Inorganica Chimica Acta, 18 (1976) 189–194

DNA–Platinum Interactions. Characterization of Solid DNA–$K_2[PtCl_4]$ Complexes

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Received October 13, 1975

A specific reaction of cis-dichlorodiammineplatinum-(II), cis-$Pt(NH_3)_2Cl_2$ with the GC planes of DNA is reported. This interaction is localized on the $N_7(G)$ and the $O_6(G)$ sites forming a chelate without proton liberation. On the other hand, the reaction of $K_2[PtCl_4]$ with DNA (41% or 72% GC) is also specific and takes place on the GC planes, but the site specificity differs from that of cis-$Pt(NH_3)_2Cl_2$ in that there is proton liberation before the saturation of the $N_7(G)$ sites. This may be due to the $N_1H(G)$ proton displacement and the formation of an intercrosslink between $N_1(G)$ and $N_3(C)$. Solid complexes obtained from the reaction of DNA and $K_2[PtCl_4]$ have been identified and characterized having the formula, Pt (DNA)Cl_2. The UV spectra of a series of DNA–Pt complexes obtained with $K_2[PtCl_4]$, $K[Pt(C_2H_4)Cl_3]$, cis-$Pt(en)Cl_2$, cis-$Pt(NH_3)_2Cl_2$, trans-$Pt(NH_3)_2Cl_2$ and $[Pt(dien)Cl]Cl$ are also reported here. Some thoughts on the mode of action of cis-$Pt(NH_3)_2Cl_2$ are presented.

Abbreviations

DNA	=	deoxyribonucleic acid
dien	=	diethylenetriamine, $H_2N–CH_2–CH_2–NH–CH_2–CH_2–NH_2$
en	=	ethylenediamine, $H_2N–CH_2–CH_2–NH_2$
C_2H_4	=	ethylene
GC	=	guanine–cytosine
AT	=	adenine–thymine
$N_7(G)$	=	nitrogen atom on position 7 of the guanine molecule
$O_6(G)$	=	oxygen atom on carbon C_6 of the guanine molecule
$N_1(G)$	=	nitrogen atom on position 1 of the guanine molecule
$N_3(C)$	=	nitrogen atom on position 3 of the cytosine molecule
$N_7(A)$	=	nitrogen atom on position 7 of the adenine molecule

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Introduction

Since Rosenberg's discovery[1] on the antimitotic properties of the platinum compounds a great deal of work has been published concerning the *in vivo* activity of these compounds. It is known that the DNA molecules are the principal targets of the platinum compounds *in vivo*[2] and *in vitro*[3] and that this interaction is responsible for the antitumour activity of the *cis*-platinum compounds. The *in vitro* platinum(II) complexation with the DNA components, *i.e.* nucleosides[4,5], nucleotides[6] and nucleic acids[7] was undertaken in this laboratory in order to differentiate the *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ interactions. We have recently shown[7a] the specificity of the GC planes towards platinum compounds using a DNA extracted from salmon sperm and containing 41% GC. Moreover, the chemical moieties corresponding to the saturation of the $N_7(G)$ sites were identified in solution[7b] and in the solid state[7c]. We present here more data on this specificity with a DNA extracted from bacteria *Micrococcus lysodeikticus* containing 72% GC[8]. The UV spectra of a series of DNA–Pt complexes have been studied and interpreted in terms of helical perturbation upon complexation.

Experimental

Apparatus

A Perkin–Elmer 403 atomic absorption spectrophotometer with a hollow cathode platinum lamp was used for the platinum analyses. The pH was measured with a Potentiograph E 336 A Metrohm Herisau and combined Metrohm electrodes type EA 121 UX calibrated with a Radiometer buffer type S 1001 for pH = 6.50 and with a monoacid potassium phthalate buffer for pH = 4.00. The reactions were run at constant temperature (37° C ± 0.1) with an Ultra Thermostat NB-34431 Colora. UV spectra were recorded at room temperature, using 1 cm path cells, with a Cary 14 spectrophotometer calibrated with a standard potassium dichromate solution (50 mg/l in 0.01*N* H_2SO_4, OD = 0.725 at 257 nm and OD = 0.535 at 350 nm).

Nucleic Acids and Platinum Compounds

Two types of DNA were used in the present study: a sodium salt of a DNA extracted from salmon sperm (Calbiochem, Los Angeles, California) containing 41% GC and a DNA extracted from bacteria *Micrococcus lysodeikticus* (Miles Laboratories, Elkhart, Indiana) containing 72% GC. The characteristics of these two types of DNA are: $\varepsilon_{(P)} = 8590$ (salmon) and 6900 (*M. lysodeikticus*) at pH = 7 with an RNA and protein content less than 4%. The platinum compounds were prepared as previously reported[7]. $K_2[PtCl_4]$ from Johnson Matthey & Mallory company was recrystallised before use.

Preparation and Characterization

The DNA–Pt complexes were prepared as previously reported[7,8]. The microanalyses (C, H, N, P, Cl) were carried out by Chemalytics, Tempe, Arizona. Sodium was determined by flame emission and platinum by atomic absorption spectrophotometry[9,10]. The water content in the solid samples was found in two ways. First by drying the complexes *in vacuo* at 60° C in the presence of P_2O_5 and second by difference from the analytical data. Both values were in excellent agreement.

Results and Discussion

We have already reported[7] the specificity of the reactivity of the GC planes with a series of platinum compounds. In order to extend this specific reaction, we have used in this work a DNA containing an almost double amount of GC planes (72%, instead of the previous DNA 41%). We have studied the reactions of *M. lysodeikticus*, 72% GC with the platinum salts *cis*-$Pt(NH_3)_2Cl_2$ and $K_2[PtCl_4]$. The choice of these two platinum compounds was made initially[7] because the curves $\Delta pH = f(Pt\ fixed)$ for these two compounds are slightly different, $K_2[PtCl_4]$ liberating protons a little bit faster than *cis*-$Pt(NH_3)_2Cl_2$, but a proton calculation indicates a specificity in both cases. In this study we have also determined the number of platinum atoms fixed per (AT, GC) and the number of protons liberated with $K_2[PtCl_4]$ at saturation of all the sites. The results are given in Table I and Figure 1. It can be seen from Figure 1 that the beginning of liberation of protons in the case of *cis*-$Pt(NH_3)_2Cl_2$ corresponds exactly to the GC content of the DNA's (0.82 salmon and 1.44 *M. lysodeikticus*). This indicates that the first platinum atom is reacting exclusively with the GC pairs. The complex obtained is a chelate[7b], with the $N_7(G)$ and $O_6(G)$ coordinating sites corresponding

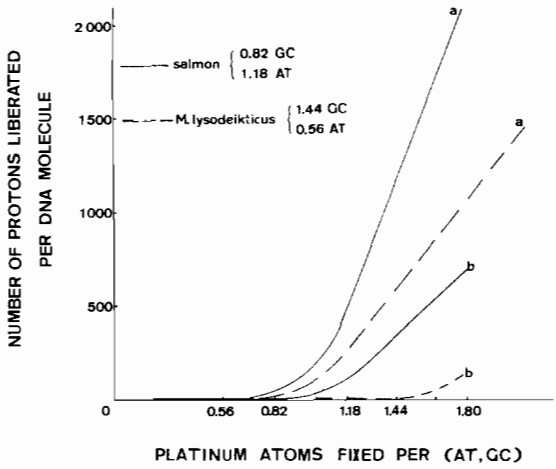


Fig. 1. Proton liberation during the complexation of the following nucleic acids: —— salmon sperm (41% GC), —— —— —— *M. lysodeikticus* (72% GC) with (a) $K_2[PtCl_4]$ (no precipitation) and (b) *cis*-$Pt(NH_3)_2Cl_2$ (precipitation).

TABLE I. Protons Liberated in the Reaction of Cis-$Pt(NH_3)_2Cl_2$ and $K_2[PtCl_4]$ with DNA *(M. Lysodeikticus)*.

Platinum Compounds	Number of Platinum Atoms Fixed per (AT,GC)	Final[a] pH	ΔpH[b]	Number of Protons Liberated per (AT,GC)	Number of Protons Liberated per DNA Molecule	% Reactivity[c] with the $N_7(G)$ Sites
	0.72	6.50	0.00	0.0000	0	100
cis-$Pt(NH_3)_2Cl_2$	1.44	6.25	0.25	0.0010	4	100
	1.75	5.80	0.70	0.0087	35	
	0.72	6.15	0.35	0.0022	9	100
$K_2[PtCl_4]$	1.44	4.55	1.95	0.1614	647	89
	1.75	4.40	2.10	0.2287	917	

[a] pH initial = 6.50. [b] ΔpH = initial pH–final pH. [c] The reactivity on a site is defined as the ratio of $\left(\frac{\text{platinum atoms introduced.}}{\text{platinum atoms fixed on this site}}\right)$ A 100% reactivity corresponds to a specific reaction.

to the formula, *cis*-$[Pt(NH_3)_2\ N_7(G)–O_6(G)]Cl_2$. Upon completion of this first attack a second reaction takes place which liberates protons. Since the liberation of protons does not correspond to the number of platinum atoms fixed (Table I), two different sites may be involved in the second platinum fixation. One of these two sites liberates protons and has been proposed[7a] to be the intercrosslink between $N_1(G)$ and $N_3(C)$ liberating the $N_1H(G)$ proton. The second site may be the $N_7(A)$ which takes place without proton liberation. From the pH values of Table I we can calculate the proton concentration by postulating the chemical reactions for the first platinum attack:

cis-$Pt(NH_3)_2Cl_2$ + DNA $\rightleftharpoons$ *cis*-$[Pt(NH_3)_2(DNA)]Cl_2$

$K_2[PtCl_4]$ + DNA $\rightleftharpoons$ $PtCl_2(DNA)$ + 2 KCl

We find that 90% of the non specific reaction takes place through $N_7(A)$ and 10% between $N_1(G)$–$N_3(C)$ for the *cis*-$Pt(en)Cl_2$, *cis*-$Pt(NH_3)_2Cl_2$ and *trans*-$Pt(NH_3)_2Cl_2$ in the case of a DNA containing 41% GC. For $K_2[PtCl_4]$ and the same DNA the values are 70% with $N_7(A)$ and 30% between $N_1(G)$–$N_3(C)$. The reactions of the *cis*-$Pt(NH_3)_2Cl_2$ and $K_2[PtCl_4]$ have been repeated in the present study with GC-enriched DNA (72% GC). The number of the GC planes is almost double with this DNA and we expect a higher reactivity with $K_2[PtCl_4]$ and also a higher proton liberation. In Table I the results for *cis*-Pt $(NH_3)_2Cl_2$ are almost identical with those previously obtained[7a]. In thc case of $K_2[PtCl_4]$ salt the saturation of the $N_7(G)$ sites equivalent to 1.44 Pt atoms fixed per (AT, GC) liberates 0.16 proton (Table I). This implies a non-specificity of the $N_7(G)$ sites. However since we have assumed that proton liberation comes from complexation with the $N_1(G)$–$N_3(C)$ sites the specificity on the GC pair is kept. For 1.44 Pt atoms fixed on the GC pairs there is 90% with $N_7(G)$ without proton liberation and 10% liberating protons from $N_1(G)$–$N_3(C)$. For the GC-enriched DNA (72%) the site specificity with $N_7(G)$ is kept with the *cis*-$Pt(NH_3)_2Cl_2$ but not with $K_2[PtCl_4]$. This result is not surprising since $K_2[PtCl_4]$ is much more reactive than *cis*-$Pt(NH_3)_2Cl_2$. A GC content of about 40% seems to be the maximum value for a site specificity with $N_7(G)$ for the $K_2[PtCl_4]$ salt. It is concluded from these experiments that a high reactivity of the platinum compound together with a high GC content in a DNA seems to lower the site specificity. This is also the case with Zeise's salt, $K[Pt(C_2H_4)Cl_3]$ which liberates protons even after a small quantity of platinum[7a] has been added to the DNA.

We have characterized the DNA–$K_2[PtCl_4]$ complexes only in the solid state, because in solution the electrodes used to determine the Cl^- concentration became erratic[7b]. The results are given in Table II and the complexes show a Cl/Pt ratio = 2 for the fixation of 0.38, 0.95 and 1.90 Pt atoms per (AT, GC). The general formula of these complexes is found to be $Pt(DNA)Cl_2$ from analytical and physical data. The specificity of $K_2[PtCl_4]$ with the GC planes was also reported by Moshkovskii *et al.*[11] using fusion curve studies and sedimentation constants. Moreover, Zakharenko *et al.*[12] have shown that DNA still possesses the ability to "melt" for a DNA–K_2 $[PtCl_4]$ in the ratio of 1 platinum atom fixed per 10 DNA bases *i.e.*, a value of 0.40 Pt per (AT, GC). With a ratio of Pt/base = 1.0, the "infusible" state is reached in 10 hours. This value corresponds to 4 Pt atoms introduced per (AT, GC) which means a fixation of 2.1 Pt atoms[7a]. These results agree with the previously proposed structures for the two types of complexes – the first complex in which only the $N_7(G)$ site or the $N_7(G)$ and the $O_6(G)$ sites are involved as in the case of *cis*-$Pt(NH_3)_2Cl_2$ and the second complex with $N_1(G)$ and $N_3(C)$. In the first complex the DNA can still "melt", whereas in the second complex the two strands are chemically retained and

TABLE II. Analytical Data of Pt–DNA (Salmon Sperm) Complexes.

Compound		Analyses (%)							
		C	H	N	P	Na	N/P	Pt	Cl
DNA[a]	Calculated	29.18	4.75	12.81	7.69	5.71	1.67	–	–
	Found	29.30	3.59	12.14	7.56	5.40	1.60	–	–
DNA[b] +	Calculated	28.69	4.18	12.65	7.56	–	1.67	4.62	1.64
0.38 $K_2[PtCl_4]$	Found	29.49	3.15	12.00	7.19	–	1.67	4.55	1.44
DNA[c] +	Calculated	26.78	3.68	11.81	7.06	–	1.67	10.55	3.84
0.95 $K_2[PtCl_4]$	Found	27.08	3.09	10.79	6.31	–	1.70	10.71	4.14
DNA[d] +	Calculated	23.83	3.07	10.51	6.28	–	1.67	17.71	7.06
1.90 $K_2[PtCl_4]$	Found	24.39	2.82	9.03	5.97	–	1.52	17.60	6.53

[a] 16 water molecules per (AT,GC). [b] 12 water molecules per (AT,GC). [c] 10 water molecules per (AT,GC). [d] 8 water molecules per (AT,GC). The number of water molecules is known with an accuracy of one per (AT,GC). The three complexes correspond to one Pt atom and two Cl atoms ($PtCl_2$).

the "infusible" state may be reached. The saturation of all the sites of a DNA extracted from salmon sperm (41% GC) with $K_2[PtCl_4]$ or $K[Pt(C_2H_4)Cl_3]$ corresponds to a fixation of six platinum atoms per (AT, GC) with the liberation of two protons. The same result is found with a DNA extracted from bacteria *M. lysodeikticus* (72% GC). The six sites are most likely the following nitrogen atoms: $N_7(G)$, $N_7(A)$, $N_1(G)$–$N_3(C)$, $N_1(A)$–$N_3(T)$, $N_3(G)$ and $N_3(A)$. The two protons could be liberated from the $N_1H(G)$ and the $N_3H(T)$ sites.

UV Spectra

The results are given in Table III and the spectra in Figure 2. All the spectra were taken with dialysed solutions. The UV spectrum obtained from the interaction of $[Pt(NH_3)_4]Cl_2$ and DNA is not given since this platinum salt does not interact with DNA at all. The spectra in general are in good agreement with those published in the case of $K_2[PtCl_4]$[12], *cis*-$Pt(NH_3)_2Cl_2$[13,14] and *trans*-$Pt(NH_3)_2Cl_2$[14]. The saturation of the $N_7(G)$ sites corresponds to a red shift of 4 to 5 nm in the case of *cis*-$Pt(en)Cl_2$, *cis*-$Pt(NH_3)_2Cl_2$, *trans*-$Pt(NH_3)_2Cl_2$ and $K_2[PtCl_4]$. The maximum red shift is obtained for a value of 1.6–1.8 Pt atoms per (AT, GC). The minimum red shift (1.5 nm) corresponds to the saturation of the $N_7(G)$ sites by [Pt(dien)Cl]Cl. The saturation of DNA with $K[Pt(C_2H_4)Cl_3]$ or $K_2[PtCl_4]$ affects the characteristic DNA band around 260 nm which is changed into a broad shoulder (see Figures 2–3e, 4e). The polymeric form of this "platinized" DNA at saturation was checked using dark field transmission electron microscopy.

In the present experiments it has been found that the site specificity of *cis*-$Pt(NH_3)_2Cl_2$ with the guanines in DNA is preserved for GC content values in the range of 41 to 72%. A useful application of this reaction is the determination of the GC content in any DNA which can be done by successive additions of the platinum compound until proton liberation begins. This was done with a DNA containing 72% GC which indicates a liberation of protons after saturation of the $N_7(G)$ sites corresponding to 1.44 Pt atoms per (AT, GC) (see Figure 1). We have also shown the specificity of the $N_7(G)$ sites for a DNA containing 41% GC[7a] (0.82 Pt atom per (AT, GC)). The detailed experimental procedure is described elsewhere[8].

An interesting application of this specificity is the localization of the individual bases in DNA by labelling them with platinum and visualizing them by dark field transmission electron microscopy. Preliminary results[8] obtained with a λ DNA and enough *cis*-$Pt(NH_3)_2Cl_2$ to saturate all the $N_7(G)$ sites of the λ DNA are very encouraging. This is extremely important in order to localize each individual base in a DNA.

In Vivo Activity

A possible mechanism of the inhibition of DNA synthesis[7b,c] by the *cis*-$Pt(NH_3)_2Cl_2$ was reported. A Pt–$N_7(G)$–$O_6(G)$ chelation has been proposed which could prevent GC base pairing and reduce from three to two the number of hydrogen bonds because of the Pt–$O_6(G)$ bond. In the present study we have investigated the extent of DNA configuration changes in the solid state due to the reaction with *cis*-$Pt(NH_3)_2Cl_2$. We have examined crystallographically several DNA and DNA–Pt fibers[8]. The fiber diagrams of DNA (A and B forms) were relatively easy to reproduce, however the Pt complexes did not give good X-ray diagrams[8]. The fixation of platinum at the $N_7(G)$–$O_6(G)$ sites of the DNA planes reduces or destroys the crystallinity of DNA (either of the A or the B form) and produces a different form which is

TABLE III. UV Spectra of Pt–DNA (Salmon Sperm) Complexes (Dialysed solutions, DNA(P) = 0.5×10^{-4} *M*, 10^{-2} *M* $NaClO_4$, pH = 7.00, t = 25° C).

Compounds	λ (nm)[a]	Compounds	λ (nm)
DNA	257	DNA + 0.82 $K[Pt(C_2H_4)Cl_3]$	260.5
		DNA + 1.18 $K[Pt(C_2H_4)Cl_3]$	262
DNA + 5 $[Pt(NH_3)_4]Cl_2$[b]	257	DNA + 1.64 $K[Pt(C_2H_4)Cl_3]$	265
DNA + 0.82 [Pt(dien)Cl]Cl[c]	258.5	DNA + 2.2 $K[Pt(C_2H_4)Cl_3]$	262.5
		DNA + 3.3 $K[Pt(C_2H_4)Cl_3]$	261.5
DNA + 0.82 *cis*-$Pt(en)Cl_2$	261.5	DNA + 6.0 $K[Pt(C_2H_4)Cl_3]$	258
DNA + 0.82 *cis*-$Pt(NH_3)_2Cl_2$	262	DNA + 0.82 $K_2[PtCl_4]$	261
		DNA + 1.18 $K_2[PtCl_4]$	263
DNA + 1.64 *cis*-$Pt(NH_3)_2Cl_2$	263.5	DNA + 1.64 $K_2[PtCl_4]$	264
DNA + 0.82 *trans*-$Pt(NH_3)_2Cl_2$	261	DNA + 1.8 $K_2[PtCl_4]$	263
DNA + 1.64 *trans*-$Pt(NH_3)_2Cl_2$	263.5	DNA + 2.2 $K_2[PtCl_4]$	262.5
DNA + 1.80 *trans*-$Pt(NH_3)_2Cl_2$	264.5	DNA + 5.9 $K_2[PtCl_4]$	257.5

[a] The accuracy is 0.5 nm; for the saturation of DNA (6 Pt) the maximum of the broad band is obtained with an accuracy of 2 nm. [b] $[Pt(NH_3)_4]Cl_2$ does not react with DNA. [c] Number of platinum atoms fixed per (AT,GC).

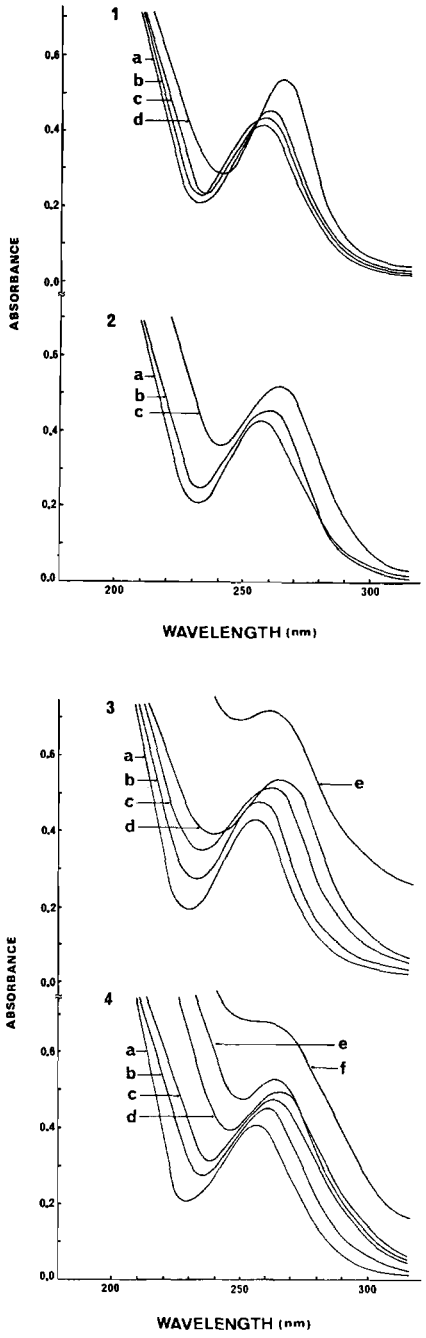


Figure 2. UV spectra of the DNA–Pt complexes (salmon sperm).
1: a) DNA, b) DNA + 0.82 [Pt(dien)Cl]Cl, c) DNA + 0.82 *cis*-$Pt(NH_3)_2Cl_2$, d) DNA + 1.64 *cis*-$Pt(NH_3)_2Cl_2$.
2: a) DNA, b) DNA + 0.82 *trans*-$Pt(NH_3)_2Cl_2$, c) DNA + 1.64 *trans*-$Pt(NH_3)_2Cl_2$.
3: a) DNA, b), c), d), e) DNA + 0.82, 1.18, 1.64, 6.0 K[Pt $(C_2H_4)Cl_3$].
4: a) DNA, b), c), d), e), f) DNA + 0.82, 1.18, 1.64, 3.3, 5.9 $K_2[PtCl_4]$.

significantly less crystalline or not crystalline at all. This perturbation could also be responsible for the inhibition of DNA replication since a conformational change of DNA may affect many of the properties of this genetic material. Naturally, breaking –HNH(C) $\cdots O_6$(G) hydrogen bonds and crystallinity changes are not completely independent, since the weakening or the breaking of hydrogen bonds could also be responsible for the conformational and stability changes of the base pairing.

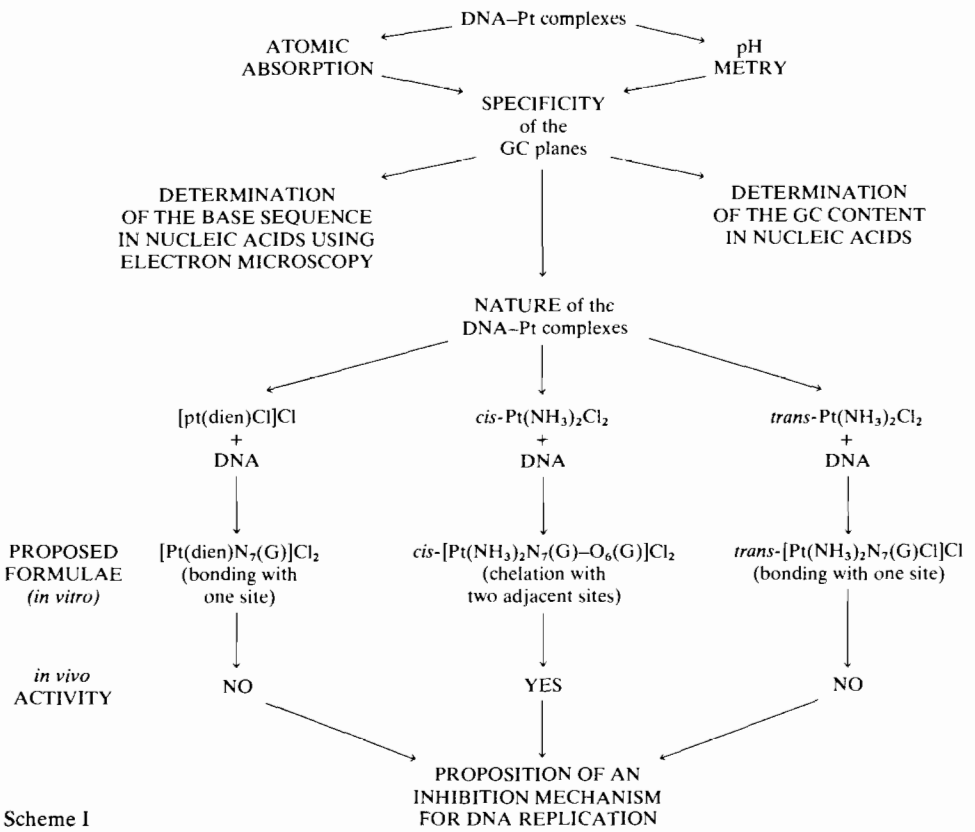
Why cis-$Pt(NH_3)_2Cl_2$?

A lot of metals have been tested for their antitumour properties *i.e.*, Co, Cu, Hg, Ni, Rh, Rb, Ag, Tl, Zn, *etc.*[15–17], but only a few platinum *cis*-compounds showed a significant antitumour activity. Why are the *cis*-compounds only active? The following are some useful characteristics which may be essential for the antitumour activity of *cis*-Platinum compounds:

1) the +2 oxydation state is kept in solution
2) the dsp^2 hybridization is not flexible and has a specific geometry which is retained
3) the high stability of the *cis*-Pt complexes formed
4) the specific distance of the two leaving groups in the *cis*-Pt complexes (3.2 Å)
5) the *cis*-chelation which has a specific geometry and reinforces stability
6) the rate exchange and the reactivity are lower than those of other compounds (the *trans*-compounds, for instance)

These points do differentiate the interaction of *cis*-$Pt(NH_3)_2Cl_2$ from that of the *trans*-$Pt(NH_3)_2Cl_2$ with DNA[7b].

One point needs comment here concerning the comparison between the *in vivo* and *in vitro* activity of $K_2[PtCl_4]$ and *cis*-$Pt(NH_3)_2Cl_2$. The *in vitro* specific reactions of these two compounds with DNA N_7(G)–O_6(G) do not parallel their *in vivo* activity. It is known that the *cis*-$Pt(NH_3)_2Cl_2$ inhibits DNA replication more than RNA and protein synthesis. This has been shown *in vitro* in human amnion AV_3 cells by Harder and Rosenberg[3], and *in vivo* in Ehrlich ascite tumour cells by Howle and Gale[2]. It was found that $K_2[PtCl_4]$, which is an ionic compound, causes bacterial death and is a highly toxic and non-active compound[18]. On the other hand, the *cis*-$Pt(NH_3)_2Cl_2$ is a very active antitumor agent which blocks cell division but does not prevent growth. The *in vivo* $K_2[PtCl_4]$ interaction does not seem to take place with DNA since the side effects (activity and toxicity) differ completely from those of the *cis*-$Pt(NH_3)_2Cl_2$. It seems more probable that *in vivo* $K_2[PtCl_4]$ reacts more effectively with proteins and enzymes. This behaviour is similar to that of the $[PtCl_6]^{2-}$ ion which causes also bacterial death and reacts almost entirely with the cytoplasmic protein, while the corresponding ammino neutral species *cis*-$Pt(NH_3)_2Cl_4$ was bound



Scheme I

to nucleic acid and to metabolic intermediates[19]. In scheme I we show a correlation between the proposed formula of the DNA–Pt complexes obtained *in vitro* and the *in vivo* activity of their corresponding platinum compounds. Some applications of the GC specificity of platinum compounds are also shown schematically.

Acknowledgments

The financial support of this investigation by the National Research Council of Canada and the Ministry of Education of Quebec is acknowledged. One of us (J.P.M.) gratefully acknowledges an N.R.C.C. Fellowship.

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