Nucleic Acids and Molecular Biology

Volume 7

Edited by
Fritz Eckstein · David M.J. Lilley

With 105 Figures

Springer-Verlag
Berlin Heidelberg New York
London Paris Tokyo
Hong Kong Barcelona
Budapest

Prof. Dr. FRITZ ECKSTEIN
Max-Planck-Institut
für Experimentelle Medizin
Hermann-Rein-Straße 3
37075 Göttingen, Germany

Prof. Dr. DAVID M. J. LILLEY
University of Dundee
Biochemistry Department
Dundee DD1 4HN
United Kingdom

Cover illustration by kind permission of B. W. Matthews

ISBN-13: 978-3-642-77952-7 e-ISBN-13: 978-3-642-77950-3
DOI: 10.1007/978-3-642-77950-3

The Library of Congress has catalogued this serial publication as follows:
Library of Congress Catalog Card Number 90-64/8/7

Typesetting: Best-set Typesetter Ltd., Hong Kong
31/3145-5 4 3 2 1 0 – Printed on acid-free paper

Preface to the Series

From its inception, molecular biology has always been a discipline of rapid development. Despite this, we are presently experiencing a period of unprecedented proliferation of information in nucleic acid studies and molecular biology. These areas are intimately interwoven, so that each influences the other to their mutual benefit. This rapid growth in information leads to ever-increasing specialization, so that it becomes increasingly difficult for a scientist to keep abreast of developments in all the various aspects of the field, although an upto-date knowledge of the field as a whole is highly desirable.

With this background in mind, we have conceived the present series *Nucleic Acids and Molecular Biology*. It comprises focused review articles by active researchers, who report on the newest developments in their areas of particular interest. The reviews are not intended to be exhaustive, but rather to place the most recent data into context. This format will allow our colleagues of familiarize themselves with new developments in areas outside their own immediate speciality, thus facilitating a more global view of their own work. Moreover, we hope sincerely that this will convey some of the excitement of the interdisciplinary nature of the study of nucleic acids and molecular biology.

This series is planned to appear annually. This period will allow us to return to important topics with sufficient frequency to cover new developments as they emerge.

FRITZ ECKSTEIN
DAVID M. J. LILLEY

Introduction to Volume 7

A substantial proportion of this volume has been devoted to the analysis of different aspects of nucleic acid-protein interactions. Following an initial chapter on the catalytic activity of *cis*-platinated DNA, the next three chapters address structural questions in prokaryotic proteins that interact with DNA. While most DNA binding proteins are based around sequence recognition by side chains from an α-helix (e.g. the 434 repressor and the restriction enzyme *Eco* RV), the MetJ repressor represents an important new class of proteins that are based on antiparallel β-sheet. An alternative use of the α-helix in sequence recognition is provided by the leucine zipper proteins, exemplified by the Fos-Jun heterodimer, where a coiled-coil plays an important role in mediating protein-protein interactions. Another very important class of DNA binding proteins is represented by the zinc fingers, yet these are also divisible into a number of distinct classes of proteins. The first to be identified contains TFIIIA as its archetypical member. The manner of the binding of this nine-finger transcription factor to its cognate binding site in the 5S rRNA gene is discussed in this volume. The steroid receptor proteins are structurally very different from the TFIIIA fingers, and are exemplified by the glucocorticoid and retinoic acid receptor proteins. We hope to cover additional zinc binding motifs in future volumes.

In addition to recognizing sequence, DNA binding proteins may also respond to, and indeed modify, the structure of DNA. *Eco* RV severely disrupts local DNA structure when bound to its target site, while the structure of the Fis protein suggests a major bending of DNA. Many of these proteins are involved in either repair or recombination. New insight has recently been gained into the enzymology of recombination in *Escherichia coli*, and in the mechanism of the site-specific recombination events that underlie DNA segregation in plasmids. Perhaps the histones may be thought of as the ultimate manipulators of DNA structure, wrapping DNA of random sequence into nucleosomes for packaging; however, this leaves the question of just how random this sequence can be, and how the nucleosomes may be positioned with respect to the DNA sequence. Further higher order structure of chromatin

and chromosomes appears to be genetically important, and the role of chromosomal attachment is considered.

RNA and its interactions are not neglected in this volume. The fundamentally single-stranded character of RNA molecules is extensively folded in secondary and tertiary structure, and an important structural element that is restricted to RNA is the pseudoknot. Still more interesting, RNA species that may be adapted to the binding of novel substrates may be selected using in vitro methods, potentially making the repertoire of RNA almost limitless. RNA-protein interactions, while not so extensively studied as those of DNA, are becoming better understood, as the studies of RNaseH and ribonucleoproteins indicate. Finally, perhaps the most complex functional RNA-protein apparatus in the cell is the ribosome, but even there we see significant progress.

We are grateful to all the authors of Volume 7 for maintaining the level of expertise, interest and clarity to which we have become accustomed in editing this series.

Spring 1993

FRITZ ECKSTEIN
DAVID M. J. LILLEY

Contents

Contributors

You will find the addresses at the beginning of the respective contribution.

DNA, *cis*-Platinum and Intercalators: Catalytic Activity of the DNA Double Helix

M. Sip and M. Leng[1]

1 Introduction

Several drugs have cellular DNA as target. Some act by binding reversibly to DNA, while others bind covalently (Waring 1981). The binding sites are located within the grooves or between the base pairs of the double helix. They vary greatly in size from one to several nucleotide residues and their intrinsic properties are modulated by the DNA molecule itself through short- and long-range interactions. Short-range interactions are dictated by the neighboring nucleotide residues which affect hydration, electrostatic potential, and the accessibility of the binding sites. Long-range interactions result from an event (DNA bending, DNA supercoiling, DNA-protein complexes) on the same DNA molecule but far away from the binding site (Wang and Giaever 1988). The covalent binding of the chemical carcinogen N-acetoxy-N-acetyl-aminofluorene to the C(8) of guanine residues in a plasmid containing a $(dC\text{-}dG)_n$ insert illustrates the importance of these interactions. In the relaxed plasmid all the guanine residues react with the carcinogen but to a different extent. When the insert is driven into the Z structure by negative supercoiling, the guanine residues within the insert lose their reactivity with the carcinogen, whereas the guanine residues at the B-Z junctions become much more reactive than the more reactive guanine residues in the rest of the plasmid (Marrot et al. 1987). More generally, the interactions between a drug and its binding site depend on the conformation of the whole DNA molecule and reciprocally the bound drug induces distortions in the double helix at the level of the binding sites and far away.

Numerous studies have been already devoted to the drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP, *cis*-platinum) which is largely used in the treatment of human tumors. It is generally accepted that the cytotoxic action of *cis*-DDP is related to its ability to react with cellular DNA (Eastman 1987; Reedijk 1987; Brabec et al. 1990; Lepre and Lippard 1990). The mechanism of *cis*-DDP antitumor activity is not yet completely understood, while *cis*-DDP is a very simple inorganic molecule consisting of only 11 atoms, 6 of which are hydrogens. It is of fundamental importance to determine how *cis*-DDP-DNA adducts interfere with the cellular machinery.

[1] Centre de Biophysique Moleculaire, CNRS 1A, Avenue de la Recherche Scientifique, 45071 Orléans Cedex 2, France

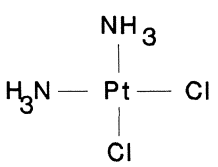
Nucleic Acids and Molecular Biology, Vol. 7
ed. by F. Eckstein and D.M.J. Lilley

Quite often, *cis*-DDP is used with other drugs in combination chemotherapy. Some of these drugs also bind to DNA albeit their binding sites and binding modes might differ from those of *cis*-DDP. It is also fundamental to understand at the molecular level how the binding of one drug to DNA can interfere with the binding of another drug. In this review, we first present some results on the reaction between DNA and *cis*-DDP which highlight the key role played by the double helix. Subsequently, some indications that the presence of DNA-intercalating compounds interfere with the binding of *cis*-DDP are reviewed. Finally, the active participation of DNA double helix is described and it is proposed that DNA double helix acts as a catalyst.

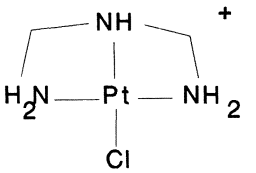
2 *cis*-Diamminedichloroplatinum(II): Reaction with DNA

2.1 DNA – cis-*DDP Adducts*

In the reaction between native DNA and *cis*-DDP (Fig. 1), different kinds of adducts are formed. The two major adducts have been characterized as d(GpG) and d(ApG) 1,2 intrastrand cross-links, representing about 65 and 25% of the bound platinum, respectively. The other adducts are d(GXG) 1,3 intrastrand cross-links, X being a base residue, d(GG) 1,2 interstrand cross-links, and monofunctional adducts. It has been noted that DNA-protein cross-links form if proteins are present during the reaction of platination (Fichtinger-Schepman et al. 1985; Eastman 1987). The same types of adducts are also formed in vivo. The formation of the adducts proceeds in two solvent-assisted reactions in sequence (Bancroft et al. 1990; Brabec et al. 1990; Lepre and Lippard 1990; Bernges and Holler 1991). The exchange of the chloro-groups of *cis*-DDP is rate-limiting in both the attack of DNA and the closure of monofunctional adducts to bifunctional adducts. The preferred initial binding site of *cis*-DDP to DNA is the N7 position of guanine residues. The main reaction steps of the reaction are summarized in Fig. 2.



cis-DDP



[Pt(dien)Cl]+

Fig. 1. Formula of *cis*-diamminedichloroplatinum(II) (*cis*-DDP) and chlorodiethylenetriamineplatinum(II) $[Pt(dien)Cl]^+$

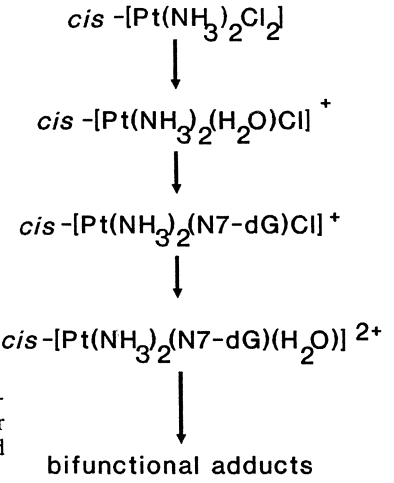


Fig. 2. Main steps of the reaction between *cis*-DDP and guanine (dG) residues in DNA. After hydrolysis of *cis*-DDP several species are formed but are not mentioned

The distribution of the adducts along the DNA has been mainly elucidated by mapping with exonucleases or DNA polymerases (Lepre and Lippard 1990). Recently, it has been shown that RNA polymerases are blocked by bifunctional lesions (Lemaire et al. 1991; Corda et al. 1991, 1992). Analysis of the RNA fragments generated by T7 RNA polymerase acting on a platinated DNA containing the insert d(TTGCTTGATTAGTTGTGT) has given the following order for the reactivity of the sites: d(ApG) > d(GC) = d(GpA) > d(GTG) (unpubl. results). These results suggest that among the minor lesions, d(GpA) 1,2 intrastrand cross-links and d(GG) 1,2 interstrand cross-links are preferentially formed. It is important to note that the percentage of interstrand cross-links represents about 5–8% of the total lesions (Hansson and Wood 1989; Jones et al. 1991).

2.2 Importance of DNA Double Helix in Reactivity

In the reaction between DNA and the monoaqua or diaqua form of *cis*-DDP, the double helix interferes with the different steps presented in Fig. 2 by (1) accessibility of the potential binding sites; the N(3) position of cytosine residues is reactive to *cis*-DDP in single-stranded DNA but not in double-stranded DNA (Fichtinger-Schepman et al. 1985; Eastman 1987); (2) distances between the potential binding sites and local flexibility of DNA; *cis*-DDP binds to the N7 position of guanine residues in Z-DNA and only monofunctional adducts are formed (Malinge and Leng 1984). The d(GG) 1,2 interstrand cross-links are formed at the d(GC/GC) sequences and not at the d(CG/CG) sequences (Hopkins et al. 1991; Lemaire et al. 1991). (3) Electrostatic potential; the high frequency of platination at the d(GpG) sites

in natural DNA excludes an equal reactivity for all the guanine residues. The electrostatic potential is not constant along the DNA molecule (Pullman and Pullman 1981) and therefore the aqua form of *cis*-DDP (which is positively charged) is preferentially attracted by the most negative electrostatic potential located at the N7 position of consecutive guanine residues; (4) pentacoordinated intermediate; in natural DNA the number of d(ApG) and d(GpA) sites is approximately the same. Nevertheless, mainly d(ApG) 1,2 intrastrand cross-links are formed. Even if the platinum atom of the monofunctional *cis*-$[Pt(NH_3)_2(N7\text{-}dG)Cl]^+$ adduct is closer to the 5′ A residue than the 3′ A residue, the closure rates of the *cis*-$[Pt(NH_3)_2(N7\text{-}dG)Cl]^+$ adduct to the bifunctional d(ApG) or d(GpA) 1,2 intrastrand cross-links are of the same order of magnitude (Payet et al. 1992). An explanation is that d(ApG) sites are recognized preferentially at d(GpA) sites because the two sites stabilize differently the pentacoordinated intermediate formed during the first coordination of the aqua species of *cis*-DDP (Laoui et al. 1988).

With respect to both the formation of monofunctional adducts and their closure to bifunctional adducts, the flexibility of DNA deserves some additional comments. As indicated in Table 1, in B-DNA the distances between the N7 position of guanine residues and between the platinum atom of the monofunctional adduct of *cis*-DDP and the N7 positions of guanine residues in d(GpG), d(GpXpG), d(GC/GC), and d(CG/CG) sequences are larger than the distance of about 3 Å required for a *cis*-DDP cross-linking reaction (Sherman et al. 1988; Lippert 1989). To accomodate these distances, the canonical conformation has to be locally distorted. This is possible because its structure is not static but dynamic. Double-stranded nucleic acids are subjected to thermally driven fluctuations resulting in transient conformations involving distortions such as bending, twisting, base-pair opening, and strand separation (Leroy et al. 1988; Ramstein and Lavery 1990). Once the monofunctional adduct is formed, a conformational change occurs (and thus the conformational fluctuations are modified) as suggested by the following results. The monofunctional platinum derivative $[Pt(dien)]^{2+}$ (a model for the monofunctional adduct of *cis*-DDP) bound at the N7

Table 1. Distances between two N7 positions of guanine residues and between a *cis*-platinum residue bound to the N7 position of a guanine residue and an N7 position of another guanine residue. Standard Arnott B-DNA conformation was developed by JUMNA. The bound platinum atom was positioned at a distance of 2 Å from the N7 position of the guanine residue on the axis bisecting the guanine C8-N7-C5 angle, in the plane of guanine residue

Sequence distance (Å)	G_1C/G_2C	CG_1/CG_2	G_1G_2/CC	G_1XG_2/CXC
$N7(G_1)\cdot N7(G_2)$	7.1	8.9	4.2	8.3
$Pt(G_1)\cdot N7(G_2)$	6.8	9.8	5.8	9.6
$N7(G_1)\cdot Pt(G_2)$	6.7	9.8	3.7	8.0

position of the G(5) residue of d(TCTCGTCTC)·d(GAGACGAGA) decreases the thermal stability of the duplex (van Garderen et al. 1989). A more recent study of several double-stranded oligonucleotides containing a single monofunctional adduct extends this finding (Brabec et al. 1992). In all cases, the double helices are distorted as revealed by chemical probes and artificial nuclease and they are thermally less stable. The effects are sequence-dependent and are largest when the 5′ and 3′ residues adjacent to the modified guanine are pyrimidine residues. The binding of $[Pt(dien)]^{2+}$ to poly(dG-dC)·poly(dG-dC) promotes the B-Z transition (Malfoy et al. 1981; Ushay et al. 1982).

In summary, all these parameters (accessibility, geometry, electrostatic potential, intermediate reactive species, flexibility) interfere with the binding of *cis*-DDP to DNA but to different extents. To emphasize this, a final example is mentioned. The reactivity of a single monofunctional adduct (*cis*-$[Pt(NH_3)_2(N7\text{-}dG)Cl]^+$) within two double-stranded oligonucleotides (22-mer), differing by their central sequences, is base sequence-dependent (Payet et al. 1992). The half-lives for closure of the monofunctional adducts to bifunctional cross-links are 14 and 3 h for the central sequences d(TGCT) and d(AGCT), respectively. In both cases the main cross-link is an interstrand cross-link. Even in the central sequence d(AGCT) the monofunctional adduct reacts preferentially with the opposite G residue (interstrand cross-link) rather than with the 5′ adjacent A residue (intrastrand cross-link).

2.3 Distortions Induced in DNA by Interstrand Cross-Links

The distortions induced in DNA by the adducts have been characterized by several techniques with special focus on the d(GpG) 1,2 intrastrand cross-link (Brabec et al. 1990; Lepre and Lippard 1990). Recently, gel electrophoresis experiments (Rice et al. 1988; Bellon et al. 1991) revealed that both d(GpG) and d(ApG) 1,2 intrastrand cross-links bend and unwind DNA by 32°–34° and 13°, respectively, but the details of the distortions at the nucleotide level are different (Schwartz et al. 1989). It is worth noting that the distortion spreads over only one or two base pairs on the 5′ side but not on the 3′ side of the d(ApG) 1,2 intrastrand cross-link. On the basis of gel electrophoresis, chemical probes, and molecular mechanics modeling data (Sip et al. 1992), it has been proposed that the cross-linking of two guanine N7 atoms on the opposite strand within the sequence d(GC/GC) by *cis*-DDP induces a bent of about 55° of the double helix toward the major groove. While the double helix conserves its average twist angle, nevertheless, a large change in the local twist values at the adduct level is observed. The distortion is localized at the platinated d(GC/GC) sequence. Stereoviews of unplatinated and platinated oligonucleotides are given in Fig. 3.

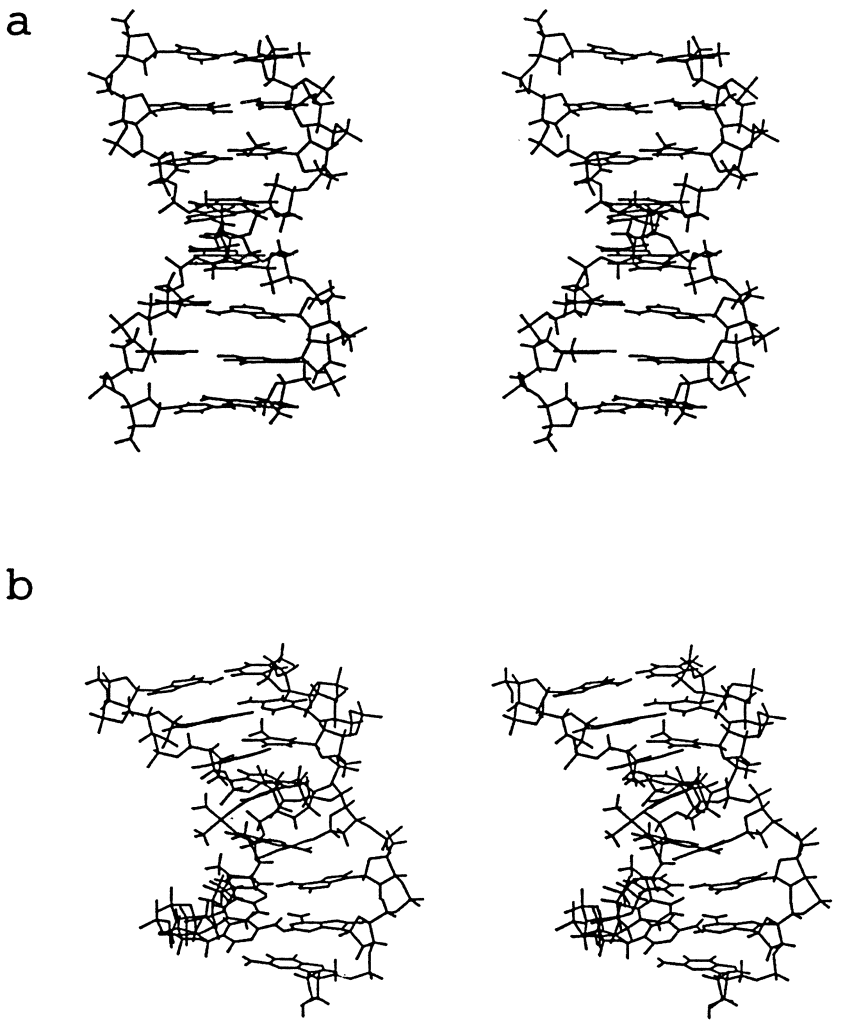


Fig. 3. **a** Stereoview of the unplatinated d(CTTGCTCT/AGAGCAAG) and **b** of the same oligonucleotide containing an interstrand cross-link between the G(4) and G(12) residues

3 N-Methyl-2,7-Diazapyrenium: Reaction with DNA

The interaction between DNA and N-methyl-2,7-diazapyrenium (MDAP; Fig. 4) has not yet been studied extensively and thus the corresponding complexes are not described in detail as, for example, the complexes between DNA and proflavine or between DNA and ethidium bromide. Nevertheless, spectroscopic and hydrodynamic measurements on MDAP and some of its derivatives show that they behave as several planar cationic

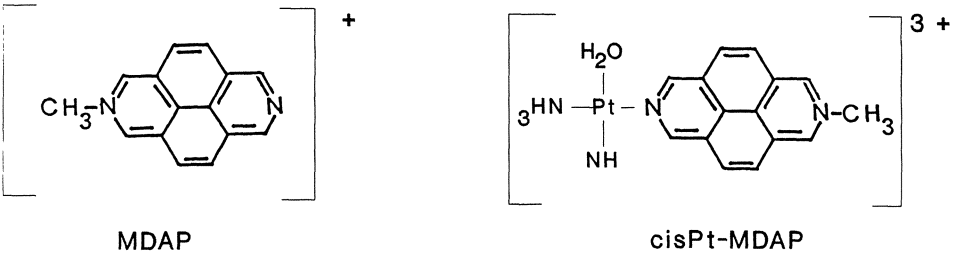


Fig. 4. Formula of N-methyl-2,7-diazapyrenium (*MDAP*) and *cis*-$[Pt(NH_3)_2$(N7-N-methyl-2-diazapyrenium)$H_2O]^{3+}$ (*cisPt-MDAP*)

molecules (ethidium bromide, proflavine, etc.) (Blacker et al. 1986; Slama-Schwok et al. 1989; Malinge et al. 1990). We assume that MDAP as ethidium bromide, proflavine, etc. binds DNA by intercalation between base pairs.

4 DNA, *cis*-DDP and Intercalators

4.1 Mutual Interference of cis-*DDP and Intercalators*

Two key experiments have indicated a clear interference between the DNA binding of intercalators and *cis*-DDP. It was first reported that the number of potential binding sites for ethidium bromide (Eth) decreases as the amount of bound *cis*-platinum residues increases (Howe-Grant et al. 1976; Macquet and Butour 1978). This can be explained satisfactorily taking into account the distortions induced in the double helix by the intra- and interstrand cross-links. The second experiment demonstrates that the distribution of the lesions along a DNA fragment is different if the reaction of platination is carried out in the presence or in the absence of Eth (Tullius and Lippard 1982).

Among others, one reason for this modulation of *cis*-DDP binding by the intercalator is the formation of a new kind of adduct (dG-cisPt-Eth) which arises from a cross-link between one of the exocyclic amino groups of Eth and the N7 atom of a guanine residue (Malinge and Leng 1986, 1988; Malinge et al. 1987; Sundquist et al. 1988). If double-stranded DNA is replaced by single-stranded DNA, this new adduct is not formed. Under the same experimental conditions but without DNA, *cis*-DDP is hardly reactive with Eth. Thus, double-stranded DNA promotes the binding of *cis*-DDP to Eth by acting as a matrix which enables a favorable orientation of the reactants. These results are summarized as follows:

$$\text{DNA} + \text{Eth} \leftrightharpoons \text{DNA}\cdot\text{Eth} \qquad \text{(reversible)}$$
$$\text{DNA} + \textit{cis}\text{-DDP} + \text{Eth} \rightarrow \text{DNA-cisPt-Eth} \qquad \text{(irreversible)}$$

Similar results were obtained with intercalators such as proflavine, ellipticine, and MDAP. In the case of the reaction between DNA, *cis*-DDP, and MDAP (the molar ratio MDAP/*cis*-DDP being larger than 1), the major adduct is dG-cisPt-MDAP (Malinge et al. 1990). The cross-linking between two purine residues becomes a minor event. On the other hand, no ternary complexes are formed with acridine or 9-aminoacridine possibly because of an unfavorable orientation of the reacting partners at the binding site. The importance of the orientation is also supported by the fact that the new type of adduct is not formed if *cis*-DDP is replaced by *trans*-DDP (Malinge and Leng 1986).

4.2 Lability of the Adducts in Double-Stranded Oligonucleotides

It is well established that under physiological conditions, once *cis*-DDP or the monofunctional derivative $[Pt(dien)]^{2+}$ is bound to DNA, the covalent adducts are stable (Lepre and Lippard 1990). Although the binding of *cis*-DDP to DNA is controlled kinetically, metal migration has only been observed within two platinated, single-stranded oligonucleotides. In the reaction between GpA and a derivative of *cis*-DDP, four species are formed. For one of them the cross-link (N7-G,N1-A) rearranges to form the cross-link (N7-G,N7-G) (Inagaki et al. 1990). In the reaction between a d-dodecamer and *trans*-diamminedichloroplatinum(II) the major 1,3 cross-link (N7-G,N7-G) rearranges to form the 1,4 cross-link (N7-G,N3-C) (Comess et al. 1990).

Within double-stranded DNA, dG-cisPt-intercalator adducts are labile. The intercalator, but not *cis*-platinum, is released in solution. The lability depends upon the chemical nature of the intercalator. For example, the dG-cisPt-MDAP adduct is sufficiently stable to be recovered and identified after complete enzymatic hydrolysis of the modified DNA (Malinge et al. 1990). A striking observation was that even after partial hydrolysis of the modified DNA, the adduct was stable; while before hydrolysis, a slow release of MDAP occurred.

The question was to determine whether the stability of dG-cisPt-MDAP was dependent upon DNA conformation. For this purpose, the stability of a single adduct within a single-stranded oligonucleotide or a double-stranded oligonucleotide was tested (Gaucheron et al. 1991). The experiment was carried out according to the following steps:

1. A single-stranded oligonucleotide containing a single guanine residue was reacted with the platinum triamine complex *cis*-$[Pt(NH_3)_2(MDAP)H_2O]$ (cisPt-MDAP). This compound, prepared in organic solvent, reacts with single- and double-stranded DNA and forms the dG-cisPt-MDAP adduct (the same adduct as in the reaction between double-stranded DNA, *cis*-DDP, and MDAP; Malinge et al. 1990).

2. The adduct within either a single- or double-stranded oligonucleotide (the latter was obtained by pairing the modified single-stranded oligonucleotide with its complementary strand) was incubated at 37 °C over various periods of time.
3. The stability of the adduct was followed by gel electrophoresis under denaturing conditions. Since MDAP bears a positive charge, its release from the adduct modifies the electrophoretic mobility of the oligonucleotide.

Even after a 48-h incubation of the single-stranded oligonucleotide at 37 °C and in 50 mM $NaClO_4$ (or 50 mM NaCl), only one electrophoretic band was detected, indicating that the adduct within the single-stranded oligonucleotide is stable. Interestingly enough in the case of the double-stranded oligonucleotide, after a few hours of incubation, two additional new products were detected. This experiment, together with parallel spectroscopic and HPLC analyses, show that two reactions occur. The main reaction is the release of the platinum-triamine complex *cis*Pt-MDAP. The other reaction is the release of MDAP. The conclusion is that the adduct is inert within a single-stranded oligonucleotide and labile within a double-stranded oligonucleotide.

This experiment was repeated in 500 mM $NaClO_4$ (Gaucheron et al. 1991). Within both the single- and double-stranded oligonucleotide the adduct is stable. It must be noted that the distortions induced in the double helix by the adduct in low and high salt conditions are different, as shown by chemical probes. Thus, an important point is that within a double-stranded oligonucleotide the adduct is inert or labile, depending on the local conformation of the double helix. All these results are summarized in Fig. 5.

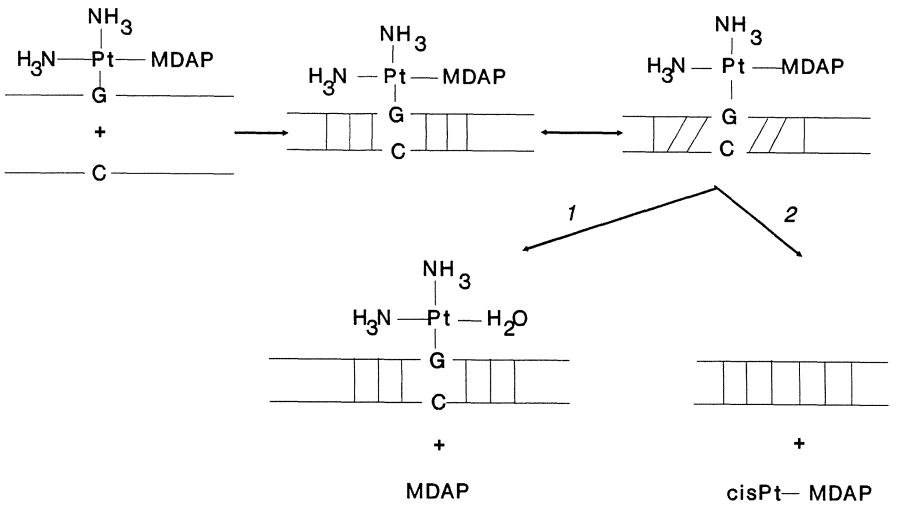


Fig. 5. Lability of the (dG-cisPt-MDAP) adduct in a double-stranded oligonucleotide

4.3 Catalytic Activity of DNA Double Helix

It is interesting to compare the reaction scheme proposed here (Fig. 4) and the classical minimal reaction scheme of a Menton – Michaelis reaction:

$$E + S \leftrightharpoons ES \rightarrow E + P.$$

In this scheme, the enzyme (E) binds the substrate (S), lowers the energy of the transition state, and promotes the catalytic event. The two reaction schemes are formally similar if one identifies the substrate and the enzyme with the adduct within the single-stranded oligonucleotide and the double-stranded oligonucleotide, respectively. The adduct occupies at least two different positions in the double helix. Only the low-salt conformation of the double helix is of low energy and promotes the catalytic event.

As previously mentioned, the amount of released cisPt-MDAP is larger than the amount of released MDAP. The relative yields depend upon the nature of the intercalator and, in some cases, only the *cis*Pt-intercalator is released (unpubl. results). As a first approximation, neglecting the release of MDAP, all the results can be summarized by the following scheme:

$$\textit{cis}\text{-DDP} + \text{MDAP} \overset{\text{DNA}}{\rightarrow} \text{cisPt-MDAP}$$

According to this scheme, DNA double helix promotes the binding of *cis*-DDP to MDAP and, subsequently, a local conformational change in the double helix initiates the release of cisPt-MDAP. We propose that DNA double helix behaves as a catalyst in this reaction.

The elementary definition of a catalyst states that the catalyst must be left unchanged at the end of the reaction. Strictly speaking, this is not the case in reactions 1 and 2 (Fig. 5). After the cleavage of the bond between platinum and MDAP, the *cis*-platinum residue remains bound to the guanine residue. Once cisPt-MDAP is released into solution, if no trapping agents are present in the solution, cisPt-MDAP can further react with any guanine residue within the double-stranded oligonucleotide. In fact, it has been demonstrated that after several hours of incubation of the modified double-stranded oligonucleotide, cisPt-MDAP was bound to the complementary strand, a strand which did not contain any adduct at the beginning of the incubation.

We do not know as yet how to explain these results. A crucial point is the conformation of DNA at the level of the adduct. Preliminary experiments done at low ionic strength suggest that MDAP is not intercalated between the base pairs, but lies in the groove.

4.4 Adduct Lability in DNA

The release of MDAP and cisPt-MDAP, first observed on double-stranded oligonucleotides containing a single adduct, occurs also with large DNA fragments containing several adducts (Anin et al. 1992).

The exchange of cisPt-MDAP among DNA molecules has been demonstrated by trapping cisPt-MDAP with single-stranded DNA. Before describing the experiment, two remarks must be made. Once cisPt-MDAP is covalently bound to single-stranded DNA, the adduct is stable. Along the cisPt-MDAP-modified DNA, the distribution of the adducts is easily revealed by mapping experiments. DNA replication by the Klenow fragment of *E. coli* DNA polymerase I is blocked by the adducts. The enzyme is also stopped by adducts of smaller size such as dG-cisPt-pyridine adducts (Hollis et al. 1991).

A double-stranded DNA was first modified with cisPt-MDAP and then mixed with the unmodified single-stranded DNA from bacteriophage M13. The mixture of the two DNAs was incubated at 37 °C and at various times aliquots were withdrawn. The single-stranded DNA from the aliquots was primed with a complementary oligonucleotide and used as a template for second strand synthesis by the Klenow fragment. At time = 0 of incubation, DNA synthesis by the enzyme generated long DNA fragments. At time = 1, 2, 3 . . . h shorter fragments were detected. The size of these fragments did not change but their quantity increased. Thus, incubation of cisPt-MDAP modified double-stranded DNA with unmodified single-stranded DNA leads to a modified single-stranded DNA which, in the presence of the Klenow fragment, behaves as cisPt-MDAP modified single-stranded DNA. In other words, cisPt-MDAP residues first bound to double-stranded DNA are released into solution and then react with the unmodified single-stranded DNA.

Another experiment, designed to follow the release of MDAP, took advantage of the property that dG-cisPt-MDAP adducts do not stop DNA transcription by T7 RNA polymerase, while the intra- and interstrand cross-links formed in the reaction between DNA and *cis*-DDP stop the enzyme (Lemaire et al. 1991; Corda et al. 1992). A DNA restriction fragment containing T7 promoter was first reacted with cisPt-MDAP and then incubated at 37 °C. At various times, aliquots were withdrawn and used as template for RNA synthesis by T7 RNA polymerase. At time = 0 of incubation, RNA synthesis generated long RNA fragments. After longer incubation times, shorter RNA fragments were detected. Their size was identical to the size of the fragments obtained when *cis*-DDP-modified DNA was used as a template. This experiment suggests that the monofunctional *cis*-platinum adducts generated by the release of MDAP react with the neighboring bases of the template and form intra- and interstrand adducts as does *cis*-DDP.

These results generalize those obtained with the modified oligonucleotides. They confirm that, due to the lability of the adducts within a double helix, cisPt-MDAP is released into solution. It can further react with any guanine residue in DNA. Thus, a given cisPt-MDAP does not necessarily remain bound to the same guanine residue. The release of MDAP generates the formation of intra- and interstrand cross-links. Finally, it has also been observed that the lability of the adducts depends upon the nature of the adjacent bases.