Synthesis and Evaluation of 8-halogenated-7-deaza-2′-deoxy-guanosine as 8-oxo-2′-deoxy-guanosine analogues

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【Introduction】

8-Oxo-2′-deoxyguanosine (8-oxo-dG) is a representative nucleoside damage that is formed by oxidation of 2′-deoxyguanosine (dG) with reactive oxygen species (ROS), and its presence has been linked to aging, cancer, etc [1]. Unlike dG, 8-oxo-dG forms stable base pairs with both 2′-deoxycytidine (dC) and 2′-deoxyadenosine (dA). Based on the base-pairing properties of 8-oxo-dG, DNA polymerases incorporate 8-oxo-dGTP opposite dA and dATP opposite 8-oxo-dG, causing AT to CG and GC to TA transversion mutations. To suppress the genotoxicity of 8-oxo-dG and protect the genome integrity, hOGG1 can excise 8-oxo-dG from 8-oxo-dG:dC base pairs within duplex DNA. And hMYH provides the defense by removing dA opposite 8-oxo-dG. To prevent the incorporation of 8-oxo-dGTP into DNA, hMTH1 hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP that is further hydrolyzed by nucleotidase (Figure 1).

Recently, some DNA repair enzymes such as DNA polymerase β and hOGG1 have been regarded as antitumor targets. Especially, hMTH1 is responsible for removing of oxidized nucleotides and required for survival of cancer cells [2]. 8-Halogenated-7-deaza-dG derivatives were designed as 8-oxo-dG analogues to elucidate the contributions of N7-H and C8-oxygen to the base pairing, replication and repair of 8-oxo-dG. In this study, I have attempted to find out functional inhibitors of DNA repair enzymes among the 8-halogenated-7-deaza-dG derivatives (Figure 2).

【Experiments and Results】

1. Synthesis and base pairing properties of 8-halogenated-7-deaza-dG derivatives.

The syntheses of 8-halogenated-7-deaza-dG derivatives were achieved via the reaction between acetylated 7-deaza-dG and N-halogenated succinimides. These compounds were incorporated into the central part of 13-mer oligonucleotides (Scheme 1). The properties of these derivatives were investigated by computational, NMR and thermal denaturing studies. The significant upfield shift of the C-2′ signals and characteristic downfield shift of H-2′ signals indicated that 8-halogenated-7-deaza-dG derivatives prefer syn-conformation in DMSO solution similarly to 8-oxo-dG (Table 1). It was shown that the base pair of 8-halogenated-7-deaza-dG with dC was destabilized compared with dG, supporting their preference for syn conformation. Unlike 8-oxo-dG, 8-halogenated-7-deaza-dG did not form a stable base pair with dA, most likely due to the lack of N7-H hydrogen bonding with dA (Table 2 and Figure 3). Therefore, the newly-designed 8-halogenated-7-deaza-dG derivatives resemble 8-oxo-dG in shape and preference for syn conformation, but they do not form Hoogsteen base pair with the opposite dA.
2. Recognition and excision of 8-halogenated-7-deazadG in DNA duplex by 8-oxo-dG glycosidase.

I next tested the recognition and excision of 8-halogenated-7-deazadG derivatives in DNA duplex by Fpg and hOGG1. After incubation of the DNA duplex containing 8-oxo-dG analogues with Fpg and hOGG1 at 37°C, β-elimination and δ-elimination products are obtained which can be reflected on the gel (Figure 4). 8-Halogenated-7-deazadG derivatives, especially 8-Cl-7-deazadG, were good glycosidase substrates for Fpg. However, 8-halogenated-7-deazadG derivatives were slightly excised by hOGG1. Quartz crystal microbalance (QCM) provided the direct observation of the time courses of interaction between 8-halogenated-7-deazadG containing duplex and Fpg or hOGG1 (Figure 5 and Table 3). In the case of Fpg, the association rate constant ($k_{on}$) for dG or 7-deaza-dG was smaller than that for 8-oxo-dG and 8-halogenated-7-deazadG, suggesting that introducing C8-oxygen or C8-halogen help to the recognition by hOGG1.
Fpg. Interestingly, the dissociation rate constants ($k_{off}$) for 7-deaza-dG derivatives were similar to 8-oxo-dG, implying the importance of the presence of hydrogen at 7-position. In the case of hOGG1, 8-oxo-dG exhibited much lower $k_{off}$ value than the other compounds, probably arising from the strong hydrogen bonding between 7-NH with Gly42 in the active site of hOGG1 (Figure 6). Although 8-Cl- and 8-Br-7-deazadG had lower $k_{off}$ value than 8-oxo-dG, they exhibited higher $k_{on}$ which resulted in the similar dissociate constant to 8-oxo-dG. Accordingly, it has been demonstrated that 8-halogenated-7-deaza-dG containing duplexes are competitive inhibitors for the glycosidase activity of hOGG1 to excise 8-oxo-dG in duplex DNA (Figure 7).

3. Synthesis and evaluation of 8-halogenated-7-deaza-dGTP.

I next synthesized 7-deaza-dGTP as well as 8-Cl-, 8-Br- and 8-I-7-deaza-dGTP using the conventional method (Scheme 2). Although 8-halogenated-7-deaza-dGTP derivatives were hardly hydrolyzed by hMTH1 (Figure 8A), they showed competitive inhibitory activity against 8-oxo-dGTP hydrolysis by hMTH1 (Figure 8B). Docking study (Autodock 4.2.6) and molecular dynamic simulation (NAMD 2.10) revealed that 8-I-7-deazadGTP may adopt anti-conformation and place itself in the almost same position as 8-oxo-dGTP. However, Trp117 in the active site of hMTH1 was shifted 2 Å as compared to its position in the complex of 8-oxo-dGMP and hMTH1, which might enhance the π-π interaction between Trp117 and 8-I-7-deazadGTP (Figure 8C). Furthermore, the IC$_{50}$ values of 8-Cl-7-deazadGTP, 8-Br-7-deazadGTP and 8-I-7-deazadGTP were determined to be 0.857, 0.496 and 0.415 μM, respectively (Table 4). And their corresponding inhibition constants ($K_i$) were respectively calculated to be 116.8, 78.2 and 61.7 nM by Lineweaver-Burk plots (Table 5). The diol compound of 8-I-7-deazadG exhibited some inhibitory effect against hMTH1 but it is 100-fold weaker than 8-I-7-deazadGTP, implying the importance of the triphosphate group. Interestingly, 8-halogenated-7-deazadGTP exhibited much higher inhibitory activities against hMTH1 than SCH51344 and (S)-Crizotinib. Therefore, it is expected that 7-deazadGTP and 8-halogenated-7-deazadGTP would show antitumor activity by targeting hMTH1.

![Figure 6. 8-Cl-7-deazadG in the active site of hOGG1.](image)

![Figure 7. Bar graph of normalized excision ability of hOGG1 in the presence of corresponding 400 nM of competitor DNA (dG, oxodG, deazadG, Cl-deazadG, Br-deazadG, Cl-deazadG, and Br-dG).](image)

![Figure 8. (A) Time course of hydrolysis of 7-deazadGTP and 8-halogenated-7-deaza-dGTP with hMTH1; (B) Lineweaver-Burk plots of 8-oxo-dGTP hydrolysis by hMTH1 in the absence of inhibitor or presence of 8-Cl-7-deazadGTP; (C) Alignment of 8-I-7-deazadGMP after simulation (Autodock 4.2.6 and NAMD 2.10) with 8-oxo-dGMP crystal structure (PDB: 3ZRO).](image)
It was found that 8-halogenated-7-deazadGTP were only slightly incorporated into DNA to pair with dC and hardly incorporated to pair with dA by KF-exo- and human polymerase β (Figure 9). Moreover, 8-halogenated-7-deazadG derivatives in duplex DNA were tested to be difficult to pair with dA during replication process. Therefore, 8-halogenated-7-deazadGTP derivatives are expected to have little side effects, further supporting their potentials as antitumor agents.

【Conclusion】
8-Halogenated-7-deaza-dG derivatives were designed as 8-oxo-dG analogues, and their syntheses and incorporations into oligonucleotides were successful. 8-halogenated-7-deaza-dG derivatives resemble 8-oxo-dG in shape and preference for syn-conformation confirmed by the DFT calculations and NMR studies, but they do not form Hoogsteen base pair with the opposite dA based on the lower $T_m$ values as compared to 8-oxo-dG. Interestingly, 8-halogenated-7-deaza-dG derivatives in duplex DNA, especially 8-Cl-7-deaza-dG, were good glycosidase substrates for Fpg and strong binders to hOGG1. Accordingly, 8-halogenated-7-deaza-dG derivatives in duplex DNA demonstrated competitive inhibition for the glycosidase activity of hOGG1 to excise 8-oxo-dG in duplex DNA. Furthermore, 8-halogenated-7-deazadGTP were successfully synthesized and demonstrated as strong inhibitors of hMTH1 at nanomolar concentrations. It is interesting that 8-halogenated-7-deazadGTP derivatives exhibited low mutagenic potential by DNA polymerases, implying that they might have little side effects. Thus, this study has clearly demonstrated that 8-halogenated-7-deazadG derivatives are potential to be used as probes and functional inhibitors of 8-oxo-dG repair enzymes.

【References】

【Publications】
2. Yin, Y.; Sasaki, S.; Taniguchi, Y. Recognition and excision properties of 8-Halogenated-7-deaza-2'-deoxyguanosine as 8-oxo-2'-deoxyguanosine analogue by Fpg and hOGG1. Submitted.