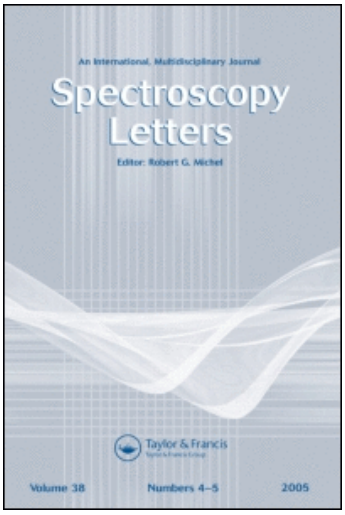

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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To cite this Article Robichaud, Annette , Jankowski, Krzysztof and Virelizier, Henri(1988) 'Study of Preferential Platination of Equimolar DNA-RNA Mixture in Solutions', Spectroscopy Letters, 21: 6, 423 — 436
To link to this Article: DOI: 10.1080/00387018808062723
URL: http://dx.doi.org/10.1080/00387018808062723

SPECTROSCOPY LETTERS, 21(6), 423-436 (1988)

Study of Preferential Platination of Equimolar DNA-RNA Mixture in Solution

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Abstract

The complexation of equimolar mixture of two biopolymers DNA and RNA by cis-platinum at $r_b = 0.1$ leads to 20% preferential binding to DNA as established by Electron Impact Pyrolytical Mass Spectrometry.

Introduction

The anti-cancer activity of cis-platinum (PDD or cis-dichlorodiamins platinum II) is related to the specific binding of this drug to the DNA. A widely accepted hypothesis concerning the mechanism of its action involves the complexation (via N-7 of guanine G) of the DNA, followed by cleavage of hydrogen bondings and shortening of the nucleic acid. The problem has been abundantly studied in vitro by using a variety of physicochemical

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techniques, including the spectroscopic ones and appropriate model compounds (1-4). The hypothesis has a basic weakness, however, it ignores simultaneous presence of several nucleophilic ligands in vivo e.g. RNA, peptides, proteins. The ligands have similar nucleophility as well as binding properties.

We have designed a simple sequence of in vitro experiments which should determine the preference, if any, of cis-platinum toward the DNA when closely related compound RNA is present in equimolar ratio.

We note that the chemical difference between RNA and DNA, that is the 2'-hydroxy group, cannot change its nucleophility. Three strongly nucleophilic heterocyclic bases are the same in both (A, G, C); the remaining pair bases (U, T) are not drastically different either and belong to the same family of pyrimidines.

Our first choice of a technique for the investigation of the preferential binding toward one or the other of the nucleic acids was ^1H NMR. We have obtained spectra in D_2O solutions for DNA and RNA models as well as for complexes of cis-platinum-DNA and cis-platinum-RNA at $r_b = 0.1$ ($r_b = 0.1$ means one Pt per ten bases) and the mixed complex cis-platinum DNA-RNA (50:50). The results do not lead to any conclusive interpretations.

By contrast, a second technique, pyrolytical mass spectrometry normally used in the polymer analytical chemistry and which we have already used in the past for the biopolymers (5-7), has produced significant results.

Experimental

The nucleic acids DNA (Herring sperm, Sigma) and RNA (Baker yeast, Sigma) have been used without previous purification. Both

Table 1

Base Abundance of Nucleic Acids

	Base	%*	%**
DNA	A	28.5	29.7
(Herring sperm)	T	28.2	29.1
	G	21.0	20.8
	C	21.0	20.4
RNA	A	15.0	15-17
(Baker yeast)	U	7.0	6-12
	G	20.5	18-21
	C	20.0	15-20
	5,6-dih U	5.0	2-6

* Deflection (mass spectrometric method[5])
**Litterature[9]

compounds have been checked via mass spectrometry for the presence of essential as well as modified bases showing usual base pattern (Table 1). The cis-platinum has been bought from Sigma.

Mass spectra have been recorded on a Riber 1010 spectrometer in the positive ion mode using electron impact to generate the ion species.

The recording and calculation of spectra were done using a PDP 8/M computer coupled to the spectrometer. Typical analytical conditions were: source temperature 200-220°C, the 5 mm filament

(Tungsten wire 60 μm, 9 turns) was heated from 300-1000°C in 40 s (current programmed from 200-600 mA, 10 mA s^{-1}). The average lifetime of the filaments under these conditions was around 20 analyses. The source was indirectly heated by a filament (variation of 10°C - for this reason the direct probe was removed after the end of the programme. The pressure measured was 10-6 Torr (average level). According to our previous studies, the diagnostic mass range was fixed at 100-300 u.

The herring sperm DNA, or yeast RNA, was cut up into 1 mm pieces and placed in the centre of the filament coil or directly glued on the coil after being poured into bidistilled water. One mm of this DNA corresponds to ca 0.5 A260 unit. The recording has been made at the maximum of TIC (8).

The $BH^{\cdot +}$ ion intensities have been computed in a usual way, each determination was repeated six times. The intensities of $BH^{\cdot +}$ ions reported here are the respective average of these six determinations (Fig. 1).

Preparation of the complexes at $r_b = 0.1$ has been modeled on Macquet procedure (3). A solution of cis-platinum 0.48 mg/ml in $NaClO_4$ 10^{-2} M was prepared. The nucleic acids were dissolved in $NaClO_4$ 10^{-2} M in a way to obtain a final concentration of mg/ml of nucleic acids and 20% (v/v) in cis-platinum. These solutions were then incubated at 37°C in the dark for 48 hours, after what they were lyophylised.

Results and Discussion

The mass spectra of both nucleic acids, Herring Sperm DNA and RNA Baker yeast, were recorded in order to produce a reference spectra for the platinated series. Recording of all the spectra has been done at the maximum of total ion current

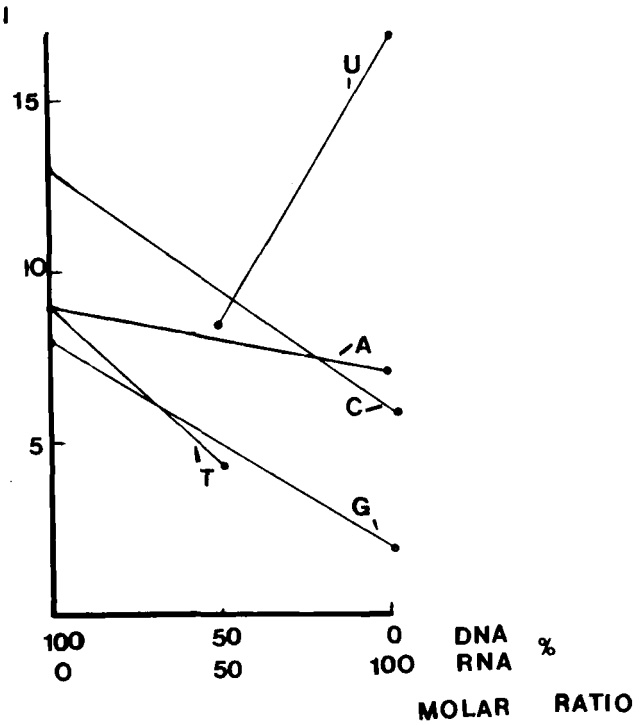

FIG. 1 CALIBRATION CURVE: I FOR BH^{+} IONS AS A FUNCTION OF DNA OR RNA MOLAR FRACTION

I FOR BH^{+} IS EXPRESSED AS $I_{BH^{+}}$ FOR DNA - $I_{BH^{+}}$ FOR DNA - Pt OR $I_{BH^{+}}$ FOR RNA - $I_{BH^{+}}$ FOR RNA - Pt AT r_B - 0.1

BH IONS (m/z): A 135, C 111, T 126, G 151, U 112

under the usual electron impact conditions. These conditions have been rigorously maintained throughout the entire series of measurements which enables the following quantitations.

i) Unplatinated DNA and RNA

The base abundance evaluation for both nucleic acids, made by using the usual deflection technique (5), and presented in

Table 1, displays good agreement between the four essential base percentage obtained and the literature values (9). It is pertinent, however, to note a significant quantity of 5,6-dihydrouridine in the spectrum of RNA. As already noted, the intensities of $BH^{\cdot +}$ ions at 151(G), 135(A), 126(T), 114(5,6 dihU), 112(U), 111(C) have been measured in six independent experiments, and Table 2 contains the respective average. In the case of RNA, five measurements only have been taken into account. The less volatile $BH^{\cdot +}$ for G (m/z 151) intensity has been evaluated using several DNA phages as references.

The abundance of U in the yeast RNA has been evaluated by comparison of three purified tRNA (Phe, Sigma) samples of known base abundances. The accuracy of the deflection method using quadrupole mass spectrometer and observing the intensities of BH positive ions is 2%. The results are shown in Fig. 2 and Fig. 3.

ii) Spectra of Pt-DNA and Pt-RNA

Both spectra have been recorded in the usual way (8). The results are shown in Fig. 4 and Fig. 5. The intensities of four $BH^{\cdot +}$ ions have been calculated and then compared to free DNA or RNA respectively (Table 2). The differences are reported in Figure 1 expressed as mole fractions of DNA or RNA in both extremities of the Figure. In such a manner we have recorded a calibration curve for the three common bases (A, G, C). The intensities of particular bases (U for RNA and T for DNA) have been calculated assuming the proportionality of I with r_b e.g. at r_b = 0.05 a half of intensities of T at r_b = 0.1 is observed. For U similar calculation has been made. The graphs of I as a function of mole fraction of the base present have been used in the evaluation of preferential binding of Pt to one of the nucleic

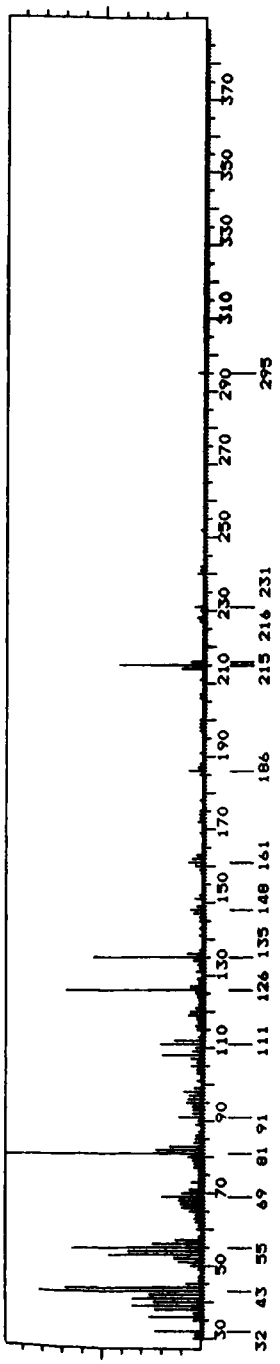

FIG. 2 HERRING SPERM DNA EI - PI MASS SPECTRUM

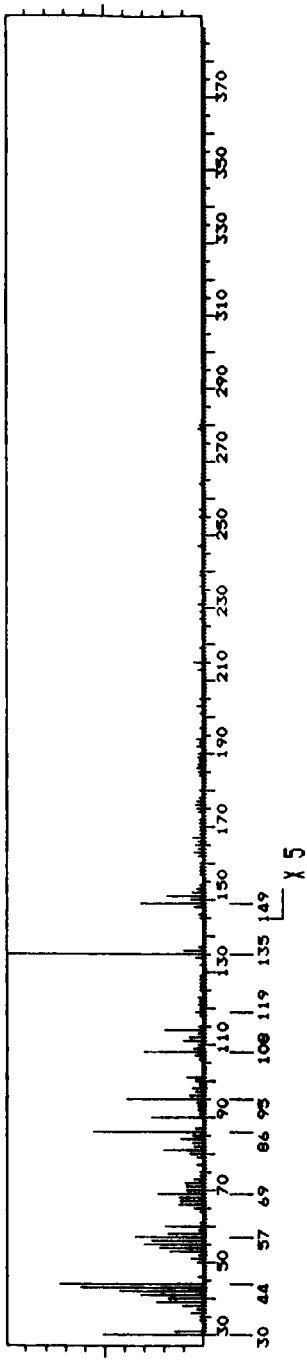

FIG. 3 BAKER YEAST RNA EI - PI MASS SPECTRUM

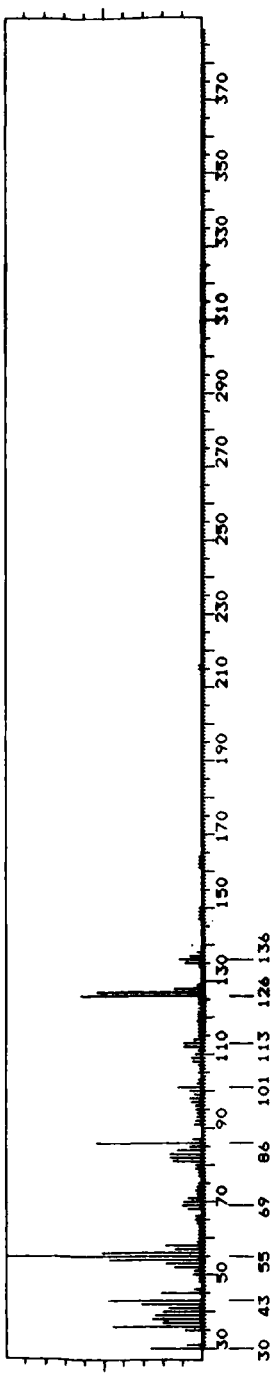

FIG. 4 DNA - Pt [illegible] 0.1 [illegible] - PI MASS SPECTRUM

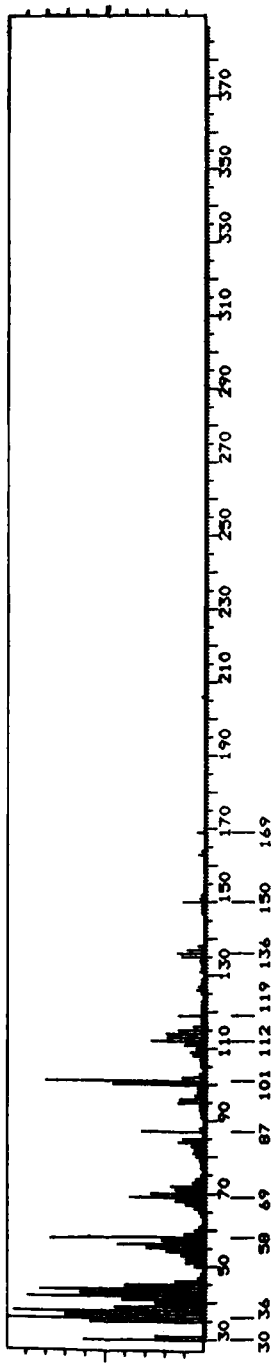

FIG. 5 RNA - Pt $r_B = 0.1$ EI - PI MASS SPECTRUM

TABLE 2

	$BH^{\cdot +}$ (m/z)		I(%)	% of Pt bound to DNA	% of Pt bound to RNA
DNA-Pt	A	135	9		
(0.1)	T	126	9		
	G	151	8		
	C	111	13		
RNA-Pt*	A	135	7		
	U	112	17		
	G	151	2		
	C	111	5		
DNA-RNA**	A	135	8.2	58	42
(50:50)-Pt	U	112	7.6	-	41
(0.1)	T	126	5.8	64	-
	G	151	4.3	61	39
	C	111	8.6	63	37
	Average			61.5	39.8

* 5,6-dih U intensity of m/z 114 ion is 5%.

** average of six recordings.

acid, this with reference to the equimolar quantities of both nucleic acids complexed with cis-platinum at $r_b = 0.1$.

iii) Spectra of Pt-DNA-RNA (50:50)

The spectrum of the equimolar DNA-RNA mixture at $r_b = 0.1$ reveals several particularities (Fig. 6). Relating of average intensities of five ions (A, G, C, T and U, $BH^{\cdot +}$ ions as in Table 1) to the calibration graph (Figure 1) enables the evaluation of

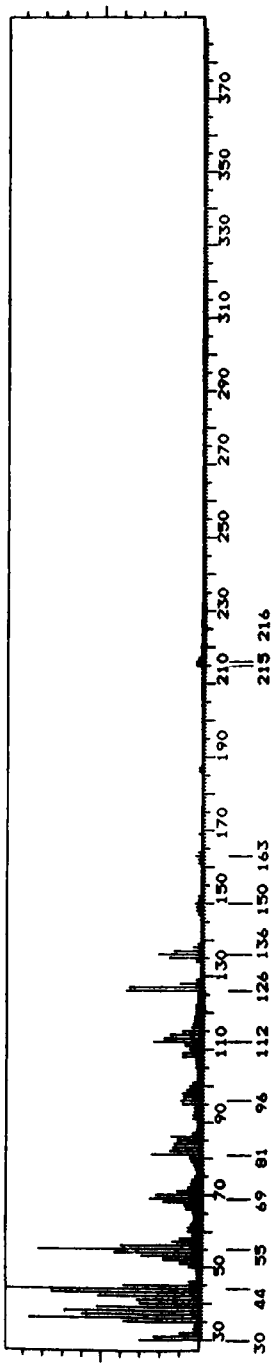

FIG. 6 DNA - RNA (50:50) - Pt $r_B = 0.1$
EI - PI MASS SPECTRUM

the percentage of cis-platinum bound to one of the nucleic acids (e.g. DNA).

Thus, we have found that the average percentage of cis-platinum bound to DNA (61.5) and to RNA (39.5). The average excess of Pt bound to DNA that is 21.5% approx. (Table 2).

Conclusion

The results obtained by using mass spectrometry indicate only a preference of bonding of cis-platinum towards DNA when the equivalent quantities of RNA are present. The present results should be considered preliminary and would be worthwhile to apply other analytical techniques in order to confirm them.

We note that Pascoe and Roberts (10) have inferred that there is a strong preferential binding in favor of the DNA. The small 20% preference towards DNA does not invalidate the hypothesis of the exclusive binding of cis-platinum to cellular ligands as being responsible for its anti-cancer action. From purely chemical standpoint, the structural similarity and almost identical nucleophility of both nucleic acids in solution imply concomittant participation of both acids in the formation of Pt complexes. Reasons for the small preference observed might lie in differences in the ion strength of solutions and solubilities of two nucleic acids.

This study shows the interesting potential of the Py-Mass spectrometry technique applied to biopolymers.

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Date Received: 03/25/88
Date Accepted: 04/29/88