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INTERACTION OF *CIS*-DIAMMINEDICHLOROPLATINUM (II) TO CHROMATIN

SPECIFICITY OF THE DRUG DISTRIBUTION

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Abstract—We have studied the interaction of the antitumoral drug, *cis*-diamminedichloroplatinum (II), *cis*-DDP, to chromatin.

Degradation of chromatin–platinum complexes with micrococcal nuclease releases the platinum bound to the linker DNA. By comparing the percentage of platinum released throughout the digestion to the percentage of acid-soluble DNA we suggest that the linker DNA is the preferential target for this drug. This is mainly the case when the amount of bound platinum is low ($r < 0.03$) and is less at higher drug concentrations.

By comparing the rate constants corresponding to the reaction of *cis*-DDP to chromatin, DNA or core particle it appears that these constants are the same. This indicates that the bound platinum is located mainly at the DNA level.

Our results are discussed with respect to the structure of chromatin and we conclude that this structure should play a role in the *in vivo* association of *cis*-DDP to DNA.

Cis-diamminedichloroplatinum (II), *cis*-DDP or cisplatin has proven to be an effective anti-neoplastic agent especially when used in combination chemotherapy [1]. Cisplatin is a drug in which the central platinum atom is coordinated to 2 chloro and 2 ammine ligands located in the *cis* position on the horizontal plane. In the cell the active forms of the complex imply the aquation of one or both chloro ligands [2]. The molecule is very electrophilic and reacts in cells with many different types of molecules bearing nucleophilic groups such as DNA, RNA and proteins [3, 4]. However, a reaction of *cis*-DDP with other cellular targets cannot be excluded and could contribute to cellular toxicity.

Much evidence from *in vitro* and *in vivo* systems suggests that the DNA may be a preferential biologic target of the platinum (II) drug [5]. Biological inactivation of platinum modified DNA has already been described [6] and platinum mutagenicity demonstrated [7, 8].

Several hypotheses have been considered for the interaction of *cis*-DDP with DNA. They all imply a bifunctional coordination of the *cis*-Pt^{II}-$(NH_3)_2$ moiety to form either an interstrand or intrastrand DNA crosslink or a DNA protein crosslink [9–11].

However, it is well known that the DNA in eukaryotic cell nuclei is not in a free state but complexed to histone and nonhistone proteins in a relatively elaborate assembly consisting of strings of nucleosomal subunits [12]. Thus in the cell the interaction of a drug with DNA depends upon chomatin structure. The chromatin is built of nucleosomes which correspond to the association of a DNA structure of about 200 bp with an octamer of the histones H_{2a}, H_{2b}, H_3, H_4, (the protein core), and with the histone H_1. Chromatin can be compacted to a certain extent, to give rise to high order structures. The presence of such structures, which more or less expose the DNA, could modulate drug binding. First of all, it is likely that the existence of histone-free linker DNA and core DNA (the latter being closely associated to the protein core) interferes with the binding of the drug to DNA. A very limited number of reports have appeared on the chromatin or nucleosome interaction with platinum compounds. In the work of Lippard and Hoeschele [13] the interaction of the *cis* and *trans* isomers of *cis*-DDP with nucleosomes and core particles was investigated.

Other authors [14] compare the effects of different phatinum coordination complexes on several physico-chemical characteristics of nucleosomes and chromatin.

In this work, we have investigated the mode of cisplatin binding to DNA in isolated chromatin and studied the effects of chromatin structure on drug distribution all along the DNA to see whether it is associated with histones (core DNA) or not (linker DNA).

MATERIALS AND METHODS

Cis-DDP was synthetised by Dr. J. P. Macquet (CNRS, Toulouse) according to the already described Dhara technique [15]. The stock solution of the chemical was prepared at a concentration of 1 mg/ml (platinum weight) in isotonic sodium chloride (0.15 M). Purity and stability of the stock solution was tested with high performance liquid

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chromatography (Waters). The aqua derivative *cis*$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ was prepared according to the method of Scovell [16].

Calf thymus DNA is obtained from Sigma Chemical Co. and is used without further purification. DNA concentrations were determined by absorption measurement [εM at 258 nm = 6650 cm M^{-1}].

"*Core particle*". The core particle preparation was a generous gift from Dr. A. Mazen (Biophysics Laboratory, IBMC, Strasbourg).

Cells. The erythrocytes were obtained from chicken circulating blood by cardiac puncture.

Nuclei isolation. All operations were conducted at 4°. Blood was homogenized in †Tris–HCl 10 mM pH 7.6, NaCl 75 mM, EDTA 24 mM, and brought to 0.1 mM in PMSF. The homogenate was centrifuged at 4000 rev min^{-1} for 5 min. The supernatant was discarded and the pellet, resuspended in the same solution. Nonidet P40 was added to the suspension at a final concentration of 0.25%. The suspension was incubated for 40 min with slight agitation, and centrifuged at 4000 rev min^{-1} for 5 min. This step was repeated 4 times and the nuclei thus obtained were used immediately.

Chromatin extraction. Chromatin extraction was performed using the process described by Muyldermans *et al.* [17]. The nuclei were resuspended in Tris–HCl 10 mM pH 7.6, NaCl 40 mM, $MgCl_2$ 3 mM, $CaCl_2$ 1 mM, to a concentration of 2×10^9 nuclei/ml. Micrococcal nuclease (Boehringer, Mannheim) was added to 150 U/ml and the nuclear suspension, incubated for 6–8 min at 37°. The reaction was stopped at 4° with 10 mM EDTA (final concentration) and the digested nuclei, dialysed 12 hr against a buffer containing Tris HCl 10^{-2} M pH 7.6, NaCl 10^{-2} M EDTA 2×10^{-4} M. After homogenization, the suspension was centrifuged 10 min at 3000 rev min^{-1}. The supernatant represented the soluble chromatin. The concentration of chromatin was expressed as its content in DNA and determined through u.v. absorption considering that 50 μg/ml of chromatin absorb 1.1 OD at 260 nm.

Chromatin digestion. The chromatin in 10 mM $NaClO_4$ pH 7.6, 2 mM $CaCl_2$ is incubated at 37° in the presence of 15 units/ml micrococcal nuclease.

The chromatin in 10 mM Tris–HCl, pH 7.6, 10 mM NaCl, 0.2 mM EDTA, 2 mM $CaCl_2$ is incubated at 37° in the presence of 5 units/ml. After 10 min of digestion, the reaction is stopped on ice by the addition of EDTA up to a final concentration of 10 mM.

The DNA is purified through two isoamyl alcohol/chloroform (1:24) extractions and, after precipitation with 2 vol. of alcohol at −20°, resuspended in the electrophoresis buffer, 5 μg of extracted DNA resuspended in 0.04 M Tris, 0.02 M sodium acetate, 2 mM EDTA, pH 7.8 are loaded on 4% polyacrylamide gels as indicated in Fig. 7 and run as previously described [18]. The gels are stained with ethidium bromide (0.5 μg/ml) and photographed under u.v. light. The negatives are scanned with a Joyce-Loebel microdensitometer.

Spectrophotometry of the chromatin–Pt complexes. The difference spectra were measured in 1-cm double-compartment cells (Hellma) with a Kontron Uvikon 810/820 spectrophotometer. The ligand (DNA, chromatin or core particle) and platinum solutions were placed in the two separated compartments. The base line was recorded. The solutions of both compartments were then mixed by stirring the sample cell, and difference spectra were recorded at different time intervals for 24 hr.

Difference of absorbance between free ligand and complexed ligand corresponding to each wavelength were stored in a Minc Digital PDP 11/23 computer; these were later analyzed by nonlinear regression using the Marquardt algorithm [19]. This algorithm allows a fast and accurate determination of the curve $g_i = f(t)$ which fit the experimental points and minimizes the function representing the sum of the squares:

$$\$ = \Sigma_i (y_i - g_i)^2$$

A FORTRAN program performing this analysis was kindly provided by Dr Rigler and Dr Nilsson from Karolinska Institut (Stockholm) and adapted to the Minc Computer.

Determination of number of platinum atoms bound to chromatin, DNA or core particle. We have studied the amount of bound platinum by incubating the ligand (chromatin, DNA or core particle) with cisplatin at different ratios of [Pt]/[ligand]. The chromatin and core particle concentrations are expressed as DNA concentration equivalent. These ratios are:

$\frac{[\text{platinum}]M}{[\text{chromatin}]M}$: 0.03, 0.06, 0.08, 0.16, 0.32, 0.40, 0.95, 1.20, 1.43, 1.83, 1.99, 3.02, 3.97

$\frac{[\text{platinum}]M}{[\text{DNA}]M}$: 0.01, 0.03, 0.13, 0.16, 0.40, 0.64, 0.95, 1.20, 1.43, 1.83, 1.99, 3.02, 3.97

$\frac{[\text{platinum}]M}{[\text{core particle}]M}$: 0.03, 0.06, 0.16, 0.40, 0.64, 0.95, 1.19, 1.43, 1.83, 1.99, 3.02, 3.97

The reaction was performed in Tris–HCl 10^{-2}M, pH 7.6 NaCl 10^{-2}M, EDTA 2×10^{-4} M, for 30 hr under light agitation. At the end of the incubation, the free platinum is eliminated through dialysis and we measure the bound platinum with an atomic absorption spectrophotometer. We then calculate r (number of platinum atoms bound per nucleotide).

Digestion of the chromatin–platinum complexes. The control chromatin or the chromatin–platinum complexes at four different ratios of platinum over chromatin ([Pt]M/[Chr]M: 0.015, 0.03, 0.08, 1.2) were digested with micrococcal nuclease: 45 U/mg at DNA at 37° in Tris–HCl 10^{-2} M, pH 7.6 NaCl 10^{-2} M, EDTA 2×10^{-4} M. For each ratio at various times of digestion, a sample of the reaction mixture (0.2 ml) was diluted in 0.8 ml at stop solution: (SAB 2.5 mg/ml, NaCl 3 M, EDTA 510^{-3} M).

After 30 min incubation at 4°, 0.1 ml of PCA 10 × N was added. After 10 min at 4° the solution

† Abbreviations used: Tris, Tris–(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethylsulfonylfluoride; BSA, bovine serum albumin; PCA, perchloric acid; r, represents the ratio of platinum bound/nucleotides of DNA. Enzyne nomenclature: Micrococcal nuclease (Ribonucleate(deoxyribonucleate)3′-nucleotidohydroxylase): EC 3.1.4.7.

was centrifuged at 5000 rev min^{-1} for 10 min at 4°. The percentage of acid soluble material in the supernatant was then derived by means of optical absorption at 260 nm:

$$\% \text{ Acid-soluble material} = \frac{OD_t - OD_0}{OD_{100\%} - OD_0}$$

OD_0 : optical absorption of supernatant at zero time; OD_t: optical absorption of supernatant at t time; $OD_{100\%}$: correspond to the 100% digestion.

100% digestion was determined by diluting at zero time 0.2 ml of the reaction mixture in 0.8 ml of stop solution and by heating it at 80° for 30 min. The resulting pellet was centrifuged for 30 min at 5000 rev min^{-1}. The supernatant represented the degradation of 100% of the DNA. The percentage of released platinum was measured by atomic absorption spectrophotometry.

Platinum analysis. Platinum assay in samples was performed using a Perkin-Elmer 560 atomic absorption spectrophotometer (AS-1 injector and HGA 500 graphite furnace equipped with pyrocoated tubes) according to the technique of Leroy *et al.* [20].

RESULTS

Quantitative determination of cisplatin bound to isolated DNA, chromatin or core particle

We measured the amount of cisplatin bound to DNA, chromatin or core particle. For each substrate Fig. 1 shows the amount of bound platinum, expressed as the ratio of bound platinum versus nucleotide concentration (r), as a function of the ratio of total platinum versus DNA. We shall notice that for a ratio of bound platinum versus DNA similar or equal to 0.025, the amount of bound platinum is the same with either chromatin or isolated DNA. By increasing the concentration of total platinum, chromatin binds more platinum than DNA, which suggests that at this level protein binding occurs. If we follow the binding of platinum to isolated histones, we observe that for low concentrations of platinum, we cannot detect any bound platinum while, by raising the platinum concentrations, platinum binding to histones appears. Table 1 shows the amount of bound platinum respectively on DNA, chromatin, core particle and isolated histones. As long as we cannot detect binding of platinum to histones, the amounts of bound platinum on chromatin or DNA are the same. When platinum binds to histone (total platinum higher than 0.1 μg per μg of histones), we can assume that the differences between the amount of platinum bound to chromatin and the amount of platinum bound to DNA represent the amount of platinum bound to the histones.

For core particle, the pattern is different since less platinum binding takes place compared to chromatin or isolated DNA, up to a value of $r = 0.1$. This may correspond to a difference in the accessibility of the DNA to the drug. The situation is reversed for higher platinum concentrations. The core particle, therefore, binds more drug than isolated DNA. Once more, this discrepancy can be correlated to platinum binding to histones.

Degradation of platinum–chromatin complexes with micrococcal nuclease

In order to check the distribution of platinum on chromatin DNA, we submitted the complexes formed between cisplatin and chromatin to a micrococcal nuclease digestion.

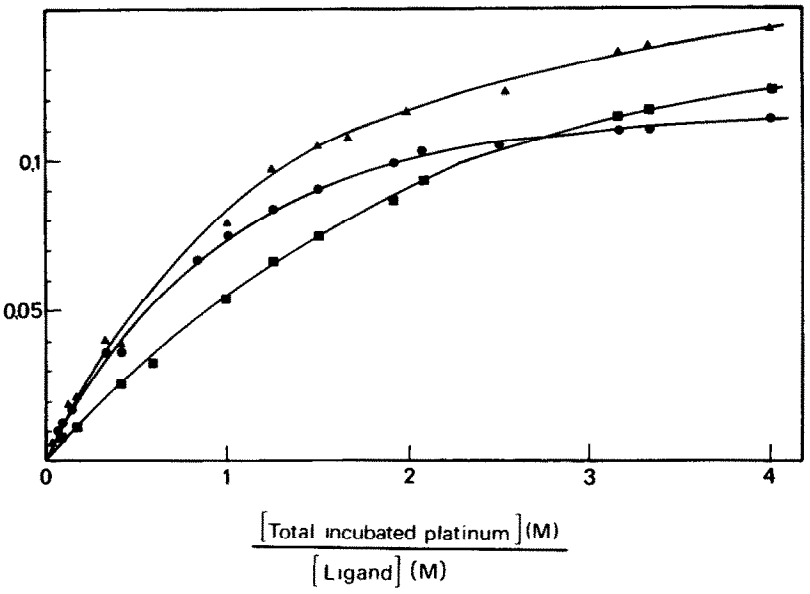


Fig. 1. Plot of r (number of platinum atoms bound per nucleotide) as a function of the ratio [Total incubated platinum]/[ligand] expressed in molarity: ●—●, ligand = DNA; ▲—▲, ligand = chromatin; ■—■, ligand = core particle. Each platinum determination has been done in triplicate (the smallest standard deviation on r was calculated to be equal to 1×10^{-4} and the highest equal to 2×10^{-3}).

Table 1. Incubation of different concentrations of platinum with 25 μg/ml of ligand (chromatin, DNA, core particle or histones). This table shows the μg of bound platinum per μg of ligand.

[Platinum incubated] μg								
[ligand] μg	0.004	0.02	0.08	0.4	0.8	1.2	2	2.4
μg of bound platinum per μg of histones	0	0	0	7.2×10^{-4}	5.2×10^{-3}	1.5×10^{-2}	3.6×10^{-2}	4.2×10^{-2}
μg of bound platinum per μg of DNA	6×10^{-4}	1.9×10^{-3}	1.1×10^{-2}	3.5×10^{-2}	5.6×10^{-2}	6.3×10^{-2}	7×10^{-2}	7.2×10^{-2}
μg of bound platinum per μg of chromatin	6×10^{-4}	3.8×10^{-3}	1.1×10^{-2}	3.6×10^{-2}	6.1×10^{-2}	7.3×10^{-2}	8.6×10^{-2}	9×10^{-2}
μg of bound platinum per μg of core particle	5.6×10^{-4}	2×10^{-3}	6.4×10^{-3}	2.3×10^{-2}	4.3×10^{-2}	5.6×10^{-2}	7.3×10^{-2}	7.6×10^{-2}

The kinetics of the enzymatic digestion of complexes corresponding to several ratios of platinum versus chromatin, are shown in Fig. 2A.

An inhibition of the degradation process appears which is a function of the amount of platinum incubated with the chromatin. If the plateau value of the digestion represents about 50% of the DNA present in the incubation mixture, the initial rate of degradation, which corresponds to the disruption of the linker DNA, is slowed down in the presence of the drug.

Such inhibition suggests that the linker DNA is a target for platinum binding. In order to get an idea whether the drug is preferentially bound to the linker DNA or uniformly distributed all along the DNA of the chromatin, we correlated the release of cisplatin

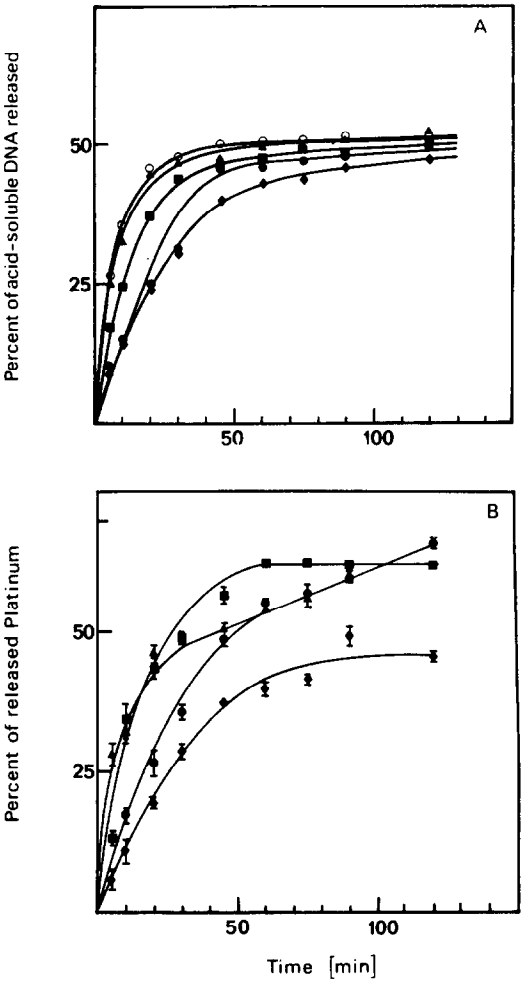


Fig. 2. (A) Enzymatic degradation of chromatin–platinum complexes in the presence of micrococcal nuclease. Percent of acid soluble DNA released: ○—○, control without drug; ▲—▲, [Pt]/[chr] = 0.015; ●—●, [Pt]/[chr] = 0.08; ■—■, [Pt]/[chr] = 0.03; ◆-◆, [Pt]/[chr] = 1.2. The concentrations were expressed in molarity. (B) Percent of released platinum during the degradation of chromatin–platinum complexes in the presence of micrococcal nuclease as a function of time: ▲—▲, [Pt]/[chr] = 0.015; ●—●, [Pt]/[chr] = 0.08; ■—■, [Pt]/[chr] = 0.03; ◆-◆, [Pt]/[chr] = 1.2.

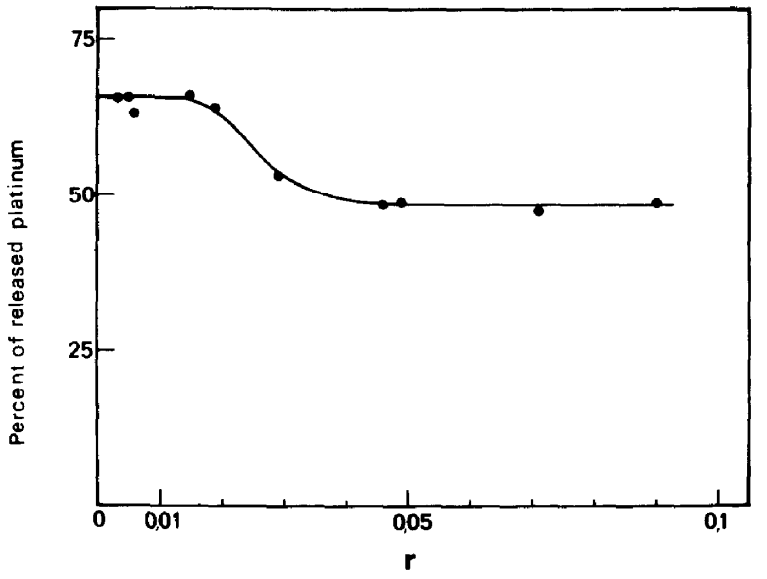


Fig. 3. Determination of the percent of platinum released, following a nuclease digestion leading to the degradation of 50% of the DNA in the chromatin–platinum complexes. These percents were plotted as a function of the *r* values (*r*: number of platinum atoms bound per nucleotide in chromatin) measured for the chromatin–platinum complexes at different ratio of incubated drug vs chromatin.

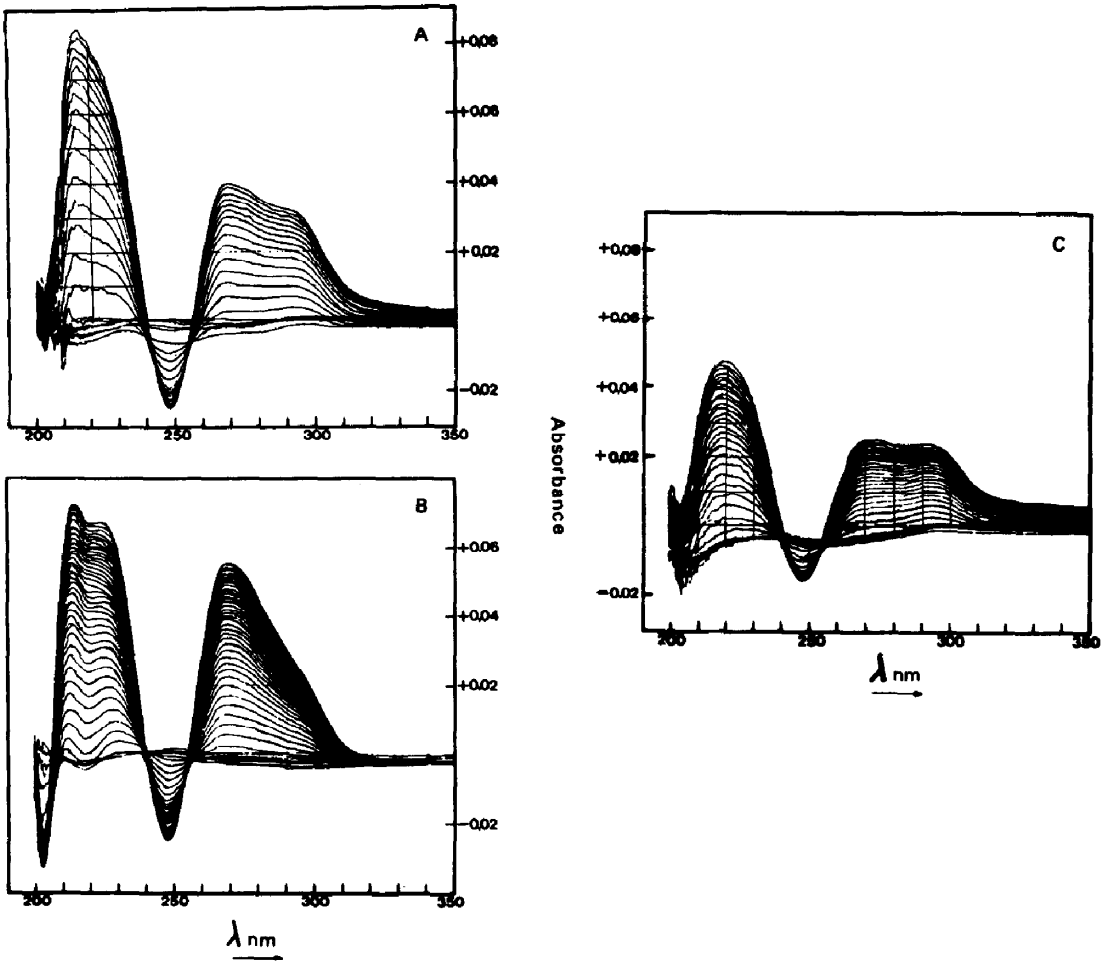


Fig. 4. Difference absorbance spectra resulting from the reaction of *cis*-$Pt(NH_3)_2Cl_2$ (2.5×10^{-5} M) with: (A) chromatin (32×10^{-5} M); (B) DNA (32×10^{-5} M); (C) core particle (32×10^{-5} M). The buffer used for these reactions is: Tris HCl 10 mM pH 7.6, NaCl 10 mM, EDTA 0.2 mM at 25°. Spectra were recorded at intervals between 0 and 40 hr.

with the release of acid soluble material. The results are shown in Fig. 2B.

For a 50% digestion of the chromatin, corresponding to the degradation of the linker DNA, and when the ratio of total platinum vs DNA is kept low, up to 65% of the bound platinum is released as free platinum. Meanwhile when the platinum concentration is increased we observe that only 45% of the bound platinum is released. In a separate experiment we have measured the percent of bound platinum released when 50% of the chromatin DNA is digested. We then plotted this percentage as a function of r (Fig. 3). It becomes apparent that a transition takes place for r equal to about 0.03. For r lower than 0.03 more than 50% of platinum is released when 50% of DNA is rendered acid-soluble which indicates that the drug is more likely to go to linker DNA than to the core-DNA since by digesting the linker DNA, two-thirds of the bound platinum is released. When r is greater than 0.03, the platinum is distributed evenly along the DNA of the chromatin, and the percentage of released platinum is equal to the percentage of released acid-soluble material. The high release of platinum for r smaller than 0.03 is related to the structure of chromatin since under the same conditions, digestion of DNA-platinum complexes with micrococcal nuclease does not give rise to any discrepancy between the amount of released platinum and the amount of released acid-soluble material.

If there is a preferential association of platinum to DNA and specially to linker DNA, we should expect to observe no differences between the rate constant corresponding to the interaction of cisplatin to chromatin and to the interaction of cisplatin to DNA.

Determination of the rate constant of the chromatin–cisplatin reaction

We measured the rate constant corresponding to the interaction between cisplatin and extracted chromatin and compared it to the rate constant corresponding to the interaction of cisplatin with DNA.

Throughout this determination, we chose conditions that would achieve a pseudo first-order reaction. We measured the rate constant by varying the chromatin concentrations. Equation (1) describes the reaction kinetics:

$$[X(P)]_t = [X(P)]_\infty (1 - e^{k_{app}xt} \qquad (1)$$

with $k_{app} = k \times [P]$ when chromatin is in excess. k equals the rate constant of the reaction and $[X(P)]_t$ is the concentration of chromatin–Pt complex formed at time t.

Figure 4A shows the difference absorbance spectra resulting from the reaction of *cis*-$Pt(NH_3)_2Cl_2$ with chromatin. Spectra were recorded at intervals between 0 and 40 hr. The reaction kinetics follow a single exponential process.

Figure 5 shows log $(OD_\infty - OD_t/OD_\infty)$ as a function of time for different values of chromatin concentration. This plot allows us to calculate K_{app}.

If the pseudo-first order model applies the apparent rate constant must be proportional to the concentration of the reactant which is in excess.

The values are reported in Table 2.

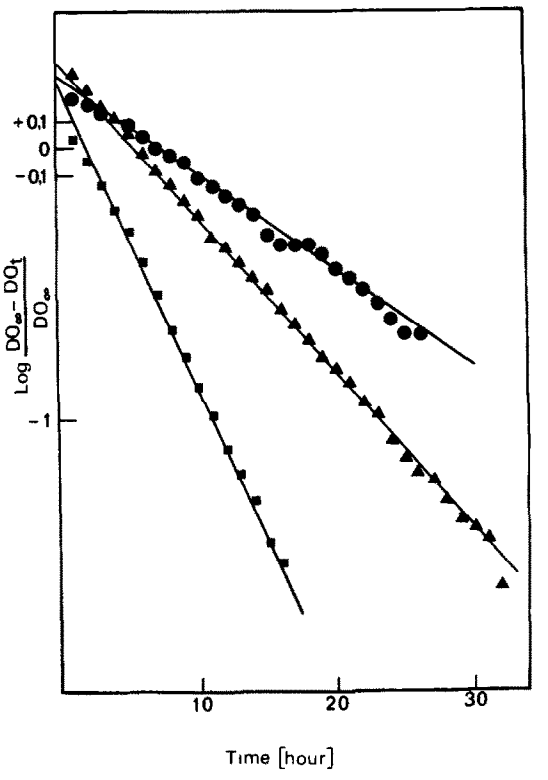


Fig. 5. Plot of log $(OD_\infty - OD_t)/OD_\infty$ as a function of time. The platinum concentration was 2.5×10^{-5} M; ●—●, [chr] = 8×10^{-5} M; ▲—▲, [chr] = 16×10^{-5} M; ■—■, [chr] = 32×10^{-5} M.

The rate constant for adduct formation deduced from these data is found to be $k_{CHR} = 0.10\ M^{-1}\ sec^{-1}$. We then measured the rate constant corresponding to the reaction between cisplatin and isolated DNA in the same conditions than between cisplatin and chromatin.

Figure 4B shows the difference absorbance spectra for the interaction of cisplatin with DNA.

The rate constant for adduct formation is found to be: $k_{DNA} = 0.083\ M^{-1}\ sec^{-1}$. The difference absorbance spectra corresponding to the interaction between cisplatin and core particle is shown in Fig. 4C and the rate constant is found to be: $k_{CP} = 0.090\ M^{-1}\ sec^{-1}$.

These results indicate that under our experimental conditions the reactions of cisplatin with chromatin, DNA or core particle exhibit similar rate constants.

Table 2. Values of the apparent rate constant (K_{appCHR}) measured by spectrophotometry for the reaction of *cis*-$Pt(NH_3)_2Cl_2$ (2.5×10^{-5} M) with different concentrations of chromatin. Concentration of chromatin is in excess over *cis*-$Pt(NH_3)_2Cl_2$.

[CHR] × 10^5M	$K_{appCHR} \times 10^5$ (sec M^{-1})
8	0.83
16	1.58
32	3

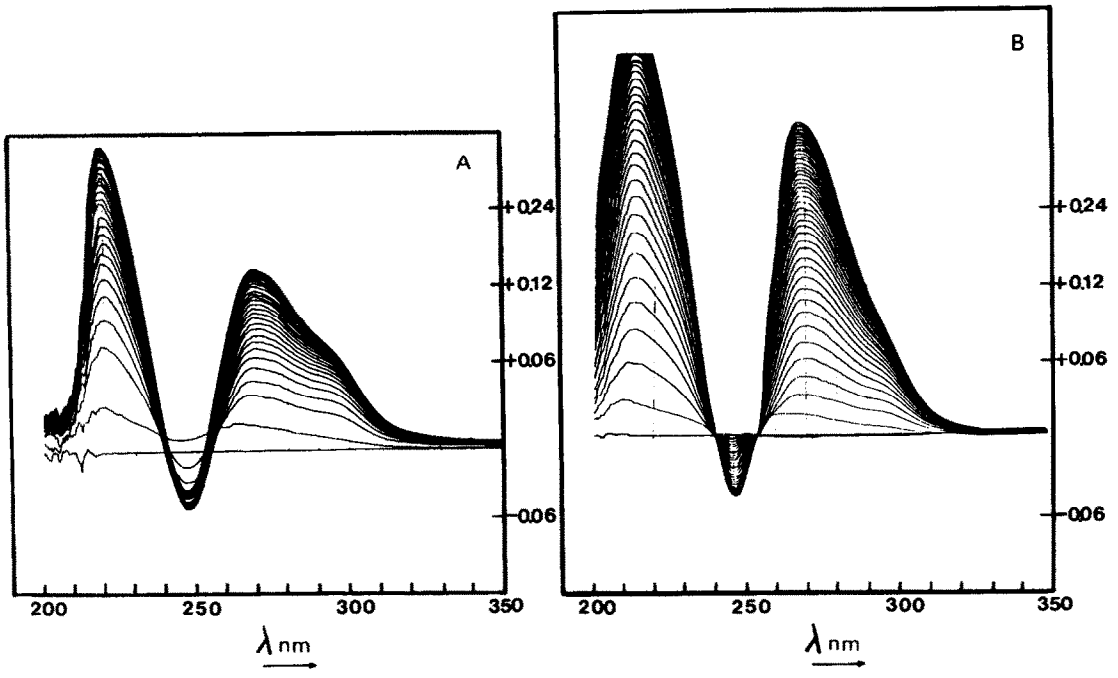
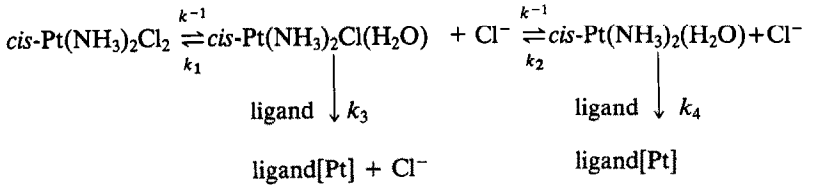


Fig. 6. Difference absorbance spectra resulting from the reaction of *cis*$[Pt(NH_3)_2(H_2O)_2]$ (2.5×10^{-5} M) with: (A) chromatin (32×10^{-5} M); (B) DNA (32×10^{-5} M). The buffer used for these reactions is: $NaClO_4$ 10 mM pH 7.6 at 25°. Spectra were recorded at intervals between 0 and 40 hr.

In these conditions, we are using the dichloride form of cisplatin and the rate constant measured is the resultant of at least two phenomena: the conversion of *cis*-diamminedichloroplatinum into the monoaqua-monochloride form or the diaqua forms and the binding of this reactive form to the ligand (Scheme I).

In these conditions, no chloride ions will compete with the platinum reaction to DNA or to chromatin. The difference absorbance spectra corresponding to these reactions are shown in Fig. 6.

The rate constant of the chromatin or DNA *cis*-$Pt(NH_3)_2(H_2O)_2$ adduct formation was determined. We measure a rate constant equals to 38.5



Scheme I

It is possible that the equality between the rate constants measured for the DNA and the chromatin does not represent an equality between the rate constants corresponding to the binding but represents a common rate constant associated with the aquation of cisplatin. In order to answer this question, which could be a serious limitation in the significance of our results, we have determined the rate constants corresponding to the interaction between the aquated form of cisplatin and DNA or chromatin in the absence of chloride ions.

We have followed the kinetics of the interactions of *cis*-$Pt(NH_3)_2$ $(H_2O)_2$ with chromatin or DNA in 10 mM $NaClO_4$ pH 7.6, at 25°.

M^{-1} sec^{-1} for the *cis*-$Pt(NH_3)_2(H_2O)_2$ – chromatin reaction and 34.2 M^{-1} sec^{-1} for the *cis*-$Pt(NH_3)_2(H_2O)_2$ – DNA reaction.

The rate constants are the same whether the diaqua forms react with DNA or with isolated chromatin. Moreover the structure of chromatin is not changed whether it is isolated in 10 mM Tris HCl, 10 mM NaCl, 0.2 mM EDTA pH 7.6 or in 10 mM $NaClO_4$ pH 7.6 as demonstrated through the determination of the rate of digestion and the repeat length of chromatin after a micrococcal nuclease digestion (Fig. 7).

The similarity in the rate constants corresponding to the reaction of platinum with DNA or chromatin

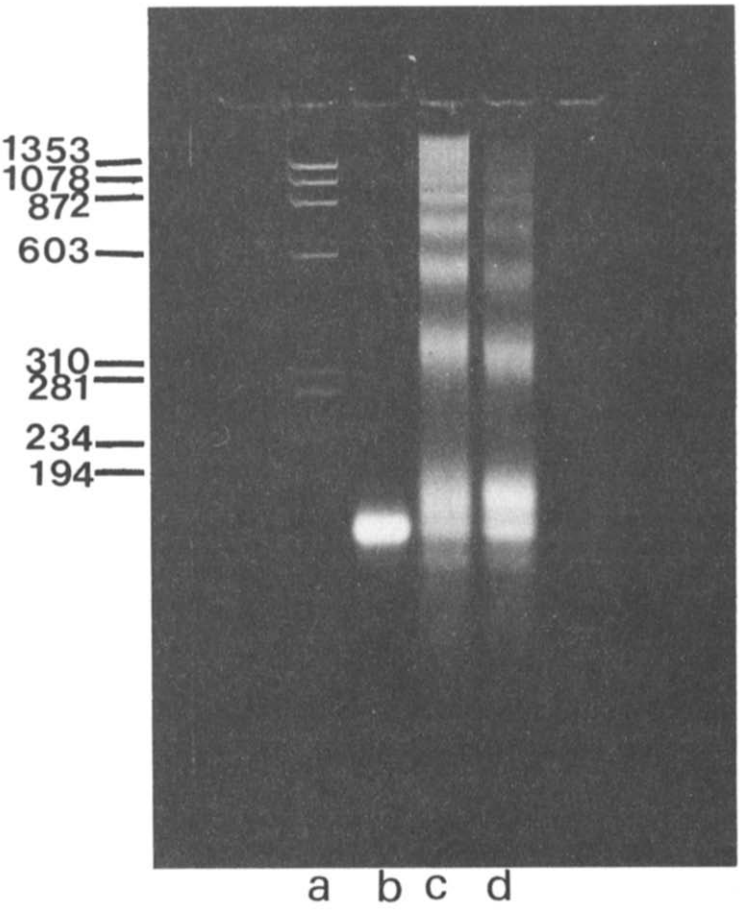


Fig. 7. 4% polyacrylamide gel electrophoresis pattern of digested chromatin: Slot a, marker DNA (ϕX174 HaeIII digest); Slot b, core particle 145 pdb; Slot c, digested chromatin in $NaClO_4$ 10 mM pH 7.6; Slot d, digested chromatin in Tris HCl 10 mM pH 7.6, NaCl 10 mM, EDTA 0.2 mM.

leads us to conclude that there is not a great difference in the nature of the binding sites of platinum either on DNA or on chromatin. The rate constant corresponding to the binding of platinum to isolated histones is found to be two times higher than the one found for DNA or chromatin. This suggests that platinum reacts mainly with the DNA whether it is histone associated or free in solution. This correlates what we observed through biochemical methods.

DISCUSSION

The measurement of the amount of platinum bound to chromatin, core particle or to DNA as a function of the ratio of total platinum versus DNA, indicates differences in the accessibility of these three substrates. At low drug concentrations ($r < 0.03$), the amounts of platinum bound to chromatin and to naked DNA are quite comparable, whereas the amount of platinum bound to core particle is lower. This observation suggests that the binding occurs preferentially at the level of the DNA linker. At higher drug concentrations ($r > 0.03$) the amount of platinum bound to chromatin and to core particle becomes higher than the one bound to naked DNA. This leads us to think that when high amounts of platinum are incubated with chromatin, a binding to the histones can appear. This correlates what is found for isolated histones. We can also assume that part of this platinum is bound to acidic proteins leading to an overbinding of platinum to chromatin compared to DNA. This assumption is unlikely since we still observe a higher amount of platinum bound to core particle than to naked DNA. Core particles during the purification process are deprived of acidic proteins, and the difference can only be accounted for by a binding of platinum to histones.

Degradation of cisplatin–chromatin complexes with micrococcal nuclease is inhibited, whereas degradation of chromatin in the presence of non-reacted platinum is not. By digesting with micrococcal nuclease the chromatin–platinum complexes corresponding to different values of r, we can follow the solubilization of the platinum bound to the DNA together with the solubilization of the DNA. For values of r smaller than 0.03, the percentage of released platinum is higher than the percentage of solubilized DNA. This indicates that the drug is not uniformly distributed over the chromatin DNA, but is preferentially bound to the linker DNA. At higher concentrations of platinum, the drug tends to be uniformly distributed all along the DNA. By com-

paring the rate of solubilization of DNA and platinum, we find that the discrepancy between the two kinetics mainly appears once a certain amount of linker DNA has already been digested. This observation suggests that the drug could be concentrated in specific regions of the linker DNA. The importance of the DNA for platinum binding, as previously suggested by physicochemical experiments [14], is verified by the determination of the rate constants of cisplatin to DNA, chromatin or core particle.

By measuring the rate constants of the interaction of cisplatin to DNA, chromatin and on core particle, a similarity arises between these three substances. The rate constant is the same, for whatever substrate we consider. Since we are working in Tris–HCl 10^{-2} M, NaCl 10^{-2} M, conditions which are classical in studying nuclease digestion of chromatin, we should be aware that we are faced to a competition between the chloride ions and the binding reaction. It is then possible that the conversion of cisplatin to aquated form, the reactive form of the drug, would be inhibited in our experimental conditions and that we observe the step limiting rate of conversion of cisplatin to aquated form. In order to rule out this limitation in interpreting our results, we have compared the rate constant measured when reacting the aqua derivative, *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ with DNA or chromatin, in perchlorate buffer. In these conditions, when only the reactive form of the drug is in the presence of the substrate, we observe the same rate constant whether the drug binds to DNA or chromatin. This suggests that the drug binds to the DNA. Another possibility is that the binding is the same in the case of chromatin or DNA because the drug completely disrupts the structure of the nucleosomes, unwrapping the DNA from the histone core. This assumption can be ruled out since electron microscope observations do not show such difference between the control and the platinum treated samples. This binding to the DNA is also supported by the fact that the same rate constants are observed when measuring the amount of bound platinum using atomic absorption spectrophotometry. Once more we are faced with the fact that the rate constants are the same for chromatin, DNA or core particle.

The specificity of the platinum binding sites to DNA and specially to linker DNA may be modulated by the compaction of chromatin structure. Such a feature might then exist *in vivo*, together with sequence specificity [21] which leads to an accumulation of drug in regions which are not compact. Since it is known that the active regions of the genome in the nucleus are under a structure which is "open" [22], these regions may therefore represent preferential targets for the binding of the drug as already demonstrated for other antitumoral drugs like bleomycin [23]. The fact that such specificity takes place with so small an amount of drug is an interesting observation which could be correlated to the pharmacological effects of these compounds observed at therapeutic doses.

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