On the Repair of Thymine and Thymidine Bromohydrins: A Possible New Model for the Damage and Repair of Nucleic Acids

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Bromohydrins (2, 3, 4, and 5), oxidatively damaged products of thymine bases, were repaired on exposure to sunlight, heat, and/or some reagents to regenerate the thymine bases. A radical mechanism is proposed for the repair reaction with sunlight and heat. A hypothesis concerning the biological significance of thymidine in deoxyribonucleic acid is presented.

Keywords oxidatively damaged product; bromohydrin; repair reaction; sunlight; heat; thymine base; radical mechanism

Oxidative damage to nucleic acids and related compounds caused by active oxygen species, such as hydrogen peroxide, hydroxy radical, superoxide anion, singlet oxygen, and lipid hydroperoxides, has recently been receiving much attention, as it may play a role in mutagenesis, carcinogenesis, and aging.^{1–6)} In relation to the oxidative damage to nucleic acids and components, it has been revealed that *cis*-thymine and thymidine diols, oxidized thymidine derivatives, are released in human and rat urine as a result of the repair of oxidatively damaged deoxyribonucleic acid (DNA).⁷⁾ Moreover, it has recently been reported that haloperoxidases such as chloroperoxidase and bromoperoxidase obtained from living cells^{8–12)} activated halide anion to the halonium cation in the presence of hydrogen peroxide¹³⁾ and catalyzed the halogenation of nitrogen-containing aromatic heterocycles such as pyrazole, uracil, thymine, and cytosine to yield the respective halogenated (oxidized) products. Chloroperoxidase, in particular, catalyzed the oxidation of thymine (1) to thymine bromohydrin (2) in the presence of potassium bromide and hydrogen peroxide.¹⁴⁾ This finding strongly suggests that thymidine in living cells can be oxidized to thymidine bromohydrin with the aid of haloperoxidase. In spite of many investigations of oxidative damage to nucleic acids, there has been little study on the repair of oxidatively damaged nucleic acids from the viewpoint of organic chemistry. Therefore, we have investigated the repair of nucleosides subjected to oxidative damage,¹⁵⁾ especially thymine and thymidine bromohydrins, as model compounds of oxidatively damaged nucleic

acids. We have briefly described the results.¹⁶⁾ The details are the subject of this paper.

During our studies on the reactions of 1,3-dimethylthymine and thymidine epoxides with amines, L-amino acid derivatives, and other nucleophiles as model reactions for nucleic acid–protein cross-links,^{17–20)} we observed that heating of the bromohydrins (3, 4, and 5) in tetrahydrofuran (THF) with nucleophiles such as thiophenol, *N*-acetyl-L-cysteine, and L-ascorbic acid often gave thymine derivatives (6, 7, and 8), corresponding to the repaired products. However, the reproducibility of the reaction was very poor. After much study, we found that the repair reaction proceeded without any reagent upon exposure of the compounds to either sunlight or heat. First, a suitable solvent for the repair reaction of the bromohydrin (3) was examined under the reaction conditions indicated in Table I. The results in Table I show that THF seems to be the most suitable solvent for this purpose. Therefore, using THF as a solvent, the repair reaction of bromohydrins (3, 4, and 5) with sunlight or heat was investigated. The results are summarized in Table II. As can be seen from Table II, the reaction proceeded with sunlight (3400 lux) or heat (entries 1 and 4), but not in the dark at room temperature. However, on adding a catalytic amount of azobisisobutyronitrile (AIBN), a radical initiator, the reaction in the dark at room temperature proceeded smoothly (entry 5) and that under sunlight was slightly accelerated (entry 2). The results also showed that galvinoxyl, a radical scavenger, could quench the reaction, with the starting material being recovered in good yield (entries 3 and 6). These results show that the repair reaction proceeds by a radical mechanism.^{21–23)} No epimerization of β -thymidine (β -7) to α -thymidine (α -7) and/or 8 occurred under the reaction conditions, indicating that α -thymidine (α -7) and 8 would be produced

Fig. 1

TABLE I. Data on the Repair Reaction of the Bromohydrin (3) in Various Solvents

Entry	Solvent	Reaction temp.	Reaction time	(6) (%)	Recovery (%)
1	DMSO	130 °C	30 min	73.0	ca. 13
2	Diglyme	130 °C	45 min	88.4	—
3	THF	50 °C	1 h	95.6	—
4	Dioxane	Reflux	21 h	84.9	—
5	CCl ₄	Reflux	20 h	25.2	ca. 47
6	Benzene	Reflux	20 h	7.2	ca. 59

Reaction conditions: addition of 1% AIBN under room light (70 lux).

TABLE II. Data on the Repair Reaction of Bromohydrins (3, 4, and 5)^{a)}

Entry	Conditions ^{b)}	Reaction time (h)	3		Reaction time (h)	4		Reaction time (h)	5		Reaction time (h)	6	
			6 (%)	Recovery (%)		7 (%)	8 (%)		7 (%)	8 (%)		7 (%)	Recovery (%)
1	A	2.5	81.7	—	1.5	42.7 (1:4.1)	16.1 (β) ^{c)}	—	1.0	32.7 (1:2.8)	8.1 (1:1.4)	—	—
2	A, AIBN	1.0	89.3	—	1.0	37.0 (1:4.0)	13.0 (β) ^{c)}	—	1.0	34.9 (1:3.0)	8.9 (1:1.7)	—	—
3	A, galvinoxyl	17.0	—	66.6	17.0	—	—	92.5	22.0	—	—	—	84.2
4	B	10.0	92.4	—	3.0	59.8 (1:2.4)	13.1 (β) ^{c)}	—	3.0	66.8 (1:1.7)	22.4 (1:1.5)	—	—
5	C, AIBN	3.0	Quant.	—	4.0	50.0 (1:2.6)	18.2 (β) ^{c)}	—	6.0	49.5 (1:2.9)	4.7 (β) ^{c)}	—	—
6	B, galvinoxyl	10.0	—	81.0	6.0	—	—	90.0	8.0	—	—	—	91.7

a) Values in parentheses indicate the proportions of α - and β -isomers. Isomers were isolated by preparative TLC in entry 1. In other entries, proportions of α and β were determined by NMR spectroscopy. b) Reaction conditions: A; under sunlight irradiation at room temperature, B; in the dark under reflux, C; in the dark at room temperature. c) A trace amount of α -isomer (α -8) was present.

Fig. 2

concomitantly when the bromohydrins (4 and 5) were converted to thymidines (7 and 8). Furthermore, this repair reaction seems to be characteristic of bromohydrins having an aminor (α -carbinol amine) moiety, because exposure of the *trans*-bromohydrin (9)²⁴⁾ to the same reaction conditions resulted in recovery of the starting material (9) quantitatively.

Subsequently, the repair reaction of thymine bromohydrin (2) to thymine (1) was investigated, the results being summarized in Table III. The repair reaction of 2, as well as the bromohydrins (3, 4, and 5), with sunlight proceeded smoothly (entries 1 and 2), whereas the reaction of 2 in THF with heat scarcely proceeded (entries 3 and 4). The repair reaction with heat finally proceeded in dimethyl sulfoxide (DMSO) in the dark at 130 °C in the presence of a catalytic amount of AIBN (entry 5). The difference of reactivity between the bromohydrins 2 and 3, 4, and 5 seems to be attributable to the presence of the N-1 substituent. Therefore, we planned to synthesize bromohydrins of 1-methylthymine (10)²⁵⁾ and 3-methylthymine (11)²⁶⁾ and to investigate their repair reactions. However, the desired bromohydrins could not be isolated because of their instability.

Next, the repair reaction of the bromohydrin (3) with

TABLE III. Data on the Repair Reaction of Thymine Bromohydrin (2)

Entry	Reaction conditions ^{a)}	Reaction time (h)	(1) (%)	Recovery (%)
1	A	4.5	82.3	—
2	A, AIBN	2.5	93.2	—
3	B	23.0	—	Quant.
4	B, AIBN	14.0	—	Quant.
5	C, AIBN	0.8	72.9	—

a) Reaction conditions: A; under sunlight irradiation at room temperature, B; in the dark under reflux, C; DMSO was used instead of THF in the dark at 130 °C.

TABLE IV. Data on the Repair Reaction of the Bromohydrin (3) with Heat and Reagents

Reagent	Equivalent	Reaction time (h)	(6) (%)
—	—	10–31	92–96
<i>N</i> -Ac-L-Cys-OMe	1.2	0.8	95
<i>N</i> -Ac-L-Cys	1.2	1.5	97
PhSH	2.4	2.3	96
Ascorbic acid	1.2	2.0	96
Ph ₃ P	1.2 + 1.2 ^{a)}	5.0	88

a) The reagent (1.2 eq) was added after 1 h.

TABLE V. Data on the Repair Reaction of the Bromohydrin (3) with Sunlight and Reagents

Reagent	Equivalent	Reaction time (h)	(6) (%)
—	—	2.5–6.5	81–96
<i>N</i> -Ac-L-Cys-OMe	1.2	6.0	81
<i>N</i> -Ac-L-Cys	1.2	6.5	87
PhSH	2.4	2.5	97

other reagents, especially biological compounds was examined. The results are summarized in Tables IV and V, indicating that addition of reagents affected the repair reaction in THF under heating in the dark (Table IV),²⁷ but did not affect the reaction under sunlight (Table V), although the reason for this remains to be clarified.

In Fig. 2, we propose a plausible mechanism for the repair reaction of the bromohydrin (4), initiated by radical scission of the C₅-Br bond. Thymidine having a vinylogous amide moiety is subject to oxidation, including halogenation, catalyzed by enzymes such as haloperoxidases as well as oxidants such as active oxygens to produce oxidized (damaged) thymidine derivatives such as thymidine diol and bromohydrin possessing an aminor moiety. These oxidized thymidine derivatives, especially thymidine bromohydrins, can be repaired by exposure to sunlight, heat, and/or other agents, regenerating the thymidines. We think that thymidine may protect other compounds *in vivo* by being oxidized (halogenated) itself and then readily undergoing repair. We consider that thymidine plays an important role, functionally and structurally, in the damage and repair process of nucleic acids.

Experimental

The proton nuclear magnetic resonance (¹H-NMR) spectra were obtained in (CD₃)₂SO-*d*₆O at 200 MHz on a JEOL FX 200 spectrometer with chemical shifts being reported in parts per million downfield from a tetramethylsilane internal standard, and coupling constants in hertz. Mass spectra (MS) were taken on a JEOL JMS 01SG-2 instrument (direct inlet) at 70 eV. Preparative thin layer chromatography (TLC) was run on 20 × 20 cm plates coated with a 0.25 mm layer of Merck silica gel PF₂₅₄ and GF₂₅₄, using chloroform containing 4% methanol (solvent A) and ethyl acetate–propan-2-ol–water (75:16:5) (solvent B). A sunlight lamp (Toshiba, DR 250/TL, about 3400 lux) was used for the repair reaction. All reactions were carried out under an argon atmosphere unless otherwise stated. The reaction mixture was concentrated to dryness under reduced pressure. The residue was purified or separated by preparative TLC using solvent A (method A) or solvent B (method B). α -Thymidine (α -7) was purchased from Sigma Chemical Company and β -thymidine (β -7) from Wako Pure Chemical Industries, Ltd.

General Procedures for the Repair Reaction of the Bromohydrin (3) with Heat in Various Solvents (Table I) A solution of the bromohydrin (3) (1 mmol) with AIBN (0.01 mmol) in a dry solvent (20 ml) was heated under room light (about 70 lux) at the temperature indicated in Table I. The residue was purified by method A to give 1,3-dimethylthymine (6), mp 153–155°C (needles from EtOH) (entries 2, 3, and 4), or 1,3-dimethylthymine (6) from the upper zone and the starting material (3) from the lower zone (entries 1, 5, and 6). Yields are given in Table I.

The Repair Reaction of Bromohydrins (3, 4, and 5) with Sunlight (Entry 1 in Table II) A solution of a bromohydrin (1 mmol) in dry THF (20 ml) was irradiated with a sunlight lamp at room temperature for the time indicated in Table II. Purification of the residue from 3 by method A gave 1,3-dimethylthymine (6). Separation of the residues from 4 and 5 by method B gave β -thymidine (β -7) from the first zone (highest zone), α -thymidine (α -7) from the second zone, β -8 from the third zone, and α -8 from the fourth zone (lowest zone). Yields and ratios are indicated in Table II. The compounds (6, α -7, and β -7) were identified by comparison

with authentic samples. 1-(2-Deoxy- β -D-erythro-pentopyranosyl)thymine (β -8), mp 218–220°C (from isopropyl alcohol)²⁸; ¹H-NMR (*d*₆-DMSO) δ : 1.77 (3H, d, *J* = 1.2 Hz, C₅-CH₃), 2.01–2.16 (2H, m, C₂-H₂), 3.57–3.66 (3H, brs, C₄-H, C₅-H₂), 3.99 (1H, brs, C₃-H), 5.84 (1H, dd, *J* = 11.2, 2.1 Hz, C₁-H), 7.57 (1H, q, *J* = 1.2 Hz, C₆-H). MS Calcd for C₁₀H₁₄N₂O₅: 242.09026. Found: 242.09033. 1-(2-Deoxy- α -D-erythro-pentopyranosyl)thymine (α -8) mp 209–213°C (from isopropyl alcohol)²⁸; ¹H-NMR (*d*₆-DMSO) δ : 1.78 (3H, d, *J* = 1.2 Hz, C₅-CH₃), 1.88–2.08 (2H, m, C₂-H₂), 3.45–3.86 (4H, m, C₃-H, C₄-H, C₅-H₂), 5.50 (1H, dd, *J* = 11.2, 2.1 Hz, C₁-H), 7.56 (1H, q, *J* = 1.2 Hz, C₆-H). MS Calcd for C₁₀H₁₄N₂O₅: 242.09026. Found: 242.08967.

The Repair Reaction of Bromohydrins (3, 4, and 5) with Sunlight and AIBN (Entry 2 in Table II) A solution of a bromohydrin (1 mmol) and AIBN (0.01 mmol) in dry THF (20 ml) was irradiated with a sunlight lamp at room temperature for the time indicated in Table II. The residue from 3 was purified by method A to give 1,3-dimethylthymine (6). The residues from 4 and 5 were separated by method B to give a mixture of α - and β -thymidines (7) from the upper zone and a mixture of α - and β -8 from the lower zone. Yields are given in Table II. The proportions of α - and β -7, and α - and β -8 were determined from the C₆-H integral values in ¹H-NMR spectroscopy.

The Reaction of Bromohydrins (3, 4, and 5) with Sunlight and Galvinoxyl (Entry 3 in Table II) A solution of a bromohydrin (1 mmol) and galvinoxyl (1.2 mmol) in dry THF (20 ml) was irradiated with a sunlight lamp at room temperature for the time indicated in Table II. The residue was purified by method A for bromohydrin (3) and by method B for bromohydrins (4 and 5) to recover the starting materials (3, 4, and 5, respectively). Recovery yields are given in Table II.

The Repair Reaction of Bromohydrins (3, 4, and 5) with Heat (Entry 4 in Table II) A solution of a bromohydrin (1 mmol) in dry THF (20 ml) was refluxed in the dark for the time indicated in Table II. Each residue was purified in the same manner as for entry 3 in Table II mentioned above. Yields are given in Table II.

The Repair Reaction of Bromohydrins (3, 4, and 5) with AIBN at Room Temperature (Entry 5 in Table II) AIBN (0.01 mmol) in dry THF (1 ml) was refluxed for 1 min, then cooled, and a solution of a bromohydrin (1 mmol) in dry THF (19 ml) was added. The mixture was stirred at room temperature in the dark for the time indicated in Table II. Each residue was purified in the same manner as for entry 3 in Table II mentioned above. Yields are given in Table II.

Reaction of Bromohydrin (9) with Room Light and Heat A solution of the bromohydrin (9) (0.1 mmol) in dry THF (3 ml) was refluxed under room light for 10 h. The solvent was removed under reduced pressure to recover the starting material (9) quantitatively.

General Procedure for the Repair Reaction of Thymine Bromohydrin (2) (Table III) A solution of the bromohydrin (2) (1 mmol) or the bromohydrin (2) (1 mmol) with AIBN (0.01 mmol) in dry solvent (20 ml) was stirred under the reaction conditions indicated in Table III. The residue was washed three times with ether–acetone (10:1) by centrifugation (3000 rpm × 5 min) to afford thymine (1) in the yields given in Table III (entries 1, 2, and 5) or to afford the starting material (2) quantitatively (entries 3 and 4).

General Procedures for the Repair Reaction of the Bromohydrin (3) with Heat and Reagents (Table IV) A solution of the bromohydrin (3) (1 mmol) and a reagent [1.2, 2.4 or 1.2 + 1.2 (1.2 mmol of reagent was added first and a further 1.2 mmol of reagent was added after 1 h) mmol] in dry THF (20 ml) was refluxed in the dark for the time indicated in Table IV. The residue was purified by method A to afford 1,3-dimethylthymine (6). Yields are given in Table IV.

General Procedures for the Repair Reaction of the Bromohydrin (3) with Sunlight and Reagents (Table V) A solution of the bromohydrin (3) (1 mmol) and a reagent (1.2 or 2.4 mmol) in dry THF (20 ml) was irradiated with a sunlight lamp at room temperature for the time indicated in Table V. The residue was purified by method A to afford 1,3-dimethylthymine (6). Yields are given in Table V.

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