9.8: Site-specifically Platinated DNA 154 154

a. The Problem

Much of the information obtained about the mechanism of action of cisplatin has been derived from experiments where Pt-DNA binding has occurred *in vivo* or *in vitro*, with the use of random-sequence DNA having all available targets for the drug. In these studies, platination is controlled by the inorganic chemistry of *cis*-DDP in the medium and the accessibility of target sites on the DNA, as already discussed in considerable detail. As such, this situation best represents drug action as it actually occurs in the tumor cell. On the other hand, the resultant spectrum of DNA adducts makes it difficult, if not impossible, to understand the structural and functional consequences of any specific adduct. In order to address this problem, a methodology has been developed in which a single platinum adduct is built into a unique position in the genome. This approach is powerful and has the potential to be extended to the study of many other metal-based drugs. In this section, we discuss the strategy used to construct such site-specifically platinated DNA molecules and the information obtained thus far from their study. Some uses have already been discussed.

b. Synthesis and Characterization

Figure 9.29 displays the map of a genome constructed by insertion of platinated or unplatinated dodecanucleotide duplexes d(pTpCpTpApGpGpCpCpTpTpCpT)•d(pApGpApApGpGpCpCpTpApGpA) into DNA from bacteriophage M13mp18. This genome was constructed in the following manner.[154] Double-stranded DNA from M13mp18 was first digested with Hinc II, a restriction enzyme that recognizes a unique six-base-pair sequence in the DNA and cleaves the double helix there, leaving a blunt-ended (no overhanging bases) cleavage site. The unplatinated dodecamer duplex was next ligated into the Hinc II site, and the DNA amplified *in vivo*. The dodecamer can insert into the genome in two different orientations, the desired one of which, termed M13-12A-Stu I, was identified by DNA sequencing. The presence of the insert in the new DNA was checked by its sensitivity to the restriction enzyme Stu I, which cleaves at the d(AGGCCT) sequence uniquely situated in the dodecamer insert, and the absence of cleavage by Hinc II the site for which was destroyed. Next, Hinc II-linearized M13mp18 replicative form (RF) DNA was allowed to form a heteroduplex with excess viral DNA (which has only the + strand in the presence of the denaturant formamide which was dialyzed away during the experiment. The resulting circular DNA has a gap in the minus strand into which the platinated d(TCTAG*G*CCTTCT) was ligated (Figure 9.30). The latter material was prepared by the methods described in Section V.D.5.a and characterized by ^{1}H NMR spectroscopy. The resulting site-specifically platinated DNA contains a single *cis*-[Pt(NH$_3$)$_2${d(pGpG)}] intrastrand crosslink built into a unique position. The methodology is general, and has been used to create other known platinum-DNA adducts site-specifically in M13mp18.

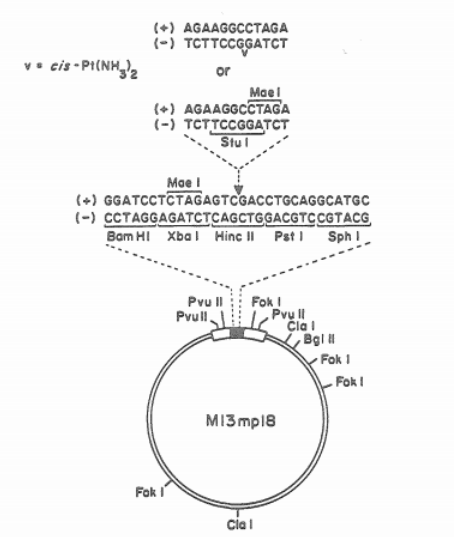

Figure 9.29 - Map of the genome created by insertion of cis-DDP platinated or unplatinated d(TpCpTpApGpGpCpCpTpTpCpT)-d(ApGpApApGpGpCpCpTpApGpA) into the Hinc II restriction site of bacteriophage M13mp18 DNA.

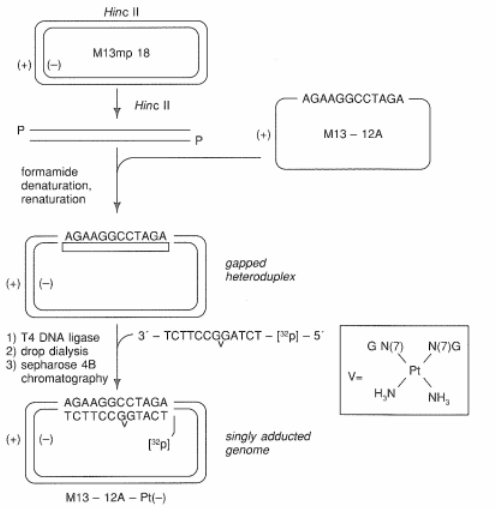

Figure 9.30 - Scheme for constructing site-specifically platinated genomes via gapped heteroduplex synthesis (reproduced by permission from Reference 154).

The chemical properties of the platinated DNA, termed M13-12A-Pt(-)-Stu I, were investigated by enzymatic, digestion and gel electrophoresis experiments. Platinum completely inhibits cleavage of the DNA by Stu I, as expected from the earlier restriction enzyme mapping studies. In addition, the *cis*- [$Pt(NH_3)_2${d(pGpG)}] and *cis*-[$Pt(NH_3)_2${d(pApG)}] intrastrand crosslinks were found to inhibit a variety of DNA polymerases, with only a small amount of bypass of the platinum lesion.[149] These results indicate that the most abundant adducts of cisplatin on DNA are able to block replication efficiently.

c. Biological Properties

When M13-12A-Pt(-)-Stu I DNA was introduced into *E. coli* cells by transformation, DNA synthesis was uninterrupted, because the cell can both repair the damage and use the unmodified (+) strand for synthesis. Consequently, a slightly different strategy was used to construct single-stranded M13-12A-Pt(+)-Stu I DNA, the details of which are available elsewhere.[154] This platinated template, in which the damage can neither be repaired nor bypassed by known mechanisms *in vivo*, was then transformed into *E. coli* cells co-plated with GW5100 cells. Under these conditions, viral DNA replication is detected by the expression of the β-galactosidase gene, which, in the presence of appropriate reagents in the medium, leads to formation of blue plaques on a clear background. The results clearly indicate that many fewer plaques appear when M13-12A-Pt(+) is introduced into the cells than when M13-12A-u(+) was employed, where u stands for unmodified DNA. In three repeats of this experiment, survival of DNA containing only a single *cis*-[Pt(NH3h{d(pGpG)}] crosslink was only 11 ± 1 percent.

These data provide unambiguous proof that the most frequent DNA adduct formed by cisplatin is toxic, capable of inhibiting replication when only a single such lesion is present on a natural DNA template of 7,167 nucleotides. The fact that as many as 10 percent of the transformed cells can bypass or repair the lesion is also of interest, and parallels the results found *in vitro*. In related work, it was found that the *cis*-[$Pt(NH_3)_2${d(pGpG)}] intrastrand crosslink is not very mutagenic, but that *cis*-[$Pt(NH_3)_2${d(pApG)}] intrastrand adducts are considerably more so. This finding is important, since mutations could lead to long-term secondary tumor production in patients treated with cisplatin. The methodology affords a way to screen new compounds that one would like to be equally effective at inhibiting replication but less mutagenic. In addition, by using repairdeficient mutant cell lines, as well as cisplatin resistant cells, one can study the effects of varying the properties of the host cells. Incorporation of site-specifically platinated DNA sequences into appropriate shuttle vectors will also facilitate investigation of toxicity, repair, and resistance in mammalian cells.

d. Prospectus

The foregoing discussion illustrates the power of site-specifically platinated DNAs as a probe of the molecular mechanism of the drug. We recall that similar strategies were employed to obtain uniquely modified DNA in the bending[92,93] and unwinding[95] experiments discussed previously. In principle, this technique can be applied to examine other aspects of the molecular mechanism of other metallochemotherapeutic agents. The requirements are a synthetic route to the uniquely modified genome, for which both the inorganic coordination chemistry and molecular biology must be amenable, an adduct stable to the biological conditions for DNA synthesis, and a method (usually genetic) for scoring the biological effects being investigated. Site-specifically platinated

DNAs allow the bioinorganic chemist to have maximal control over the genetics and should continue to provide valuable information about the molecular mechanism of action of cisplatin.
