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

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論文審査の結果の要旨

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. 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]. In this study, 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, and efforts have been devoted to find out functional inhibitors of DNA repair enzymes among the 8-halogenated-7-deaza-dG derivatives (Figure 1).

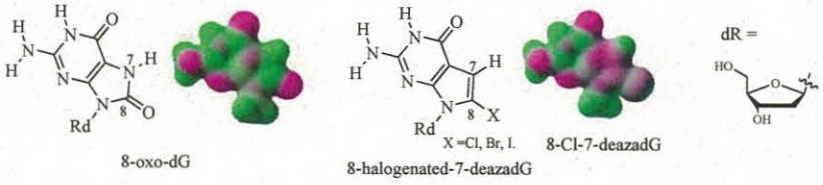

Figure 1. Structures of 8-oxo-dG and 8-halogenated-7-deazadG derivatives.

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. 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. 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. 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.

The recognition and excision of 8-halogenated-7-deazadG derivatives in DNA duplex were investigated using Fpg and hOGG1. 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. Kinetic properties of the reaction were analyzed by quartz crystal microbalance (QCM). 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 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. Although 8-Cl- and 8-Br-7-deazadG had lower k_{off} value than 8-oxo-dG, they exhibited higher k_{on}, resulting 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.

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

The triphosphate derivatives, 7-deaza-dGTP, 8-Cl-, 8-Br- and 8-I-7-deaza-dGTP, were synthesized and tested for the reactivity for hMTH1. It should be noted that 8-Halogenated- 7-deaza-dGTP derivatives were hardly hydrolyzed by hMTH1, but showed competitive inhibitory activity against 8-oxo-dGTP hydrolysis by hMTH1. Therefore, it is expected that 7-deazadGTP and 8-halogenated-7-deazadGTP would show antitumor activity by targeting hMTH1. 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. 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 and synthesized as 8-oxo-dG analogues, and their chemical and biological properties were evaluated. 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. 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. Remarkable finding was that 8-halogenated-7-deazadGTP exhibited strong inhibition against hMTH1 at nanomolar concentrations, suggesting their potentials as antitumor agents.

Thus, this study has been approved to deserve a doctorate degree.

References

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Publications

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3. Yin, Y.; Sasaki, S.; Taniguchi, Y. The triphosphate derivatives, 7-deaza-dGTP, 8-Cl-, 8-Br- and 8-I-7-deaza-dGTP, as competitive inhibitors for hMTH1, in preparation.