Easy to Get In, Hard to Get Out: X-ray Structure and Mechanism of RNA Polymerase II Stalled at an Antineoplastic Monofunctional Platinum-DNA Adduct

Cancer is a class of diseases in which abnormal cells divide without control and invade other normal tissues. Cancer cells can spread to other locations of the body via the bloodstream and lymph systems. Cancer is the second most common cause of death in the US and accounts for more than 1,500 Americans deaths a day based on NCI Cancer Statistics 2010.

Cisplatin, *cis*-diamminedichloroplatinum(II), is widely used and among the most effective cancer chemotherapy drugs used to treat various types of cancers including sarcomas, small cell lung cancer, ovarian cancer, lymphomas, and germ cell tumors.[1,2] It is the first member of a class of platinum-based anticancer drugs, which also includes carboplatin and oxaliplatin. The DNA template for transcription is the major target for these drugs. These drugs attack DNA and form bifunctional intra- or interstrand DNA cross-links, triggering a variety of cellular processes including transcription inhibition with attendant apoptosis. However, a major limitation of the use of this agent is the development of drug resistance within tumors.

A great number of compounds were synthesized and screened for anticancer activities to avoid resistance to conventional bifunctional platinum-based drugs. Among these compounds, *cis*-diammine(pyridine)chloroplatinum(II), or "pyriplatin", displayed significantly different anticancer properties and activity spectrum. Pyriplatin exhibits unique chemical and biological properties, forming monofunctional DNA adducts that can inhibit transcription and better escape nucleotide excision repair, a major drug resistance mechanism, by removing the drug-induced DNA damage.[3] The detailed molecular mechanism by which cells process DNA modified by pyriplatin is not understood. We take a combined biochemical and X-ray structural approach to investigate the molecular mechanism of pol II transcription inhibition by a site-specific monofunctional pyriplatin-DNA lesion.

Using diffraction data collected at SSRL synchrotron Beam Line 11-1, we solved the crystal structure and obtained the first structural snapshot of an RNA polymerase II (pol II) elongation complex encountering a site-specific pyriplatin-DNA lesion. We discovered that the pyriplatin-DNA adduct is located above the bridge helix, a key structural element for pol II translocation along the DNA duplex. Intriguingly, we found out that the adduct adopts a significantly different conformation within the pol II active site compared to that in duplex DNA.[3] Hydrogen bonds and van der Waals interactions with pol II residues and the DNA backbone orientates the pyriplatin-DNA adduct, requiring it to adopt this specific configuration.

Because transcription inhibition is an important component in the mechanism of action of platinum anticancer drugs, we investigated the effect of this site-specific pyriplatin-DNA adduct on the kinetics of pol II transcription elongation. Surprisingly, pol II could efficiently incorporate a matched CTP opposite to pyriplatin-damaged guanosine template at a rate comparable to that of non-damaged templates. No further extension of RNA transcripts as observed beyond the damage site. To understand the nature of the pol II complex stalled at the pyriplatin-DNA adduct, we then solved the X-ray crystal structure of pol II elongation complex after CTP incorporation opposite the platinated guanosine residue (Figure 1). Intriguingly, the conformation of the pyriplatin-DNA adduct changes significantly upon incorporation of CTP. The damaged guanosine rotates into the pol II active site and serves as a template for base pairing with the matched substrate, while the pyridine group of this

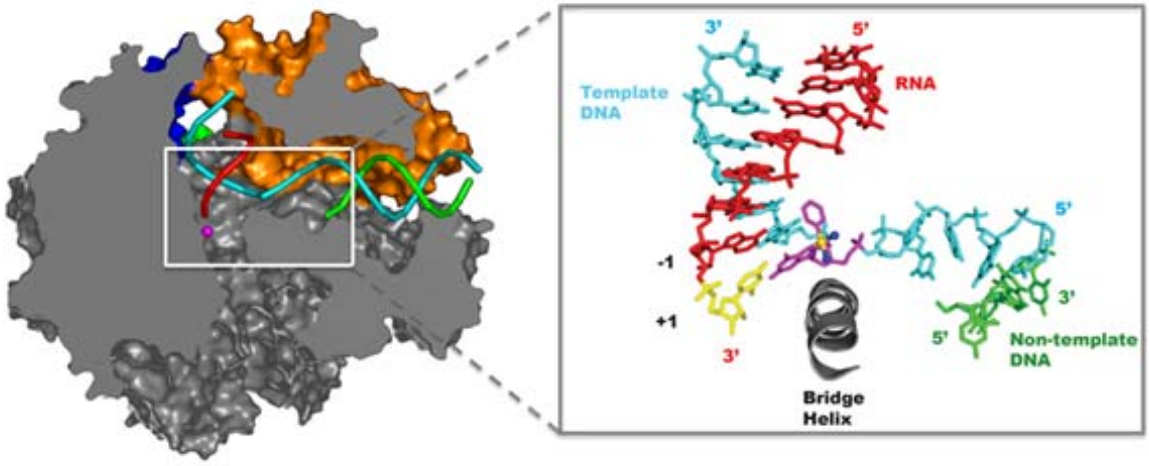

Figure 1. RNA Pol II stalled by a monofunctional pyriplatin-DNA adduct.

unit now faces toward 3'-direction of template DNA and the ammine group trans to pyridine is directed toward the bridge helix and forms hydrogen bonds with highly conserved pol II residues in bridge helix. This structure also provides the structural framework for understanding the "mysterious" unconventional structure activity relationship for monofunctional platinum drug candidates. The presence of a pyridine or other bulky group in the *cis* configuration is important for restricting the rotation range of the platinum ligand, rendering it a strong steric block to pol II translocation.

The present structural and biochemical results provide important insights into the transcription stalling process at the monofunctional pyriplatin-DNA adduct. The stalling mechanism of monofunctional platinum drugs of the pyriplatin family is significantly different from the mechanism of transcription inhibition by cisplatin and UV-induced 1,2-intrastrand cross-links. For the latter two DNA-modifications, a translocation barrier prevents delivery of damaged bases to the active site and/or leads to misincorporation of NTPs against the damage site, respectively. Monofunctional pyriplatin-damaged residues, on the other hand, can cross over the bridge helix and be accommodated in the pol II active site. The correct CMP nucleotide can be efficiently incorporated against the damaged guanosine. Blockage of the subsequent translocation from this position after incorporation of the cytosine nucleotide leads to inhibition of the RNA polymerase.

To summarize, we report here the first structure of a pol II transcribing complex stalled at a site-specific monofunctional DNA adduct, revealing a novel mechanism of transcription inhibition by this kind of genome damage. The results establish a basis for SARs that govern the anticancer drug potential of monofunctional platinum-based DNA damaging agents. Specific interactions between pol II active site residues and the platinum ligands are revealed, providing a structural framework for the rational design of more potent monofunctional pyriplatin analogues.

Primary Citation

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