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FT–IR Spectroscopic Evidence of Sugar Ring Conformational Changes in GpC and CpG on Platination and Intercalation

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Abstract

An FT–IR spectroscopic study concerning changes in the conformation of sugar in the dinucleotides; GpC and CpG, on platination and intercalation is presented. The results are compared with the FT–IR spectral data of 5′-CMP, 5′-GMP, 3′-GMP and their metal adducts. The spectra of free GpC, free CpG, proflavine-GpC, proflavine-CpG, and *cis*-$[Pt(NH_3)_2(GpC)_2]^{2+}$ exhibit the diagnostic band at 800 cm^{-1} which was assigned to a sugar phosphate vibrational mode and diagnostic of C3′-*endo* sugar pucker. In the case of 9-aminoacridine-GpC and *cis*-$[Pt(NH_3)_2(CpG]^+$ the diagnostic bands of the C2′-*endo* and C3′-*endo* conformations are observed at 810–820 cm^{-1} and near 800 cm^{-1} respectively. The results are in good agreement with X-ray data. The infrared diagnostic bands are important for distinguishing the sugar pucker conformational changes.

Introduction

Recently the sugar conformational analysis of *cis*-$[Pt(NH_3)_2$(oligonucleotide)] adducts has been reported by high field nuclear magnetic resonance spectroscopy [1]. In this respect, further information has been provided by the X-ray analysis of some of the intercalator-dinucleotide adducts [2b], as well as the Raman studies of a number of nucleosides [3], mononucleotides [3, 4], dinucleotides [4, 5], polynucleotides [5, 6], RNA [5] and DNA [5]. In a recent study by FT–IR spectroscopy the two most common sugar conformations, C2′-*endo* and C3′-*endo* have also been discussed [7].

In this paper, the FT–IR spectra of the mononucleotides 5′-CMP, 5′-GMP, 3′-GMP, and their cadmium and cis-platin adducts, have been studied together with the dinucleotides; GpC and CpG and their adducts with cis-platin and intercalating agents. The results for the corresponding systems have been compared with ^{1}H NMR and X-ray crystallographic data.

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Experimental

Preparation of the Crystalline Complexes

The Cd(5′-CMP)$(H_2O)_2$ [8], proflavine-CpG [2], *cis*-$[Pt(NH_3)_2(GpC)_2]^{2+}$ and the chelated *cis*-$[Pt(NH_3)_2(CpG)]^+$ complexes were synthesized according to literature [1g, 2, 8, 9]. The crystals of proflavine-GpC and 9-aminoacridine-GpC adducts were prepared from an aqueous solution of proflavine hemisulphate, 9-aminoacridine hydrochloride and the ammonium salt of GpC.

FT–IR Spectroscopic Measurements

The FT–IR spectra were recorded in the 1000–600 cm^{-1} region with DIGILAB FTS-15C/D Fourier Transform Infrared Interferometer equipped with deuterated triglycine sulfate detector (DTGS) (Infrared Associates, New Brunswick, N.J.), a KBr beam splitter and the Globar source. The spectra were obtained as KBr pellets and the resolution was 4 to 2 cm^{-1}.

Results and Discussion

FT–IR Spectra of 5′-CMPNa$_2$ and its Cadmium Complex

The FT–IR spectra of 5′-CMPNa$_2$ and its Cd complex are shown in Fig. 1. The spectrum of 5′-CMPNa$_2$ exhibits the marker band at 820 cm^{-1}. This band which was also observed in the infrared spectra of 5′-GMPNa$_2$ (at 821 cm^{-1}) [7d] and at 826 cm^{-1} for 5′-IMPNa$_2$ [7d], is assigned to the C2′-*endo anti gauche–gauche* (*gg*) sugar ring pucker of a sugar–phosphate vibrational mode. From Raman studies it is known that a band in the 805–816 cm^{-1} region is associated with the existence of an A-form of DNA or C3′-*endo* sugar ring pucker and the band

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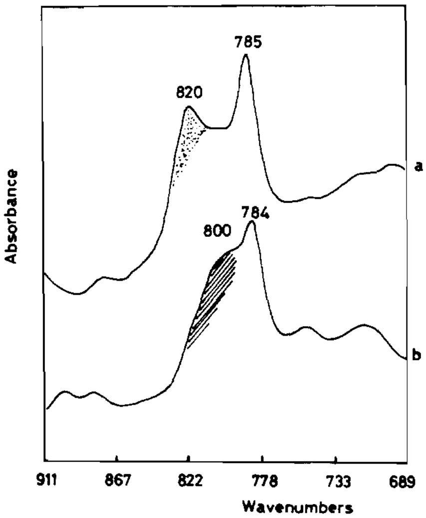

Fig. 1. FT–IR spectra of (a) 5′-CMPNa_2 and (b) [Cd(5′-CMP)(H_2O)]H_2O.

at 835 cm^{-1} is characteristic of the B-form or the C2′-*endo* conformation [4–6, 10]. In contrast, the FT–IR spectrum of the complex Cd(5′-CMP)$(H_2O)_2$ shows the band at 800 cm^{-1} with the absence of the 820 cm^{-1} band. The X-ray analysis of this complex on the other hand has shown that the sugar moiety has the C3′-*endo anti* conformation and the *gg* rotation about the C4′–C5′ bond, while the Cd atom is bound to N3 of the base and to one of the oxygens of the phosphate group [11]. Furthermore, the 800 cm^{-1} band of Cd(5′-CMP)$(H_2O)_2$ has also been observed in the infrared spectra of Cd(5′-GMP)·$8H_2O$ [7d, 12] and 5′-GMP free acid [7d, 13]. Therefore, it is believed to be diagnostic of the C3′-*endo anti gg* sugar pucker; in agreement with X-ray data [12, 13].

FT–IR Spectra of GpC and its Intercalator Complexes with Proflavine and 9-Aminoacridine

X-ray analyses of the dinucleotides NaGpC [14], CaGpC [15] have shown that the bases are *anti*, the sugars are C3′-*endo* and the C4′–C5′ bond rotation is *gg*. In the present work, the FT–IR spectra of NH_4GpC, proflavine-GpC, and 9-amino-acridine-GpC are shown in Fig. 2. The spectra of NH_4GpC and proflavine-GpC show only the C3′-*endo* marker band at 806 and 802 cm^{-1}, respectively. On the other hand, as is shown in Fig. 3 the structure of NH_4GpC consists of the 3′-GMP(Gp–) and 5′-CMP(–pC) fragments. The FT–IR spectra of the corresponding fragments have been studied previously [7] in the compounds, *cis*-$[Pt(NH_3)_2(3'\text{-GMP})_2]^{2+}$ and Cd(5′-CMP)$(H_2O)_2$, respectively. The conformation of the sugar moiety in *cis*-$[Pt(NH_3)_2(3'\text{-GMP})_2]^{2+}$ and Cd(5′-CMP)$(H_2O)_2$ is C3′-*endo anti gg* and therefore the corresponding infrared bands were observed at 793 and 800 cm^{-1}, respectively [7]. By the same analogy, the infrared modes at 806 cm^{-1} for NH_4GpC and 802 cm^{-1} for the proflavine-GpC complex are assigned to the sugar–phosphate vibrational mode of the furanose ring and are characteristic of the C3′-*endo anti gg* conformation.

The 9-aminoacridine–GpC complex on the other hand shows the two bands at 813 and 800 cm^{-1}. The band at 813 cm^{-1} which was also observed in the spectrum of 5′-CMPNa_2 is assigned to the vibrational mode of the C2′-*endo* conformation, whereas the latter is attributed to the C3′-*endo* conformation.

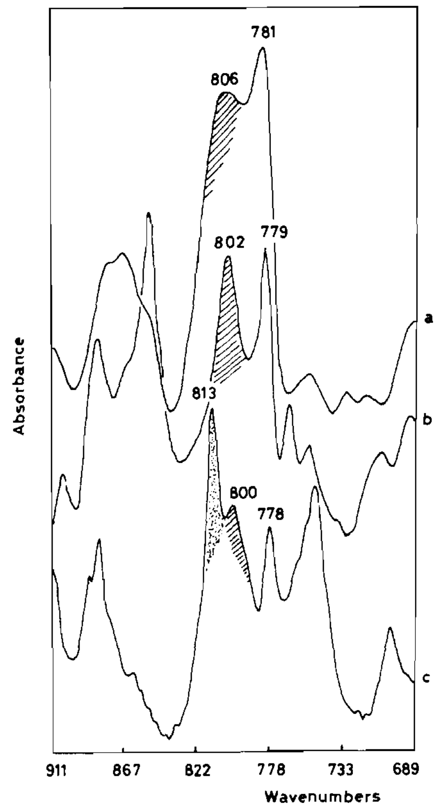

Fig. 2. FT–IR spectra of (a) NH_4GpC, (b) proflavine-GpC, and (c) 9-aminoacridine-GpC.

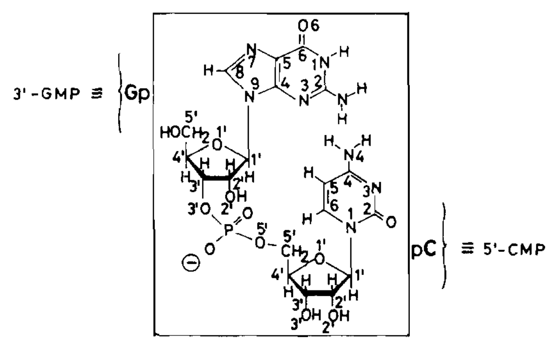

Fig. 3. Structural relationships and site numbering in GpC^-, 3′-GMP-(Gp–), and 5′-CMP-(pC).

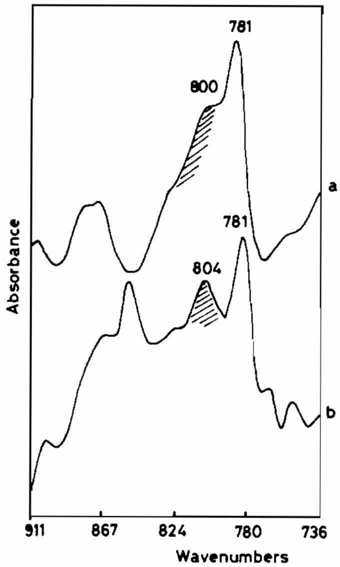

Fig. 4. FT–IR spectra of (a) NH_4CpG and (b) proflavine-CpG.

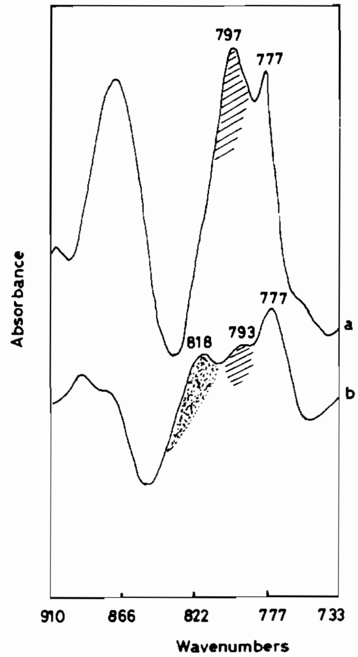

Fig. 5. FT–IR spectra of (a) *cis*-$[Pt(NH_3)_2(GpC)_2]^{2+}$ and (b) *cis*-$[Pt(NH_3)_2(CpG)]^+$.

FT–IR Spectra of CpG and its Proflavine-CpG Adduct

The FT–IR spectra of NH_4CpG and its proflavine-CpG adduct are shown in Fig. 4. The marker bands at 800 and 804 cm^{-1}, are diagnostic of C3'-*endo* conformation for the sugar moiety. A shoulder band with medium intensity near 824 cm^{-1} for NH_4CpG also suggests the presence of the C2'-*endo* conformation. The dinucleotide NH_4CpG consists of the 3'-CMP(Cp–) and 5'-GMP(–pG) moieties. So far, unlike 5'-GMP [7], there has not been any vibrational study on the sugar conformation of 3'-CMP moiety. Our results therefore indicate that the presence of C2'-*endo* conformation for NH_4CpG is associated with the 3'-CMP(Cp–) fragment. The X-ray analysis of proflavine-CpG adduct on the other hand indicates that the two sugars are C3'-*endo*, and the orientation around the C4'–C5' bond is *gauche–gauche* [2]. This suggests that our assignment concerning the sugar pucker is in good agreement with the X-ray data.

FT–IR Spectra of the Adducts cis-[Pt(NH$_3$)$_2$-(GpC)$_2$]$^{2+}$ and cis-[Pt(NH$_3$)$_2$(CpG)]$^+$

It is known that at high CpG concentrations (10^{-2} M) the platinum atom coordinates to N7 of guanine in two CpG molecules [1g]. However, at low concentrations (10^{-4} M) CpG reacts with *cis*-$[PtCl_2(NH_3)_2]$ to give the adduct *cis*-$[Pt(NH_3)_2$-$(CpG)]^+$, where the platinum is bound to the N3-cytidine and N7-guanine coordination sites. The FT–IR spectrum of *cis*-$[Pt(NH_3)_2(GpC)_2]^{2+}$ is shown in Fig. 5. The marker band at 797 cm^{-1} is diagnostic of the C3'-*endo* sugar puckering. In the chelate *cis*-$[Pt(NH_3)_2(CpG)]^+$ the marker band for the guanine C2'-*endo* sugar puckering is quite intense and is observed at about 818 cm^{-1} (see Fig. 5b) while the band at 793 cm^{-1} is assigned to the cytidine C3'-*endo anti* conformation. This shows a change of the guanine sugar pucker on complexation from C2'-*endo* to C3'-*endo* in the case of *cis*-$[Pt(NH_3)_2$-$(CpG)_2]^{2+}$ (see ref. 1g). As for the chelate; *cis*-$[Pt(NH_3)_2(CpG)]^+$ the guanine furanose remains in the C2'-*endo* conformation whereas the cytidine sugar pucker changes from C2'-*endo* to C3'-*endo* as suggested by the FT–IR spectra.

The line observed in the range; (775–785) cm^{-1} is tentatively assigned to a base vibration [19, 20]. Peticolas and collaborators, on the other hand believe that strong contributions from symmetric O–P–O stretching could be equally responsible for the appearance of a band in the corresponding region [21].

The sugar conformational study of *cis*-$[Pt(NH_3)_2$-$(CpG)]^+$ in aqueous solution has been reported by proton NMR spectroscopy [1g]. The absence of the coupling constant of the cytidine ribose $J_{1'2'}$ value in the spectra shows that the cytidine ribose adopts a 100% C3'-*endo* conformation, whereas the guanine ribose has a predominantly C2'-*endo* conformation ($J_{1'2'}$ = 6.0–7.7 Hz). This conformational change was also observed for platinum chelation (GN7–GN7) in the GpG sequence [7, 16b]. It is interesting to note that both intercalation and *cis*-$Pt(NH_3)_2{}^{2+}$ binding to N7 of guanine change the sugar conformation of the dinucleotide to fit the complex.

As a conclusion, it seems that the binding of the anticancer drugs (intercalating or chemically bound) with d(GpG), d(GpC), or d(CpG) sequences in DNA may destroy the backbone sugar conformation of DNA by changing the sugar pucker to accommodate the strain caused by the presence of the drug. The above examples, show that the conformational changes of the sugar pucker from C2'-*endo* to C3'-*endo* or *vice versa* are taking place in order to accommodate and stabilize the drug-dinucleotide adduct. Recently a similar sugar flexibility has also been predicted by molecular mechanics calculations [17] and from the crystal structure of the adduct of the antitumor drug cis-platin with d(GpG) [18]. Further studies of small duplex DNA fragments modified by anticancer drugs will help in confirming or infirming this hypothesis.

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