Reaction of nucleic acids and cis-diamminedichloroplatinum(II) in the presence of intercalating agents

[eosidium bromide/proflavine/acridine/trans-diamminedichloroplatinum(II)]

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ABSTRACT The reaction of cis-diamminedichloroplatinum(II) and several synthetic or natural double-stranded polydeoxyribonucleotides has been carried out in the presence of such intercalating agents as ethidium bromide, proflavine, and acridine. After incubation of the reaction mixtures at 37°C for 24 hr, some ethidium or proflavine, but no acridine, molecules are tightly bound to nucleic acids. Tightly bound cis-diamminedichloroplatinum(II) is replaced by trans-diamminedichloroplatinum(II). Competition experiments between cis-diamminedichloroplatinum(II), poly(dG-dC), and poly(dG)-poly(dC) or poly(dA-dT) show that the presence of ethidium bromide, proflavine, or acridine interferes with the presence of nucleic acids. These results might help to explain the synergism for drugs used in combination with cis-diamminedichloroplatinum(II) and in the design of new chemotherapeutic agents.

Numerous works have been devoted to the study of the binding of the antitumor drug cis-diamminedichloroplatinum(II) [cis-Pt(NH3)2Cl2] to DNA. cis-Pt(NH3)2Cl2 is clinically active against many different neoplasms and it is often used in combination with other anticancer drugs, such as doxorubicin (adriamycin) or bleomycin. Several studies have reported enhanced effects for combination drug therapy, including synergy (1).

The mechanism of action of cis-Pt(NH3)2Cl2 is not yet known, but it is often thought that its antitumor activity is related to its binding to DNA (2–5). In the reaction of cis-Pt(NH3)2Cl2 and DNA, guanine residues are the preferred binding sites but adenine and cytosine residues also react, which leads to the formation of various types of adducts (6–11). On the other hand, for a given DNA, the nature of the adducts depends upon DNA conformation. In the reaction of cis-Pt(NH3)2Cl2 and poly(dG-m5dC) in the Z conformation, a monodentate adduct is formed. In the reaction of cis-Pt(NH3)2Cl2 and poly(dG-m5dC) in the B conformation, a bidentate adduct is formed (12).

The conformations of B-DNA and Z-DNA are very different (13, 14). To know whether smaller conformational changes of DNA could also play a role in the nature of the adducts, we have carried out the platination of nucleic acids in the presence of intercalating compounds. It has been already reported that new cis-Pt(NH3)2Cl2 binding sites were detected by means of exonuclease III when DNA was incubated with the intercalating agent ethidium bromide before the addition of cis-Pt(NH3)2Cl2 (15, 16). We show here by competition experiments between various nucleic acids that the preferential binding of cis-Pt(NH3)2Cl2 to (dG)4(dC)4 sequences is abolished when the reaction is carried out in the presence of ethidium bromide, proflavine, and acridine. In addition, we show that the specificity of ternary complexes [nucleic acid-cis-Pt(NH3)2-dye] strongly depends upon the nature of the dye. In the ternary complexes, ethidium and proflavine are tightly bound to the nucleic acids. They cannot be removed from the nucleic acids, as judged by several assays, such as extraction with organic solvent, filter assay at acid pH, or thin-layer chromatography (TLC) at basic pH. By these three assays, acridine is completely removed. It is suggested that cis-Pt(NH3)2 cross-links guanine residues and ethidium or proflavine but not acridine.

MATERIALS AND METHODS

Calf thymus DNA and poly(dG)·poly(dC) (purchased from Boehringer Mannheim), poly(dG-dC), poly(dA-dC)·poly(dG-dT), and poly(dA-dT) (purchased from Pharmacia) were treated twice with phenol and then precipitated with ethanol. Stock solutions of nucleic acids were made in 10 mM NaClO4/1 mM phosphate buffer, pH 7.5. Unlabeled ethidium bromide (Sigma) and [6-14C]ethidium bromide (18.3 mCi/mmol; 1 Ci = 37 GBq) (Centre Energie Atomique, France), acridine (Fluka), and 3,6-diaminoacridine (proflavine, Sigma) were used without any further purification. cis-Pt(NH3)2Cl2 and trans-Pt(NH3)2Cl2 were kindly provided by J. L. Butour. The synthesis of poly(d[8-14C]G-dC) was performed as described (12).

The reaction with cis-Pt(NH3)2Cl2, the nucleic acids, and the intercalating agents was carried out as follows. An appropriate volume of the intercalating agent was first added to the nucleic acid solution (0.5 mM). After 5 min, a freshly made solution of cis-Pt(NH3)2Cl2 was added and the reaction mixture was incubated at 37°C. The molar ratio of bound ethidium per nucleotide was determined by the following three assays. (i) Filter assay: the nucleic acid of the reaction mixture was precipitated on a microfiber glass filter (Whatman) by a 5% solution of trichloroacetic acid (Merck). The filter was washed and then dried, and the radioactivity of [14C]ethidium bound to the nucleic acid was counted with an LKB 1216 counter. (ii) TLC was run on plates of Silica gel 60 F 254 (Merck) with the solvent system isopropanol/ammonia/water, 6:3:1 (vol/vol). The radioactivity of the different spots was then determined. (iii) Butanol extraction: the salt concentration of the reaction mixture was adjusted to 0.2 M

Abbreviations: cis- and trans-Pt(NH3)2Cl2, cis- and trans-diamminedichloroplatinum(II); $r_n$, molar ratio drug/nucleotide.
NaClO₄. The mixture was extracted four times with water-
saturated 1-butanol and then dialyzed against 0.2 M NaClO₄/1 mM phosphate buffer, pH 7.5, for 2 hr in the cold. The
Pt content of the samples was determined with an atomic absorption
spectrophotometer as described (12).

Results

Reaction in the Presence of Ethidium Bromide. It is known
that cis-Pt(NH₃)₂Cl₂ binds to poly(dG-dC) in the B con-
formation. The main adduct in this platinated polymer, named
poly(dG-dC)-cis-Pt(NH₃)₂, arises from a cross-link between the
N-7 of two guanine residues (6, 17). The stability of the
complex and the nature of the adducts are quite different when
platination of poly(dG-dC) is carried out in the presence of
ethidium bromide.

Formations of the Ternary Complexes. To a solution of
poly(dG-dC) was added first ethidium bromide; cis-Pt
(NH₃)₂Cl₂ was added 5 min later and then the reaction
mixture was incubated at 37°C. After a few hours of incubation,
and ethidium was tightly bound to poly(dG-dC). Tightly
bound ethidium is defined as ethidium that cannot be
removed from poly(dG-dC), as judged by extraction with
butanol, by filter assay at acid pH, or by TLC at basic pH.
Even in more drastic conditions, the molar ratio ƞₕ of tightly
bound ethidium per nucleotide was unchanged, as determined
by the filter assay on the reaction mixture or on the reaction
mixture first diluted 10 times with 2 M NaClO₄ and
then extracted 3 times with butanol. As shown in Fig. 1, ƞₕ
increased as a function of the time of incubation and after 16
hr remained constant over a long period of time. Within
the experimental error, ƞₕ was the same when the salt concen-
tration of the reaction mixture was 5 or 50 mM NaClO₄.
Similar results were obtained when poly(dG-dC) was re-
placed by poly(dG)-poly(dC), poly(dA-dC)-poly(dG-dT), or
natural DNA (not shown). If cis-Pt(NH₃)₂Cl₂ was replaced by
trans-Pt(NH₃)₂Cl₂, all of the added ethidium bromide was
removed (Fig. 1).

After incubation of ethidium bromide and poly(dG-dC) or
poly(dG-dC) at 37°C for 16 hr, no tightly bound ethidium was found, as judged by the three assays. More-
over, in the absence of poly(dG-dC), no reaction between
ethidium bromide and cis-Pt(NH₃)₂Cl₂ was detected by TLC or
by absorption.

As shown in Fig. 2, for a given quantity of cis-Pt(NH₃)₂Cl₂,
the amount of tightly bound ethidium depends upon the
amount of added ethidium bromide (ƞₕ was determined after
20 hr of incubation of the reaction mixture by filter assay). On
the other hand, the amount of Pt bound to poly(dG-dC) was
almost the same in the absence or in the presence of ethidium
bromide. Similar results were obtained when poly(dG-dC)
was replaced by poly(dG)-poly(dC), poly(dA-dC)-poly(dG-
dT), or natural DNA. After incubation of the reaction mix-
tures at 37°C for 20 hr, ƞₕ (ethidium) and ƞₕ (Pt) were

FIG. 1. Kinetics of binding of ethidium bromide (EtdBr)
to poly(dG-dC) in the presence of cis-Pt(NH₃)₂Cl₂ ( ) or
trans-Pt(NH₃)₂Cl₂ ( ). Solvent, 5 mM NaClO₄/1 mM phosphate buffer, pH
7.5; temperature, 37°C. The input molar ratios cis-Pt(NH₃)₂Cl₂, trans-Pt(NH₃)₂Cl₂, and ethidium bromide per nucleotide = 0.08,
0.08, and 0.30, respectively. The amount of tightly bound ethidium
per nucleotide, ƞₕ, was determined by filter assay.

FIG. 2. ƞₕ, molar ratio of tightly bound ethidium ( ) or bound
cis-Pt(NH₃)₂Cl₂ ( ) to nucleotide residue of poly(dG-dC) as a function of
the input molar ratio ethidium bromide (EtdBr) per nucleotide
residue. The input molar ratio, cis-Pt(NH₃)₂Cl₂ per nucleotide, =
0.08. The reaction mixture was incubated at 37°C for 24 hr. Solvent,
5 mM NaClO₄/1 mM phosphate buffer, pH 7.5. The values of ƞₕ were
determined by filter assay.
change with non-tightly bound ethidium bromide, as suggested by the results shown in Fig. 1. The exchange was demonstrated by the following experiment. Poly(dG-dC), [14C]ethidium bromide, and cis-Pt(NH$_3$)$_2$Cl$_2$ were incubated at 37°C for 20 hr. The non-tightly bound [14C]ethidium was removed by extraction with butanol in the cold and then was replaced by unlabeled ethidium bromide. At 37°C, the amount of tightly bound [14C]ethidium decreased (Fig. 3) but the total amount of tightly bound labeled and unlabeled ethidium remained constant, as determined by absorption after butanol extraction. It took several hours for the total exchange.

Pt Exchange. There was no exchange of cis-Pt(NH$_3$)$_2$ bound to the ternary complex. After 20 hr of incubation of poly(d-[14C]G-dC), ethidium bromide, and cis-Pt(NH$_3$)$_2$Cl$_2$ (the input molar ratios cis-Pt(NH$_3$)$_2$Cl$_2$/nucleotide and ethidium/nucleotide = 0.08 and 0.30, respectively), a 2-fold excess of poly(dG-dC) and ethidium bromide (input ratio ethidium/nucleotide = 0.30) was added and then the solution was incubated at 37°C for 24 hr. The amount of cis-Pt(NH$_3$)$_2$ bound to poly(d-[14C]G-dC) (determined by enzymatic hydrolysis of the polymer after removal of the tightly bound ethidium) before and after the addition of poly(dG-dC) was the same.

Characterization of Platinated Poly(dG-dC). Some properties of poly(dG-dC)-cis-Pt(NH$_3$)$_2$ and of platinated poly(dG-dC) after complete removal of the tightly bound ethidium from poly(dG-dC)-cis-Pt(NH$_3$)$_2$-ethidium complex have been compared. Both platinated samples behaved similarly, as judged by circular dichroism [the circular dichroism spectra of poly(dG-dC) and poly(dG-dC)-cis-Pt(NH$_3$)$_2$ (6.05) are different (17, 20)], by the binding to antibodies to Z-DNA [poly(dG-dC)-cis-Pt(NH$_3$)$_2$ is well recognized by the antibodies to Z-DNA (20)], and by the nature of the adducts (determined after enzymatic hydrolysis), mainly (deoxyguanosine)-2-cis-Pt(NH$_3$)$_2$ (6, 16) (results not shown).

**Reaction in the Presence of Proflavine.** Only a few experiments have been performed with proflavine. In the first approximation, proflavine behaves like ethidium bromide. After 20 hr of incubation of proflavine, cis-Pt(NH$_3$)$_2$Cl$_2$ and poly(dG-dC) or poly(dG)poly(dC) or poly(dA-dC)poly(dG-dT) or natural DNA, some proflavine molecules were tightly bound to the nucleic acid. All of the proflavine was removed when there was no cis-Pt(NH$_3$)$_2$Cl$_2$. At 37°C, the ternary complex nucleic acid-cis-Pt(NH$_3$)$_2$-proflavine was relatively unstable and behaved like the ternary complex nucleic acid-cis-Pt(NH$_3$)$_2$-ethidium. There was no tightly bound proflavine if cis-Pt(NH$_3$)$_2$Cl$_2$ was replaced by trans-Pt(NH$_3$)$_2$Cl$_2$. Finally, in the absence of nucleic acid, no reaction of cis-Pt(NH$_3$)$_2$Cl$_2$ and proflavine was detected by absorption or TLC.

**Reaction in the Presence of Acridine.** After incubation of poly(dG-dC) or natural DNA with acridine and cis-Pt(NH$_3$)$_2$Cl$_2$ at 37°C for 20 hr (same experimental conditions as in Fig. 1) followed by extraction with butanol and dialysis, there was no tightly bound acridine but some Pt was bound to the nucleic acid. Thus, acridine and proflavine behave quite differently.

Several experiments were performed with platinated poly(dG-dC) after removal of acridine by extraction with butanol and dialysis in the cold. In an initial experiment, the platinated poly(dG-dC) was incubated at 37°C for 20 hr and then ethidium bromide or proflavine were added. No tightly bound dye was found. In a second experiment, the platinated poly(dG-dC) was mixed with proflavine or ethidium bromide at 4°C (input ratio dye/nucleotide = 0.3) and then incubated at 37°C. The amount of tightly bound dye increased as a function of time and then remained constant. A plateau was reached only after 7 hr (not shown).

Acridine stabilizes an intermediate form in the ternary complex poly(dG-dC)-cis-Pt(NH$_3$)$_2$-ethidium. This complex was incubated at 37°C in the presence of acridine (input molar ratio acridine/nucleotide = 0.3). As shown in Fig. 4, $r_0$, (tightly bound ethidium) decreased as a function of time. After 20 hr of incubation, acridine was extracted with cold butanol, ethidium bromide was added (input molar ratio = 0.3), and the solution was incubated at 37°C. The value of $r_0$ increased and, after several hours of incubation, reached a value slightly smaller than that at the beginning of the experiment.

**Preferential Binding of cis-Pt(NH$_3$)$_2$Cl$_2$.** Competition experiments between cis-Pt(NH$_3$)$_2$Cl$_2$ and several double-stranded polydeoxyxynucleotides have been performed. In all of these experiments poly(d-[14C]G-dC) was taken as reference. The same amount of cis-Pt(NH$_3$)$_2$Cl$_2$ was added on the one hand to poly(d-[14C]G-dC) and on the other hand to an equimolar mixture of poly(d-[14C]G-dC) and poly(dG)poly(dC) or poly(dA-dT) in the absence or in the presence, respectively,

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**Fig. 3.** Kinetics of release of tightly bound ethidium to poly(dG-dC) at 4°C (a) and at 37°C (b, +, o). The squares (b) are relative to the amount of bound cis-Pt(NH$_3$)$_2$. Solvent, 10 mM NaClO$_4$/1 mM phosphate buffer, pH 7.5. The solutions were extracted at various times with cold butanol (b) or were not extracted (b, +). Unlabeled ethidium bromide was added [input ratio ethidium bromide (EtdBr)/nucleotide = 0.3]. The values of $r_0$ were determined by filter assay and by visible absorption.

**Fig. 4.** Kinetics of release of tightly bound ethidium to poly(dG-dC) in the presence of acridine. The complex was incubated at 37°C in the presence of acridine (input ratio acridine/nucleotide = 0.3). After 20 hr, the solution was extracted with cold butanol and then [14C]ethidium bromide was added [input ratio ethidium bromide (EtdBr)/nucleotide = 0.25]. Solvent, 5 mM NaClO$_4$/1 mM phosphate buffer, pH 7.5. The filter assay was used for determination of $r_0$. 
of dye (ethidium bromide, proflavine, or acridine). The amount of cis-Pt(NH$_3$)$_2$Cl$_2$ bound to poly(d$^{14}$C)G-dC) was determined by TLC after enzymatic hydrolysis of the polynucleotide by the tightly bound dye having been first removed by incubation at 37°C for 80 hr. The results were summarized in Table 1 (the percentages are known within ±5%). In the absence of dye, cis-Pt(NH$_3$)$_2$Cl$_2$ bound preferentially to poly(dG)poly(dC) as compared to poly(dG-dC) and preferentially to poly(dG-dC) as compared to poly(dA-dT). In the presence of dye, cis-Pt(NH$_3$)$_2$Cl$_2$ bound equally well to poly(dG-dC) and to poly(dG)poly(dC). In the competition experiments with poly(dA-dT) and ethidium bromide, all of the cis-Pt(NH$_3$)$_2$Cl$_2$ bound to poly(dG-dC).

**DISCUSSION**

The reaction of cis-Pt(NH$_3$)$_2$Cl$_2$ with nucleic acids proceeds through loss of chloride ions to form hydrolysis products and the limiting step is the extent of hydrolysis (1-5). In the *in vitro* reaction with nucleic acids the most frequent lesions are a cross-link between two bases and only a small proportion of monofunctional adducts has been detected, mainly for short incubation times (6-11). In platinated DNA, the lesions prevent the binding of ethidium bromide (21).

After incubation of poly(dG-dC), cis-Pt(NH$_3$)$_2$Cl$_2$, and ethidium bromide for about 16 hr at 37°C, some ethidium molecules form a very stable complex with poly(dG-dC) and cannot be removed by extraction with butanol, by filter assay at acid pH, by TLC at basic pH, or even by a combination of extraction with butanol and filter assay. The molar ratio $r_b$ (tightly bound ethidium per nucleotide) depends upon the ethidium bromide and cis-Pt(NH$_3$)$_2$Cl$_2$ per nucleotide input ratios. For ratios of 0.3 and 0.8, respectively, almost all of the added cis-Pt(NH$_3$)$_2$Cl$_2$ is bound to poly(dG-dC), and bound cis-Pt(NH$_3$)$_2$ and tightly bound ethidium molar amounts are about the same.

After incubation of ethidium bromide and poly(dG-dC) or poly(dG)poly(dC)-cis-Pt(NH$_3$)$_2$, all of the dye can be removed by the three assays. Thus, the ternary complexes formed in the reaction of cis-Pt(NH$_3$)$_2$Cl$_2$ and poly(dG-dC) in the presence of ethidium bromide are different from those formed between platinated or unplatinated poly(dG-dC) and ethidium bromide. Similar conclusions were obtained from the study of ternary complexes in which poly(dG-dC) was replaced by poly(dG)poly(dC), poly(dA-dC)poly(dG-dT), and natural DNA. There was no tightly bound ethidium when cis-Pt(NH$_3$)$_2$Cl$_2$ was replaced by trans-Pt(NH$_3$)$_2$Cl$_2$.

The stability of the ternary complex poly(dG-dC)-cis-Pt(NH$_3$)$_2$-ethidium (all of the non-tightly bound ethidium having been removed by extraction with cold butanol) has been studied at two temperatures. At 4°C, only a small amount of ethidium is released with time. At 37°C, almost all the tightly bound ethidium is released after about 100 hr, whereas the amount of bound Pt remains constant. After complete loss of the tightly bound ethidium, the platinated poly(dG-dC) has all of the characteristics of poly(dG-dC)-cis-Pt(NH$_3$)$_2$, as judged by circular dichroism, to the antibodies to Z DNA, and by the nature of the adducts.

The ternary complex poly(dG-dC)-cis-Pt(NH$_3$)$_2$-ethidium is even less stable in the presence of thiourea, which is known to have a high affinity for Pt (22). Previous results have shown that the presence of ethidium during the platination of DNA makes easier the removal of Pt by KCN (16).

Tightly bound ethidium in the ternary complex poly(dG-dC) cis-Pt(NH$_3$)$_2$-ethidium can exchange with free ethidium. On the other hand, there is no exchange of the bound Pt even in the presence of an excess of poly(dG-dC). These results can be summarized as follows:

\[
\text{Poly(dG-dC) + cis-Pt(NH}_3\text{)Cl}_2 \rightarrow \text{poly(dG-dC) - cis-Pt(NH}_3\text{)Cl}_2.} 
\]

\[
\text{Poly(dG-dC) + cis-Pt(NH}_3\text{)Cl}_2 + \text{ethidium} \rightarrow \text{poly(dG-dC) - cis-Pt(NH}_3\text{)Cl}_2 - \text{ethidium}.} 
\]

\[
\text{Poly(dG-dC) - cis-Pt(NH}_3\text{)Cl}_2 - \text{ethidium} \Rightarrow \text{poly(dG-dC) - cis-Pt*(NH}_3\text{)Cl}_2 + \text{ethidium}.
\]

\[
\text{Poly(dG-dC) - cis-Pt*(NH}_3\text{)Cl}_2 \rightarrow \text{poly(dG-dC) - cis-Pt(NH}_3\text{)Cl}_2.} 
\]

In poly(dG-dC)-cis-Pt(NH$_3$)$_2$ prepared according to reaction 1, the main adduct arises from a cross-link between two guanine residues (6, 17). In poly(dG-dC)-cis-Pt(NH$_3$)$_2$-ethidium (reaction 2), ethidium is tightly bound and cis-Pt(NH$_3$)$_2$ is not bound to the bases as in poly(dG-dC)-cis-Pt(NH$_3$)$_2$. This is suggested by the reaction with thiourea and because there is no tightly bound ethidium when the dye is added to an already formed poly(dG-dC)-cis-Pt(NH$_3$)$_2$. Moreover, in poly(dG-dC)-cis-Pt(NH$_3$)$_2$-ethidium there is about one ethidium per platinum. Tightly bound ethidium can exchange with non-tightly bound ethidium (reaction 3). The exchange is slow (several hours) and the intermediate compound is poly(dG-dC)-cis-Pt*(NH$_3$)$_2$. Incubation of poly(dG-dC)-cis-Pt*(NH$_3$)$_2$ in the absence of ethidium leads to poly(dG-dC)-cis-Pt(NH$_3$)$_2$ (reaction 4).

Some experiments have been performed in the presence of proflavine and acridine. Proflavine behaves like ethidium bromide. By contrast, after incubation of acridine, cis-Pt(NH$_3$)$_2$Cl$_2$, and poly(dG-dC) (or natural DNA) reaction mixture at 37°C for 16 hr, no tightly bound acridine has been detected, although cis-Pt(NH$_3$)$_2$Cl$_2$ reacts with the nucleic acid. After removal of the dye and further incubation, the platinated poly(dG-dC) behaves as poly(dG-dC)-cis-Pt(NH$_3$)$_2$. If the incubation is carried out in the presence of ethidium bromide, some ethidium is tightly bound. Thus,

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### Table 1. Competition experiments between cis-Pt(NH$_3$)$_2$Cl$_2$, poly(dG-dC), and poly(dG)poly(dC) or poly(dA-dT) in the absence or in the presence of ethidium bromide, proflavine, or acridine, respectively

<table>
<thead>
<tr>
<th>% bound Pt</th>
<th>To poly(dG-dC) poly(dG)poly(dC)</th>
<th>To poly(dG-dC) poly(dG)poly(dC) or poly(dA-dT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(dG-dC), poly(dG)poly(dC)</td>
<td>23</td>
<td>77</td>
</tr>
<tr>
<td>Poly(dG-dC), poly(dG)poly(dC), acridine</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td>Poly(dG-dC), poly(dG)poly(dC), proflavine</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>Poly(dG-dC), poly(dG)poly(dC), ethidium bromide</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Poly(dG-dC), poly(dA-dT), poly(dG-dC)</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Poly(dG-dC), poly(dA-dT), ethidium bromide</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

In all experiments, the input molar ratio cis-Pt(NH$_3$)$_2$Cl$_2$ per nucleotide = 0.08 and the input molar ratio ethidium bromide, proflavine, or acridine per nucleotide = 0.30.
acridine can stabilize an intermediate form of platinated poly(dG-dC) that is able to bind ethidium tightly. This is also shown by the study of poly(dG-dC)-cis-Pt(NH₃)₂-ethidium in the presence of acridine. At 37°C, \( r_p \) (tightly bound ethidium) decreases as a function of time. After removal of acridine and of released ethidium by butanol extraction in the cold and then addition of ethidium bromide, one again gets a ternary complex poly(dG-dC)-cis-Pt(NH₃)₂-ethidium in which the amount of tightly bound ethidium is almost equal to that at the beginning of the experiment. These results argue strongly that this intermediate form and poly(dG-dC)-cis-Pt*(NH₃)₂ (reaction 3) are identical.

At this point, it is necessary to discuss how cis-Pt(NH₃)₂Cl₂ binds to poly(dG-dC) in the presence of the dyes. In the presence of ethidium or proflavine, it can be excluded that cis-Pt(NH₃)₂ cross-links two guanine residues, as already discussed. On the other hand, after complete removal of the tightly bound ethidium, the platinated poly(dG-dC) behaves like poly(dG-dC)-cis-Pt(NH₃)₂. Moreover, the Pt residues do not exchange in the ternary complexes. These results suggest strongly that in the presence of the dyes, one function of Pt is bound to N-7 of guanine residues. In the ternary complexes poly(dG-dC)-cis-Pt(NH₃)₂-ethidium or -proflavine the molar amounts of tightly bound dye and Pt are almost equal. This suggests that Pt is involved in the binding site of the tightly bound dye. These platinated sites interact quite differently with the two related molecules acridine and proflavine. The association and dissociation rates of ethidium (or proflavine) and platinated binding sites are very slow. It is proposed that a bidentate adduct (guanine-ethidium or -proflavine) is formed and that the slow step of the reaction is the binding of Pt-guanine adduct to the dye. In this reaction, the nucleic acid can be considered as a matrix achieving a favorable orientation of the reactants. In the absence of nucleic acid, no reaction between cis-Pt(NH₃)₂Cl₂ and the dye has been detected (this work; refs. 15, 16).

Several results strongly suggest that cis-Pt(NH₃)₂Cl₂ binds to DNA in a sequence-dependent manner (7, 9, 17, 23–25). By means of exonuclease III, new cis-Pt(NH₃)₂Cl₂ binding sites have been detected by platination treatment of DNA in the presence of ethidium (15, 16). By competition experiments between various double-stranded polynucleotides and cis-Pt(NH₃)₂Cl₂ in the presence of ethidium bromide, proflavine, or acridine, we show that the dyes interfere with the preferential binding of cis-Pt(NH₃)₂Cl₂ to (dT)ₙ-(dC)ₙ sequences. For example, in the absence of ethidium bromide, more cis-Pt(NH₃)₂Cl₂ binds to poly(dG)poly(dC) than to poly(dG-dC) and more binds to poly(dG-dC) than to poly(dA-dT). In the presence of ethidium bromide about the same amount of Pt is now bound to poly(dG-dC) and to poly(dG)poly(dC). In the competition experiment between poly(dG-dC) and poly(dA-dT), there is no Pt bound to poly(dA-dT). It is possible that in vivo intercalating agents redirect cis-Pt(NH₃)₂Cl₂, which might increase the therapeutic efficiency of cis-Pt(NH₃)₂Cl₂.

In conclusion, in the reaction of cis-Pt(NH₃)₂Cl₂ and nucleic acids, intercalating dyes such as ethidium bromide, proflavine, and acridine prevent cis-Pt(NH₃)₂Cl₂ from binding preferentially to (dG)ₙ-(dC)ₙ sequences. The stability of the ternary complexes nucleic acid-cis-Pt(NH₃)₂-dye depends strongly upon the nature of the dye. More generally, since many compounds intercalate into DNA, one expects large differences not only in the distribution of bound Pt but also in the interactions between bound Pt and the intercalated compound as a function of the chemical nature of the intercalating compound, the base sequence, and the conformation of DNA. The study of the ternary complexes might help to explain the synergism for drugs used in combination with cis-Pt(NH₃)₂Cl₂ and in the design of new chemotherapeutic agents.

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