

Biological Chemistry Group

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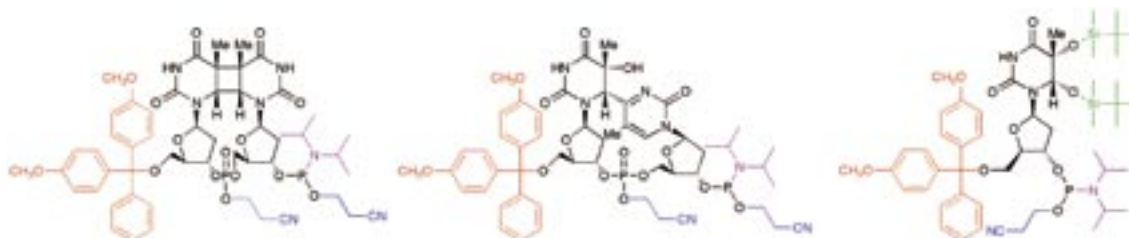
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Chemical Synthesis of Damaged DNA

Since DNA, the genetic material, is an organic molecule, it is vulnerable to chemical reactions with both endogenous and exogenous substances in cells. The products are called DNA lesions, and they induce genetic mutations, which result in carcinogenesis and cell death. We have been studying the chemical synthesis of lesion-containing DNA and its application to molecular biology. Among the DNA lesions, we are especially interested in UV-induced photoproducts and oxidatively damaged bases because these types of lesions inevitably occur in organisms living on the earth.

Oligonucleotides, i.e., single-stranded fragments of nucleic acids, are synthesized on a solid support using phosphoramidite chemistry. Chain elongation is performed by the coupling of a nucleoside 3'-phosphoramidite with the adjacent 5'-hydroxyl function in the presence of tetrazole as an activator, followed by oxidation to phosphotriester with iodine. Therefore, lesion-containing oligonucleotides can be synthesized if a building block bearing the damaged base and the phosphoramidite moiety is prepared. We reported the syntheses of the building blocks of the cyclobutane pyrimidine dimers (CPDs) formed at the TT and TU sequences by UV irradiation, the pyrimidine(6-4)pyrimidone photoproducts formed at TT and TC, the Dewar valence isomer of the (6-4) photoproduct, and (5*R*,6*S*)- and (5*S*,6*R*)-thymine glycols. These lesions were successfully incorporated into oligonucleotides.



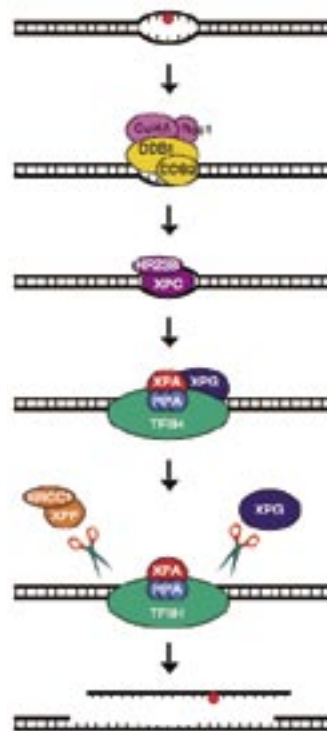
Building blocks of the CPD, the (6-4) photoproduct, and (5*R*,6*S*)-thymine glycol (from left)

Application of Synthetic DNA to Molecular Biology

Oligonucleotides containing various lesions synthesized by our group have been used for biological studies in our own research and in collaboration with biologists all over the world. One of the subjects is nucleotide excision repair (NER). Defects in NER cause hereditary diseases, with the most famous one being xeroderma pigmentosum (XP). We searched for proteins that recognized the (6-4) photoproduct in human cells using a synthetic DNA duplex as a probe, and the major factor was the UV-damaged DNA-binding (UV-DDB) protein, which is inactivated by a mutation in XP group E (XPE) cells. We characterized the binding of this protein and concluded that it recognized the kinkable nature of damaged DNA. Another study of NER is a collaborative work with Prof. Fumio Hanaoka's group on the damage recognition of the XPC protein. It was found recently that ubiquitylation by the Cul4A-Roc1 complex plays a critical role in the transfer of the UV lesion from the UV-DDB protein to the XPC-HR23B complex. Another collaboration with Prof. Hanaoka's group is on DNA polymerases responsible for translesion synthesis (TLS). Using CPD-containing oligonucleotides, they found that human DNA polymerase η (the XPV protein) could bypass this lesion, incorporating the correct nucleotides. There are many collaborative studies with other groups on the TLS polymerases, and some of them are still in progress. We are also studying base excision repair and the photoreactivating enzymes in collaboration

with various researchers in the fields of biochemistry, structural biology, and bioinformatics. The principal collaborator is Prof. John A. Tainer of the Scripps Research Institute, USA.

As part of our own research, we searched for a low-molecular-weight compound that could bind to DNA containing the (6–4) photoproduct. Such a molecule may be used to develop an artificial repair system for this type of lesion, which can prevent skin cancer in XP patients. Since distamycin A, a natural antibiotic known as a minor groove binder, gave a good result in preliminary experiments, we analyzed the binding of this drug to damaged DNA. Distamycin A could discriminate between the (6–4) photoproduct and the CPD, but it also bound to several other types of damaged DNA. From the experimental results, we concluded that this drug bound to damaged DNA in the same way as to the normal target site, by recognizing the chemical structure of the minor groove. We are now testing other types of compounds. In addition, we are developing a molecular sensor that can detect the NER activity in cell extracts and in complete cells.



The NER pathway

References (main papers in 2004–2007)

- (1) Chemical synthesis and translesion replication of a *cis-syn* cyclobutane thymine–uracil dimer. Kohei Takasawa, Chikahide Masutani, Fumio Hanaoka and Shigenori Iwai, *Nucleic Acids Res.*, **32** (5), 1738-1745 (2004).
- (2) Preferential *cis-syn* thymine dimer bypass by DNA polymerase η occurs with biased fidelity. Scott D. McCulloch, Robert J. Kokoska, Chikahide Masutani, Shigenori Iwai, Fumio Hanaoka and Thomas A. Kunkel, *Nature*, **428** (6978), 97-100 (2004).
- (3) Binding of distamycin A to UV-damaged DNA. Aki Inase, Takashi S. Kodama, Jafar Sharif, Yan Xu, Hirohito Ayame, Hiroshi Sugiyama and Shigenori Iwai, *J. Am. Chem. Soc.*, **126** (35), 11017-11023 (2004).
- (4) High-efficiency bypass of DNA damage by human DNA polymerase Q. Mineaki Seki, Chikahide Masutani, Lee Wei Yang, Anthony Schuffert, Shigenori Iwai, Ivet Bahar and Richard D. Wood, *EMBO J.*, **23** (22), 4484-4494 (2004).
- (5) UV-induced ubiquitylation of XPC protein mediated by UV-DDB-ubiquitin ligase complex. Kaoru Sugawara, Yuki Okuda, Masafumi Saijo, Ryotaro Nishi, Noriyuki Matsuda, Gilbert Chu, Toshio Mori, Shigenori Iwai, Keiji Tanaka, Kiyoji Tanaka and Fumio Hanaoka, *Cell*, **121** (3), 387-400 (2005).
- (6) Preferential formation of (5*S*,6*R*)-thymine glycol for oligodeoxyribonucleotide synthesis and analysis of drug binding to thymine glycol-containing DNA. Tatsuhiko Shimizu, Koichiro Manabe, Shinya Yoshikawa, Yusuke Kawasaki and Shigenori Iwai, *Nucleic Acids Res.*, **34** (1), 313-321 (2006).
- (7) Synthesis and characterization of oligonucleotides containing 2'-fluorinated thymidine glycol as inhibitors of the endonuclease III reaction. Yusuke Doi, Atsushi Katafuchi, Yoshie Fujiwara, Kenichi Hitomi, John A. Tainer, Hiroshi Ide and Shigenori Iwai, *Nucleic Acids Res.*, **34** (5), 1540-1551 (2006).
- (8) Conserved XPB core structure and motifs for DNA unwinding: Implications for pathway selection of transcription or excision repair. Li Fan, Andrew Arvai, Priscilla K. Cooper, Shigenori Iwai, Fumio Hanaoka and John A. Tainer, *Mol. Cell*, **22** (1), 27-37 (2006).
- (9) Chemical synthesis of oligodeoxyribonucleotides containing the Dewar valence isomer of the (6–4) photoproduct and their use in (6–4) photolyase studies. Junpei Yamamoto, Kenichi Hitomi, Takeshi Todo and Shigenori Iwai, *Nucleic Acids Res.*, **34** (16), 4406-4415 (2006).
- (10) Characterization of distamycin A binding to damaged DNA. Aki Inase-Hashimoto, Shinya Yoshikawa, Yusuke Kawasaki, Takashi S. Kodama and Shigenori Iwai, *Bioorg. Med. Chem.*, **15**, in press (2007).

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