DOI: 10.1002/cbic.200800733

Protein Recognition of Platinated DNA

Peter J. Sadler*[a]

Platinum-based drugs are widely used for the treatment of cancer.[1] They include the first- and second-generation drugs cisplatin and carboplatin, which are used for treating various types of cancers, including sarcomas, carcinomas and lymphomas. More recently oxaliplatin (Scheme 1) was introduced, and is mainly used for the treatment of colorectal cancer in combination with fluorouracil. Oxaliplatin is already a billion-dollar earner, an indication of the success of platinum drugs in the clinic.

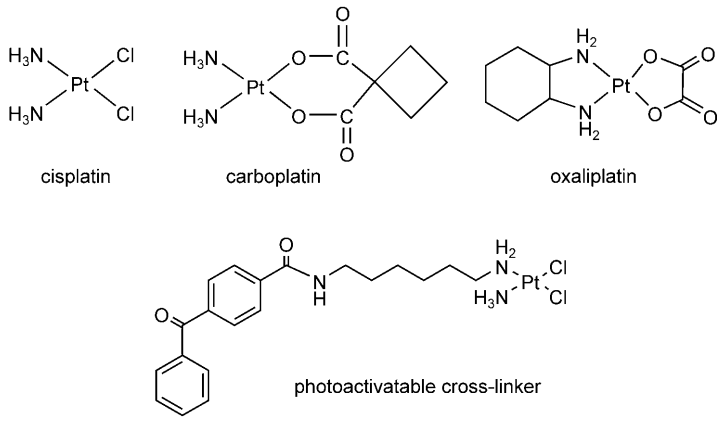
The mechanism of action of platinum drugs revolves around attack on cancer-cell DNA. Bonds between Pt^{II} and guanine N7, which is readily accessible in the major groove of DNA, are very strong, and intrastrand crosslinking of two adjacent guanines by square-planar Pt^{II} results in bending and unwinding of the DNA.

This dramatic structural change in DNA induced by platination triggers programmed cell death (apoptosis), a series of downstream events that result from protein and enzyme recognition of the damaged DNA.[2]

An elegant example of one initial protein-recognition event is the complex between GG-platinated DNA and high mobility group (HMG) protein. HMG protein binds to bent DNA and inserts a phenylalanine side chain between the G bases in the platinated crosslink.[3]

Protein recognition of platinated DNA does not only lead to cell death, but also to cell survival, to resistance. Nucleotide-excision-repair, for example, provides a mechanism for removing platinated adducts from DNA.

Hence it becomes critical to gain a better understanding of which proteins recognise platinated DNA and how they process it. Such knowledge can contribute to the design of more effective drugs, to drugs which avoid cross-resistance, have fewer side effects and are active against a wider spectrum of cancers.



Scheme 1. Some platinum drugs in clinical use, and the structure of the cisplatin analogue bearing a photoactivatable pendant benzophenone cross-linker. This agent forms intrastrand d(GpG) and d(GpTpG) crosslinks (chloride substitution by G).

Lippard et al.[4] have addressed the problem of detecting and identifying proteins that bind to platinated DNA in a range of cancer cells by synthesising a cisplatin analogue in which one of the NH_3 ligands is replaced by an amine with a tail containing benzophenone (Scheme 1), which can be photoactivated with 365 nm-wavelength light.

The authors platinated oligonucleotides site-specifically so as to form 1,2- or 1,3-intrastrand GG crosslinks, exposed them to nuclear extracts from cervical, testicular, pancreatic and bone cancer cells, and then irradiated them to induce the protein–DNA crosslinks. The benzophenone fragment gives rise to a diradical that attacks any nearby protein backbone or side chain.

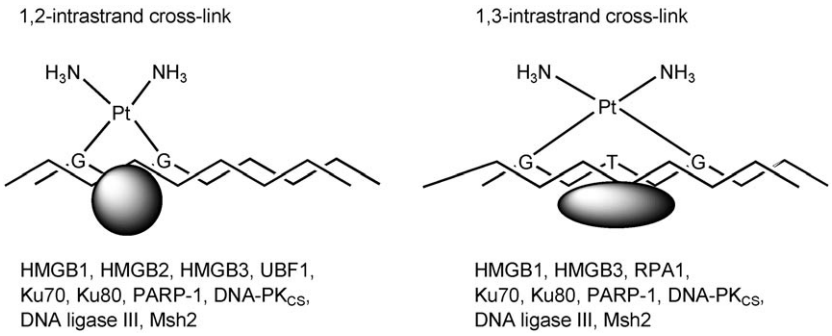
In general, protein recognition of platinated DNA was similar for cells with different sensitivities to cisplatin; this suggests that the variable responses of cells are due to more than just DNA-damage recognition.

The proteins identified include DNA repair factors as well as HMG proteins, including some that preferentially recognise either 1,2-d(GpG) or 1.3-d(GpTpG) crosslinks (Scheme 2). For example, the nucleotide-excision-repair protein RPA1 appears only to recognise the latter. Particularly interesting is the finding that poly(ADP-ribose) polymerase-1 (PARP-1) binds to both types of intrastrand crosslink. PARP-1 can recruit DNA ligase III to the damage site and might facilitate repair of the platinated DNA. This finding is relevant to the use of cisplatin and PARP-1 inhibitors in combination therapy.

The reported work was carried out on relatively short platinated DNA duplexes (25–40 base pairs), and it is interesting to ask whether the same proteins recognise platinated DNA in cells where the DNA is wrapped around histone proteins in nucleosomes. The histones in nucleosomes are thought to modulate DNA platination.[5] The photoaffinity technology should also allow detection at low levels of proteins involved in processing platinum-induced DNA damage in cells.

It will also be interesting to apply this photoaffinity method to the recognition of other types of platinum-induced DNA

[a] *Prof. P. J. Sadler*
Department of Chemistry, University of Warwick
Gibbet Hill Road, Coventry CV4 7AL (UK)
E-mail: p.j.sadler@warwick.ac.uk



Scheme 2. Some of the proteins identified by photocrosslinking reactions as recognising platinated DNA.

damage. For example to DNA adducts with di- and trinuclear platinum complexes,[6] *trans* diamine complexes[7] and photoactivated Pt^{IV} diazido complexes,[8] all of which are capable of producing distinctly different types of DNA lesions.

It is clear that protein recognition of platinated DNA plays a major role in the cellular processing of platinum anticancer complexes. It seems likely that the specific identification of the proteins and enzymes involved will eventually lead to the design of even more effective platinum drugs and to improved understanding of the molecular basis for their mechanism of action, including new insights into platinum resistance and unwanted side effects.

Keywords: antitumor agents · cisplatin · DNA · proteins

[1] P. J. Sadler, C. Muncie, M. Shipman, *Biological Inorganic Chemistry: Structure and Reactivity* (Eds.: I. Bertini, H. B. Gray, E. I. Stiefel, J. S. Valentine), University Science Books, New York, **2006**, pp. 95–135.
[2] Y. Jung, S. J. Lippard, *Chem. Rev.* **2007**, *107*, 1387–1407.
[3] U.-M. Ohndorf, M. A. Rould, Q. He, C. O. Pabo, S. J. Lippard, *Nature* **1999**, *399*, 708–712.
[4] E. R. Guggenheim, D. Xu, C. X. Zhang, P. V. Chang, S. J. Lippard, *ChemBioChem* **2009**; DOI: 10.1002/cbic.200800471.
[5] B. Wu, P. Dröge, C. A. Davey, *Nat. Chem. Biol.* **2008**, *4*, 110–112.
[6] A. Hegmans, J. Kasparkova, O. Vrana, L. R. Kelland, V. Brabec, N. P. Farrell, *J. Med. Chem.* **2008**, *51*, 2254–2260.
[7] G. H. Bulluss, K. M. Knott, E. S. Ma, S. M. Aris, E. Alvarado, N. Farrell, *Inorg. Chem.* **2006**, *45*, 5733–5735.
[8] F. S. Mackay, J. A. Woods, P. Heringová, J. Kaspárková, A. M. Pizarro, S. A. Moggach, S. Parsons, V. Brabec, P. J. Sadler, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 20743–20748.

Received: November 7, 2008
Published online on November 28, 2008

