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A Prebiotic Role for 8-Oxoguanosine as a Flavin Mimic in Pyrimidine Dimer Photorepair

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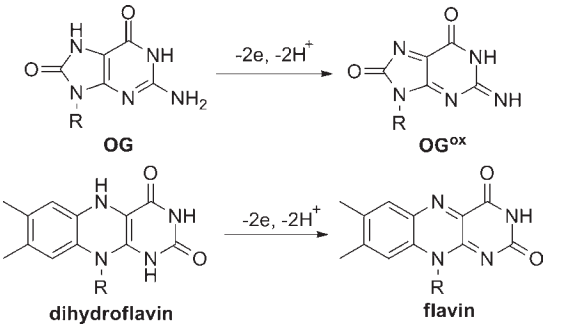
Supporting Information

ABSTRACT: Redox-active enzyme cofactors derived from ribonucleotides have been called "fossils of the RNA world," suggesting that early catalysts employed modified nucleobases to facilitate redox chemistry in primitive metabolism. Here, we show that the common oxidative damage product 8-oxo-7,8-dihydroguanine (OG), when incorporated into a DNA or RNA strand in proximity to a cyclobutane pyrimidine dimer, can mimic the function of a flavin in photorepair. The OG nucleotide acts catalytically in a mechanism consistent with that of photolyase in which the photoexcited state of the purine donates an electron to a pyrimidine dimer to initiate bond cleavage; subsequent back electron transfer regenerates OG. This unusual example of one form of DNA damage, oxidation, functioning to repair another, photodimerization, may provide insight into the origins of prebiotic redox processes.

The RNA world hypothesis suggests that ancient life evolved from the catalytic chemistry of RNA oligomers.[1] Numerous in vitro selection experiments now demonstrate the concept that RNA can catalyze a wide range of chemical reactions including ligation, hydrolysis, and C−C bond formation.[2,3] Early life would have also required redox reactions to support metabolism, but neither the four RNA bases nor the canonical amino acids are very redox active. Instead, present-day proteins employ nucleotide cofactors (NADH, $FADH_2$, pterins, etc.) to facilitate oxidation and reduction. These "fossils of the RNA world"[4] likely evolved from the four ribonucleotide bases, A, C, G, and U, or coevolved as separate nucleotide components.

A recent hypothesis places the dinucleotide cofactors at a critical juncture called the Initial Darwinian Ancestor (IDA).[5] Guanine is the most redox active base, but its one-electron reduction potential ($E°_7$ = 1.3 V vs NHE) is still too high to be effective in catalysis. Similarly, the simple hydrolysis products of G arising from either N^2 deamination (xanthine) or hydrolytic opening of the imidazole ring (Fapy-G) show only modestly lower reduction potentials, around 1.1 V. (See Supporting Information, Figure S8 and Table S2.) In contrast, 8-oxo-7,8-dihydroguanine (OG), the common oxidative damage product of G in DNA and RNA, has a greatly reduced redox potential of 0.74 V, representing a nearly 600 mV reduction in $E°$ at pH 7; at pH 9, the value is even lower, 0.5 V.[6] OG is readily formed from G via ionizing radiation or Fenton-like reactions that produce $HO^•$, conditions that are plausible on early Earth.[7] In fact, given the likely complexity of primordial synthesis of G,[8] OG may have been more plentiful than G. We therefore hypothesize that, prior to the evolution of more sophisticated cofactors such as flavin adenine dinucleotide, a simple and abundant derivative of guanine, namely, OG, could have played the role of a redox coenzyme in RNA-based catalysis (Scheme 1).

Scheme 1. Structural Similarity of OG and Dihydroflavin and Their Oxidized Forms



To investigate this hypothesis, we designed experiments that would test the electron-transfer capability of OG as a substitute for the flavoenzyme photolyase. The role of the flavin cofactor in photolyase has been investigated extensively, and there is consensus that the photoexcited state of $FADH^-$ transfers an electron to the cyclobutane thymine dimer (T═T) in bound duplex DNA, resulting in rapid cleavage of the σ bonds and back electron transfer to the flavin radical.[9−11] The process regenerates an undamaged TT-containing DNA strand as well as the original flavin cofactor. In parallel with a lower redox potential than the natural nucleosides, OG also has significant absorbance above 300 nm,[12] a region in which DNA and RNA oligomers have essentially no absorbance. It therefore appeared feasible to photoexcite the OG base specifically in an oligomer using wavelengths >300 nm, and to examine the reversion of the cyclobutane dimer to two undamaged thymidines.

For ease of synthesis and comparison to other work, initial studies were conducted in DNA oligomers using 8-oxo-7,8-dihydro-2′-deoxyguanosine (designated "O" in sequences) at positions in a 22-mer strand that placed it near a T═T dimer that was either synthesized and purified in an 18-mer strand or installed via the T═T phosphoramidite.[12] The difference in strand length permitted direct analysis of the quantities of T═T-containing strands versus repaired TT strands in the presence of the longer OG-containing oligomer by denaturing HPLC conducted at 70 °C (Figure 1A). Duplex **1A** has an OG:A base pair

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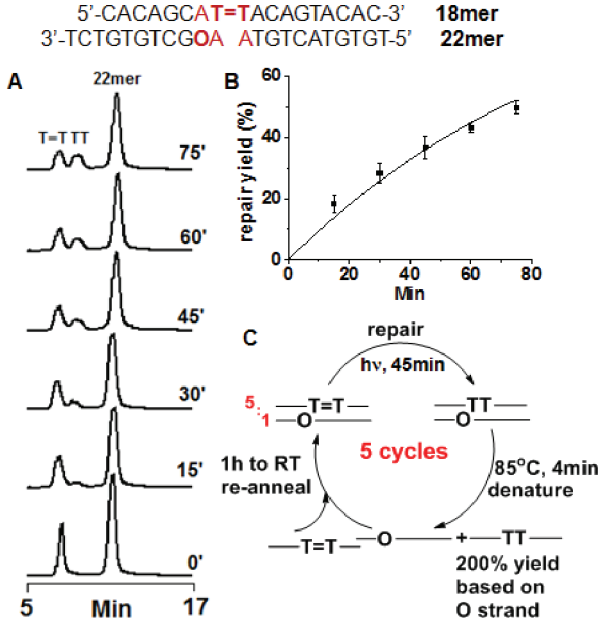

Figure 1. Photorepair (λ_{max} = 313 nm) of T=T cyclobutane dimer in an 18mer strand of DNA annealed to an O-containing 22mer. (A) Denaturing HPLC (70 °C) analysis of strands as a function of time. (B) Repair demonstrates first-order kinetics. (C) Turnover catalysis is shown by repair of a 5.4:1 mixture of T=T and O-containing strands in a light/heat/cool cycle leading to ~200% yield of repair based on OG.

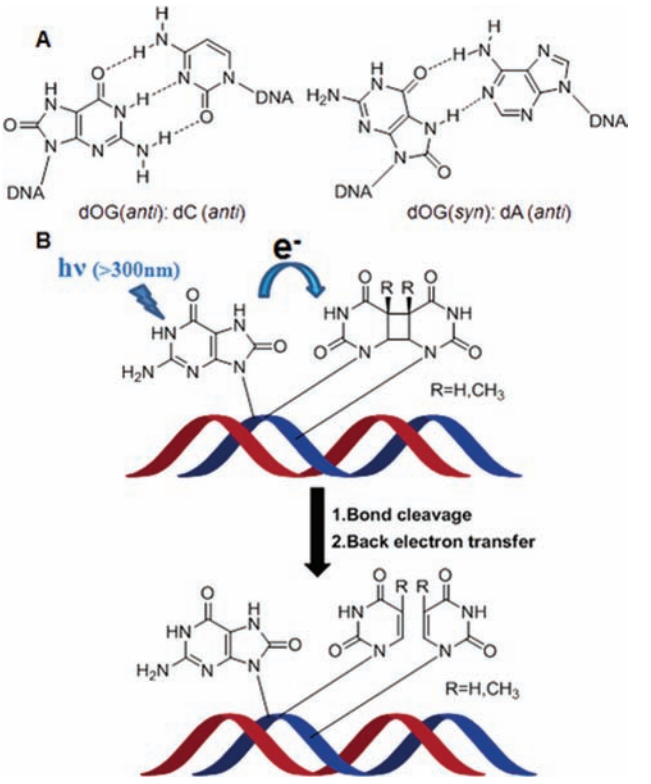

Figure 2. Repair of T=T by OG in duplex DNA. (A) OG can form a stable Watson−Crick base pair with C or, in the syn conformation, pair with A. (B) Studies herein support a photolyase-type mechanism in which the excited state of OG transfers an electron to T=T (or U=U) effecting cleavage of the cyclobutane; back electron transfer regenerates OG and the repaired pyrimidines.

(Figure 2A) positioned directly 5′ to the T=T site although OG is located in the strand complementary to the dimer. Irradiation of the duplex using a 40 W UVB light source (λ_{max} = 313 nm) and a polystyrene filter to remove wavelengths <300 nm led to repair of the T=T dimer in a process that showed first-order kinetics and a rate constant of 1×10^{-2} min^{-1} at 22 °C (Figure 1B). Control experiments in DNA duplexes without OG present resulted in no detectable repair (<5%, Supporting Information Figure S2),[12] indicating the dependence of the repair process on OG.

To better understand the catalytic role of OG, the OG-containing strand was isolated after ~50% repair and reanalyzed by ion-exchange HPLC. Under these conditions, the oxidation products of OG are readily separable; comparisons with authentic standards indicated that OG remained intact in the DNA strand (Figure S5).[12] Thus, any reactive intermediates formed during photorepair, such as $OG^{+\bullet}$, revert to OG during the course of the reaction. Furthermore, the OG-containing strand showed turnover catalysis. In this experiment, a 5.4:1 ratio of T=T and OG-containing strands was subjected to a reaction cycle of irradiation, thermal denaturation, and reannealing. Each irradiation period, 45 min, was sufficient to effect approximately 40% repair of the bound strand, and the final yield of repair after 5 cycles was ~200% based on OG (Figure 1C).

The dependence of the repair process on base pair and sequence context provided insight into the mechanism of repair. OG can form stable base pairs with either C or A, depending upon the anti or syn orientation of the OG base with respect to the glycosidic bond (Figure 2A), and these base pairs cause very little change in stability or structure of DNA duplexes.[13] Surprisingly, the OG:A base pair was about 3-fold more efficient than the Watson−Crick OG:C base pair in repairing the thymine dimer in both interstrand and intrastrand duplexes (**1A** vs **1C**, **4A** vs **4C**, Figure 3). One explanation of this data is based on the finding that the G:C base pair has a shortened excited state lifetime due to proton-coupled electron transfer.[14−17] If quenching of the OG* excited state also occurs via the assistance of the relatively acidic N1−H proton transfer to N3 of C, the OG:C base pair would exhibit a reduced excited state lifetime and therefore be less efficient than OG:A in donating an electron to the nearby T=T (Figure 2B). In addition, computations of ionization potentials predict a lower value for OG:A compared to OG:C.[18] Internal electron transfer in the OG:A base pair would be less efficient than in an OG:C base pair, because the N7−H of OG is less acidic and because purine bases have lower electron affinity than pyrimidine bases.[14]

Strand, directional and distal effects on thymine dimer repair by OG were also investigated by changing the location and orientation of the OG:A base pair in the vicinity of the T=T dimer. The highest rate of repair was observed when OG (with A opposite) was located immediately 5′ to the T=T lesion and in the same strand (Figure 3, duplex **4A** vs **3**); in this case, 85% repair was observed in 75 min. The same 5′ preference was also exhibited when the orientation of the base pair was reversed, placing OG in the opposite strand (Figure 3, **1A** vs **2**). These data agree with the observation of Rokita and co-workers who found that formation of T=T in duplex DNA using 254 nm light was inhibited to some extent by the presence of a G nucleotide at the 5′ side of the TT sequence.[19] In addition, we found a 4- to 5-fold preference for location of the OG nucleotide in the same strand as the lesion, consistent with the higher efficiency of intrastrand

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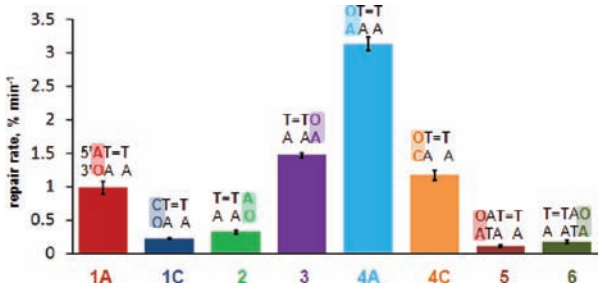

Figure 3. Repair rates at 22 °C for various sequence contexts for O and T═T. The complete sequence of duplex **1A** is shown in Figure 1; see Table S1 for others.[12] Repair rates were obtained from fitting the repair yields to the first-order exponential curve. Error bars indicate the standard deviation of at least 3 experiments.

electron transfer (Figure 3, **4A** vs **1A**, **4C** vs **1C**, **3** vs **2**).[20,21] The presence of T═T is known to cause disturbance of the duplex DNA and destacking at the thymine dimer site also has an effect on charge migration through duplex DNA.[22] NMR and crystal structures of thymine dimer-containing DNA show that though the 3′ side of T═T still retains good hydrogen bonding, the phosphate backbone changes to the B_{II} conformation upon formation of T═T, which can destack the base at this site.[23,24] Therefore, the higher repair efficiency when OG is located at the 5′ side of T═T may result from better base stacking at this position, which would in turn facilitate formation of an exciplex between OG as an electron donor and the adjacent T═T acceptor.[25]

The preference for OG-mediated repair from the immediate 5′ side of the T═T dimer was reversed when the repair was attempted from a longer distance. Insertion of an A:T base pair between the OG donor and T═T acceptor yielded sequences **5** and **6** (Figure 3), respectively. For these sequences, when OG was present in a 5′ orientation with respect to T═T, the presence of the intervening base pair led to a 25-fold reduction in rate, while only an 8-fold reduction in rate was observed from the 3′ direction (Figure 3, **4A** vs **5**, and **3** vs **6**); indeed, duplex **6** is now somewhat more reactive than duplex **5**. We propose that the thymine dimer is repaired reductively via electron transfer from OG* to T═T in a manner analogous to flavin-dependent photorepair with the enzyme photolyase. This constitutes an excess electron transfer (EET) mechanism in the DNA duplex, which has been shown to have a 3′ to 5′ directional preference[20,21] due to the asymmetric overlap of frontier molecular orbitals of the two adjacent bases.[26] Taken together, the studies of strand, direction and distance effects of OG-catalyzed photorepair of thymine dimers support an electron transfer mechanism of repair, analogous to that of photolyase, with the caveat that the immediate adjacency of the donor−acceptor pair creates a special preference for the 5′−3′ orientation because of enhanced exciplex formation resulting from better base stacking.

As reported, EET still occurs in double-stranded DNA containing a structural disturbance,[27] as well as in single-stranded DNA, although only over a short distance.[28] Conversely, hole transfer seems not to migrate through single-stranded DNA at all.[29] Thus, to further support the EET mechanism, we investigated thymine dimer repair by OG in single-stranded DNA in which there was an intervening dA between OG and T═T. The results showed that T═T is moderately repaired in ssDNA, reaching to 19% after 90 min irradiation in strand **5S** (see Table S1 for sequence).[12] Without OG present, no detectable repair was observed after 150 min of irradiation. The retention of activity of OG in single-stranded DNA is consistent with the EET mechanism in this system.

Table 1. Single Time Point Repair Yields for Cyclobutane Pyrimidine Dimers in Various Strand Contexts Based on the Sequence Context of Duplex 1A

entry	substrate	irradiation time (min)	yield (%)
1	$DNA^{T=T}$-DNA^{O}	75	50
2	$RNA^{U=U}$-DNA^{O}	150	40
3	$RNA^{U=U}$-RNA^{O}	150	12

The relevancy of OG as a primordial flavin requires that its photorepair activity also operate on uracil dimers in RNA. To this end, we synthesized the cyclobutane photodimer in an RNA sequence analogous to duplex **1A** such that a U═U dimer was installed adjacent to an A:O base pair, although the photosynthetic method required that OG be present in the opposite strand where it is 3-fold less reactive. Table 1 compares the yields of T═T versus U═U photorepair in the **1A** sequence context for the DNA:DNA, RNA:DNA, and RNA:RNA duplexes. While less efficient in the A form helices in which base stacking is dramatically altered compared to B form DNA, the photorepair of U═U by OG is still clearly evident.

We have demonstrated that OG, a common base oxidation product in nucleic acids, can trigger cyclobutane pyrimidine dimer repair using wavelengths of light red-shifted from the normal absorption spectrum of DNA or RNA. A related example of such a repair process is the work of Sen and co-workers[30,31] who generated a DNAzyme capable of photorepair of a bound thymine dimer substrate; in that case, a very different motif, a G quartet, appears to be responsible for repair rather than the flavin analogue OG. In addition, Carell and co-workers have demonstrated that the photolyase protein is not necessary for repair of T═T; synthetic incorporation of a flavin into the DNA stack also effects photorepair.[32,33]

Although more detailed analysis of the photophysical events surrounding this phenomenon are clearly warranted, the context effects on repair kinetics support a catalytic mechanism involving excess electron transfer from OG to the pyrimidine dimer in a fashion analogous to that of the flavin-dependent photolyases. This is an unusual example of one form of DNA damage serving to repair another. While the formation of both OG and T═T is common in present-day solar DNA damage, the relative amounts of these modifications in the prebiotic world are unknown. Nevertheless, conditions favoring OG formation at the same time as cyclobutane pyrimidine dimers could have driven the further evolution of purine nucleotides toward flavin-like activity. The overall similarity of OG and flavin chemistry suggests that nature may have adopted this close relative of the guanine base as a step toward organic-based redox metabolism, possibly as a component of the IDA, prior to the appearance of modern enzyme cofactors.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures, complete DNA sequences studied, kinetic fitting plots, and additional HPLC analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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