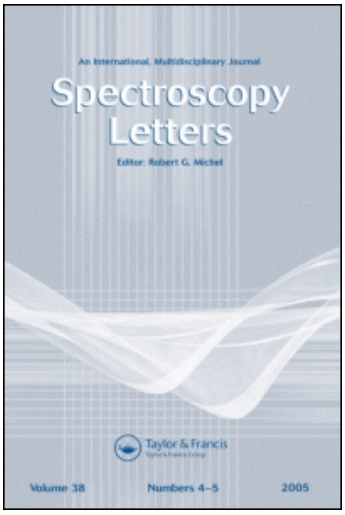

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Study of Platination of Nucleic Acids Using Atomic Absorption and Mass Spectrometry

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STUDY OF PLATINATION OF NUCLEIC ACIDS USING ATOMIC ABSORPTION AND MASS SPECTROMETRY

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Abstract

The partition of cis-platinum between a mixture of DNA and RNA as well as transport of DNA cis-platinum complexes via synthetic membranes were studied using atomic absorption and mass spectrometry.

Introduction

The interaction of action of the cis-platinum (DDP or cis-diaminedichloro platinum II) (1) with DNA has been widely investigated by different physico-chemical methods (1-3). Among others, atomic absorption (4) (AA) and more

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recently mass spectrometry (MS) have been used to study the platination levels of DNA and the structures of the complexes between nucleic acids and the compound 1 (3, 5). Both techniques have proven themselves, in this respect, as valuable qualitative or quantitative analytical tools. For instance these techniques have rendered possible quantification of platinum (AA) or bases of nucleic acids DNA or RNA (MS).

In this series of experiments we have compared these methods with regard to two applications: platination of DNA and RNA and transport of Pt-DNA complexes across synthetic membranes. The results are presented in the three following sections:

i) determination of platination of a mixture of DNA and RNA and separation of individual Pt-DNA and Pt-RNA complexes using atomic absorption spectrometry

ii) determination of platination level of the same mixtures by mass spectrometry

iii) use of atomic absorption and mass spectrometry in the study of transport of DNA-Pt complexes via synthetic membranes.

Results

i) Determination of platination of a mixture of DNA and RNA after separation of individual Pt-DNA and Pt-RNA complexes using atomic absorption spectrometry

In our previous Py-MS (pyrolytical mass spectrometry) investigation of the preferential binding of 1 to DNA or RNA when equimolar quantities of both nucleic acids were present, a small excess of platinum complexed by DNA was reported (5). However, a reexamination of this experiment has shown that the presence of free and uncomplexed nucleic acids and differences in their solubilities could affect our interpretation. The experiment therefore was redesigned where one of the two nucleic acids had been separated from the mixtures by an enzymatic digestion.

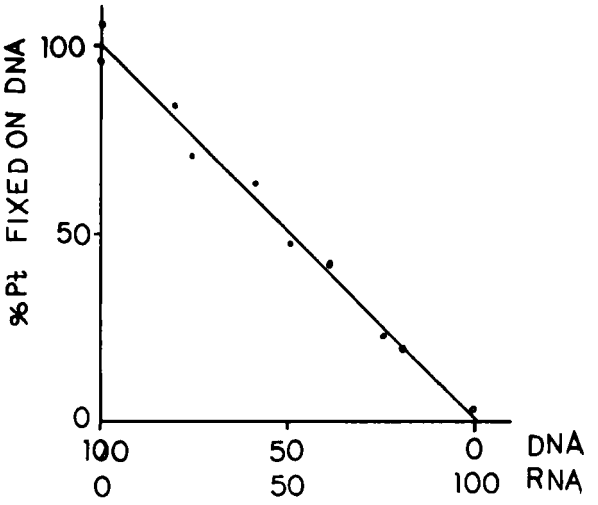

Fig. 1 Percentage of Pt fixed on the DNA as determinated by atomic absorption

Mixtures of DNA and RNA at different molar ratios (DNA:RNA 100:0, 80:20, 75:25, 60:40, 50:50, 40:60, 25:75, 20:80 and 0:100) were exposed to $\underline{1}$ r_B = 0.1*. After quantitative fixation of platinum on the nucleic acids, RNA was degraded by RNase and the resulting ribonucleotides were separated from the DNA by column chromatography. The concentration of platinum on the DNA was quantified using atomic absorption and mass spectroscopies. In order to ascertain these results a series of DNA-RNA mixtures were examined (DNA:RNA 100:0, 80:20, 75:25, 60:40, 50:50, 40:60, 25:75, 20:80 and 0:100). Taking into account the precision of this method ($\pm$2%), the results (Table 1, Figure 1) do not show any preferential binding of cis-platinum to either nucleic acid when equimolar quantities of both were present (Pt distribution between DNA:RNA 49:51, from Fig. 1 graphs, calculations Table 1).

* r_B stands for molar ratio of bound Pt per nucleotide.

TABLE 1

Percentage of Pt Fixed on the DNA as Determined by AA

DNA / RNA Molar Ratio	% Pt on DNA
100 : 0	106, 96
80 : 20	85
75 : 25	72
60 : 40	64
50 : 50	48
40 : 60	43
25 : 75	24
20 : 80	21
0 : 100	0

Measured % Pt-DNA at 50:50 from the statistical data: 48.9%

(% Pt-DNA = 0.97 x, ρ = 0.996)

ii) Determination of platination for the DNA-RNA mixtures by the EI-PI mass spectrometry

The series of nucleic acid mixtures previously described were examined using the technique of mass spectrometry (3, 4, 7) before and after ribonuclease digestion. The comparison of the results for the series of six DNA-RNA mixtures examined this time (DNA:RNA 100:0, 80:20, 60:40, 40:60, 20:80, 0:100) leads again to the conclusion that there is no apparent preferential binding toward either DNA or RNA (Table 2, Figure 2). The equimolar mixture of both nucleic acids showed again an equal degree of platination toward the two nucleophiles DNA:RNA, (52:48 from mass spectrometric experiments using Fig. graphs, calculated as per Table 2).

TABLE 2

I for $BH^{\cdot+}$ ions for DNA-RNA mixtures
(common bases only)*

DNA/RNA Ratio	Base	A	C	G	T/U Ratio
100:0		94	65	27	-
80:20		86	56	34	2.56
60:40		73	46	39	1.29
40:60		64	41	42	0.35
20:80		52	32	43	0.07
0:100		41	26	49	-
Measured % Pt-DNA at 50:50 from the statistical data		49	53	53	-

average 52

% Pt-DNA (base A) = -76.9 + 1.86 x (ρ 0.99)
% Pt-DNA (base C) = -63.2 + 2.55 x (ρ 0.99)
% Pt-DNA (base G) = 236 - 4.76 x (ρ 0.97)

* $I = \frac{I_{BH^{\cdot+}}}{\sum BH^{\cdot+}}$ $BH^{\cdot+}$ ions for bases are (a.m.u.): A-adenine 135, C-cytosine 111, G-guanine 151, T-thymine 126 and U-uracil 112.

I at Pt-DNA 50:50 is read from the graph, e.g.:
$BH^{\cdot+}$ A = $\frac{\sum I_{BH^{\cdot+}} \text{ at } 100:0 \text{ and } 0:10}{2}$

iii) Atomic absorption and mass spectrometry study of transport of the Pt-DNA complexes across synthetic membranes

The dialysis of r_B 0.1 cis-platinum-DNA complex as well as cis-platinum (1) alone across synthetic membranes was investigated. The nylon pellets or cellulose synthetic membranes are often used as a model for the natural membranes transport studies, in spite of the fact that they do not offer the same retention of the reactive molecules (1, 6). In the present case the sample of

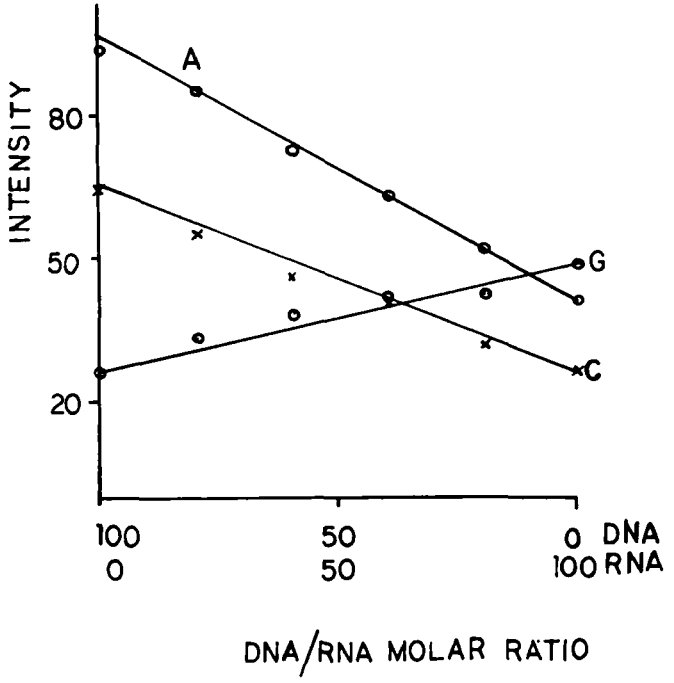

Fig. 2 Mass spectra of DNA-RNA mixtures - calibration curve for base $BH^{\cdot +}$ ions, at DNA:RNA 50:50 52% of platination of DNA (average) is observed

Pt-DNA (1 ppm or 2 ppm) placed in the cellulose or benzoylated cellulose nylon bag showed the same Pt concentration on both sides of the membrane (0.5 or 1 ppm approximately) after 48 hours as determined by the atomic absorption spectroscopy (Table 3).

The Pt-DNA complex placed in the cellulose sacks on the other hand showed total retention of the complex inside the membrane. Using solutions of up to 8 ppm of Pt no trace of metal is detected outside the membrane after 48 hrs. Positive ion mass spectrometry results confirm these observations. After the lyophilisation, the membrane-enclosed solution shows the usual presence of four essential bases ($BH^{\cdot +}$ ions), reinforcing the hypothesis that the Pt-DNA complexes do not migrate via membranes and are not modified by this operation.

TABLE 3

Migration of platinated complexes via synthetic members
(in % of Pt used in the experiment)

Membrane	Membrane transport characteristics	Percentage of 1		Pt-DNA (r_B = 0.1)	
		inner	outer	inner	outer
Cellulose	Membrane Retention of MW 12KD	50	50	100	0
Benzoylated Cellulose	Membrane Retention of 2KD	40	40	80	0
Nylon 12	Pellets Pore size 0.45 μm	-	45	80	0

The same set of experiments repeated for benzoylated cellulose membranes of different permeability (Table 3) lead to the same conclusion. It is worth noticing that the benzoylated membranes and nylon pellets as well as all plastic vessels used in these experiments absorb significant quantities of DNA in particular. For this reason use of all glass system is advisable.

Experimental

Material and methods

All chemicals used in the present studies were procured from Sigma Chemicals (St. Louis, Miss., U.S.A.) except the sodium perchlorate which was obtained from Fluka (Buchs, Switzerland). The Salmon sperm DNA and ribonuclease A (RNase) type XII-A were also obtained from Sigma while Baker yeast RNA was from Boehringer (Mannheim, Germany). The atomic absorption spectra were recorded on Perkin Elmer-5000 (flame, nitrous oxide-acetylene or air-acetylene) and Perkin Elmer 603 graphite furnace spectrometers equiped with Pt-hollow cathode lamps. The absorption wavelength of 267 nm or 265.9 nm was

employed for platinum determinations. Standard curves were plotted using stock standard aqueous solutions of 1 (0.5-8.0 ppm or 0.5-20 ppm for PE 5000 and 0.06-1 ppm for PE 603 apparatus). These standard curves correspond to 1 to 75 ng/ L of the cis-platinum for PE 5000 and 0.0625-0.25 ng/μL for the PE 603 apparatus). The UV spectra were recorded on UV Beckman-35 and Zeiss PMQ II spectrophotometers and the PI-EI pyrolytical mass spectra were obtained with Riber 1010 spectrometer (70 eV, 200-600^{o}C) (CEN Saclay, France), using the previously described experimental procedures (7). The reference herring sperm DNA (Sigma) was used to calibrate the mass spectra. All mass spectrometric results are an average of six independent measurements. The cellulose and benzoylated cellulose membranes were bought from Sigma, while the nylon 12 pellets 0.45 μm was from Aldrich Chemicals.

Preparation of homogeneous Pt-nucleic acid complexes

Appropriate quantities of DNA and RNA were mixed together in variable molar ratios (DNA/ RNA 100:0, 80:20, 75:25, 60:40, 50:50, 40:60, 25:75, 20:80 and 0:100). The nucleic acid solutions of 0.5 mg per ml in 10^{-2} M sodium perchlorate were mixed with 1 in order to obtain r_B = 0.1, and incubated in the dark for 48 hrs at 37^{o}C. The concentration of 1 was checked by atomic absorption (graphite furnace) and that of the nucleotides by ultraviolet and mass spectroscopies.

Separation of complexes

The mixed complexes were incubated in the presence of (beef pancrease) RNase (final concentration of 4.2 mg mL^{-1} in 50 mM sodium acetate at the pH 5.5) at 37^{o}C for 24 hrs, conditions which quantitatively digest the RNA. Samples of resulting nucleotides were then passed on the G-50 gel (fine) (Pharmacia, Sweden) column (115 cm x 1 cm OD). The elution with 10^{-2} M sodium perchlorate was performed and fractions collected with LKB Bromma 2070 ultrovac fraction collector (fraction volume 1.0 mL, 0.5 mL min^{-1}), into glass

vials to avoid the adhesion of the nucleic acids to plastic test tubes. The elution profile was established using UV 260 nm absorption band. The fraction were analyzed by extinction coefficients used to determine the concentrations of DNA and RNA atomic absorption in order to calculate their specific r_B. All fractions corresponding to a particular complex type were combined together and after lyophilisation examined by mass spectrometry (Table 2).

Membrane transport

The complexes Pt-DNA at r_B = 0.1 in original solutions prepared as described previously or standard 1 solutions were dialyzed by cellulose or benzoylated cellulose membranes for 48 hrs at 37°C against distilled water. The inner and outer solutions were analyzed by atomic absorption or after lyophilisation by mass spectrometry. The starting cis-platinum concentration was adjusted at 8 ppm approximately (Table 3).

Conclusion

The quantitation of Pt-DNA and Pt-RNA complexes using two spectroscopic methods leads to the conclusion that there is not preferential binding of the metal toward either of these two closely related nucleophiles. This observation combined with our previous findings (3, 8-10) justifies further investigation of the metal-nucleic acids by the mass spectroscoopy. This study also shows lack of mobility of the Pt-DNA complexes across membranes, as showed in this study adds further to the controversy about the possible mechanism of action of this potent anticancer agent. The mass spectroscopy technique is simpler from the purely analytical standpoint, (e.g. demands less calibration and permits to study metal binding to DNA and RNA mixtures without previous enzymatic digestion in the similar to atomic absorption platinum concentration range).

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