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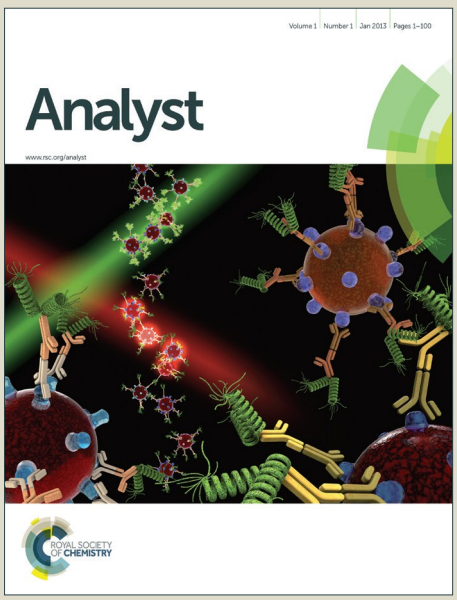

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ARTICLE

A new insight into the interaction of cisplatin with DNA: ROA spectroscopic studies on the therapeutic effect of the drug

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Raman optical activity (ROA) spectroscopy has been applied for the first time to study the interaction of cisplatin with DNA. The knowledge about the structure of DNA-metal ions cross-linking and hence the mechanism of the drug action is fundamental for the development of new antitumor drugs. At the same time, there is an urgent need to search for new methods of monitoring of that effect at the therapeutic dose of a drug. We have demonstrated that ROA spectroscopy is a sensitive technique with capability to follow the structural alteration of the whole DNA molecule upon drug binding via a direct observation of transformation undergoing within chiral sugar moieties. A ROA profile delivers a clear evidence of a partial transition from the B-DNA to the A-form due to the formation of cisplatin-DNA cross-links.

Introduction

Nowadays, many transition metal complexes have been commonly used as antitumor drugs and diagnostic agents[1–5]. Despite of their high efficiency, the newer, safer and more effective pharmaceuticals have been looked for. There are increasing demands for maximizing clinical benefits and minimising side effects, bypassing drug resistance and individualization of drug therapy to the patient. The understanding of the molecular mechanism of interactions between deoxyribonucleic acid (DNA) and metal ions is an essential part of the anticancer drug development.

We focus our interest on cisplatin (cis-DDP, $Pt(NH_3)_2Cl_2$), the first clinically applied platinum antitumor drug[6], widely used for treatment of many types of cancers e.g. testicular cancer, ovarian, bladder cancer, squamous cell lung cancers and small-cell carcinoma[7,8]. Cisplatin interacts with DNA within cell nucleus, inducing cell death via apoptosis. It reacts with the DNA bases, primarily with guanine (G) and adenine (A). The most preferred site of platinum binding are the guanine-N(7) and adenine-N(3), as the most nucleophilic atoms in DNA[5,6,9]. Cis-DDP forms mainly intrastrand 1,2-GG (~65%) and 1,2-AG adducts (~25%). However, adducts involving cisplatin intrastrand crosslinks to 1,3-GXG as well as different interstrand crosslinks are also possible Platinum complexes cause significant distortion of DNA double helical structure[5,6,10,11]. Crystallographic studies have showed that the Pt adducts induce a global bending toward the major groove in DNA, local unwinding of the helix, widening and flattering of the minor groove[11]. Conformational changes occurring upon cisplatin binding have been observed also by AFM[10], NMR[12] and VCD spectroscopy[13,14]. It has been found that the transition of the native B-form into other DNA forms occurs as a result of platination. The formation of a Z-form stabilizing an effect of cisplatin has been postulated[15,16], but the formation of this left-handed double helix has been not yet confirmed[13]. Raman spectroscopic studies have revealed only a partial transition from the B- to the A-form[17] whereas X-ray crystallographic results have showed that DNA intercalated with cisplatin adopts predominantly the A-form[18]. Although DNA-cisplatin interactions have been widely investigated by a numerous techniques, a full explanation of this process has still remained unclear. It should be also stressed here that all these studies have been conducted for the drug interacting with DNA at higher concentrations than the therapeutic dose.

The purpose of this work is to demonstrate capability of Raman Optical Activity (ROA) spectroscopy in assessing changes in DNA structure caused by the interaction with cisplatin. To the best of our knowledge, this is the first time, when the ROA spectrum of DNA affected by platinum(II)-containing drug is presented. Moreover, the first ROA spectra of DNA and RNA were recorded in 1998 by Bell and co-workes[19], and since that time, no further work has been undertaken on this topic.

ROA spectroscopy has been already proved to be a very sensitive tool for structure exploration of biomolecules that provides complementary information to other analytical techniques. A major advantage of ROA is effectiveness to examine chirality of biomolecules in solution and to determine their absolute configuration[20–23]. Chiroptical methods follow up even slight conformational and stereochemical changes in secondary and tertiary structures of studied macrobiomolecules. ROA spectra of nucleic acids provide

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Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

information on sugar ring conformation, base-stacking arrangement of base rings and mutual orientation of the sugar and base rings[19,21,23–25]. Based on that, the authors are convinced that ROA spectroscopy is able to expand knowledge about the interactions between DNA molecules and antitumor drugs.

We investigated herring sperm DNA type XIV (Sigma D6898). This popular spectroscopic standard of DNA characterises a similar average base content like calf thymus DNA (G-C 42%, A-T 58%) but exhibits higher solubility in water and physiological buffers than the latter. From this reason, we collected ROA spectrum of DNA without ultrasonification of solution. Omitting this step in the sample preparation is crucial for preserving the native structure of nucleic acid, because ultrasonification leads to degradation of DNA by breaking hydrogen bonds and ruptures of single- and double-strands of the helix[26]. Thus, we recorded Raman and ROA spectra of relatively long DNA molecules (av. ~700 bp vs <50 bp[19]) in their native, double-stranded B-DNA form. Moreover, we investigated DNA-platination at two concentrations of cisplatin, i.e. 0.01 and 0.02 molar ratio of cis-Pt/nucleotide, used commonly as therapeutic doses. We found that ROA spectroscopy is a very sensitive technique to study metal-DNA binding process in a very low concentration of the intercalator, in the nearly-physiological conditions. For the first time, we show a detailed comparative analysis of ROA spectra of DNA before and after the cis-DDP treatment and we propose ROA spectroscopic markers of the drug-DNA interactions.

Experimental

DNA samples were prepared by dissolving 50 mg of lyophilised herring sperm DNA (Sigma-Aldrich) in 1.0 mL MOPS (3-(N-morpholino)propane sulfonic acid) buffer (pH = 7.2, c = 50 mM). The ionic strength of the DNA solution was approx. 0.1. Prior to the spectral analysis, samples were filtered by a syringe filter with a 0.45 µm Nylon membrane. The concentration of DNA was determined spectrophotometrically by measuring an absorbance value at 260 nm, according to the procedure of Gallagher et al.[27].

Cisplatin (cis-diammineplatinum (II) dichloride) was added to DNA solution from an original solution (1.0 mg mL^{-1}) manufactured by Accord Healthcare Limited, UK. Cisplatin-DNA solutions were prepared in two molar ratios of Pt/nucleotide, 0.01 and 0.02. All samples were prepared in ultrapure water (18.2 MΩ, Milli-Q system).

Raman and ROA spectra were collected simultaneously on a ChiralRAMAN-2X spectrometer (BioTools Inc.) with an excitation wavelength at 532 nm and output laser power of 600 mW. All spectra were recorded in the range of 2000-50 cm^{-1} with a spectral resolution of 7 cm^{-1}. Data collection time was 24 hours for all spectra. No melting of any sample was observed. Three spectra of triplicates were collected.

Then, spectra were processed using a OriginPro 9.1 software (OriginLab Corp.). After background subtraction, spectra were smoothed using a second-order Savitzky-Golay filter with a 15-point window. The corrected spectra were next normalised to a band at 1097 cm^{-1} (the symmetric stretching phosphate mode), which is often used as an intensity standard[17,28–30].

Results and discussion

Raman and ROA spectra in the 1800 – 600 cm^{-1} region of DNA and its complex with cisplatin at the molar ratio of cis-Pt/nucleotide = 0.01 are presented in Figure 1. Spectra in the entire spectral range from 2000 to 50 cm^{-1} are presented in

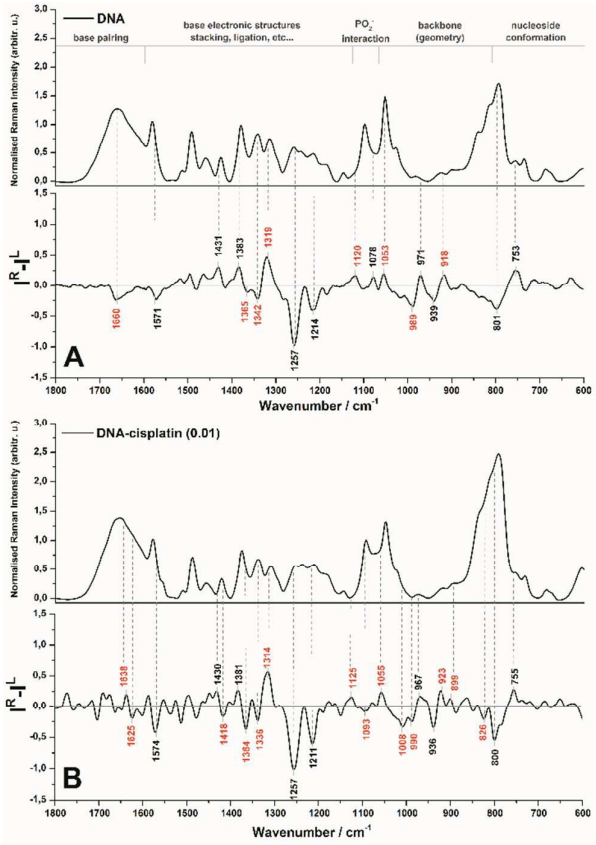

Fig.1. Backscattered SCP Raman and Raman optical activity (ROA) spectra of DNA (A) and DNA complex with cisplatin at 0.01 molar ratio of Pt/nucleotide (B).

Figure S1 (ESI), however the low-wavenumber region is overlapped by bands of the fused silica of quartz cuvettes used for spectra collection.

Spectral regions sensitive to various conformational features of DNA are labelled in Fig. 1 according to Duguid et al.[31] while the most dominant spectral changes are labelled in red. A detailed assignment of the observed Raman and ROA bands of herring sperm DNA and its Pt complex is given in Electronic Supplementary Information (Table S1).

An analysis of Raman spectra of DNA and DNA treated with cis-DDP leads to the conclusion that cisplatin binds to DNA and guanine is the most preferred site of platination. Upon Pt-

binding the predominant spectral changes are observed specifically for the stretching modes of the guanine ring (1579, 1490, 1341 and 1311 cm^{-1})[31,32]. Moreover, bands originating from hydrogen bonding between G-C[17,31–35] may be also correlated with cisplatin biadducts appealing. The lack of alterations in Raman features assigned to dA (1513 cm^{-1})[32,34–36], dT and dC (1185 cm^{-1})[37] confirms no or weak interactions between Pt and adenine and pyrimidines. This observation is rather expected since we used a relatively low cis-Pt/nucleotide ratio (0,01), and consequently N7-guanine monoadducts are created at the first step of the cross-linking process. Raman spectra of DNA and its Pt-complex also show the formation of crosslinks by cis-DDP causing marked conformational transformation of DNA. A decrease in intensity of bands characteristic for C2'-endo conformation of sugar moieties (843 and 684 cm^{-1}) and an increase of features assigned to C3'-endo (815 and 605 cm^{-1})[17,19,31,35,29], endorse the conclusion of the B- to A-DNA transition, reported previously by Vrána et al.[17]. However, the suggested Z-form[37,38] is not noticed in our work.

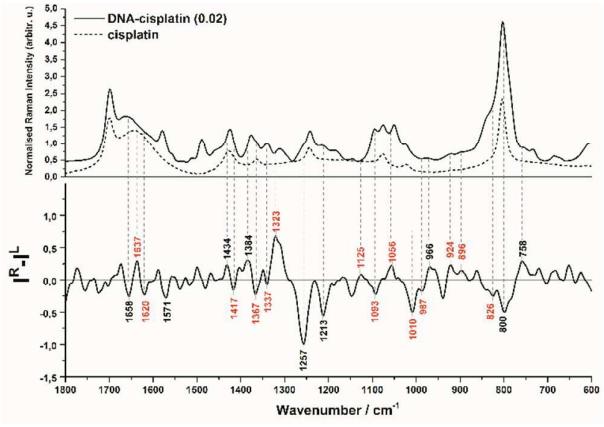

Fig. 2. Backscattered SCP Raman and Raman optical activity (ROA) spectra of the DNA complex with cisplatin at 0.02 molar ratio of Pt/nucleotide (solid). Raman spectrum of the cisplatin solution is given as the reference (dash).

It is worth stressing here that ROA spectrum of herring sperm DNA (c.f. Fig. 1A bottom) shows some differences in comparison to ROA spectrum of calf thymus DNA – the only one spectrum reported so far by Bell and co-workers[19]. Likely, the observed differences result from several issues associated with sample preparation and collection of ROA spectra such as compensation of Raman bands, background correction, differences in hydration and aggregation of both types of DNA. First of all, we do not observe in the ROA spectrum recorded here a strong positive band at ca. 1480 cm^{-1} (see Fig. 1A bottom), which has been found in ROA spectra of ultrasonicated calf thymus DNA, tRNA[19] and other RNA types[30]. The Raman counterpart is assigned to guanine and it is found to be sensitive to sugar-base torsion angles and base-base stacking interactions[31,32,34–36]. In addition this band exhibits a lower intensity in ROA spectrum in Mg^{2+}-free $tRNA^{Phe}$, which adopts an open clover leaf form upon binding of magnesium ions. A stabilization role of Mg^{2+} for quaternary structure of tRNA is a commonly known fact[19]. Hobro et al.[30] have also observed a weak signal at ca. 1480 cm^{-1} in ROA spectrum of RNA 37-nucleotide based on EMC IRES domain I (internal ribosomal entry site (IRES) from encephalomyocarditis virus), which exhibits a complex cloverleaf type structure. In turn, Blanch et al.[39] has associated the absence of this band in ROA spectrum of viral RNA with a single-strand helix present in that nucleic acids, proposing an origin of the signal from vibrations of bases within the base-paired regions of $tRNA^{Phe}$. Therefore, the fact, that this band is not observed in our work can be primarily explained by the presence of a long chain of herring sperm DNA as well as by preservation of the native, double-stranded form of the helix.

An evidence of a partial transition from the B-DNA to A-form upon the interaction with cis-DDP is clearly visible from the ROA profile. A spectral marker of a A-type double helical form is a negative-positive-negative triplet at 1093, 1055, 1008 cm^{-1} (Fig. 1B, bottom). Negative components of that triplet are more intense in the spectrum of DNA treated with a higher concentration of cisplatin (Fig. 2, bottom). A similar signature has been found in ROA spectra of A-type polynucleotides[24,25], and Mg^{2+}-free tRNA (1089, 1047, 992 cm^{-1}), which adopt mainly A-form by losing C2'-endo conformation of sugar moieties[19,30,40,41]. Next, ROA spectrum of untreated DNA shows the presence of a broad negative peak with maximum at 989 cm^{-1} (Fig. 1A) which disappears after addition of cisplatin and a doublet at 1008 and 990 cm^{-1} occurs (Fig. 1B). Intensity of the 1008 cm^{-1} band increases along with an increase of the drug dose (Fig.2). A similar effect is noticed for a negative band at 1093 cm^{-1}. We propose that these features are ROA markers for the transformation of C2'-endo conformation into C3'-endo. In turn, a positive band at ca. 918 cm^{-1} is consider to be an indicator of C2'-endo pucker in a molecule of nucleic acids[19,24,25,40,41]. This band is assigned to the stretching vibrations of the deoxyribose ring in Raman spectrum of herring sperm DNA[32] and is still observed after platination, see Figs. 1B and 2. Its presence indicates the incomplete transition of DNA from B- to A-form. Upon the interaction with cisplatin, a broadening and shift to the higher wavenumbers of this band is found and followed by occurring of a weak band at 899 cm^{-1}. In addition, the spectral profile in the region of 1550-1200 cm^{-1} represents changes in sugar pucker conformation from C2'endo – C3'endo. This is manifested by decreasing intensity of a positive band at 1319 cm^{-1} and appearing of a negative band at 1418 cm^{-1} [24].

Next, an increase in intensity of a ROA negative doublet at 1364 and 1336 cm^{-1} in cisplatin-bound DNA and changes in their intensity ratio confirm spatial rearrangement of mutual orientation of the sugar and base rings around the glyosidic link[24,41]. Raman counterparts in DNA spectrum are mainly assigned to vibrations of guanine and adenine[32,34,35]. This observation supports the formation of 1,2-GG and 1,2-AG cisplatin adducts. At the current stage of our studies, we are not able to identify the type of crosslink. However, only a little decrease in intensity of a band at 1120 cm^{-1}, which is specific

for adenine[32,34,35], suggests that guanine residues are rather involved in the crosslink formation.

Another spectral feature of the drug action on chiral structure of DNA is a weak negative-positive doublet at ca. 1638 and 1625 cm^{-1}, characteristic for both doses of cisplatin, see Figs. 1B and 2. This doublet has been proposed as a ROA marker of right-handed helix of RNA[30]. In turn, the ~1750–1550 cm^{-1} region exhibits the presence of ROA bands attribute to bases involved in stacking arrangements[41]. For example, a weak positive band at 1495 cm^{-1} per analogue to Raman band is assigned to guanine, while a band at 1462 cm^{-1} to adenine and thymine (see Table S1). Changes observed in this region identify distortions of base stacking interactions and disruptions of hydrogen bonding due to cross-linking of cisplatin to a DNA molecule, c.f. Fig. 1.

We summarise the most prominent markers specific for DNA-cisplatin interaction in Table 1.

Table 1. Changes in the position of ROA bands observed for the interaction of cisplatin with sperm herring DNA.

DNA	DNA-cisplatin (r = 0.01)	DNA-cisplatin (r = 0.02)	Assignment
	wavenumber/cm^{-1}		
	+1638 w	+1637 m	B-DNA -> A-form
	-1625 w	-1620 m	
	-1418 w	-1417 w	C2'endo –> C3'endo
-1365 w	+1364 m	+1367 m	dT, dA, dG
-1342 m	-1336 m/w	-1337 w/vw	dG, dA
+1120 w	+1125 w	+1125 vw	dA
	-1093 vw	-1093 w	
+1053 w	+1055 w	+1056 w	
	-1008 m	-1010 s	C2'endo –> C3'endo
-989 m	-990 w	-987 w	
	+899 w	+896 w	
+918 w	+923 w	+924 w	

r – molar ratio of Pt/nucleotide. ROA band: - negative, + positive. Band intensity: vs – very strong, s – strong, m – medium, w – weak, vw – very weak. Components: dA – deoxyadenosine, dC – deoxycytidine, dG – deoxyguanosine, dT – thymidine, d - deoxyribose

Conclusions

We demonstrate that ROA spectroscopy is a powerful technique to monitor DNA conformational changes occurring upon drug binding. Our results clearly show sensitivity of this technique in following structural alteration of the whole DNA molecule upon cisplatin action. A direct observation of DNA transformation is identified by structural rearrangement within chiral sugar moieties. A wide application of ROA spectroscopy for such challenging task as the determination of the drug action on chiral molecules is to some extend limited by requirements of the sample preparation and problems with spectra collection. This technique requires high concentration and high purity of a sample. In addition commercially available instrumentation employs only an excitation wavelength at 532 nm that can induce high fluorescence background obscuring week ROA signal. Despite this, our work shows the description of chiral nature of a given biomolecule like nucleic acid or a receptor provides a complementary insight into to the drug action.

Acknowledgements

This study was supported by European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme [grant coordinated by JCET-UJ, No POIG.01.01.02-00-069/09] and by National Centre of Science [DEC-2013/08/A/ST4/00308].

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