table 1). In accord with the previous result of reductive repair of dTg,[4a] the photoreduction afforded dT in selectivities of 6–43%, depending on the concentration of dTg. The result implies that structural differences between the reduced flavins ($FADH^-$ and $FMNH^-$) do not affect the photoreactivity, since both decomposition of dTg and the selectivity of generated dT were independent of the flavin structure even in a higher concentration range of dTg. As proposed previously,[4a] successive two-electrons reduction should be necessary for reproducing dT from dTg (see Scheme 1). At a high concentration of dTg, the second reduction of the intermediate 5-yl radical by photoexcited flavins is kinetically less favorable than that at a lower concentration of dTg, and thus the 5-yl radical could generate some byproducts via hydrogen abstraction from dTg molecules, which might result in low selectivity of dT (Table 1). Considering potential structural changes of FAD into $FADH_2$ by the preirradiation in the presence of EDTA, this result may raise the possibility that once fully reduced flavin is formed (open form, Scheme 2), the intermolecular interaction between reduced flavin and adenosine is abolished, making $FADH^-$ equally reactive as $FMNH^-$. Measurement of qualitative fluorescence spectra of the reduced flavins in the presence of dTg was unsuccessful, because they are less fluorescent than the oxidized forms, as reported previously.[9]

In addition, both $FADH^-$ and $FMNH^-$ showed similar photoreactivities toward 4-mer long oligodeoxynucleotides bearing Tg in the sequence (5′-XXTgX-3′). Our previous attempts of photoreduction by $FADH^-$ demonstrated restoration of Tg in various yields depending on the sequence. As shown in Table 2, $FMNH^-$-sensitized reduction also yielded repaired oligodeoxynucleotide in a maximum yield of 22% for 5′-GATG-3′. For the sequence, about 30% of the products corresponded to the thymine-containing oligodeoxynucleotide, which is comparable to the selectivity of dT from dTg (Table 1, ~30%). On the other hand, restoration of thymine in the sequences 5′-CGTgA-3′ and 5′-GGTgG-3′ was not observed under the current conditions. Reduced forms of the flavins possess high oxidation potentials enough to induce one-electron reduction of all four nucleobases (A, T, G, C).[10] Previous experiments of intramolecular reductive electron transfer in DNA have shown that cytosine is a good electron acceptor and the corresponding radical anion ($C^{-\bullet}$) could be readily protonated by the counterpart guanine in duplex DNA.[11] It is not clear that ($C^{-\bullet}$) could be protonated also in ssDNA (5′-CGTgA-3′), but it is likely that electron is trapped by cytosine moiety in the sequence, which could



Scheme 2. Photoreduction of dTg by $^*FADH^-$.

Table 1. Photoinduced reduction of thymidine glycol by flavins. Various concentrations of thymidine glycol in Ar-saturated phosphate buffer solution (pH 7.0) containing flavins (0.1 mM) were UV-irradiated (>300 nm) for 5 h

dTg/mM	Conversion of dTg/%	Yield of dT/%	Selectivity of dT[a]/%
FAD			
0.1	44	14	32
0.5	30	8.4	28
5	24	5.8	24
20	19	1.1	6
FMN			
0.1	45	14	31
0.5	27	12	43
5	20	4.4	22
20	12	1.2	10

[a] (Yield of dT)/(conversion of dTg).

Table 2. Photoinduced reductive repair of thymine glycol in single-stranded oligodeoxynucleotides[a]

Sequence	Conversion of XXTgX/%	Yield of XXTX/%	Selectivity[b]/%
GG(Tg/T)G	17 (62)[c]	0 (0)[c]	0 (0)[c]
GA(Tg/T)G	72 (62)	22 (37)	31 (60)
GG(Tg/T)A	72 (61)	12 (41)	17 (67)
GA(Tg/T)A	96 (85)	14 (46)	15 (54)
CG(Tg/T)A	32 (54)	0 (15)	0 (28)

[a] Single-stranded DNA (66 μM) in phosphate buffer solution (pH 7) was exposed to UV-light in the presence of FMN (0.1 mM) and EDTA (20 mM) at 24 °C for 90 min.

[b] (Yield of XXTX)/(conversion of XXTgX).

[c] Values in parentheses are obtained by photoreduction with FAD (Ref. 4a).

be competing with the electron migration along the strand, and thus lowering the reactivity of Tg. It is also expected that reduction of guanine is less efficient because of the high reduction potential ($E_{red} < -3.00$ V vs SCE),[12] especially in the sequence of 5′-GGTgG-3′, which is well consistent with the reactivity obtained in this study. As discussed in our previous report,[4a] 6-hydroxy-5,6-dihydrothymin-5-yl radical generated from Tg could be further reduced by adjacent guanines due to high electron affinity of the radical, as a result, the photolysis may yield guanine modifications in the sequence. In addition, it has been predicted that the 5-yl radical adds to the adjacent guanines to form cross-linked products.[13,14] For that reason, we attempted a photolysis of Tg-containing oligodeoxynucleotide possessing multiple guanines (5′-CTTGGGTgGCT-3′) in the presence of flavins. Sodium dithionite or EDTA was employed for reducing FAD into $FADH^-$ in the present reaction. After piperidine-catalyzed hydrolysis, the products were analyzed by gel electrophoresis, but no alkaline-labile products of guanine were detected (Supplementary data). Photoreduction by phenothiazine (PTZ, $^*E_{ox} = -2.7$ V),[4,15] an alternative electron donor for avoiding the possibility of reduction of the transient guanine radical cation by excess dithionite, did not give any notable products. Consistent with the results with the G-rich 4-mer long oligodeoxynucleotides, repair of Tg was not observed in the longer sequence, which suggests that the G-rich oligodeoxynucleotides are intrinsically less reactive toward photoexcited reduced forms of the flavins.

With regard to interaction between electrons and DNA containing modified nucleic acid bases, we have demonstrated that Tg in DNA sequences does not prevent reductive electron migration along the duplex.[4a] To compare electron affinities between dTg and dT by reductive fluorescence quenching analysis, 1-aminopyrene (AP, $^*E_{ox} = -2.6$ V) in 50% acetonitrile aqueous solution was employed as a photoinduced electron donor. Upon excitation of AP at 365 nm, fluorescence intensity ($\lambda_{max} = 443$ nm, $\tau_0 = 5.2$ ns)[16] decreased as increasing the amount of dT, and the Stern–Volmer plot gave a quenching rate constant of $k_q = (9.8 \pm 0.5) \times 10^{10}\ M^{-1}\ s^{-1}$ (Supplementary information). On the other hand, a slightly lower quenching rate constant [$k_q = (3.4 \pm 0.1) \times 10^9\ M^{-1}\ s^{-1}$] was obtained for dTg suggesting that reduction potential of Tg is more negative than that of dT. The lower electron affinity of Tg might be the reason for the efficient electron migration through Tg-containing oligodeoxynucleotides.[4a]

In conclusion, photoinduced reduction of dTg and Tg-containing oligodeoxynucleotides sensitized by flavins was investigated by quantitative product analysis and fluorescence quenching. Although oxidized form of FAD may discriminate the structural difference between dTg and dT with relatively weak interactions, this did not affect photoinduced reductive repair of Tg into dT. As demonstrated above and in our previous attempts, both $FADH^-$ and $FMNH^-$ can induce repair of Tg in single-stranded DNA in a sequence-dependent manner. We anticipate that electrons could migrate a short distance until they are trapped by cytosine or Tg in the sequences, on the other hand, guanine could diminish the efficiency of electron attachment to the strands because of its low electron affinity. Our fluorescence quenching experiment showed that electron affinity of dTg is lower than that of dT, and thus which affects reductive electron migration in DNA. Electron affinity of DNA lesions is possibly important to understand DNA modification mechanisms involved in the early stages of DNA damage formation by ionizing radiation and UV-light.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.038.

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