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The oxidation of 8-oxo-7,8-dihydroguanine by iodine

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ABSTRACT

8-Oxo-7,8-dihydroguanine was specifically oxidized by iodine with aqueous KI. Under acidic conditions, the major product was dehydro-guanidinohydantoin. Under basic conditions, two diastereoisomers of spirohydantoin were chiefly obtained. In addition, unstable diimine was detected for the first time.

Guanine bases are hot spots for DNA damage by oxidation, and >20 lesions are known to result from guanine due to reactive oxygen or nitrogen species and hole-transfer reactions.[1–3] Among the types of DNA damage, 8-oxo-7,8-dihydroguanine (8oxoG) (Fig. 1) is widely known as a typical oxidation marker. However, since the redox potential of 8oxoG is significantly lower than that of guanine,[4] 8oxoG can be further oxidized by several oxidizing reactions.[1–3] Iodine is an oxidizing agent and is reportedly capable of oxidizing uric acid.[5] Although the structure of 8oxoG is similar to that of uric acid, to the best of our knowledge, the oxidation of 8oxoG by iodine has not been reported. Since the redox potential of iodine (0.54 V vs NHE) is similar to that of 8oxoG (0.58 V vs NHE),[6] 8-oxo-7,8-dihydro-2′-deoxyguanosine was completely oxidized, but UV-undetectable products seemed to be generated.[7] Since the ionization potential of thymine is the highest among the four DNA base,[8] 2′-deoxythymidine was not oxidized by iodine.[9] Therefore, we used oligomers containing UV-detectable thymine. In this study, we report for the first time that iodine can oxidize 8oxoG in nucleotides and found that different products were formed under acidic and basic conditions.

A 3mer DNA oligomer, T8oxoGT, was synthesized by the standard phosphoramidite method and used as a substrate containing 8oxoG. T8oxoGT (50 μM) in 10 mM sodium phosphate (pH 5.7) was reacted for 60 min at room temperature with I_2 (250 μM) and KI (5 mM), and the reaction mixture was subjected to HPLC analysis (Fig. 2A). T8oxoGT was completely reacted, and at least four products were detected. Each product was isolated by HPLC and analyzed by high resolution electrospray mass spectra (ESI-MS).[10]

The major peak in Figure 2A had mass corresponding to [8oxoG – 12],[11] and the major product was slowly degraded to a product with mass [8oxoG – 35].[12] This degraded product corresponded to the product that was also detected at 17 min in Figure 2A. As judged by a previous report,[13] the former product was the oligomer containing dehydro-guanidinohydantoin[14] (Ghox), and the latter product was the oligomer containing oxaluric acid (Oxa). The yield of TOxaT based on the starting material (T8oxoGT)

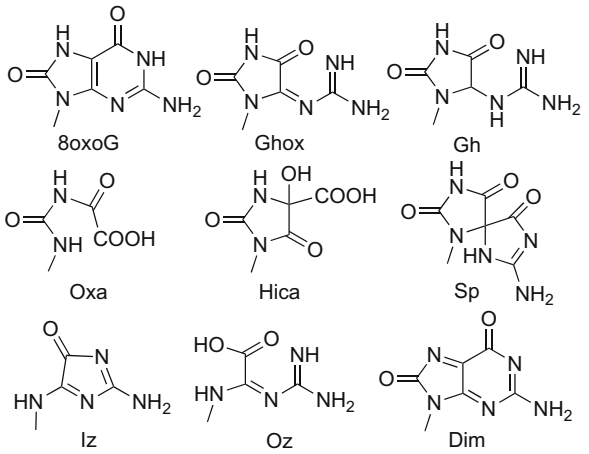

Figure 1. Structures representing DNA damages of only the base portion.

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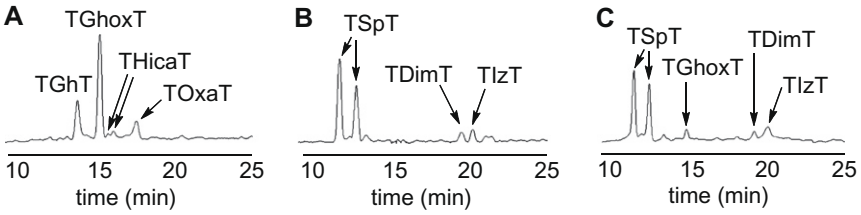

Figure 2. Oxidation of T8oxoGT by I_2/KI. Samples were analyzed by HPLC with a 5C18-MS column (Nacalai Tesque, 5 μm, 150 × 4.6 mm, elution with a solvent mixture of 10 mM TEAA (pH 7), 3–7% CH_3CN/30 min at a flow rate of 1.0 ml/min) and monitored at 260 nm absorbance. T8oxoGT (50 μM) in 10 mM sodium phosphate buffer ((A) pH 5.7, (B) pH 7.7 or (C) pH 7.0) was reacted for 60 min with 250 μM I_2 and 5 mM KI. Yields based on the absorbance of the starting material, T8oxoGT, were calculated. (A) TGhoxT, TGhT, TOxaT and THicaT were obtained in 29%, 14%, 8% and 2% yields, respectively. (B) TSpT, TIzT and TDimT were obtained in 49%, 5% and 4% yields, respectively. (C) TSpT, TIzT, TDimT and TGhoxT were obtained in 36%, 5%, 2% and 2% yields, respectively.

was lower than that of TGhoxT, and the low yield of TOxaT is attributed to the shorter incubation time rather than the half-life of Ghox. Therefore, the reaction time can influence the preparation of Ghox or Oxa.

The peak detected at 14 min in Figure 2A was stable and had mass [8oxoG − 10].[15] Moreover, the loss of CH_5N_3 was observed in the fragment mass. CH_5N_3 corresponds to a guanidium fragment, and a similar loss had been reported previously.[16] Thus, this peak was identified as the oligomer containing guanidinohydantoin (Gh), TGhT.

In addition to Ghox, Oxa and Gh, we noted that 4-hydroxy-2,5-dioxo-imidazolidine-4-carboxylic acid (Hica)[17] was detected in Figure 2A. To date, the only known product with mass [8oxoG − 7] is Hica,[3] and losses of 44 Da and 18 Da correspond to CO_2 and H_2O.[18] Hica is known to be the product of 8oxoG oxidation by peroxynitrite and $KHSO_5/CoCl_2$,[18] but the generation of Hica by iodine oxidation indicates that Hica is likely to be widely generated by several oxidizing agents.

Collectively, the oxidative reaction using iodine led to the generation of Oxa, Gh and Hica from 8oxoG, with Ghox as the major product under acidic conditions (Fig. 2A). In contrast, under basic conditions (pH 7.7) (Fig. 2B), four different peaks were observed.

Two major peaks in Figure 2B had the same mass, [8oxoG + 16],[19] and were identified as the oligomer containing spirohydantoin (Sp), TSpT. Two separate peaks were attributed to diastereoisomers as previously reported.[20] Thus, oxidation using iodine under basic conditions is suitable for the preparation of Sp.

The peak detected at 20 min in Figure 2B had mass [8oxoG − 55][21] and was degraded to a product with mass [8oxoG − 37].[21] The former product was also generated from the flavin-catalyzed photooxidation of guanine,[22,23] and the loss of CO_2 from the latter product was observed.[24] Thus, each product was identified as the oligomer containing 2,5-diamino-4*H*-imidazol-4-one (Iz) or 2,2,4-triamino-5(2*H*)-oxazolone (Oz).

Of note, a new product was detected at 19 min in Figure 2B, and had mass [8oxoG − 2].[25] The HPLC profile in Figure 2B indicates that the yield of the peak based on T8oxoGT was 4%, but this product was no longer detected at a reaction time of 4 h.[26] This unstable product is considered to be the proposed species 'diimine'[27] (Dim). Although Dim has been described previously,[20,27–32] it has not been detected directly. We show here for the first time the direct detection and instability of Dim.

Under neutral conditions (pH 7.0), four products were detected (Fig. 2C), a result similar to that under basic conditions (Fig. 2B). However, detection of TGhoxT was slight, and the mixed products were obtained at conditions between pH 5.7 and pH 7.7. Thus, under neutral conditions, neither Hica nor Dim was predominantly generated, and other new products were not detected.

When the concentration of iodine was lower and the reaction time was shorter, T8oxoGT was only partially oxidized under acidic conditions (Fig. 3A); TGhT was the major product and TGhoxT was not observed (Fig. 3A). This result differed from the result in Figure 2A. Moreover, TGhoxT was predominantly generated by a large excess of iodine (Fig. 3B). These different results can be attributed to the oxidation of TGhT to TGhoxT by iodine (Fig. 3C) as well as to oxidation by Na_2IrCl_6.[16] Thus, Gh is unstable under oxidative conditions, and iodine oxidation is unsuitable for high-yield preparation of Gh.

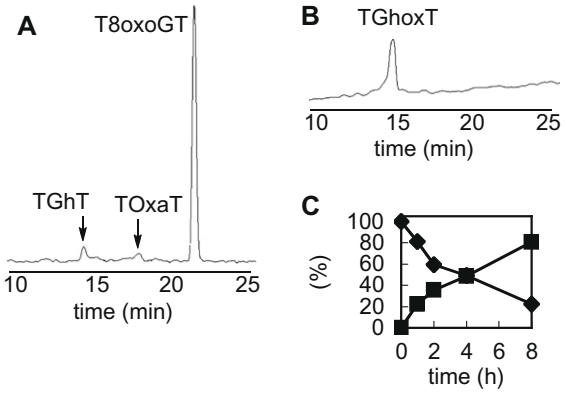

Figure 3. Oxidation of TGhT by I_2/KI. The samples were analyzed by HPLC as in Figure 2. Yields based on the absorbance of the starting material were calculated. (A) T8oxoGT (50 μM) in 10 mM sodium phosphate buffer (pH 5.7) was reacted for 5 min with 25 μM I_2 and 0.5 mM KI. TGhT and TOxaT were obtained in 5% and 3% yields, respectively. (B) T8oxoGT (50 μM) in 10 mM sodium phosphate buffer (pH 5.7) was reacted for 60 min with 2.5 mM I_2 and 50 mM KI. TGhoxT was obtained in 42% yield. (C) Time course of TGhT oxidation with I_2/KI. TGhT (0.75 μM) in 10 mM sodium phosphate buffer (pH 5.7) was oxidized for 0, 1, 2, 4 and 8 h by 250 μM I_2 and 5 mM KI. Diamonds indicate the percentage of TGhT, and squares indicate TGhoxT.

Despite the fact that 8oxoG was oxidized by iodine, 8oxoG was not degraded by iodine oxidation during automated DNA synthesis. When the reaction time of iodine oxidation during automated DNA synthesis was extended from 10 s to 1 min, 89% of the oligomer containing 8oxoG was not oxidized. The low reactivity by iodine may be attributable to the protection of 8oxoG by the isobutyryl group; in fact, 66% of N^2-isobutyryl-8-oxo-7,8-dihydro-2′-deoxyguanosine remained unoxidized at a reaction time of 1 min.[33]

In conclusion, we found that 8oxoG is oxidized by iodine and iodine with KI can be used as reagents for post-modification of 8oxoG: the products are dependent on acidic or basic conditions. Additionally, Dim was detected directly for the first time. Thus, preparations of Ghox, Oxa and Sp by iodine oxidation can be exploited for future biological studies on DNA damages.

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