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Communication

Dynamics of 8-Oxoguanine in DNA: Decisive Effects of Base Pairing and Nucleotide Context

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ABSTRACT: 8-Oxo-7,8-dihydroguanine (oxoG), an abundant DNA lesion, can mispair with adenine and induce mutations. To prevent this, cells possess DNA repair glycosylases that excise either oxoG from oxoG:C pairs (bacterial Fpg, human OGG1) or A from oxoG:A mispairs (bacterial MutY, human MUTYH). Early lesion recognition steps remain murky and may include enforced base pair opening or capture of a spontaneously opened pair. We adapted the CLEANEX-PM NMR protocol to detect DNA imino proton exchange and analyzed the dynamics of oxoG:C, oxoG:A, and their undamaged counterparts in nucleotide contexts with different stacking energy. Even in a poorly stacking context, the oxoG:C pair did not open easier than G:C, arguing against extrahelical base capture by Fpg/OGG1. On the contrary, oxoG opposite A significantly populated the extrahelical state, which may assist recognition by MutY/MUTYH.

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8-Oxo-7,8-dihydroguanine (oxoG) is a prominent DNA oxidation product (Figure 1).[1,2] In human cells, the background oxoG level is ∼1/10^6 guanines, increasing upon oxidative stress.[2,3] DNA polymerases often incorporate dAMP opposite oxoG producing G:C → T:A transversions[4] that dominate SBS18 and SBS36 cancer mutation signatures.[5] OxoG prefers the *syn* orientation due to the steric repulsion between O^8 and deoxyribose.[6] Base pairing with C stabilizes oxoG in *anti*,[7] with the clash alleviated by the sugar−phosphate backbone adjustment from BI to BII conformation.[8] However, upon rotation around the glycosidic bond, oxoG forms a Hoogsteen oxoG(*syn*):A(*anti*) pair[6,9] (Supporting Information Figure S1). In contrast, G:A mispairs are intrinsically flexible, accepting both G(*anti*):A(*anti*) and G(*anti*):A(*syn*) conformations.[10,11] Molecular dynamics simulations show that oxoG mispaired with A flips spontaneously from *anti* to *syn* but remains *anti* opposite to C.[12] Accordingly, the introduction of an 8-oxo group thermodynamically destabilizes the G:C pair but stabilizes the G:A mispair.[13] The presence of oxoG facilitates DNA bending[14] but increases its torsional rigidity.[15]

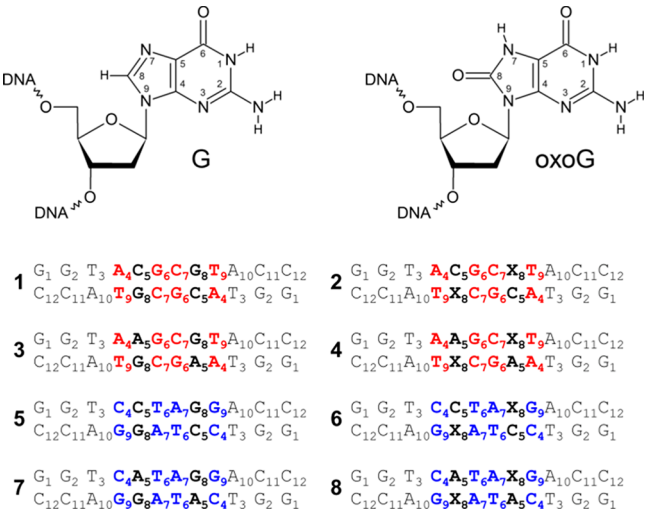

Figure 1. Structures of G and oxoG (X) and the duplexes used in this study. (red) Nucleotides forming a poorly stacking context. (blue) Favorably stacking nucleotides.

To avoid mutations, Fpg/OGG1 must selectively recognize oxoG:C but not oxoG:A, whereas MutY/MUTYH recognizes oxoG:A but not oxoG:C. A problem still unsolved is whether these proteins share some common oxoG recognition mechanism. Structural, kinetic, and computational data suggest that oxoG discrimination from G starts when the enzyme encounters the damaged base pair and everts oxoG into an extrahelical pocket.[17,19,20] Lower stability of the damaged pair may be a major factor early in the recognition, and Fpg was suggested to buckle the oxoG:C pair to facilitate its opening.[20,21] However, a rival model was proposed for another glycosylase, UNG, which removes uracil from DNA. NMR measurements of base pair opening and closing rates showed

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that UNG delays closing but does not affect opening, suggesting that U is captured in spontaneously opened base pairs.[22−24]

Despite the potential importance of base pair dynamics for oxoG recognition, experimental information about this lesion is limited. Imino proton exchange measurements indicate that the opening rates and extrahelical lifetimes of oxoG:C and G:C pairs are similar and are ∼2 orders of magnitude lower than for U:A.[25,26] However, the dynamics of oxoG:A and the influence of the nucleotide context on the oxoG dynamics remain unstudied.

Base pair opening and closing rates can be estimated using transfer of magnetization from water to measure imino proton exchange (Supporting Information Text), with a caveat that the exchange can already occur at ∼30° base eversion so the open state is not necessarily fully extrahelical.[27] This technique permits determination of exchange rate constants in the range common for imino protons in double-stranded DNA. Among several approaches to transfer of magnetization from water (WEX,[28] WEX II,[29] MEXICO,[30] 2D NOESY/EXSY,[31] etc.), the CLEAN chemical exchange (CLEANEX-PM)[32] is popular when following protein amide protons due to its superior ability to suppress artifacts associated with intramolecular nuclear Overhauser effects (NOEs). Here we have modified the CLEANEX-PM protocol to detect DNA imino proton exchange (Supporting Information Text, Supporting Information Figures S2−S7) and applied it to investigate the dynamics of oxoG:C and oxoG:A. As base pair opening is influenced by stacking with the neighboring pairs,[33] we have designed duplexes based on known G stacking energies,[34] placing G or oxoG into poorly stacking C[G/oxoG]T or well-stacking A[G/oxoG]G contexts. The target base was in position 8 of a self-complementary 12-mer (Figure 1) to minimize end effects.[8]

The exchange rate constant k_{ex} relates to the base pair opening and closing rate constants k_{op} and k_{cl} as

$$k_{ex} = \frac{k_{op}\alpha(k_0 + k_B[B])}{k_{cl} + \alpha(k_0 + k_B[B])} \quad (1)$$

where k_B is the rate constant of proton transfer from an isolated nucleotide to an acceptor (exchange catalyst) B, αk_0 is the exchange rate constant from an open pair without the catalyst, and α accounts for the difference between an isolated nucleotide and an open pair.[35] As an exchange catalyst, we chose 2,2-difluoroethylamine (DFEA), which ionizes at physiological pH (pK_a = 7.45 at 25 °C) and proved useful in NMR measurements of base pair dynamics.[22] If $k_{cl} \gg \alpha(k_0 + k_B[B])$ (the EX2 regime, e.g., at low [B]), then

$$k_{ex} = \frac{k_{op}\alpha(k_0 + k_B[B])}{k_{cl}} = K'_{eq} \times (k_0 + k_B[B]) \quad (2)$$

where the apparent equilibrium constant $K'_{eq} = \alpha K_{eq} = \alpha k_{op}/k_{cl}$ can be extracted as a fitting parameter. If, on the contrary, $k_{cl} \ll \alpha(k_0 + k_B[B])$ (as at high [B]), the apparent exchange rate achieves its upper limit $k_{ex} = k_{op}$, and an exchange occurs at each base pair opening event. Between these limits, one can establish k_{op}, the apparent closing rate constant $k'_{cl} = k_{cl}/\alpha = k_{op}/K'_{eq}$, and K'_{eq}.

To follow base pair dynamics, we determined k_{ex} at different DFEA concentrations at 10 °C (Figure 2, Supporting Information Figures S8−S11). The data were fitted by eqs 1 or 2 to extract k_{op} and K'_{eq} and calculate k'_{cl} (Table 1, Supporting Information Table S1).

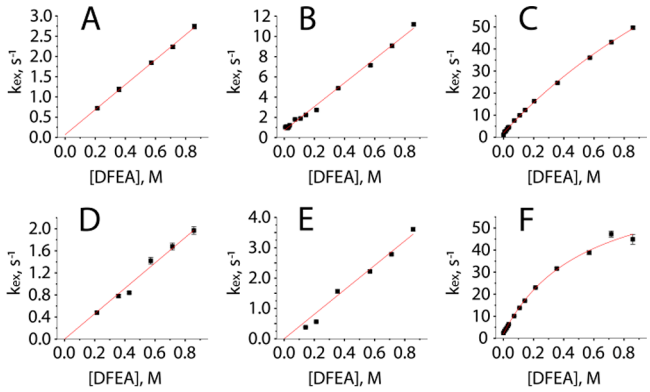

Figure 2. Dependence of k_{ex} on DFEA concentration for G_8 and oxoG_8 in duplexes **1** (A), **2** (B), **4** (C), **5** (D), **6** (E), and **8** (F). Lines show data fitting by eqs 1 (C, F) or 2 (A, B, D, E).

In the Watson−Crick pairs (G:C, oxoG:C; duplexes **1**, **2**, **5**, **6**) we followed the exchange of N1 imino protons. OxoG N7 imino proton, exposed to the DNA major groove, was observed as a separate signal at ∼10 ppm only in the C[oxoG]T context without DFEA, and it was missing in the A[oxoG]G context presumably due to the fast exchange with water protons. Both G:C and oxoG:C pairs demonstrated the EX2 regime with closing much faster than the exchange. Hence, only the apparent equilibrium constants could be compared. In the poorly stacking C[G/oxoG]T context, oxoG was slightly more stable intrahelically ($K'_{eq} = 0.141 \times 10^{-6}$ for oxoG vs 0.158×10^{-6} for G). In the well-stacking A[G/oxoG]G context, both G and oxoG were even less exposed, and the difference between them widened ($K'_{eq} = 0.048 \times 10^{-6}$ for oxoG vs 0.118×10^{-6} for G). Thus, even if oxoG:C pairs destabilize the duplex thermodynamically, this is not due to their easier opening. Our equilibrium constants are similar to those reported for the A[G/oxoG]T context by Every and Russu[25] (0.21×10^{-6} for oxoG, 0.31×10^{-6} for G). Interestingly, in that work rate constants could be recovered, likely due to the different catalyst employed (NH_3), and were similar for oxoG (k_{op} = 38 s^{-1}, $k'_{cl} = 18 \times 10^7$ s^{-1}) and G (k_{op} = 47 s^{-1}, $k'_{cl} = 15 \times 10^7$ s^{-1}). Crenshaw et al. measured somewhat higher equilibrium constants (1.6×10^{-6} for oxoG, 1.8×10^{-6} for G) in the A[G/oxoG]A context with a glycine catalyst but were also unable to extract k_{op} and k'_{cl}.[25]

The situation changed drastically in the adenine-containing mispairs. G_8 N1 imino protons in G:A duplexes **3** and **7** were not observed presumably due to their high solvent accessibility and fast exchange. The only measurable N1/N3 imino protons belonged to G_6 in duplex **3** and to G_2, T_3, and G_9 in **7** (Supporting Information Figures S9, S11, Supporting Information Table S1). In contrast, oxoG N7 imino protons engaged in Hoogsteen base pairing were well-resolved. OxoG:A-containing duplexes **4** and **8** showed a kinetic regime that allowed extraction of both k_{op} and k'_{cl} (Table 1). The opening rate constant was expectedly higher for the poorly stacking context (∼2.4-fold). Surprisingly, closing also accelerated ∼4.6-fold in the C[oxoG]T context, making oxoG overall 1.9-fold more stable intrahelically than in A[oxoG]G. Conceivably, base pair opening could allow the flanking A and G to stack across the gap and keep oxoG from retracting into the helix. Compared with oxoG:C, the equilibrium is shifted by 3−4 orders of magnitude toward the open state in the oxoG:A mispair.

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Table 1. Kinetic, Equilibrium, and Thermodynamic Parameters of G and oxoG Obtained from CLEANEX-PM Spectra[a]

Duplex	k_{op} (s^{-1})	k'_{cl} (10^4 s^{-1})	K'_{eq} (10^{-6})	T_m (K)	ΔH (kJ·mol^{-1})	$\Delta G°_{298}$ (kJ·mol^{-1})	$\Delta S°_{298}$ (kJ·mol^{-1}·K^{-1})
1	EX2[b]	EX2	0.158 ± 0.003	340.6 ± 0.2	−466 ± 15	−73.7 ± 1.9	−1.32 ± 0.06
2	EX2	EX2	0.141 ± 0.003	335.7 ± 0.4	−491 ± 22	−70.5 ± 2.5	−1.41 ± 0.08
4	182.7 ± 17.1	31.85 ± 3.14	573.6 ± 17.9	331.1 ± 0.3	−478 ± 19	−63.2 ± 1.9	−1.39 ± 0.07
5	EX2	EX2	0.118 ± 0.009	336.9 ± 0.4	−556 ± 30	−79.6 ± 3.5	−1.60 ± 0.11
6	EX2	EX2	0.048 ± 0.003	336.4 ± 0.3	−575 ± 33	−81.0 ± 3.8	−1.66 ± 0.12
8	75.6 ± 5.1	7.00 ± 0.75	1080 ± 89	329.6 ± 0.4	−565 ± 41	−69.6 ± 4.0	−1.66 ± 0.15

[a]For duplexes **3** and **7**, imino protons were not identified, and complex temperature profiles of the ^{1}H signal prevented determination of melting parameters. [b]EX2, individual constants cannot be determined because the closing rate greatly exceeds the exchange rate.

As calorimetry-based studies indicate DNA destabilization by oxoG:C,[13] we have tracked temperature dependences of the chemical shift of nonexchangeable thymine methyl protons in our duplexes.[36] Both T_3 and T_6/T_9 showed two well-resolved ^{1}H signals in the characteristic 1−2 ppm region, one of which was used to extract thermodynamic parameters from a two-state model[37] (Figure 3, Supporting Information Figures S12, S13, Supporting Information Text). Generally, the results were consistent with the calorimetric data: oxoG slightly decreased T_m of Watson−Crick duplexes, while the Hoogsteen duplexes were even less stable (Figure 3, Table 1). However, the difference between G:C and oxoG:C in the well-stacking context (**5** vs **6**) was negligible. Interestingly, ΔH in poorly stacking (**1**, **2**, **4**) and well-stacking (**5**, **6**, **8**) duplexes closely coincided within each group and differed by ∼90 kJ·mol^{-1} between the groups. Since the calorimetric study[13] employed a poorly stacking C[G/oxoG]C context, it appears that oxoG:C is not always destabilizing. Consistent with the imino proton exchange data and the expected instability of G:A mismatches, duplexes **3** and **7** showed complex temperature dependences of ^{1}H lines preventing straightforward extraction of thermodynamic parameters.

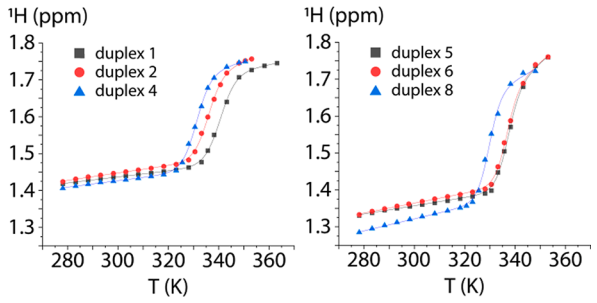

Figure 3. Temperature dependences of the chemical shifts of thymine methyl protons for poorly stacking (left) and well-stacking (right) contexts. Curves, data fitting by eq S13.

Overall, our data indicate that the destabilizing effect of oxoG:C in poorly stacking contexts cannot be explained by easier opening of the damaged base pair. However, in duplex **2**, the equilibria of base pairs neighboring the damaged ones (G_6 and T_9) are shifted toward the open state in comparison with the undamaged duplex **1**, while the near-terminal base pairs (G_2 and T_3) behave similarly in both duplexes (Supporting Information Table S1). We surmise that subtle conformational changes in the oxoG:C pair, such as the BI → BII backbone shift relieving the O^8−O5′ collision,[8] could spread over the adjacent nucleotides and facilitate their opening, which destabilizes the whole duplex. In the well-stacking context, oxoG:C was not destabilizing, indicating that stacking compensates for the penalty associated with the O^8 substituent. Indeed, an extended π-system of oxoG might improve stacking compared with regular purines. Damaged base pairs in our duplexes were separated by only two normal pairs, which may allow some dynamic coupling between the lesions. However, in a molecular dynamic study of G:A in several sequence contexts[10] this mispair affected the duplex conformation mostly over positions ±1 with minor disturbances at ±2 and almost none beyond. Since G:A is the least stable of the mispairs we have analyzed, the distance effect of the others might be even more limited.

Our results may have implications for the mechanism of oxoG search by DNA glycosylases. It is unlikely that oxoG paired with C is captured when extrahelical, since it appears even more stable than G inside the helix. Thus, OGG1 and Fpg would have to enforce oxoG flipout into the active site. In contrast, oxoG is much more available extrahelically when mispaired with A. This observation may relieve contradictions between the biochemical and structural data for MutY: while in all crystal structures oxoG is intrahelical, fast kinetics and cross-linking studies support extrahelical recognition.[38] The rate and equilibrium constants for oxoG:A (Table 1, duplexes **4** and **8**) can be compared with those measured with DFEA for T and U, for which extrahelical capture by UNG is suggested: k_{op} = 35 s^{-1}, k'_{cl} = 180 × 10^4 s^{-1}, K'_{eq} = 20 × 10^{-6} for T:A, k_{op} = 200 s^{-1}, k'_{cl} = 700 × 10^4 s^{-1}, K'_{eq} = 27 × 10^{-6} for U:A, and k_{op} = 650 s^{-1}, k'_{cl} = 130 × 10^4 s^{-1}, K'_{eq} = 500 × 10^{-6} for T:6-methylpurine (a pair with only one hydrogen bond).[23,26] Thus, oxoG in oxoG:A is even more accessible extrahelically than a destabilized thymine. We hypothesize that a spontaneously everted oxoG may be captured by MutY/MUTYH as an early substrate recognition step.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.2c11230.

Additional experimental details, description of proton exchange formalism and NMR experiments, supplementary discussion, as well as supplementary Tables and Figures including additional experimental results (PDF)

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Author Contributions

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

Fpg, DNA-formamidopyrimidine glycosylase; OGG1, 8-oxoguanine DNA glycosylase; MutY (or human MUTYH), adenine DNA glycosylase; UNG, uracil-DNA glycosylase; dAMP, deoxyadenosine monophosphate

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