Chemical Synthesis and Bypass studies of Site-Specific Damaged DNAs

Pratibha P. Ghodke
Department of Biochemistry, Vanderbilt University School of Medicine, United States
pratibhaghodke39@gmail.com

Abstract:
DNA is subject to continuous damage from exogenous and endogenous sources such as UV light and reactive oxygen species. If left unrepaired, DNA damage (adduct or lesion) can lead to mutations, which in turn are considered to be major issues in cancer, aging, and teratogenesis. To prevent genomic instability, the cell continues replication across such damaged sites in DNA *via* translesion synthesis (TLS). Such DNA damage tolerance by low-fidelity polymerases can be very error-prone. Therefore, DNA damage tolerance research is essential to understand the potential consequences on human health. For studies of pre-mutagenic DNA adducts, oligonucleotides containing site-specific modifications are required. Accordingly, we have developed a robust protocol to synthesize naturally occurring damaged DNAs containing site-specific modifications at the N^2-position of deoxyguanosine (dG) employing phosphoramidite chemistry. Primer extension studies showed that *Escherichia coli* Pol IV is highly efficient in bypassing these N^2-dG adducts. Studies with the lucidin DNA adduct (LdG, structural analog) revealed that human DNA polymerase κ (hpol κ) can bypass the adduct in an error-free manner but replication by hpol η was compromised. Crystal structure of LdG damaged DNA in a complex with hpol κ and an incoming dCTP reveals the formation of a hydrophobic pocket in the active site of the enzyme to accommodate LdG. For TLS, the peptides from the active site of DNA repair protein O^6-alkylguanine DNA-alkyltransferase (AGT) were crosslinked to the DNA using the post-oligomerization approach. It involves the conjugate addition of a thiol-modified DNA with the dehydroalanine moiety-containing peptides. TLS studies indicated that both hpol η and κ can bypass a DNA peptide crosslink in an error-free manner with high efficiency. In addition to this, post-oligomerization approach that involves the copper-catalyzed azide-alkyne cycloaddition (CuAAC) was utilized for the synthesis of DNA-protein crosslinks. Overall, the studies provided detailed insights into the replication across from DNA damages such as the limited miscoding of the LdG adduct and the DNA-peptide crosslink in humans.

References:

1. Sale, J. E.; Lehmann, A. R.; Woodgate, R. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 141.
2. Ghodke, P. P.; Harikrishna, S.; Pradeepkumar, P. I. *J. Org. Chem.* **2015**, *80*, 2128.
3. Ghodke, P. P.; Bommisetti, P.; Nair, D. T.; Pradeepkumar, P. I. *J. Org. Chem.* **2019**, *84*, 1734.
4. Yockey, O.; Jha, V.; Ghodke, P. P.; Xu, T.; Xu, W.; Ling, H.; Pradeepkumar, P. I.; Zhao, L. *Chem. Res. Toxicol.* **2017**, *30*, 2023.
5. Ghodke, P. P.; Guengerich, F. P. *J. Biol. Chem.* **2021**, *297*, 101124.
6. Ghodke, P. P.; Gonzalez, G. V.; Wang, H.; Johnson, K. M.; Sedgeman, C. A.; Guengerich, F. P. *J. Biol. Chem.* **2021**, *296,* 100444
7. Ghodke, P. P.; Albertolle, M. E.; Johnson, K. M.; Guengerich F. P. *Curr. Protoc. Nucleic Acid Chem.* **2019**, *76*, e74.