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MASS SPECTROMETRY STUDY OF DNA-CISPLATIN COMPLEXES : PERTURBATION OF GUANINE-CYTOSINE BASE-PAIRS

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SUMMARY

The combination of Pyrolysis and Mass Spectrometry (Py-MS) is used to analyse a series of DNA-*cis*-$Pt(NH_3)_2Cl_2$ (cisplatin) complexes. The fragmentation pattern of DNA is essentially maintained in the DNA-cisplatin complexes. However, when 5 cisplatin molecules are bound to 1000 DNA bases, the 150 amu fragment which corresponds to G-1H and the 149 amu fragment of dC increase in intensity, while no change is observed for the A and T fragments. This result indicates that the G-C base-pair is perturbed upon cisplatin binding and strongly suggests that this is the primary site of interaction.

INTRODUCTION

One of the most striking problem encountered in nucleic acid research is the detection of minor alterations on the polymer due for instance to intercalated molecules between base-pairs, or covalent attachment on sugar or base residues, or ionic interaction with phosphate residues. These modifications are usually of great importance since they could result in a fundamental different *in vivo* behaviour of the nucleic acid. Charnock and Loo (1) and Wiebers (2) have successfully submitted DNA and polydeoxyribonucleotides to mass spectrometry without pretreatment such as derivatization, chemical or enzymatic hydrolysis. The two fundamental papers of Wiebers'group (3,4) clearly demonstrate that this technique has become an important tool in both detection and identification of nucleotides. The DNA fragmentation under pyrolytic and electron impact conditions proceeds *via* two steps : 1. cleavage of the phosphodiester bonds and 2. cleavage of the nucleoside, giving ion products which can be used as diagnostic ions for base fragments (4) (Fig. 1).

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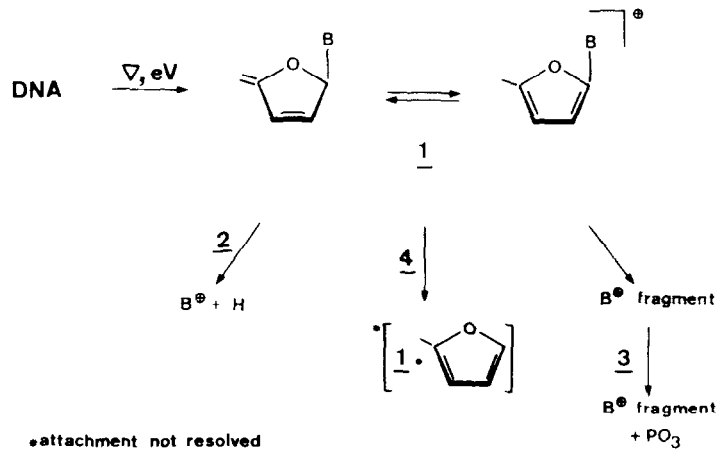

Figure 1 : Fragmentation pattern (1-4) for polydeoxyribonucleotides.

This last fragmentation has been well studied for different underivatized and derivatized nucleosides (5). The identification of base fragments is certainly the easiest technique of structure determination mainly because preliminary degradation is not required and the quantity of compound used remains very small (DNA concentrations varying from 0.01 to 1.0 $A_{260\ nm}$ units). In this paper, we report a study of underivatized salmon sperm DNA and DNA-cisplatin complexes corresponding to r_b^a = 0.001, 0.002, 0.005, 0.01, 0.10 and 0.15.

MATERIALS AND METHODS

The preparation of the DNA-cisplatin complexes has already been reported (6). Only glassware was used throughout this study in order to avoid a possible plastic contamination. The mass spectra were recorded on an Hitachi Perkin-Elmer RM-50 spectrometer. Aqueous solutions of DNA and DNA-cisplatin complexes in $NaClO_4$ 10 mM were freeze-dried and introduced *via* a direct inlet system . A temperature of 260°C was necessary in order to obtain a partial vaporisation of the products. The spectra were recorded using an ionizing energy of 70 eV at 2 x 10^{-7} torr and a source temperature of 200°C. The mass spectra were recorded several times and good reproducibility of the results is obtained only if pyrolytic conditions are rigorously respected. The tracing and the monitoring of diagnostic ions and the calculations were done using a HP-2100A minicomputer (7). The peak ratios for DNA were stored in the computer for the 100-300 amu range. The automatic recall of these data has been performed *via* the m/e ratios of peaks for each base, *via* intensity (± 2 %) and *via* all DNA peaks (self-tuning on 21 selected ions).

a. r_b = number of platinum atoms bound per nucleotide

TABLE I : DIAGNOSTIC IONS IN THE 100 - 300 amu RANGE, m/e (I,%)
SALMON SPERM DNA

BASE	ASSIGNMENT OF FRAGMENT ION PEAKS						
	2	3		1	4	Others
DNA						
dA	108 (4.7)	135 (38.1)	186 (3.3)	172 (3.1)	215 (3.0)	295 (0.5)	
dG	188 (34.3)	151 (9.5)	202 (9.0)		231 (3.0)		
dC		111 (8.8)	162 (1.1)	148 (2.5)	191 (8.8)		149 (2.9)
dT	117 (100.0) *	126 (4.7)			206 (12.0)		
DNA-CISPLATIN : r_b = 0.001							
dA	108 (5.3)	135 (49.5)	186 (4.1)	172 (8.3)	215 (12.5)	295 (3.0)	
dG	188 (37.5)	151 (12.0)	202 (11.0)		231 (16.0)		
dC		111 (10.7)	162 (3.0)	148 (1.5)	191 (3.5)		
dT	117 (100.0) *	126 (3.0)			206 (2.5)		
DNA-CISPLATIN : r_b = 0.002							
dA	108 (6.0)	135 (47.0)	186 (4.0)	172 (8.0)	215 (11.0)	295 (2.0)	
dG	188 (41.0)	151 (10.0)	202 (12.0)		231 (8.0)		150 (0.0)
dC		111 (10.0)	162 (4.0)	148 (2.0)	191 (4.0)		149 (0.0)
dT	117 (100.0) *	126 (4.0)			206 (2.0)		
DNA-CISPLATIN : r_b = 0.005							
dA	108 (5.5)	135 (43.0)	186 (4.0)	172 (5.0)	215 (10.0)	295 (3.0)	
dG	188 (35.0)	151 (11.0)	202 (7.0)		231 (8.0)		150 (14.0)
dC		111 (16.0)	162 (6.0)	148 (10.0)	191 (8.0)		149 (8.0)
dT	117 (100.0) *	126 (6.0)			206 (7.0)		
DNA-CISPLATIN : r_b = 0.01							
dA	108 (5.5)	135 (40.0)	186 (5.0)	172 (5.0)	215 (7.0)	295 (3.0)	
dG	188 (34.0)	151 (11.4)	202 (8.0)		231 (7.0)		150 (45.0)
dC		111 (20.0)	162 (8.0)	148 (11.0)	191 (13.0)		149 (27.0)
dT	117 (100.0) *	126 (8.5)			206 (11.0)		
DNA-CISPLATIN : r_b = 0.10							
dA	108 (7.5)	135 (47.5)	186 (10.0)	172 (10.0)	215 (10.0)	295 (7.0)	
dG	188 (47.0)	151 (17.0)	202 (20.0)		231 (6.0)		150 (30.0)
dC		111 (21.0)	162 (14.0)	148 (12.0)	191 (16.0)		149 (24.0)
dT	117 (100.0) *	126 (14.0)			206 (14.0)		
DNA-CISPLATIN : r_b = 0.15							
dA	108 (10.0)	135 (82.0)	186 (7.0)	172 (29.0)	215 (21.0)	295 (16.0)	
dG	188 (62.0)	151 (22.0)	202 (34.0)		231 (20.0)		150 (31.0)
dC		111 (31.0)	162 (21.0)	148 (44.0)	191 (26.0)		149 (35.0)
dT	117 (100.0) *	126 (42.0)			206 (19.0)		

* The intensity of the dT fragment corresponding to m/e = 117, has been assigned as 100 % in this range.

RESULTS

In Table I are listed the mass values of the peaks corresponding to the different fragments (1-4) observed in the 100-300 amu range with their rela-

tive intensities. These fragments are formed under pyrolytic conditions followed by electron impact for salmon sperm DNA and a series of DNA-cisplatin complexes. As one can see, the fragmentation pattern for the DNA-cisplatin complexes is similar to that of DNA. Three interesting observations can be noticed from these spectra. First, for the complex corresponding to r_b = 0.005 (Table I), a fragment is observed with a mass value of 150. The exact mass value calculated for $C_5N_5H_4O$, which represents G-1H (the mass value for G being 151) is 150.0416. The observed mass value for this fragment is 150.0447 $\pm$ 0.0006. From this result we can conclude that this fragment represents the guanine molecule which has lost one hydrogen atom. This fragment is not detected for r_b = 0.002 which corresponds to the detection limit of the technique. When $r_b \geq 0.005$ this fragment is always present. Second, the intensity of the 149 fragment corresponding to a C fragment (m/e = 148) (3) plus one hydrogen atom, increases significantly for $r_b \geq 0.005$, *i.e.* the r_b value where the guanine loses one hydrogen atom. The identity of this 149 amu fragment was confirmed by its exact mass value, m/e = 149.0244 $\pm$ 0.0004. This result excludes the possibility of a plastic contaminant (like phthalate) since its exact mass value is 149.0147. Third, the intensity of adenine (m/e = 135) and thymine (m/e = 126) fragments which correspond to the bases are highly increased for $r_b > 0.10$. These variations in intensity *vs* r_b are represented in Figure 2.

DISCUSSION

Different techniques, like buoyant densities (8), chromatographic studies (9) and circular dichroism spectra (6) have shown a preferential binding of cisplatin to the G-C base-pairs in DNA. This work presents another evidence of the G-C participation in the binding when DNA is complexed with cisplatin. Since this study was only limited to the fragments found in the 100 - 300 amu range (where the peak intensities were significant), the complex base +cisplatin was not considered (too low volatility). Only the fragments corresponding to the bases modified by cisplatin were analyzed. The

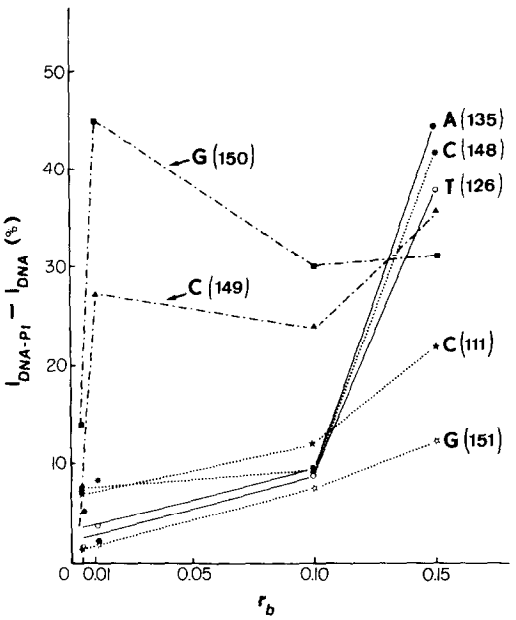

Figure 2 : Variations of the intensity of DNA (salmon sperm) peaks corresponding to the base fragments (A, T, G, C) upon cisplatin complexation.
I_{DNA-Pt} = relative intensity of a fragment in a DNA-cisplatin complex.
I_{DNA} = relative intensity of the same fragment in DNA.

fragment due to the guanine moiety (m/e = 151) was observed to be accompanied by a peak one mass unit lower (m/e = 150) for an r_b value as low as 0.005. The intensity of this new fragment relative to thymine 117 peak equals 14 % (Table I). Two explanations of the origin of this fragment which corresponds to G-1H are possible.

First, a H may transfer from G to C in the DNA-cisplatin complex as a consequence of the cisplatin fixation on G followed by a labilization of one H. It must be mentioned that this fragment is very weak (less than 1 %) in uncomplexed DNA. When this phenomenon is observed, a fragment corresponding to C + 1 H (m/e = 149) increases in intensity (Fig. 2). The second explanation also consistent with the results obtained in mass spectrometry, would be a proton liberation in solution during the complexation (10) for low r_b's. In both cases, hydrogen bonds between G and C must be weakened. In fact, a number of physico-chemical techniques recently reviewed (10),

have shown the destabilization of the double helix upon cisplatin binding. Thermal denaturation studies indicate a decrease of 2°C of the Tm even for r_b = 0.005 which could be the result of hydrogen bond breakage. Moreover, a linear decrease of the Tm (2.5°C) is observed until an r_b of 0.01. The intercalation of ethidium bromide in the DNA-cisplatin complexes (11) is prevented linearly with increasing r_b. One molecule of cisplatin fixed to DNA inhibits the intercalation of one ethidium bromide molecule. This means that cisplatin has locally destroyed the intercalation site formed by two base-pairs. Cisplatin was also found to significantly decrease DNA viscosity (manuscript in preparation). These three results strongly suggest that the G and C molecules are less or no longer hydrogen bonded when cisplatin is fixed to G, and seem to favour the second explanation involving a proton liberation in solution. Preliminary data using the same technique with *trans*-$Pt(NH_3)_2Cl_2$ and $[Pt(dien)Cl]Cl$[b] indicate no H displacement from G.

The only evidence of the A-T participation in the binding was found with cisplatin and salmon sperm DNA (Fig. 2) for $r_b > 0.10$. The intensity increase of the A and T base fragments (m/e = 135, 126) indicates that this base-pair is involved in the binding of cisplatin to DNA when $r_b > 0.10$. This is in qualitative agreement with the results of Munchausen and Rahn (9) who found a cisplatin interaction on guanine bases first followed with an interaction on adenine bases. The critical r_b value of 0.10 also corresponds to the maximum of the hyperchromic effect on the positive band found in circular dichroism with cisplatin (6).

In conclusion, mass spectrometry brings another evidence of the G-C preferentiality for cisplatin complexed with DNA at low r_b. The most interesting feature of this technique is its high sensitivity : 5 cisplatin molecules bound to 1000 nucleotides are detected on G-C base-pairs. We hope to use mass spectrometry for demonstrating the G-C preferentiality of *cis*-platinum compounds *in vivo*.

b. dien = diethylenetriamine

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