Inner and outer complexes of Pt-coordination compounds with DNA probed by SERS spectroscopy

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Surface enhanced Raman scattering (SERS) spectroscopy has been used to study the interfacial behaviour of DNA modified by *cis*-$Pt(NH_3)_2Cl_2$, (*cis*-DDP) and [Pt-(dien)Cl]Cl bidentate and monodentate platinum coordination compounds, respectively. Two stereochemical configurations of Pt–DNA complexes can be deduced from the adsorption behaviour of the Pt adducts. The antitumoral inactive [Pt-(dien)Cl] Cl forms an outer complex whereas the antitumoral active *cis*-DDP favours an inner complex.

DNA–Pt complex *Stereochemical configuration* *SERS spectroscopy* *Interfacial behavior*

1. INTRODUCTION

Since the discovery of the antitumoral properties of some platinum coordination compounds, much work has been carried out to study the molecular basis of their action [1]. It has been established that the primary cellular target for the interactions is DNA. Therefore, most of the experimental work has concerned the binding modes of the antitumoral Pt compounds, typified by *cis*-$Pt(NH_3)_2Cl_2$ (*cis*-DDP). However, the antitumoral activities of Pt compounds are very sensitive to changes in the stereochemistry of the Pt complexes. Parallel studies with antitumor-inactive Pt compounds are thus important to characterize modes of binding relevant to the anticancer activity of Pt complexes. Several physicochemical techniques [1,2] and biochemical methods [3–5] have been applied to clarify the structure-activity relation of the Pt compounds. The results suggest that specific strand cross-links of the DNA through covalent bonds of Pt compounds to the nucleic bases could be responsible for the antitumoral activity. Particularly, it has been demonstrated that *cis*-DDP reacts via intrastrand cross-linkings between neighbouring guanine bases [6,7]. Recently [8–10] we reported the application of a new spectroelectrochemical method in the conformational study of nucleic acids. Surface enhanced Raman scattering (SERS) spectroscopy is based on the enhanced Raman scattering signals from absorbed products, in our case DNA, at the surface of a silver electrode. The high sensitivity of the surface Raman signals, enhanced by a factor up to 10^6, and their specificity connected to the interfacial origin allow us to study the interfacial behaviour of the DNA. Here are reported the SERS results obtained with DNA modified by *cis*-$Pt(NH_3)_2Cl_2$ and [Pt-(dien)Cl]Cl, being, respectively, bidentate antitumoral active and monodentate antitumoral inactive platinum compounds. The presence or absence of specific SERS Raman bands of platinum compounds yields information concerning the stereochemistry of platinum binding to DNA.

2. MATERIALS AND METHODS

SERS spectra were obtained using the 514.5 nm excitation line from a Spectra Physics model

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164-09 argon ion laser. Laser power at the sample was approximately 200 mW. A general description of the spectroelectrochemical system was given in [10]. A silver disk working electrode is used for the surface experiments. The electrochemical etching of the Ag surface, a prerequisite for the enhancement of the Raman scattering [11], consisted of 3 successive rapid (50 $mV \cdot s^{-1}$) cyclovoltammetric scans from starting potential E_s −0.2 V to +0.2 V *vs* an Ag/AgCl reference electrode. High M_r calf thymus DNA was purchased from Boehringer (Mannheim, FRG) and purified by phenol extraction. *cis*-Dichlorodiammineplatinum(II), i.e., *cis*-$Pt(NH_3)Cl_2$ or *cis*-DDP, and chlorodiethylenetriaminoplatinum(II) chloride, [Pt(dien)Cl]Cl (fig.1) were a gift from J.-P. Macquet. Quantitative fixation of platinum compounds on DNA and platinum analysis were given in [3]. The ratio of bound platinum atoms per nucleotide is indicated by r_b.

3. RESULTS

The Raman spectroscopy of Pt compounds in aqueous solution shows that the Pt–N stretching vibration occurs in the 500–600 cm^{-1} region. It must be noted that in a nucleic base–platinum complex, the 4 Pt–N vibrations are indistinguishable [12]. In the case of DNA–Pt complexes, these specific Raman bands can thus be used to monitor the extent of the complexation. Fig.2 compares the SERS spectra of native DNA (middle, B) and DNA complexed with [Pt-(dien)-Cl]Cl (top, A), *cis*-$Pt(NH_3)_2Cl_2$ (bottom, C) in the restricted region from 430 to 640 cm^{-1} characteristic of the Pt–N vibration. The SERS spectra of DNA–[Pt(dien)Cl]Cl complexes clearly show a direct dependence of the Raman intensity of Pt–N vibrations on r_b. But in the same spectral region, the SERS spectra of DNA–*cis*-$Pt(NH_3)_2Cl_2$ show no similar Raman band ($r_b \leqslant 0.05$). DNA–*cis*-$Pt(NH_3)_2Cl_2$, however, is adsorbed at the electrode surface as confirmed in fig.3 by the presence of other Raman signals from the adsorbed DNA [8,9]. The appearance in the SERS spectra of a strong band at about 245 cm^{-1} attributed to silver/adsorbed phosphate group vibrations is a clear indication of the adsorption [9,10].

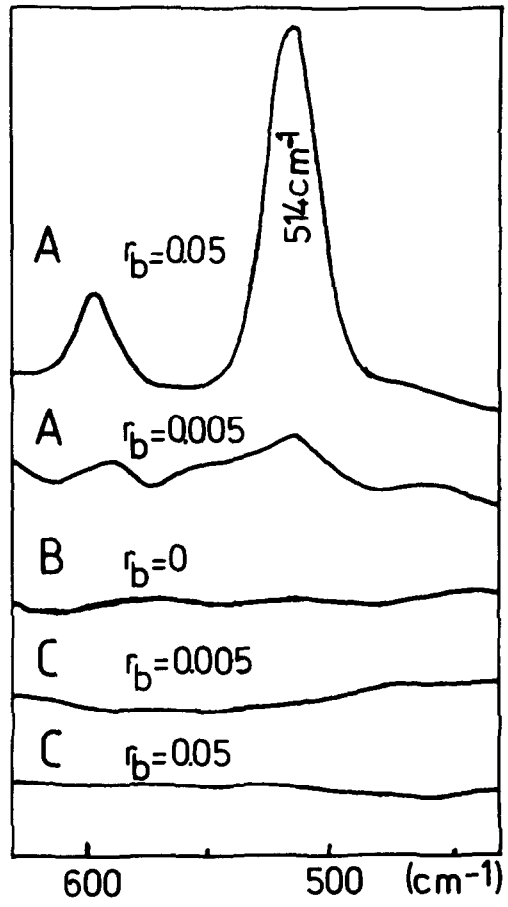

Fig.2. SERS spectra in the range of Pt–N vibration frequencies of native CT DNA (B), modified CT DNA by [Pt-(dien)Cl]Cl (A) and *cis*-$Pt(NH_3)_2Cl_2$ (C). DNA concentration 200 $\mu g \cdot ml^{-1}$, 0.15 M KCl, 10^{-3} M cacodylate (pH 6.8). Inserted r_b values represent the number of platinum atoms bound per nucleotide.

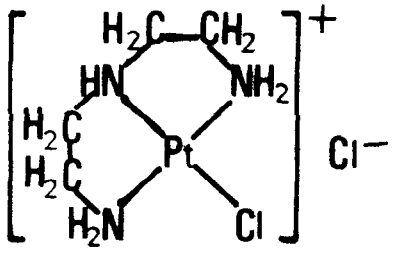

Fig.1. Structural formula of [Pt-(dien)Cl]Cl.

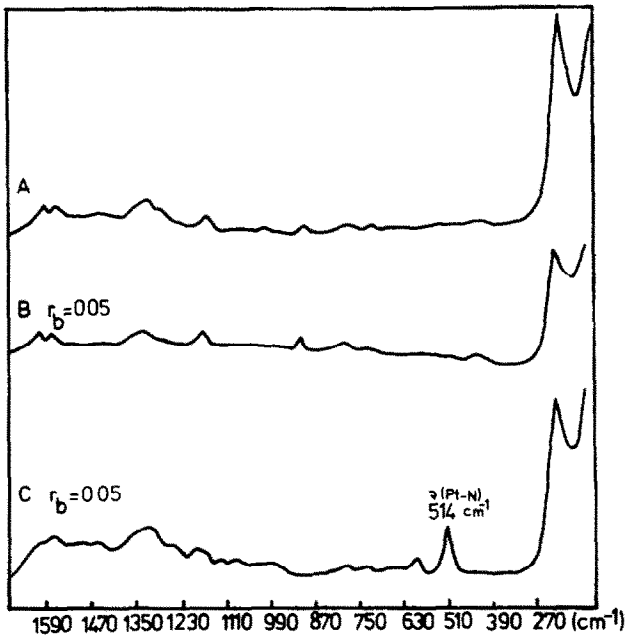

Fig.3. SERS spectra of native CT DNA (A) and modified CT DNA by *cis*-$Pt(NH_3)_2Cl_2$ (B) and [Pt-(dien)Cl]Cl (C). Other experimental conditions as in fig.2.

4. DISCUSSION

Before interpretation of the apparent discrepancy in fig.2 of the signal intensities of Pt–N vibrations on r_b in both modified DNAs, it must be kept in mind that SERS spectroscopy measures specific interfacial signals. Indeed, the high sensitivity of this technique is limited to the nucleic acid components in contact with the electrode surface [9]. Thus, in the case of adsorbed nucleic acids with a double-stranded helical structure, the outside lying phosphate–ribose backbone gives major Raman bands in the SERS spectrum. It follows that the interfacial behaviour of DNA–Pt complexes is exclusively probed through the silver electrode detector. Considering now the SERS results, it is observed that the accessibility of Pt adducts to the electrode surface depends on the nature of the Pt-coordination compounds. In other words, it can be assumed that the stereochemical configurations of the two Pt–DNA complexes are different. Complex formation with the monodentate [Pt(dien)Cl]-Cl would be located at the surface of the helical structure. A monovalent binding, oriented towards the aqueous solution, favours SERS detection. However, a complex formation with the bidentate *cis*-$Pt(NH_3)_2Cl_2$ does not produce any detectable Raman bands of the Pt–N vibrations. The absence of SERS signals suggests a localization inside the helical structure, out of range of the SERS enhancement factors. The bivalent binding of *cis*-$Pt(NH_3)_2Cl_2$ for $r_b \leqslant 0.05$ would hinder a direct accessibility of the Pt adducts to the interfacial interactions. Here it must be noted that for r_b = 0.2, weak SERS signals of the platination can be detected. For high r_b values, it is not excluded that a destabilization of the DNA favours the accessibility of the Pt adducts [2]. For low r_b values, the two differing modes of binding of Pt compounds also support other physicochemical results. Indeed, the orientation of the DNA–[Pt(dien)Cl]-Cl adducts towards the surrounding solution can be explained by a monovalent mode of binding of Pt compounds to the readily accessible N_7 of guanine bases [2]. The outer position of the guanine adducts also stabilises the Z-conformation in poly(dG-dC)·poly(dG-dC) modified by [Pt(dien)Cl]Cl [13]. Although the N_7 of guanine residues has also been demonstrated as a predominant binding site with *cis*-DDP [2], the bifunctional nature and *cis* configuration of the Pt drug imply steric constraints in the complexation. Thus, the detected intrastrand cross-linkings between neighbouring guanines are accompanied by large conformational changes of DNA [2]. Local distortions of the DNA–Pt structures, such as kinks or 'bulge out' conformations, have been proposed to play a role in the replication, enzymatic repair of the lesions and mutation induction properties of *cis*-DDP [14–16]. The inner position for the bulky DNA–*cis*-DDP adducts in the helical structure demonstrated by the present results can be a causal factor of these conformational changes. Furthermore, considering the higher mutagenic properties of *cis*-DDP in comparison with [Pt-(dien)Cl]Cl [17], speculation about the action mechanism for Pt drugs can be presented. Consideration of the accessibility of guanine-Pt adducts as a signal for repair mechanisms is also possible. A defective DNA repair, caused by a failure to recognize DNA–*cis*-DDP complexes embedded in the DNA structure, could be linked to biological activities of *cis*-DDP. Whereas in the case of [Pt-(dien)Cl]Cl, the direct accessibility to

the guanine adducts would facilitate their recognition and elimination. Although this hypothesis requires further studies with other Pt drugs, the present SERS study already shows the importance of the stereochemical aspect of Pt–DNA complexes in the interfacial behaviour of Pt-modified DNA.

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