

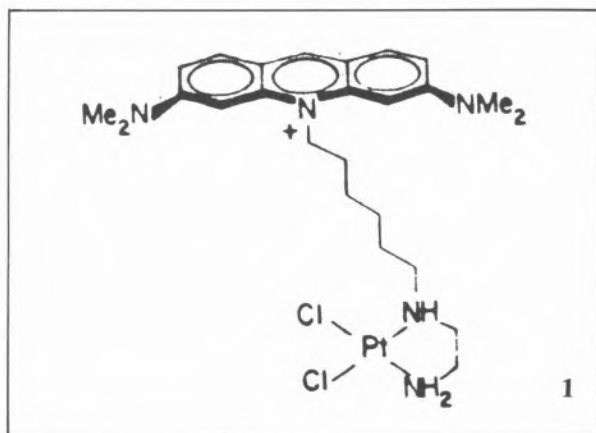


PS5.63 — TU

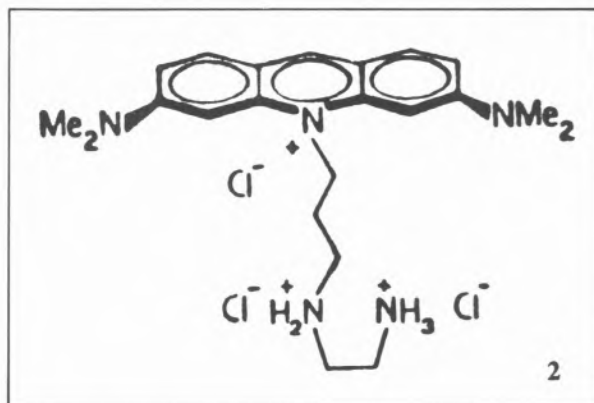
BRUCE E. BOWLER
STEPHEN J. LIPPARD
Department of Chemistry
Massachusetts Institute of Technology
Cambridge MA 02139
U.S.A.

EFFECTS OF LINKED AND EXTERNAL INTERCALATORS ON THE BINDING OF PLATINUM ANTITUMOR DRUGS TO DNA

Previously we showed that DNA intercalators, such as ethidium bromide, could alter the mode and position of binding of the platinum antitumor drug, *cis*-diamminedichloroplatinum(II) (*cis*-DDP), to the double helix [1]. These results led us to synthesize a molecule with an intercalative drug linked to a platinum complex by a hexamethylene chain [2], **1**, as a more sensitive probe of these effects. We have more recently synthe-



sized the related platinum binding ligand, **2**, in which a trimethylene chain links the acridine orange and ethylenediamine portions of the molecule [3]. When attached to platinum this new compound will allow us to probe the effects of chain length on the interaction of both ends of the molecule with DNA.



Exonuclease III mapping [4] was used to obtain information about the DNA binding sites of compound **1** on the same 165 base-pair (bp) restriction fragment from plasmid pBR322 employed in our previous *cis*-DDP/DNA mapping experiments [1a,4]. As a control we studied the binding sites of dichloroethylenediamineplatinum(II), [Pt(en)Cl₂], which is closely related to **1**, on the 165 bp fragment. We find that [Pt(en)Cl₂] binds to this restriction fragment at virtually the same oligo(dG) regions as does *cis*-DDP. Data for compound **1** also reveal binding at the same oligo(dG) sequences. Two other interesting results are observed, however. The 165 bp fragment contains a G₆CG₂ sequence near the 3'-end which is not observed as an exonuclease III stop at low levels of bound *cis*-DDP or [Pt(en)Cl₂] per nucleotide. Only when bound in the presence of the intercalator ethidium bromide are low levels of *cis*-DDP able to stop Exo III digestion at this sequence. Low levels of compound **1**, bound in the absence of ethidium bromide, definitely show stopping at this site. Thus the attached intercalator enhances platinum binding in this region in a manner similar to that by which externally added ethidium bromide enhances *cis*-DDP binding. This result probably involves relaxation of the steric requirements for platinum binding at this sequence through the conformational changes induced by intercalator binding to the DNA.

The other intriguing result is that additional stops are seen with compound **1** that correlate with (dG)₂ sequences on the unlabeled strand. For *cis*-DDP binding to the 165 bp restriction fragment, Exo III was found to map only those oligo(dG) binding sites on the labeled strand. The presence of the linked intercalator facilitates detection of

platinum bound on the unlabeled strand. This difference is ascribed to the more extensive interactions, both covalent (Pt) and intercalative (acridine), of compound 1.

In summary, exonuclease III mapping of compound 1 binding to DNA has shown that the attached intercalator alters the binding pattern of the platinum moiety in a manner analogous to that of an externally added intercalator. The presence of the attached intercalator also allows detection of binding sites on the unlabeled strand.

REFERENCES

- [1] a) T.D. TULLIUS, S.J. LIPPARD, *Proc. Natl. Acad. Sci. USA*, **79**, 3489 (1982);
b) C.M. MERKEL, S.J. LIPPARD, *Cold Spring Harbor Symp. Quant. Biol.*, **47**, 355 (1982).
- [2] B.E. BOWLER, L.S. HOLLIS, S.J. LIPPARD, *J. Am. Chem. Soc.*, **106**, 6102 (1984).
- [3] E. WHANG, B.E. BOWLER, S.J. LIPPARD, unpublished results.
- [4] T.D. TULLIUS, S.J. LIPPARD, *J. Am. Chem. Soc.*, **103**, 4620 (1981).



PS5.64 — TH

J.L. VAN DER VEER

G.J. LIGTVOET

A.R. PETERS

J. REEDIJK

Department of Chemistry

Gorlaeus Laboratories

State University Leiden

P.O. Box 9502, 2300 RA Leiden

The Netherlands

BINDING OF CIS-PLATINUM COMPOUNDS TO NUCLEOBASES, NUCLEOTIDES AND OLIGONUCLEOTIDES

For a better understanding of the specific interactions of *cis*-platinum(II) and *cis*-platinum(IV) compounds with DNA, detailed studies of the binding of such Pt compounds to nucleobases,

nucleotides and oligonucleotides are of great interest [1].

Although it is now generally accepted that binding of platinum compounds has a kinetic preference to guanine N7 sites, other bindings do also occur and may play an important rôle under certain conditions [2]. Using 9-ethylguanine as a simple analog of the GMP part in DNA, it was possible to study Pt(II) binding to guanine N1 and the — unexpected — rapid isomerisation to the N7 binding mode at acid pH values. Studying reactions of Pt(IV) compounds with 9-methylhypoxanthine and 5'-GMP, the major products appeared to be N7-coordinated Pt(II) adducts, although in a few cases spectroscopic indications for Pt(IV) adducts were found. Although it is not yet clear whether the reduction occurs before, during or after the platinum coordination to 5'-GMP, these results support the idea that *in vivo* reduction is essential for the antitumor activity of potential Pt(IV) antitumor drugs. To obtain more information about the chelation and kinetics of the so-called «GNG-*cis*Pt chelates» (platinum bound to both G's with a non-coordinating nucleobase in between), the DNA-trinucleotides pGpGpG, GpApG and GpTpG were reacted with *cis*-PtCl₂(NH₃)₂ (*cis*Pt). NMR studies indicate that the pGpGpG trimer yields only pGpGpG(N7(1),N7(3)) while GpApG gives 80% GpApG(N7(1),N7(3)) and 20% GpApG(N7(2),N7(3)). HPLC studies showed that this ratio is temperature independent. Reaction of the monofunctional platinum compound [Pt(dien)Cl]Cl with these two trimers gives 100% pGpGpG(N7(1)), while 30% GpApG(N7(1)) and 70% GpApG(N7(3)) was obtained in the second case. *cis*Pt reaction with GpTpG resulted in only one chelate: GpTpG(N7(1),N7(3)). Apparently a GG-chelate is preferred by *cis*Pt over a GNG-chelate, but if no neighbouring G is available after the first binding, kinetic preference determinates the ultimate chelate, *i.e.* binding to AG may occur.

In vitro studies concerning *cis*Pt-DNA interactions indicate that next to GG-, AG-chelation is most favoured [3]. This led to the study of the reactivity of *cis*Pt towards the decamer TpCpTpCpApGpTpCpTpC, and the stability of this decamer with its complement GpApG-