system with the π electrons of the Fe–C bond to form a ferracycloalkenone adduct from which follow generation of acyl π complexes, σ,π-allyl complexes, and/or cycloalkenones.

Thus, iron pentacarbonyl emerged as a genuine and most efficient "homodienophile" of remarkable stereospecific and regiospecific characteristics. Vinylcyclopropanes and divinylcyclopropanes, on the other hand, emerged as novel sources of four and six π electrons suitable for metal coordination.

The novel features characterizing the interaction between zerovalent transition-metal and multi-σ,π-electron systems opens a new vista of chemical research of great synthetic interest.

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Platinum Complexes: Probes of Polynucleotide Structure and Antitumor Drugs

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The study of biological macromolecules is facilitated by the attachment of metal ions that serve to probe their structures and dynamic properties. Examples include paramagnetic shift reagents,[1] fluorescent probes,[2] and energy-transfer donor–acceptor metal ion pairs.[3] Metal ions may serve[4] as substitution probes, illustrated by the replacement of zinc in metalloenzymes with magnetically and spectroscopically more rich metals such as cobalt or copper,[5] or as addition probes, exemplified by the use of electron-dense metals to assign phases during protein x-ray crystal structure determinations.[6] Heavy metal reagents may also be employed as electron microscope stains. A long-standing objective has been to sequence DNA by first attaching a heavy metal to a base-specific site in the polymer, for example all the guanine residues, and then to read the positions of the labeled bases along the polymer chain by electron microscopy.[7] Successive application of this method to other bases would provide the entire sequence.[8]

Platinum complexes offer certain advantages as biological probe reagents. They are moderately soluble in water, are kinetically stable, and, unlike some earlier third-row transition metals, do not form insoluble hydrated oxides at neutral pH. The known chemistry of platinum in the +2 and +4 oxidation states is extensive,[9] and it is now relatively easy to design and synthesize complexes having desired properties. Platinum also appears to be better behaved in the electron microscope than other heavy metals, notably mercury.

Interest in the aqueous chemistry of platinum and its binding to polynucleotides was greatly aroused by the discovery that *cis*-dichlorodiammineplatinum(II), DDP, has antitumor properties.[10] This simple compound, which has no carbon atoms, is currently used in many hospitals throughout the world in the treatment of ovarian, testicular, and other forms of cancer.[11] Although studies have shown that DDP inhibits DNA synthesis,[12] the primary site of action in the cell is unknown. Nevertheless, attention has focused on platinum–DNA interactions about which there now exists a substantial and still growing body of knowledge.

Three specific themes will be discussed in this Account. Connecting these themes is the basic coordination chemistry of platinum, an essential ingredient in each. The major objectives are (1) to sequence polynucleotides by electron microscopy following heavy metal attachment at base-specific sites and (2) to understand the mode of action of the antitumor platinum drugs. As with the Holy Grail of medieval

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(1) (a) K. G. Morallee, E. Nieboer, F. J. C. Rossotti, R. J. P. Williams, A. V. Xavier, and R. A. Dwek, *Chem. Commun.*, 1132 (1970); (b) C. R. Jones and D. R. Kearns, *J. Am. Chem. Soc.*, **96**, 3653 (1974).
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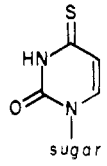
legend, the joy thus far has been in the searching. For although these two objectives have not yet been achieved, there have been some delightful findings along the way.

Platinum Phosphorothioate Binding: A General Method for the Heavy Metal Labeling of Specific Nucleotides

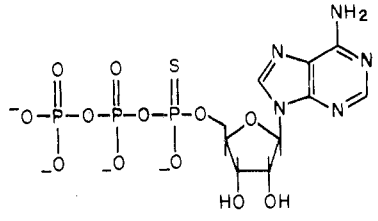
The first theme involves the selective attachment of platinum to sulfur atoms incorporated at base-specific sites in RNA and DNA. This chemistry provides heavy atom labeled nucleic acids for study by electron microscopy and other methods. To achieve a highly specific and quantitative labeling of nucleotides with a heavy metal reagent requires the proper choice of target atom. Since the available donor atoms in polynucleotides are generally class a, or hard, ligands,[13] it appeared that a soft donor such as sulfur might afford the desired selectivity. In order to test this strategy, the binding of mercury[14] and platinum[15] reagents to *E. coli* $tRNA^{Val}$ was studied. This polynucleotide contains a single sulfur atom in the form of a 4-thiouridine (s^4U)



4-thiouridine, s^4U

residue at the eighth position from the 5′ end. Using equilibrium dialysis methods, ^{14}C-labeled *p*-chloromercuribenzoate (PCMB) was shown to bind only to the sulfur donor site in the tRNA under appropriate conditions.[14b] Very similar results were reported for two additional sulfur-containing tRNAs.[16]

The next task was to find a way to incorporate sulfur at base-specific sites in a polynucleotide. The answer was provided following reports[17] that phosphorothioate-containing nucleotides could be enzymatically incorporated into RNA or DNA in place of the normal nucleotides. For example, using ATPαS and UTP as



ATPαS

substrates, RNA polymerase, and poly[d(A-T)] as template, the double-stranded, alternating heteropolymer poly(ŞA-U) can be synthesized. In this polymer every adenosyl residue contains a terminal sulfur atom

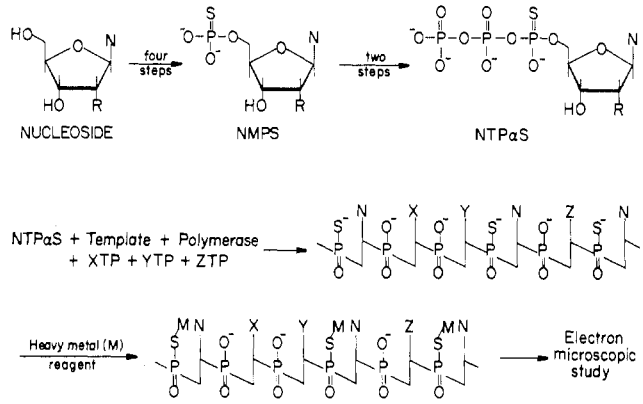


Figure 1. General scheme for heavy-metal staining of polynucleotides for study by electron microscopy and other methods.

in the form of a 5′-phosphorothioate group. More recently, double-stranded φX-174 and fd viral DNAs having one normal and one phosphorothioate labeled strand have been synthesized.[18] It has also been possible to substitute two of the four nucleotides with their phosphorothioate analogues.

With a means available to introduce sulfur atoms at base-specific sites in a polynucleotide, it remained to determine whether the desired heavy metal labeling could be achieved. Double label experiments using [^{35}S]poly(ŞA-U) and [^3H]chloroterpyridineplatinum(II) showed platinum to bind selectively and quantitatively to the sulfur atoms of the phosphorothioate groups under conditions where no binding to [^{14}C]poly(A-U) could be detected in control experiments.[19] Subsequent studies with phosphorothioate-containing fd-DNAs have resulted in platinum labeling of the sulfur sites.[20] Additional work, including attempts to see the platinum atoms in the scanning transmission electron microscope (STEM) at the Brookhaven National Laboratories, is currently in progress.

Figure 1 summarizes the general scheme for heavy metal staining of polynucleotides. Applied to the sequencing problem, this approach has the advantage over alternative methods[21] because (i) the same chemistry is used to label each of the bases, (ii) placement of the heavy metal atoms on the polymer backbone facilitates the correct location of the labeled nucleotide in the sequence, and (iii) it is possible to vary the complex and/or metal atom provided that soft, class b reagents are employed. Yet to be optimized is the stability of the metal–sulfur linkage, both in solution and in the STEM. Phosphorothioate groups in polynucleotides may be useful for heavy metal labeling applications other than sequencing, some of which are now being explored.

Metallointercalation Reagents

The second topic covers platinum metallointercalation reagents. The discovery that platinum complexes with planar, aromatic ligands can bind intercalatively to DNA has led to their use as heavy atom probes. Intercalation is a mode of binding first introduced[22] to

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(17) (a) H. Matzura and F. Eckstein, *Eur. J. Biochem.*, **3**, 448 (1968); (b) F. Eckstein and K. H. Scheit, *Proced. Nucleic Acid Res.*, **2**, 665 (1971); (c) F. Eckstein, private communication.

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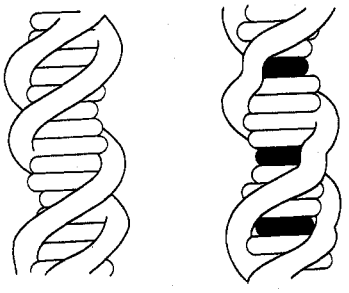
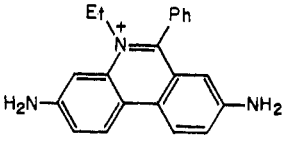


Figure 2. Diagrams showing the secondary structure of double-stranded DNA in the absence (left) and presence (right) of intercalated dyes, represented as shaded disks. Intercalation requires unwinding and lengthening of the DNA duplex (reproduced by permission from ref 23a. Copyright 1972 John Wiley & Sons, Inc.).

explain the strong affinity for double-stranded DNAs of planar, aromatic, cationic heterocycles such as ethidium, proflavin, and related acridine dyes. As



ethidium (Eth)

shown in Figure 2, the normal Watson–Crick DNA duplex is unwound when an intercalator inserts between adjacent base pairs. Intercalation is now widely accepted as a principal interaction mode for drugs and antibiotics, such as actinomycin D, with DNA,[23] and may be involved in protein–nucleic acid recognition sites.[24] There are still a number of detailed questions about the intercalation process that remain unanswered, however, such as the spatial orientation of the intercalator with respect to the helix axis and the distribution of intercalators along the duplex at saturation binding. As will be discussed, platinum intercalation reagents have provided substantial information about the latter question.

The first hint that a platinum complex might bind by intercalation to a polynucleotide was the discovery[15] that $[(terpy)PtCl]^+$ (Figure 3) could shift the 335-nm band of s^4U in *E. coli* $tRNA^{Val}$ under conditions where PCMB did not. This result was rationalized by proposing that the platinum complex altered the tertiary structure of the tRNA by intercalating at a nearby site and facilitating bond formation between platinum and the sulfur atom of the s^4U base. It is interesting that subsequent studies[25] of ethidium with tRNAs have shown the major intercalation site to be between base pairs AU_6 and AU_7, adjacent to the sulfur-containing s^4U base in *E. coli* $tRNA^{Val}$.[25a] The structure of a 2:2 intercalation complex between chloroterpyridineplatinum(II) and adenosine 5′-monophosphate (Figure

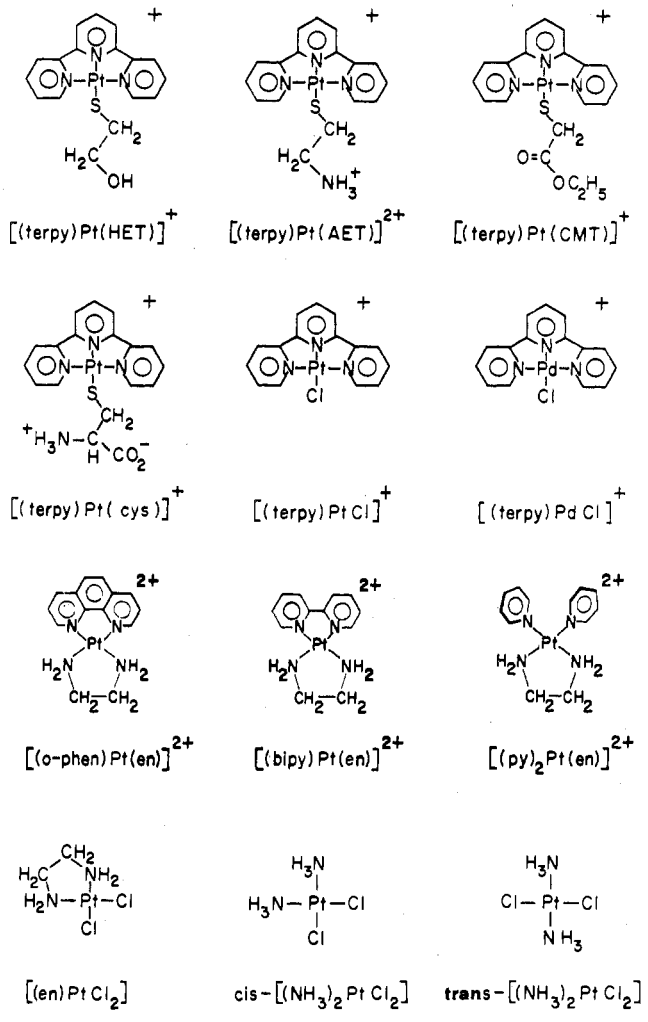


Figure 3. The structures and nomenclature for platinum complexes discussed in this Account (reproduced from ref 29).

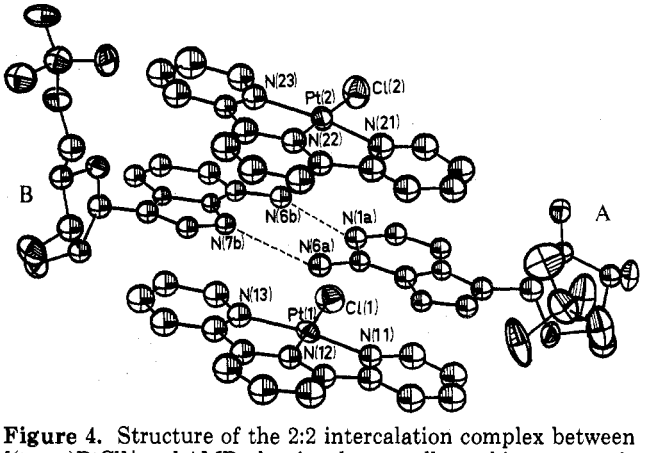


Figure 4. Structure of the 2:2 intercalation complex between $[(terpy)PtCl]^+$ and AMP^- showing the crystallographic asymmetric unit. (Reprinted with permission from ref 26. Copyright 1977, The Chemical Society, London.)

4) illustrates in a model system features of the probable geometry of the intercalation site of $[(terpy)PtX]^+$ in polynucleotides.[26]

The intercalative binding of terpyridineplatinum(II) complexes to DNA has been established by several independent methods. In Figure 5 are shown fluorescence Scatchard plots in which the effect of

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(25a) **Note Added in Proof**: A recent x-ray crystallographic study (M. Liebman, J. Rubin, and M. Sundaralingam, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 4821 (1977)) of an ethidium–tRNA molecular complex showed the dye to be stacked over the tertiary structural base pair U8-A14 and not to be intercalated. The proximity to the s^4U base site is noteworthy in the present context.

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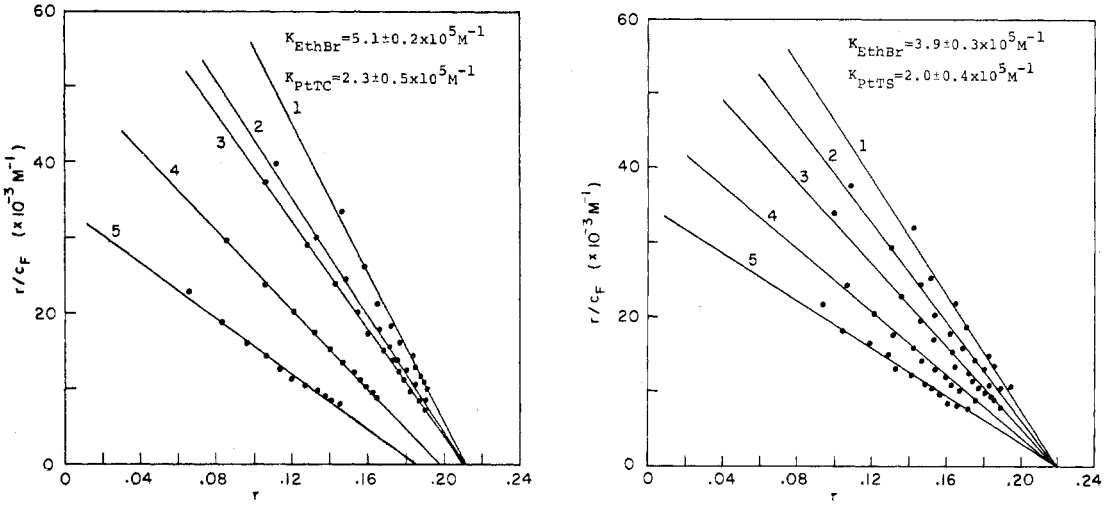

Figure 5. (left) Fluorescence Scatchard plots of EthBr binding to calf thymus DNA in the presence of increasing concentrations (lines 2–5 of $[(terpy)PtCl]^+$, ≡ PtTC. (right) Similar plots in the presence of increasing concentrations of $[(terpy)Pt(SCH_2CH_2OH)]^+$, ≡ PtTS. See ref 27 for details.

platinum reagents upon the binding of increasing amounts of ethidium bromide to calf thymus DNA is monitored.[27] The fluorescence of ethidium bromide is greatly enhanced upon intercalation and can be used to measure r, the ratio of bound Eth per nucleotide. The binding may be approximately fit to the Scatchard equation,[28]

$$r/c_f = K(n - r) \quad (1)$$

where c_f is the concentration of free Eth, n is the number of binding sites, and K is the binding constant. From a plot of r/c_f vs. r, both K and n can be determined. Addition of increasing portions of $[(terpy)PtCl]^+$ affects the fluorescence Scatchard plot of ethidium by first changing the slope ($-K$) and then both the slope and the intercept (n) (Figure 5). The former behavior results from competitive inhibition of Eth binding by the platinum reagent which can also occupy intercalation sites. The new slope, K', is described by the expression

$$1/K' = 1/K_{EthBr} + K_{Pt}/(K_{EthBr}[Pt]_f) \quad (2)$$

in which K_{EthBr} and K_{Pt} are the respective binding constants for these reagents and $[Pt]_f$ represents the free platinum concentration.[29] When sufficient $[(terpy)PtCl]^+$ is added the intercept shifts, a result ascribed to covalent binding of platinum to the DNA with loss of chloride ion. This binding mode disrupts the duplex structure and diminishes the number of available intercalation sites. In order to minimize this covalent interaction, the chloride ion was replaced by the kinetically more inert 2-hydroxyethanethiolate (HET) ligand[27,29,30] (eq 3). As expected, the complex

$$[(terpy)PtCl]^+ + HSCH_2CH_2OH + OH^- \rightarrow [(terpy)Pt(SCH_2CH_2OH)]^+ + H_2O + Cl^- \quad (3)$$

$[(terpy)Pt(HET)]^+$, which has been isolated and fully characterized[31] as its nitrate salt, competitively inhibits

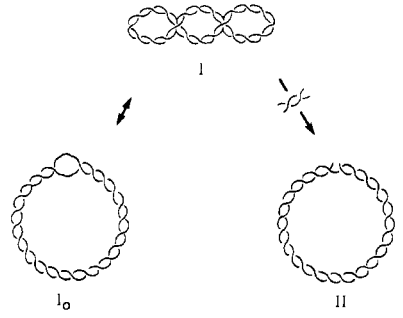

Figure 6. Closed circular DNA having several superhelical turns (form I), closed circular relaxed ($\tau = 0$) DNA (form I_0), and nicked (form II) DNA.

the binding of ethidium to DNA over the entire platinum concentration range (Figure 5). Single photon counting measurements of the fluorescence lifetime of Eth bound to DNA in the presence of the platinum reagents indicated that the ethidium fluorescence was not being quenched by a Förster energy-transfer mechanism.[29] It should be noted that the n value obtained from the fluorescence Scatchard plots reveals that the maximum number of bound Eth molecules is ~0.22 per nucleotide, or 44% of the available (one per base pair; see Figure 2) sites. We shall return to this point shortly.

Closed circular duplex DNA has also been employed to demonstrate the intercalative binding of platinum metallointercalation reagents such as $[(terpy)Pt(HET)]^+$. In this DNA (form I, Figure 6)[32] the total winding of the two strands (α), consisting of the usual Watson–Crick duplex turns (β) plus an additional number of superhelical or tertiary turns (τ), must remain constant in the absence of a sugar phosphate backbone chain scission, or "nick" (eq 4). Any process,

$$\alpha = \beta + \tau \quad (4)$$

such as intercalation, which unwinds the duplex in a closed circular DNA must change the number of superhelical turns according to eq 5. Using a nicking–

$$\Delta\tau = -\Delta\beta \quad (5)$$

closing enzyme,[33] a closed circular DNA having no

(27) K. W. Jennette, S. J. Lippard, G. A. Vassiliades, and W. R. Bauer, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 3839 (1974).
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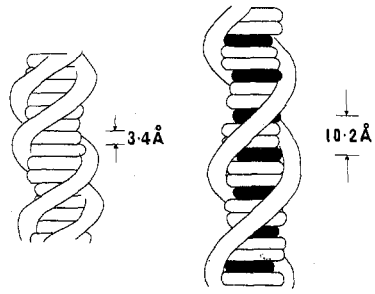

Figure 7. Normal (left) and intercalatively saturated (right) DNAs illustrating the nearest-neighbor exclusion model of the binding of an intercalator to a double-stranded polynucleotide (reproduced from ref 36).

superhelical turns (form I_0, $\tau = 0$) can be prepared from superhelical DNA (I). DNA I_0 will have the same hydrodynamic properties as the corresponding nicked circle (form II), which is not subject to the topological constraint (Figure 6). The band sedimentation behavior of a mixture of PM 2 DNAs I_0 and II was studied[27] in the presence and absence of [(terpy)Pt(HET)]$^+$. Addition of the platinum reagent separates the closed circular from the nicked DNA by unwinding the duplex and altering τ, a process strongly indicative of intercalation.[27] Intercalative binding of [(terpy)Pt(HET)]$^+$ to DNA II also occurs, but does not affect its sedimentation velocity. Further studies of this metallointercalator with superhelical DNA have shown that it unwinds the duplex to an extent experimentally indistinguishable from that of ethidium bromide.[15,27]

The tendency of planar platinum(II) complexes to form one-dimensional columnar stacks in their solid lattices, the aromaticity and size of the terpy ligand, the positive charge on the complex, and its planarity all contribute to the ability of [(terpy)Pt(HET)]$^+$ to bind intercalatively to DNA. Studies of a variety of related compounds (Figure 3) with DNA reveal that thiolatoterpyridineplatinum(II) complexes, [(o-phen)Pt(en)]$^{2+}$, and [(bipy)Pt(en)]$^{2+}$ intercalate but that [$(py)_2$Pt(en)]$^{2+}$ does not.[29,34] In the bis(pyridine) complex, the two pyridine rings cannot rotate into the platinum coordination plane owing to stereochemical interactions between the ortho ring hydrogen atoms. This complex therefore serves as a nice control on the methods used to assay for intercalation.

Intercalation of a compound into the duplex both lengthens and stiffens the DNA, a process that can be monitored by an increase in the specific viscosity. Addition of [(terpy)Pt(HET)]$^+$ to calf thymus DNA raises the specific viscosity up to $r \sim 0.22$, after which no further change occurs.[27] The failure of this and other intercalators to bind more than 44% of the available sites can be explained by the nearest-neighbor exclusion model (Figure 7).[35] Here the presence of an intercalator between two base pairs excludes the next nearest-neighbor sites. Promulgation of this effect along the entire DNA molecule would result in occupancy of 50% of the intercalation sites at saturation. The observed value of ~43–44% results from the random, noncooperative occupancy of inter-base-pair sites, subject only to neighbor exclusion of adjacent positions but not requiring the next allowed sites to be filled.[35a,36] Equation 6, which may be used to calculate this value,[35a]

$$2n = (1 - e^{-2})/2 \qquad (6)$$

was derived for the distribution of vacancies on a line of points from which pairs of adjacent points are selected at random until only isolated single points remain.[37]

As may be seen from Figure 7, intercalators bound to DNA according to the nearest-neighbor exclusion principle will distribute at 10.2-Å intervals along the helix axis. Using fiber x-ray diffraction methods, this 10.2-Å periodic spacing of platinum intercalation reagents has been detected,[34,36] providing direct confirmation of the neighbor exclusion model. The x-ray fiber patterns of calf thymus DNA containing intercalated [(terpy)Pt(HET)]$^+$ exhibit well-defined layer lines at 10.2 Å that are absent[38] in similar photographs of ethidium-bound DNA and of B-form DNA itself. The difference is ascribed to the fact that the metallointercalator has twice the x-ray scattering power of either a base pair or an organic intercalator. The latter contributes to the 3.4-Å meridional reflections and completely disrupts the characteristic helical diffraction pattern of the DNA, without revealing its presumed 10.2-Å periodicity (see ref 34 for further discussion).

The fiber x-ray studies demonstrate the utility of a platinum metallointercalation reagent as an addition probe. Attempts to measure the distribution of the platinum atoms along the duplex by the STEM technique are now being undertaken. Samples of [(terpy)Pt(HET)]$^+$ have also been supplied to crystallographers studying tRNA, oligonucleotides, and proteins that bind intercalating dyes to evaluate its potential usefulness as a heavy-metal isomorphous replacement reagent. The [(terpy)Pt(HET)]$^+$ complex (called "platinum red" by the investigators) has also been shown to inhibit genetic recombination in pneumococci.[39] Platinum metallointercalation reagents are now available commercially.[40]

Antitumor Platinum Drugs, Platinum Blues, and the Binding of Platinum Complexes to DNA

In this final section, antitumor platinum drugs, platinum blues, and the covalent binding of platinum complexes to DNA are discussed. Here the structural characterization of a platinum blue has provided new insights into the possible mode of action of platinum-containing drugs. The antitumor platinum drugs and the manner in which they might exhibit their activity are outlined in Figure 8.[41] The relatively low

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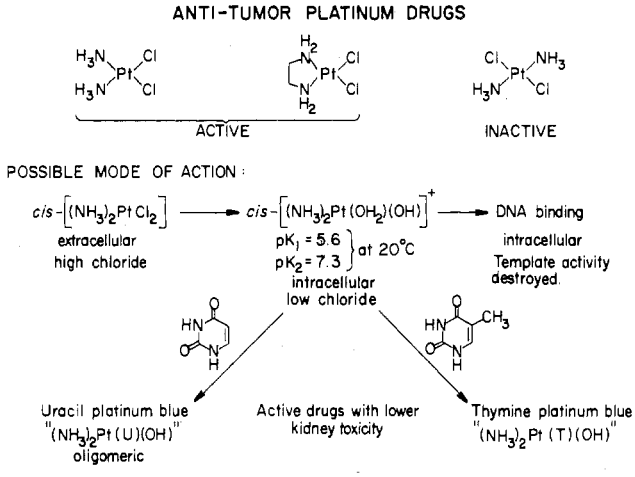

Figure 8. Platinum complexes exhibiting antitumor properties.

concentrations of platinum and its potential target sites in a biological milieu argue in favor of a solvent-assisted, dissociative kinetic pathway for DDP to manifest its antitumor activity. The high concentration (~0.10 M) of chloride ion in plasma will suppress the reactivity of the drug, lowering its toxicity. Following passive diffusion through the cell membrane, the drug can become activated by losing chloride ion (in the cytoplasm the chloride ion concentration is ~4 mM) to form $[(NH_3)_2Pt(OH_2)(OH)]^+$ and related hydrolysis products.[42] The aquo complex will bind more readily to cellular targets such as DNA, since water is a good leaving group. The bound platinum presumably kills both normal cells and tumor cells, the effect on the latter being more deleterious for reasons that are not yet entirely clear. Reaction of the DDP hydrolysis products with uracil, thymine, and other amides leads to the formation of blue solids that exhibit antitumor activity with lower nephrotoxicity than DDP (Figure 8).[43,44] Until recently the chemical composition and structures of these "platinum blues" were uncertain. Several related *cis*-$(amine)_2Pt(X)_2$ complexes are currently undergoing clinical trials as antitumor drugs.[11]

A number of specific questions concerning the antitumor platinum drugs can be raised. What is the mode of binding to DNA, covalent or intercalative? What are the geometric and electronic structures of the platinum blues? What are the binding sites on DNA? Why is the cis isomer active and the trans isomer inactive? Studies in our laboratory have provided information pertaining to each of these points and will now be summarized. We stress, however, that a complete understanding of the antitumor activity of DDP and related drugs is far from being realized.

Fluorescence Scatchard plots of the binding of EthBr to calf thymus DNA in the presence of DDP and platinum uracil blue (PUB) are shown in Figure 9. The failure of these reagents to alter the slopes of the plots indicates that their binding mode is not intercalative.[27]

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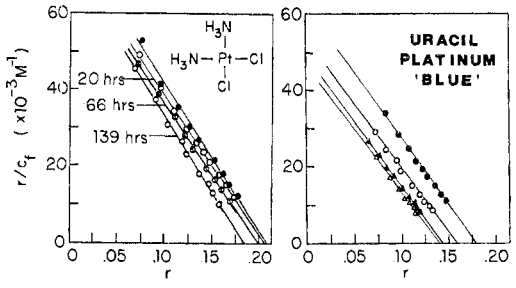

Figure 9. Fluorescence Scatchard plots of EthBr binding to 10 μM calf thymus DNA (●) in 0.05 M Tris, pH 7.5, 0.2 M NaCl, in the presence of increasing concentrations of PUB, r_f values 0.15 (○), 1.1 (▲), and 1.9 (△) or, for DDP, at r_f ~0.84 at various incubation times as shown. The symbol r_f represents the formal ratio of added platinum per nucleotide. The data for PUB are unpublished results of M. Howe-Grant and S. J. Lippard, while that for DDP comes from ref 29.

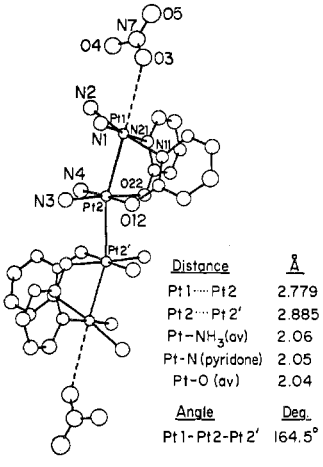

Figure 10. The structure of *cis*-diammineplatinum α-pyridone blue. Three additional nitrate anions in the crystal lattice are not shown.

Studies with closed circular DNAs support this conclusion.[29,45] The shift in intercept on the abscissa shows both DDP and PUB to bind covalently, however. In the presence of 0.2 M chloride ion, the binding of the former is slow and changes can still be observed after several days of incubation of the reagent with DNA. The platinum blue reaction is kinetically more rapid.

Little is presently known about the specific covalent attachment sites of DDP and PUB on DNA, although various studies have implicated N-7 and possibly O-6 of guanine as likely candidates.[46] DDP has been shown to bind more readily to closed circular than to nicked DNA,[29] a result that suggests interaction with a partially unwound duplex. It is possible that donor atoms normally involved in base pairing, for example N-1 of guanine, might be coordinating to platinum. Very recent work[47] reveals that DDP can change the number of superhelical turns in closed circular DNA in a manner analogous to that of an intercalator. Few if any other reagents have ever been found to exhibit such behavior. Understanding this binding mode is likely to enhance substantially our knowledge of the interaction of DDP with duplex DNA.

Platinum pyrimidine blues (Figure 8) are members of a larger class of blue compounds, reported as early

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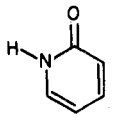
as 1908,[48] that form in aqueous solutions of platinum with amide ligands.[49] The failure to obtain good single crystals of these compounds (see ref 49) precluded their structural characterization by x-ray diffraction methods for many years. Contributing to this difficulty is the fact that, at least for the pyrimidine subclass, the blues exist in aqueous solution as mixtures of oligomers with varying chain lengths comprised of as many as 20 linked platinum atoms.[50] Recently, however, a crystalline platinum blue was synthesized using α-pyridone as the



α-pyridone

amide ligand.[51] The structure of this compound is shown in Figure 10. Two *cis*-diammineplatinum units are bridged by two α-pyridonate ligands. The terminal platinum atoms are coordinated to the deprotonated amide nitrogen atoms and the internal platinum atoms bind the exocyclic oxygen atoms. Two of these dimeric units are further linked by platinum–platinum bonding and by four NH···O hydrogen bonds across a crystallographically required center of symmetry. The charge of +5 on the tetranuclear chain complex reveals the formal oxidation state of the platinum atoms to be 2.25, accounting for the net Pt–Pt bonding interactions (Figure 10). Electron spin resonance and bulk magnetic susceptibility measurements[52] over the range $4.2 < T < 300$ K show the compound to obey the Curie–Weiss law with one unpaired electron per four platinum atoms. The effective magnetic moment is 1.83 μ_B per tetranuclear unit and the broad ESR spectrum exhibits multiplets centered at $g_{\parallel} \sim 2.0$, $g_{\perp} \sim 2.4$. Comparisons of solid and frozen-solution ESR spectra of *cis*-diammineplatinum α-pyridone blue (PPB) with each other and with similar spectra of PUB and related pyrimidine blues[53] strongly suggest structural similarities. It seems likely that all platinum blues will be mixed-valent, amidate-bridged oligomers with metal–metal interactions.

Aqueous solutions of PUB and PPB are unstable, judging by changes with time in the intensity of the blue absorption band and of ESR signals. This instability notwithstanding, it has been possible to obtain detailed information about the binding of PUB with DNA.[54] Incubation of closed circular and nicked PM-2 DNAs with PUB at 37 °C in various concentrations of NaCl and Tris buffer led to covalent binding and, ultimately, to precipitation of the DNA. The DNA could be resolubilized in high salt (>2 M) buffer and assayed by analytical equilibrium buoyant density and sedimentation velocity determinations. One such experiment was conducted using ^{14}C-labeled platinum uracil blue and [^{3}H]DNA. A large buoyant density shift vs. control DNA (no incubation time) was observed and attributed to covalent binding of platinum. The [^{14}C]PUB was not associated with the [^{3}H]DNA band, however, and it is likely that the uracil–platinum bond is broken in the process of binding platinum to the DNA. Alternatively, a platinum component in PUB not containing bound uracil may bind covalently to the DNA. Addition of cyanide can reverse the PUB-induced buoyant shift by 85%, a result that suggests that the remainder of the platinum is very tightly bound.

It has recently been found that solutions of platinum blues are stabilized by the addition of ammonium salts.[55] This discovery, together with the tight DNA binding exhibited by PUB, suggests a possible explanation for the greater antitumor activity of the cis compared with the trans isomer of DDP. Substitution of chloride ion in *cis*-DDP by a heterocyclic ring nitrogen atom of one of the bases will tend to labilize the ammine ligand trans to it in the coordination plane. Loss of the ammine groups would make available two additional DNA binding sites. Thus DDP (or PUB) could ultimately shed all four ligands in binding to DNA, giving a very tight complex not reversed either by addition of excess cyanide ion or, conceivably, by any intracellular DNA repair mechanism. Substitution of the chloride ion in *trans*-DDP would not labilize the ammine groups to the same degree. Loss of ammonia from nucleotide–platinum(II) ammine complexes has been suggested previously.[56]

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