The Repair of Thymine and Thymidine Bromohydrins, Models of Damaged Nucleic Acids, by a Copper(II) and Ascorbic Acid System

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Bromohydrins (**1**), (**3**), (**4**), and (**5**), damaged products of thymine derivatives, were repaired smoothly to regenerate the original thymine derivatives when exposed to ascorbic acid in the presence of a catalytic amount of Cu^{2+} in water at room temperature.

The oxidation of nucleic acids and related compounds by active oxygen species has been the focus of attention for the past decade.[1—6] These species are suspected of playing a role in mutagenesis, carcinogenesis, and aging. In relation to the oxidative damage of nucleic acids and components, a recent study has shown that haloperoxidase obtained from *Caldariomyces fumago* can catalyse the oxidation of thymine into thymine bromohydrin in the presence of potassium bromide and hydrogen peroxide.[7] This finding suggests that thymidine in living cells can be oxidized to thymidine bromohydrin with the aid of haloperoxidases. In spite of many investigations on the oxidative damage of nucleic acids, there has been little study on the repair of the oxidatively damaged nucleic acids from the viewpoint of organic chemistry. Therefore we have aimed at the repair of thymine and thymidine bromohydrins as model compounds of oxidatively damaged nucleic acids.

We have already reported that thymidine bromohydrin was repaired *via* the intermediate (A) by sunlight or heat in tetrahydrofuran (THF),[8] but this reaction hardly proceeded at all in water. Since this repair reaction included a radical mechanism,[8] we have tried the use of transition metals known to perform electron transfer in biological systems. We now report the repair of the bromohydrins by the action of ascorbic acid (AA) and copper(II).

We explored the repair of 1,3-dimethylthymine bromohydrin (**1**) with metals (Cu, Co, Fe, Mo, or Mn), which are known to participate in redox reactions in living cells. In a typical procedure, a suspension of (**1**), $CuSO_4$, and AA in water was stirred under argon atmosphere at room temperature. The product (**2**) was isolated by preparative t.l.c. The results showed that the repair reaction did not occur with Co, Fe, Mo, Mn, or AA and the respective metal ions.† However, as can be seen from Table 1, the reaction proceeded rapidly with 2.4 equiv. of $CuSO_4$ and AA (Entry 1), and proceeded even with a catalytic amount of $CuSO_4$ with 2.4 equiv. of AA (Entry 4). However, it did not proceed when AA or $CuSO_4$ alone was used (Entry 5 and 6). The repair reaction also proceeded smoothly within 5 min with AA and $Cu(OAc)_2$ which contained a counter anion other than SO_4^{2-} (Entry 7).

Table 1. The repair reaction of (**1**) by the Cu^{2+}–ascorbic acid (AA) system.

Entry	$CuSO_4$ (equiv.)	AA (equiv.)	Reaction time	TMU (**2**) %	Recovery %
1	2.4	2.4	5 min	99.0	—
2	1.2	2.4	14 h	73.6	9.2
3	0.5	2.4	21 h	72.3	13.2
4	0.1	2.4	36 h	64.5	21.0
5	—	2.4	36 h	—	quant.
6	2.4	—	36 h	—	quant.
7[a]	2.4	2.4	5 min	80.4	—

[a] $Cu(OAc)_2$ was used instead of $CuSO_4$.

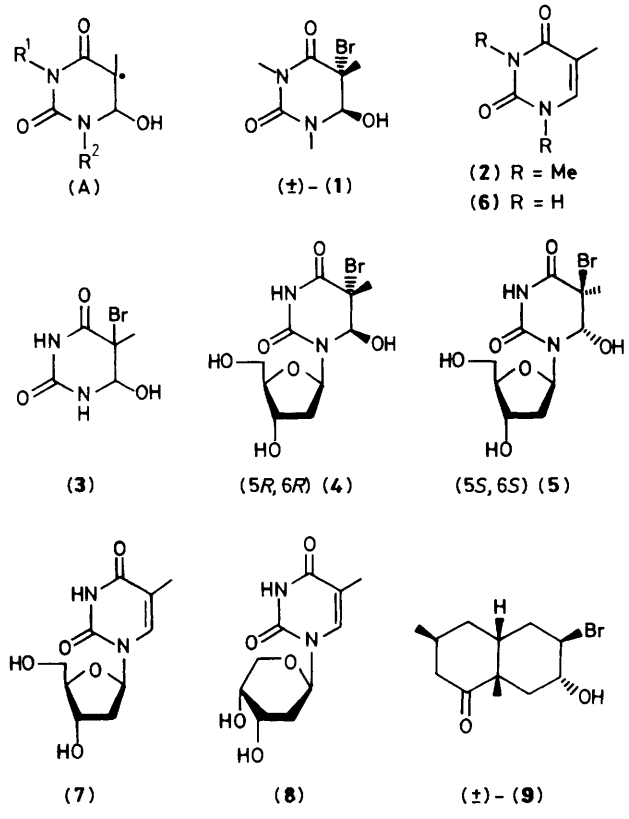
† $CoCl_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $FeCl_3$, MoS_2, MoO_3, $MnSO_4 \cdot 4$—$5H_2O$, and $Mn(acac)_3$ were used (acac = acetylacetonato).

Table 2. The repair reaction of (**1**) by Cu^{+}.

Entry	CuCl (equiv.)	AA (equiv.)	Reaction time	TMU (**2**) %	Recovery %
1	2.4	2.4	20 h	89.3	4.4
2	2.4	—	36 h	74.3	10.6

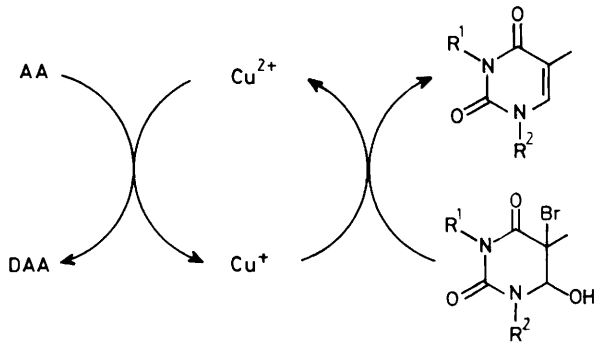
Table 3. The repair reactions of (**3**), (**4**), (**5**), and (**7**) by the Cu^{2+}–AA system.

Starting material	$CuSO_4$ (equiv.)	AA (equiv.)	Reaction time	(**7**) %	(**8**) %	Thymine %
(**3**)	2.4	2.4	5 min	—	—	92.9
(**4**)	2.4	2.4	5 min	61.9	14.0	15.1
(**5**)	2.4	2.4	5 min	57.5	16.8	14.3
(**7**)	2.4	2.4	2 h	90.7	—	8.3



To examine the active species of this reaction, we used CuCl instead of Cu^{2+}–AA (Table 2). Table 2 shows that the reaction proceeded *via* either Cu^{+} alone or Cu^{+}–AA together, but was slower than that with Cu^{2+}–AA. Moreover, water-soluble Cu^{+} is unstable in water and leads immediately to disproportionation to Cu and Cu^{2+}.[9] Since the reaction with Cu^{2+}–AA was very fast, it was thought that the active species was fresh Cu^{+} generated *in situ* in the media. DNA and nucleic acid bases are known to be damaged by Cu^{2+}, AA, and O_2,[10–18] where a hydroxyl radical is thought generally to be the active species. To determine whether the active species of this repair reaction is the hydroxyl radical or not, the reaction of (**1**) with Fenton's reagent ($FeSO_4$–H_2O_2) was examined. Since there was no repaired product (**2**) at all, the hydroxyl radical was excluded as an active species in the repair reaction by Cu^{2+}–AA.

Next, we investigated the repair reaction of thymine bromohydrin (**3**), (5*R*,6*R*)-thymidine bromohydrin (**4**), and



Scheme 1

(5*S*,6*S*)-thymidine bromohydrin (**5**), which might be present in living cells as oxidatively damaged products. Thymine bromohydrin (**3**) gave thymine (**6**) in high yield, and thymidine bromohydrins (**4**) and (**5**) gave thymidine (**7**) accompanied by thymine (**6**) and pyranose-form thymidine (**8**)[8] (Table 3). The reaction of thymidine (**7**) with Cu^{2+}–AA did not give (**8**) at all but a small amount of thymine (**6**) (Table 3); therefore (**8**) might be produced in the repair process of (**4**) and (**5**) as reported already.[8] As the reaction of bromohydrin (**9**) with Cu^{2+}–AA gave the starting material (**9**) in high yield, this repair reaction may be characteristic of thymine and thymidine bromohydrins.

In conclusion, thymine and thymidine bromohydrins turned out to be readily repaired in water at room temperature by the Cu^{2+} and AA system. Since both Cu^{2+} and AA are widely distributed in biological systems, this type of repair of thymine and thymidine bromohydrins might occur in living cells.

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