Photoreduction of Thymine Glycol sensitized by Aromatic Amines in Aqueous Solution

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Photolyses (λ_{ex} > 280 nm or λ_{ex} 300 nm) of thymine glycol (TG) (1mM) in the absence and presence of aromatic amines (1mM) have been performed in aqueous solution at pH 7.0. The photoconversion of TG was quite inefficient without aromatic amines, but was remarkably promoted by the addition of the amines. The main products in the amine-sensitized photolysis were thymine (T) and 6-hydroxy-5,6-dihydrothymine (6HODHT). With the exception of *NNN'N'*-tetramethyl-*p*-phenylenediamine (TMPD), the amine-sensitized reaction of TG was quenched by a triplet quencher, biacetyl. The yield of T increased on decreasing the half-wave oxidation potential ($E^{ox}_{1/2}$) of the aromatic amines. The yield of 6HODHT in the photolysis with aniline increased on increasing the concentration of sodium formate added as a hydrogen-atom donor. The effects of $E^{ox}_{1/2}$ and sodium formate on the product yields were consistent with an intermediacy of 6-hydroxythymin-5-yl radicals (6HOT·) produced by reductive dehydroxylation of TG.

The reaction of pyrimidine bases with radicals produced by radiolysis of water, *i.e.* ·OH, H·, and e^-_{aq}, has been extensively investigated as a model for the radiation damage of nucleic acids.[1,2] Hydroxyl radicals add to C-5 of pyrimidine bases in preference to C-6, *e.g.* 65% (for thymine), 80% (for uracil), and 87% (for cystosine) of ·OH formed by γ-irradiation account for the addition reaction to C-5 of pyrimidine.[3–5] Recent pulse radiolysis experiments[3,4] and product analyses [6–8] have shown that 5-hydroxypyrimidin-6-yl radicals as the products of ·OH addition to C-5 are oxidized by nitro compounds or high-valent transition metal ions to 6-hydroxypyrimidine cations, which subsequently undergo either hydrolysis to form glycols or proton elimination to form isobarbituric acids.

In contrast to the 6-yl radicals, only a few reports[3,4] of the fate of 6-hydroxypyrimidin-5-yl radicals under deoxygenated conditions are available as the 6-yl radicals are simultaneously formed in higher yield than the 5-yl radicals in conventional radiolysis systems. In a previous paper we reported that radiolytic reduction of 5-bromo-6-hydroxy-5,6-dihydrothymine in aqueous solution leads selectively to the formation of 6-hydroxythymin-5-yl radicals (6HOT·), which subsequently undergo one-electron reduction by a low-valent transition metal ion such as Fe^{2+} to product T *via* the anion intermediate.[9]

In this work we performed the photoreaction of thymine glycol (TG; *cis*-5,6-dihydroxy-5,6-dihydrothymine) in aqueous solution by utilizing aromatic amines as sensitizers. A mechanism for the amine-sensitized photoreduction of TG with the formation of intermediate 6-hydroxythymin-5-yl radicals is discussed in terms of the effects of half-wave oxidation potential of amines and the addition of formate ion on the product distribution.

Experimental

Materials.—Thymine glycol (TG; *cis*-5,6-dihydroxy-5,6-dihydrothymine)[10] and 6-hydroxy-5,6-dihydrothymine (6HODHT)[11] were synthesized according to reported methods. Thymine (T), 5,6-dihydrothymine (DHT), and the aromatic amines aniline, *p*-toluidine, *o*- and *p*-anisidine, *o*- and *p*-phenylenediamine, and *NNN'N'*-tetramethyl-*p*-phenylenediamine (TMPD) were obtained from Nakarai and used without further purification. All the other reagents were of the best available grades. Predistilled water was purified by distillations from alkaline permanganate and then without additives.

Photoreaction.—Typically, an aqueous solution (5 ml) of TG (1mM) and an aromatic amine (1mM) adjusted to pH 7.0 ± 0.1 (phosphate buffer 10 mM) was purged with Ar for 20 min and sealed off. The solution in a Pyrex glass tube (cut-off wavelengths below 280 nm) was irradiated at room temperature with a merry-go-round apparatus equipped with a 400 W high-pressure mercury arc (Eiko-sha 400). Dark reaction of TG with aromatic amines was negligible under these conditions.

Reaction Quantum Yield.—Monochromatic light at 300 nm was isolated from a Philips high-pressure mercury arc (SP-500) using a JASCO model CT-25N grating monochromator. The intensity of the incident light was determined as 2.39×10^{-7} einstein ml^{-1} min^{-1} using a ferrioxalate actinometer ($\Phi_{Fe^{II}}$ 1.24 at wavelengths of 297—313 nm).[12] An aqueous solution (4 ml) of TG (1mM) and an aromatic amine in a quartz cell (1 cm path length) was purged with Ar, sealed off, and then irradiated for 20 min with the 300 nm mercury line at room temperature. The quantum yield (Φ) for the photoreaction was obtained as the ratio of the number of molecules converted or produced to that of quanta absorbed by aromatic amines over the irradiation.

H.p.l.c. Analysis.—The irradiated solution (100 μl) was subjected to h.p.l.c. analysis, using a Shimadzu model LC-3A high-performance liquid chromatograph equipped with an ODS-type column (4 mm × 15 cm) of Machery Nagel Nucleosil $5C_{18}$. For analysing photolysis products of TG, water containing 5 vol % methanol was delivered as the mobile phase at a flow rate of 0.8 ml min^{-1}. 40 or 70 vol % methanol–water was used for the analysis of amines. The eluants were monitored from the u.v. absorbance at 210 or 260 nm, using a Shimadzu model SPD-2A spectrophotometer.

Spectroscopic Measurement.—U.v. absorption spectra were recorded on a Shimadzu UV-200S spectrophotometer. Fluorescence spectra were measured by a JASCO FP-550A fluorescence spectrophotometer. The fluorescence lifetime of 50μM-TMPD in Ar-purged aqueous solution (pH 7.0) was determined by a single-photon time-coincidence method with a PRA 510B fluorometer and a Hitachi multichannel analyser.

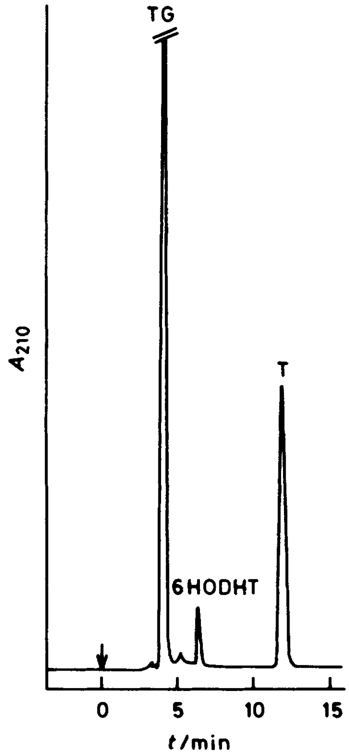


Figure 1. Representative h.p.l.c. chart of an irradiated TG solution with monitoring wavelength at 210 nm. An aqueous solution of TG (1 mM) irradiated with TMPD (1mM) for 45 min was subjected to h.p.l.c. Neither TMPD nor TMPD$^{+\bullet}$ was eluted under these conditions (see text)

The fluorescence decay was observed through a Fuji SC-42 filter, which cut off wavelengths below 408 nm and had 50% transmittance at 429 nm.

Results and Discussion

Product Analysis.—Figure 1 shows a representative h.p.l.c. chart of the irradiated solution when eluates were monitored by the u.v. absorbance at 210 nm. The major products were thymine (T) and 6-hydroxy-5,6-dihydrothymine (6HODHT) as confirmed by comparison of retention times with authentic samples. When eluates were monitored at 260 nm, only T was detected consistent with the u.v. spectral data that T has an absorption maximum at 265 nm with ε 7.9 × 10^3 l mol^{-1} cm^{-1}.[13] In contrast, 6HODHT has an absorption tail at wavelengths below 240 nm and negligibly small extinction coefficient at 260 nm.

The aromatic amines used or their photodegradation products were eluted with much longer retention times than that of T. Hence, we could evaluate the yields of T and 6HODHT by h.p.l.c.

Photolysis with TMPD.—Aqueous solutions of TG (1mM) were irradiated (λ_{ex} > 280 nm) without and with TMPD (1mM). Figure 2 shows variations in the concentrations of TG, TMPD, and products T and 6HODHT as a function of irradiation time. The conversion of TG was quite inefficient without TMPD (*ca.* 2% conversion on 120 min irradiation), as is expected from negligibly small extinction coefficient of TG at wavelengths above 280 nm, *e.g.* ε_{280nm} 10 l mol^{-1} cm^{-1} (see also Figure 4). It is also noted that no evidence for the formation of T or 6HODHT was obtained by h.p.l.c. in the photolysis without aromatic amines.

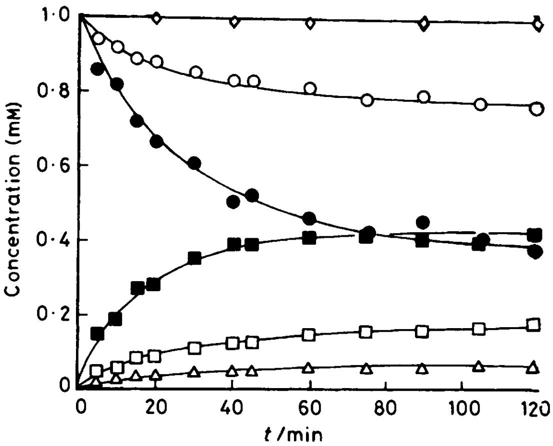


Figure 2. Variation of the concentrations of TG, TMPD, and products with irradiation time in the photolysis (λ_{ex} > 280 nm) of aqueous TG solution containing TMPD: ○, TG; □, T; △, 6HODHT; ●, TMPD; ■, TMPD$^{+\bullet}$. Concentration change in TG in the photolysis without TMPD is also shown with ◇

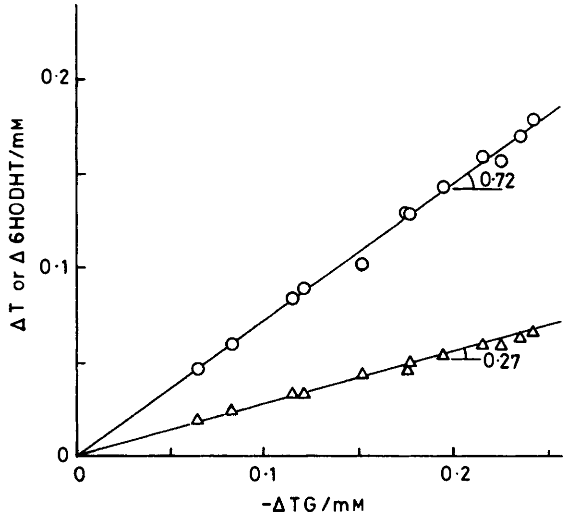
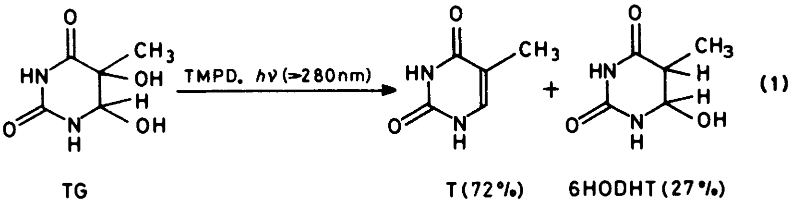


Figure 3. Relationship between the amount of TG decomposed (−ΔTG) and those of ○, T (ΔT) and △, 6HODHT (Δ6HODHT) produced in the photolysis of TG (1mM) with TMPD (1mM)

In contrast, the addition of TMPD (1mM) led to highly efficient conversion of TG (*ca.* 24% conversion over 120 min of irradiation) with formation of T and 6HODHT. The concentrations of products T and 6HODHT as well as that of TG varied in nonlinear fashion with irradiation time, but the selectivities of T (72%) and 6HODHT (27%) based on converted TG were constant throughout the reaction [Figure 3 and reaction (1)]. The decreased conversion rate of TG with irradiation time is largely attributable to an inner-filter effect of TMPD$^{+\bullet}$ formed by the irradiation as follows.

The initially colourless aqueous solution of TG and TMPD turned intense purple during irradiation, due to the formation of TMPD$^{+\bullet}$ which was confirmed by the characteristic u.v. absorption maxima,[14] $\lambda_{max.}$ 325, 565, and 610 nm (Figure 4). The concentration of TMPD$^{+\bullet}$ was determined from the u.v. absorbance at 565 nm using ε 1.25 × 10^4 l mol^{-1} cm^{-1},[3,15] and is plotted as a function of irradiation time in Figure 2. Since the absorption band of TMPD$^{+\bullet}$ centred at 325 nm overlaps the absorption tail of TMPD (Figure 4), it is apparent that



(1)

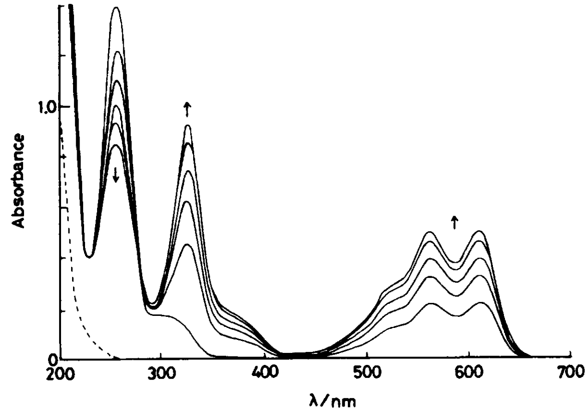
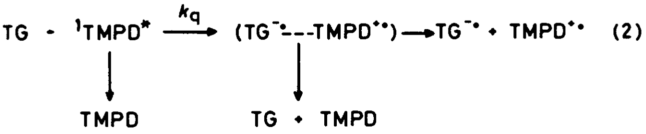


Figure 4. U.v. absorption spectra of aqueous TG solution irradiated with TMPD for 0, 10, 20, 30, 45, and 75 min. The spectra were measured after 10-fold dilution of the irradiated solutions. For comparison, u.v. spectrum of TG (0.1mM) is also shown with broken line

$TMPD^{+\cdot}$ exerts an inner-filter effect on the TMPD-sensitized photoreaction of TG. Moreover, the inner-filter effect is expected to be more pronounced with the accumulation of $TMPD^{+\cdot}$, thereby diminishing the light intensity effective for the excitation of TMPD [see reaction (2)].

The formation of $TMPD^{+\cdot}$ upon irradiation indicates strongly that reaction (1) is the result of a one-electron reduction of TG by photoexcited TMPD. Since the fluorescence of TMPD was quenched by TG with the Stern–Volmer constant of $14\ \mathrm{l\ mol^{-1}}$, electron transfer from excited singlet TMPD to TG is likely to occur in the initial step [reaction (2)].



$$\begin{tikzcd}
TG + {}^{1}TMPD^{*} \arrow[r, "k_q"] \arrow[d] & (TG^{-\cdot}\text{---}TMPD^{+\cdot}) \arrow[r] \arrow[d] & TG^{-\cdot} + TMPD^{+\cdot} \\
TMPD & TG + TMPD &
\end{tikzcd} \qquad (2)$$

By reference to the fluorescence lifetime of TMPD (5.1 ns), the quenching rate constant k_q was evaluated as $2.8 \times 10^{9}\ \mathrm{l\ mol^{-1}\ s^{-1}}$ which is in the diffusion-controlled limit. Similar electron-transfer quenching of triplet TMPD was unlikely in view of the quenching experiment with biacetyl (see Figure 5). On the other hand, laser-photolysis studies[16,17] have revealed that absorption of one photon by TMPD is enough to result in ionization to $TMPD^{+\cdot}$ *via* a semi-ionized or Rydberg state ($^{1}TMPD^{\dagger}$ in a polar solvent like water [reaction (3)]. We also

$$TMPD \longrightarrow {}^{1}TMPD^{\dagger} \longrightarrow TMPD^{+\cdot} + e^{-}_{aq} \qquad (3)$$

observed that photolysis ($\lambda_{ex} > 280$ nm) of TMPD (1mM) produces $TMPD^{+\cdot}$ even in the absence of TG. Thus, it may be possible that TG is reduced to $TG^{-\cdot}$ by way of the reaction with photoejected electron e^{-}_{aq}, similar to the γ-radiolysis in aqueous formate solution under deaerated conditions.* However, the failure of biacetyl to quench the reaction (see Figure 5) discounted the direct reaction of TG with e^{-}_{aq}. In view of the reactivity of e^{-}_{aq} towards TG as characterized in the radiolytic reduction system, this result also implies that the

Table 1. Quantum yields for TG conversion [Φ(−TG)] and T formation [Φ(T)] in the photolysis (λ_{ex} 300 nm) with aromatic amine[a]

Amine	Φ(−TG)	(% conversion)	Φ(T)	(% yield)[b]
Aniline	0.113	(18)	0.018	(16)
p-Toluidine	0.051	(19)	0.009	(18)
o-Anisidine	0.041	(11)	0.005	(12)
p-Anisidine	0.033	(16)	0.003	(9)
TMPD	0.014	(7)	0.007	(50)

[a] On irradiation in a quartz cell at room temperature, $[TG]_0 = [amine]_0 = 1.0$mM, pH 7.0, irradiation time 20 min. [b] Based on TG converted.

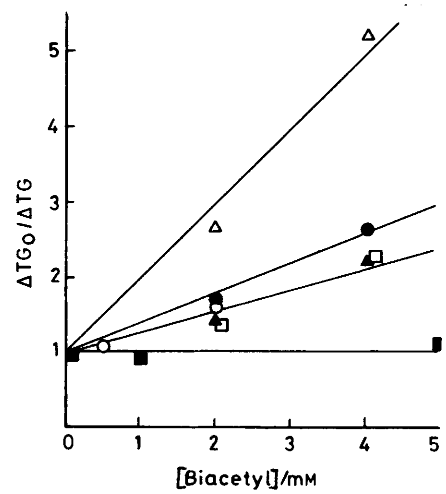


Figure 5. Stern–Volmer plots of TG conversion ($\Delta TG_0/\Delta TG$) from the photolyses (45 min) of TG (1mM)–amine (1mM)–biacetyl system, where ΔTG_0 and ΔTG designate the TG conversion in the absence and presence of biacetyl, respectively: ○, aniline; ●, *p*-toluidine; △, *o*-anisidine; ▲, *p*-anisidine; ■, TMPD; □, *p*-phenylenediamine

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* TG is reduced to T and 6HODHT upon γ-irradiation of a deaerated aqueous solution containing excess of sodium formate at pH 7.0 (S. Nishimoto, H. Ide, N. Otsuki, K. Nakamichi, and T. Kagiya, *J. Chem. Soc., Perkin Trans. 2*, 1985, 1127). Under these conditions, hydrated electron e^{-}_{aq} is responsible for the reduction of TG. In a separate experiment radiolytic reduction was found to be quenched by biacetyl as a good electron scavenger; the linear Stern-Volmer plot of TG conversion as in Figure 5 gave a quenching constant of $640\ \mathrm{l\ mol^{-1}}$. One of the referees is thanked for his suggestion of this quenching experiment.

Table 2. Photoreaction (λ_{ex} > 280 nm) of thymine glycol (TG) sensitized by aromatic amines[a]

Amine	$E^{Ox}_{1/2}$/V[b]	% Conversion TG	% Yield[c] T	% Yield[c] 6HODHT	6HODHT:T
Aniline	0.70	37	22	10	0.45
p-Toluidine	0.44	42	26	16	0.62
o-Anisidine	0.34	27	17	13	0.76
p-Anisidine	0.26	48	18	10	0.56
o-Phenylenediamine	0.06	29	59	33	0.56
TMPD	−0.14	18	72	27	0.39
p-Phenylenediamine	−0.16	32	71	26	0.37

[a] In aqueous solution at pH 7.0, $[TG]_0$ = $[amine]_0$ = 1.0mM, irradiation time 45 min. [b] Half-wave oxidation potential relative to Ag–0.1M-Ag$^+$ couple in acetonitrile.[19] [c] Based on TG converted.

quantum yield for e_{aq}^- [Φ(e_{aq}^-)] becomes negligibly small in the presence of 1mM-TG; the reported Φ(e_{aq}^-) values in the photolysis of TMPD in water[16] are 0.035 at λ_{ex} 347 nm and 0.070 at λ_{ex} 265 nm. It is therefore presumable that TG intercepts not only ^{1}TMPD* but also semi-ionized ^{1}TMPD† to produce TG$^{-\cdot}$ and TMPD$^{+\cdot}$, thereby diminishing the electron ejection.

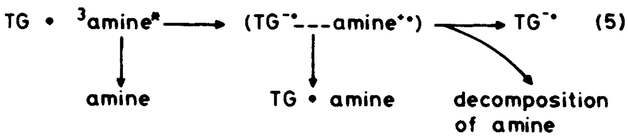
In order to clarify the TMPD-sensitized reduction of TG quantitatively, the quantum yield for the TG conversion [Φ(−TG)] was also determined. For this purpose an aqueous solution of TG (1mM) and TMPD (1mM) was irradiated for 20 min with a 300 nm mercury line (see also Experimental). Under conditions of 7% TG conversion for the TMPD-sensitized system (Table 1) the inner-filter effect of TMPD$^{+\cdot}$ was essentially negligible. Thus, we obtained a much smaller Φ(−TG) value with TMPD, 0.014, as listed in Table 1. This result indicates that the quenching of ^{1}TMPD* or ^{1}TMPD† by TG may involve a non-electron-transfer process as well as electron transfer.

Photolysis with Various Aromatic Amines.—In order to clarify mechanistic details of the reductive conversion of TG into T and 6HODHT, we performed further photolyses (λ_{ex} > 280 nm) of TG (1mM) in aqueous solution at pH 7.0 in the presence of various aromatic amines (1mM).

Including TMPD, all the aromatic amines examined were found to be effective for promoting the conversion of TG, while their promoting efficiencies were quite different (Table 2). Similar results were also obtained for the photolysis with monochromatic light at λ_{ex} 300 nm (Table 1). With the exception of using TMPD, these amine-sensitized photoreactions were quenched by the addition of biacetyl, a typical triplet quencher (E_T 234 kJ mol^{-1}),[18] and linear Stern–Volmer plots were obtained with varying concentrations of biacetyl up to 5mM (Figure 5).‡ Since Stern–Volmer constants for the fluorescence quenching of the amines (0—20 l mol^{-1}) were one order of magnitude smaller than those for quenching of the sensitized photoreaction (280—800 l mol^{-1}), the interception of excited singlet amines by biacetyl would be minor under these conditions.

Based on the above quenching experiments and results in the photolysis with TMPD, we concluded that the reactions of TG in the presence of TMPD and the other aromatic amines are initiated by one-electron reduction to form a common intermediate TG$^{-\cdot}$ with different mechanisms. Thus, electron-transfer quenching by TG of excited singlet TMPD, ^{1}TMPD* and/or ^{1}TMPD† results in TG$^{-\cdot}$, whereas that of the excited

$$\text{amine} \xrightarrow{(\lambda_{ex} > 280\,\text{nm})} {}^1\text{amine}^* \xrightarrow{\text{i.s.c.}} {}^3\text{amine}^* \qquad (4)$$



triplet state of the other amines (^{3}amine*) accounts for TG$^{-\cdot}$ formation. Failure to obtain evidence for the formation of amine$^{+\cdot}$ *via* reaction (5) suggests that compared with TMPD$^{+\cdot}$ the cation radicals are very unstable in water.

The Φ(−TG) values (0.113—0.033) for the sensitization of aniline, *p*-toluidine, *o*- and *p*-anisidine were greater than that (0.014) for the TMPD-sensitized system (Table 1). These results seem to be associated with the stability of the intermediate cation radicals. The rapid decomposition of amine$^{+\cdot}$ will depress the reverse electron-transfer from TG$^{-\cdot}$ to amine$^{+\cdot}$, thereby facilitating TG$^{-\cdot}$ formation [reaction (5)]. Accordingly, the variation of Φ(−TG) with amines reflects not only the differences in population of the triplet state (^{3}amine*) and the triplet life-time but also the stability of amine$^{+\cdot}$ in water.

Mechanism of Product Formation.—Although considerable scatter was noted for the TG conversion in the photolysis with amines at λ_{ex} > 280 nm, the yield of T based on converted TG was strongly dependent on the half-wave oxidation potential ($E^{Ox}_{1/2}$ *versus* Ag–0.1M-Ag$^+$ couple in acetonitrile) of amines.[19] Thus, the yield of T increased with decreasing the $E^{Ox}_{1/2}$ value (Table 2, Figure 6). A similar trend was also observed for the photolysis at λ_{ex} 300 nm (Table 1). The characteristic $E^{Ox}_{1/2}$ dependence suggests that an intermediate species, possibly derived from TG$^{-\cdot}$, is involved in these reaction systems and undergoes one-electron reduction by ground-state aromatic amines as a key reaction leading to T.

We have demonstrated[9] that 5-bromo-6-hydroxy-5,6-dihydrothymine (BrHODHT) undergoes reductive debromination to give 6-hydroxythymin-5-yl radical (6HOT$^\cdot$) selectively [X = Br in reaction (6)]. The resulting 6HOT$^\cdot$ can be converted into T *via* one-electron reduction by a low-valent transition metal ion such as Fe^{2+} [reaction (7)]. In this light, a mechanism for the conversion of TG$^{-\cdot}$ into T in the presence of aromatic amines is proposed as follows: the TG$^{-\cdot}$ first liberates OH$^-$ to produce 6HOT$^\cdot$ [reaction (6; X = OH)], and consecutively the one-electron reduction of the 6HOT$^\cdot$ by ground-state aromatic amines leads to T *via* the anion intermediate (6HOT$^-$) [reaction (7)]. The efficiency of the 6HOT$^-$ formation in reaction (7) may be dependent on reducing ability of the aromatic amines as measured by the $E^{Ox}_{1/2}$ value. According to this mechanism, the conversion of TG into T involves, on the

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‡ Quenching experiments for the photoreaction sensitized by *o*-phenylenediamine could not be performed due to the dark reaction of the amine with biacetyl.

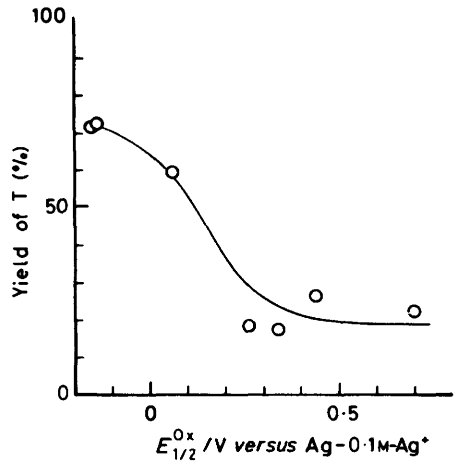
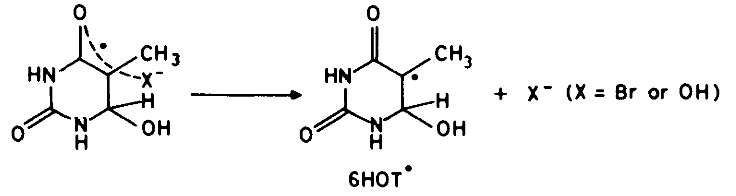


Figure 6. Dependence of the T yield on the half-wave oxidation potential ($E^{ox}_{1/2}$ *versus* Ag–0.1M-Ag$^+$ couple) of aromatic amines. Data points are based on the values in Table 2

whole, two one-electron reductions, *i.e.* (i) one-electron reduction of TG by excited aromatic amines [reactions (2) and (5)] and (ii) that of 6HOT$^•$ by ground-state aromatic amines [reaction (7)]. Further support for the participation of aromatic amines in the reaction (7) is the fact that 6HOT$^•$ produced by $^•$OH addition to C-6 of thymine oxidizes TMPD to TMPD$^{+•}$.[3] The significant increase in the yield of TMPD$^{+•}$ in TG–TMPD system, compared with a control system without TG, can also be rationalized by the formation of TMPD$^{+•}$ *via* reaction (7) in addition to reaction (2).



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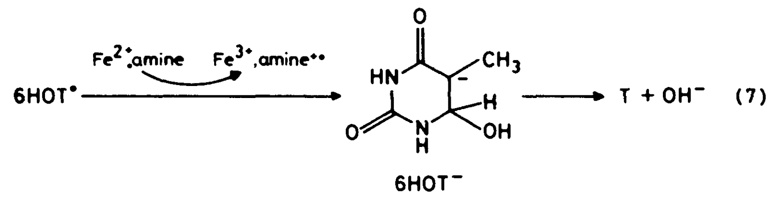

(7)

In competition with the reduction of 6HOT$^•$ to 6HOT$^-$ by aromatic amines the autoreaction of 6HOT$^•$ can occur, although the resulting products have not yet been characterized. In such a competition, the reduction to 6HOT$^-$ will become more important on decreasing the $E^{ox}_{1/2}$ value of amines. Furthermore, the formation of T *via* dehydration of 6HODHT is minor under the present conditions, since the 6HODHT was very stable with half-life time of *ca.* 8 days at pH 7.0 (24 °C). This is also the case at pH 3.0 as shown previously.[9]

The yield of 6HODHT also increased slightly on decreasing the $E^{ox}_{1/2}$ value (Table 1). This result cannot be explained in terms of the possible protonation of 6HOT$^-$, as shown in our previous experiments with BrHODHT,[9] *i.e.* protonation of 6HOT$^-$ is negligible even at pH 3.0 and the elimination of OH$^-$ is favoured [reaction (7)]. Another rationale for discounting the protonation process of 6HOT$^-$ is that the ratio of the yields of 6HODHT and T (6HODHT:T) was not constant but varied with the amine (Table 2). If both 6HODHT and T were formed from a common intermediate 6HOT$^-$, 6HODHT:T would be independent of the amine used. Consequently, 6HODHT is presumable to be produced by hydrogen-atom abstraction of 6HOT$^•$ from appropriate hydrogen-atom donors like aminyl radicals which could be formed *via* proton elimination from amine$^{+•}$. Further studies will be needed for an understanding of amine-dependent formation of 6HODHT.

Table 3. Effect of varying concentrations of formate ([HCO_2^-]) on the photolysis (λ_{ex} > 280 nm) of TG with aniline[a]

[HCO_2^-]/mM	% Conversion of TG	% Yield of product[b] T	6HODHT
0	37	22	10
1	35	22	10
010	40	22	12
100	38	17	31
200	41	13	48
500	45	6	71
1 000	45	4	90

[a] In aqueous solution at pH 7.0, $[TG]_0$ = $[aniline]_0$ = 1.0mM, irradiation time 45 min. [b] Based on TG converted.

In contrast to TG, product T did not undergo reduction to 5,6-dihydrothymine (DHT) during the photolysis of TG (1mM) with TMPD (1mM) at λ_{ex} > 280 nm, *e.g.* 76% of TG was converted with a 64% yield of T upon irradiation for 10 h. As characterized previously in the radiolysis of aqueous formate solutions, T reacts readily with e^-_{aq} to produce DHT *via* disproportionation of dihydrothymyl radicals.[20] Thus, the inert behaviour of T in the TMPD-sensitized photoreaction system is also consistent with the conclusion that photoejected electron is not involved in the reduction of TG to TG$^{-•}$.

Effect of Formate Ion as Hydrogen-atom Donor.—In order to obtain further evidence for the formation of 6HOT$^•$ *via* reaction (6), we studied the effect of sodium formate, as a hydrogen-atom donor, on the amine-sensitized photoreaction of TG. It is well known that formate ions donate a hydrogen atom to $^•$OH and $^•$H and produce H_2O and H_2, respectively. For this purpose we chose aniline which has a higher $E^{ox}_{1/2}$ value (0.7 V) as a sensitizer, by which the undesirable reduction of 6HOT$^•$ to 6HOT$^-$ [reaction (7)] could be minimized. Thus, the addition of sodium formate increased the yield of 6HODHT up to 90% ([HCO_2^-] 1M), without changing the TG conversion to a significant extent. At the same time the yield of T was decreased to 4% (Table 3). The increased yield of 6HODHT with increasing [HCO_2^-] clearly indicates the formation of 6HOT$^{\bullet}$, which can be converted into 6HODHT by hydrogen-atom abstraction from HCO_2^- [reaction (8)]. The decreased yield of

$$6HOT^{\bullet} + HCO_2^- \longrightarrow 6HODHT + CO_2^{-\bullet} \qquad (8)$$

T is also consistent with the efficient conversion of 6HOT$^{\bullet}$ into 6HODHT, which may prevent the reductive conversion of 6HOT$^{\bullet}$ into $6HOT^-$ as a precursor of T.

Acknowledgements

We thank Dr. S. Ito for the fluorescence lifetime measurement.

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Received 30*th July* 1984; *Paper* 4/1334