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T15

Formation of Chelates between *cis*-Diamminedichloroplatinum(II) and either GG or GCG Units in Oligonucleotides and DNA

J. REEDIJK, A. M. J. FICHTINGER-SCHEPMAN and J. H. J. DEN HARTOG

Department of Chemistry, Gorlaeus Laboratories, State University Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands

After the discovery of the cytostatic properties [1] of *cis*-diamminedichloroplatinum(II) and its subsequent successful use as an anti-tumor drug [2], a large number of investigations on structure–activity relations and on the mechanism of action have evolved [3]. The main target of the drug is now generally accepted to be the chromosomal DNA in the cell, and a specific binding to this DNA is supposed to be a crucial step in cell death [4].

Early investigations have shown that the kinetically most favoured binding site is the N7 atom of the guanine bases, although other sites (such as N7 of adenine, N1 of adenine and of guanine after deprotonation, N3 of cytidine) also bind to metal ions and in particular to platinum [5, 6].

Since *cis*-$PtCl_2(NH_3)_2$ possesses two binding sites after complete hydrolysis [7], there has to be a second binding in addition to guanine N7. The following possibilities have been put forward in the literature:

(a) The formation of a specific chelate with one guanine base [8] through N7 and O6.

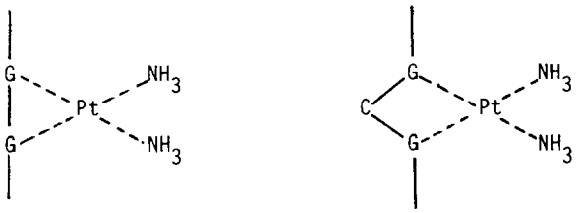
(b) The formation of an interstrand crosslink between two bases in opposite strands of DNA [9].

(c) The formation of an intrastrand crosslink between two bases in the same strand of DNA [10].

(d) The formation of a crosslink between DNA and a protein residue [11].

As has been summarized elsewhere [12], most results point towards intrastrand crosslinks as the most likely target responsible for the antineoplastic activity, although a key rôle for the other possibilities cannot be completely ruled out. Because several single-stranded oligonucleotides are commercially available or can be synthesized rather easily, we are working on the synthesis and characterization of adducts formed between *cis*-$Pt(NH_3)_2^{2+}$ and oligonucleotides. The results of this work are compared with those obtained from mutation studies in bacterial cells (specific hotspots for *cis*Pt-induced base-pair substitutions) [13], and with those from enzymatically hydrolysed *cis*-Pt treated salmon sperm DNA [14].

From a series of GG- and GCG-containing di-, tri-, tetra-, hexa- and octanucleotides the following two specific intrastrand crosslinks have been unequivocally found so far:



Mutation studies have indicated that apart from GCG sequences, GAG sequences may also occur [13]. This has not yet been investigated in oligonucleotides. The occurrence of GG-Pt chelates has also been recently found by other groups [15, 16].

Acknowledgements. These investigations have been supported in part by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO). Financial aid was also obtained through grants of the Koningin Wilhelmina Fonds (Dutch organization for fight against cancer), Amsterdam, The Netherlands (grants MBL 79-1; MBL 83-1; MG 79-68). Thanks are also due to Profs. J. H. van Boom and co-workers (for gifts of oligonucleotides), C. Altona and J. C. Chottard (for helpful discussions concerning the interpretation of the NMR spectra) and to Drs. A. T. M. Marcelis, G. W. Canters, P. H. M. Lohman, J. Brouwer, P. van de Putte and C. A. G. Haasnoot (for technical assistance and helpful discussions).

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T16

Conformation of d(ATGG)–*cis*Pt(NH_3)$_2$ in Aqueous Solution by Proton Magnetic Resonance Spectroscopy

S. TRAN-DINH, J. M. NEUMANN

Service de Biophysique C.E.N. Saclay, 91191 Gif sur Yvette, Cédex, France

J. P. GIRAULT, J. C. CHOTTARD

Laboratoire de Chimie, Ecole Normale Supérieure, 75231 Paris Cédex 05, France

T. HUYNH-DINH and J. IGOLEN

Unité de Chimie Organique, Institut Pasteur, 75724 Paris Cédex 15, France

The conformation of an adduct d(ATGG)–*cis*Pt resulting from the interaction of the antitumor *cis*Pt(NH_3)$_2$Cl$_2$ with a short DNA fragment dApTpGpG has been investigated by ^{1}H NMR at 500 MHz and at various temperatures. The chemical shift and coupling constant measurements confirm the CD and UV results that divalent platinum binds covalently to the N-7 atoms of the two adjacent guanines in the same strand as in the case of diguanosine monophosphates [1, 2]. Analysis of the coupling constants between the deoxyribose protons (Fig. 1) shows that the sugar ring of the internal dG adopts the N conformation ($C_{3'}$-endo) (91% N) while the external dG and the other residues adopt the S conformation ($C_{2'}$-endo) (70–80% S). In the case of unplatinated oligomer, the S conformation is largely predominant (>70%) for all residues of d(ATGG).

The relaxation time and nuclear Overhauser measurements indicate that the orientation of the two guanines is *anti*, in agreement with the previous results obtained for the dimers r(GpG)–*cis*Pt, d(GpG)–*cis*Pt [1, 2]. Surprisingly, on decreasing temperature from 80 to 25 °C, the $H_{1'}$, resonance of the internal dG (Fig. 1) and the $H_{4'}$ resonance of the internal dT (not shown) shift and broaden substantially and finally disappear at t<40 °C. These results suggest that d(ATGG)–*cis*Pt tends to associate with neighbouring molecules and the conformation of this adduct changes with temperature. It seems likely that at low temperature the base protons (H-6 and CH_3) of dT are situated

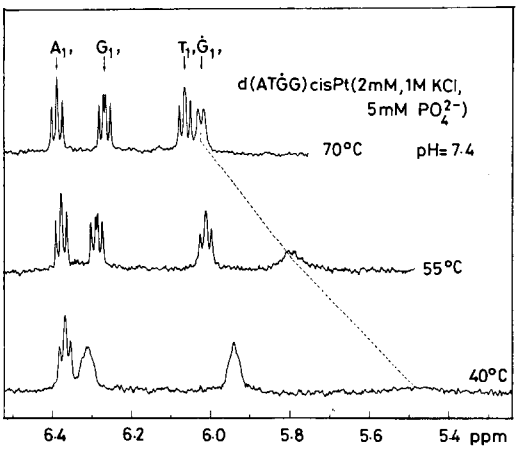

Fig. 1. 500 MHz ^{1}H NMR spectra of the $H_{1'}$ protons of d(ATGG)–*cis*Pt in aqueous solution at various temperatures.

'inside' the adenine ring whereas the sugar protons of dT are very close to the guanine ring of the internal dG.

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T17

Structural Studies on Metal-ATP Complexes: X-Ray Structures of Mg(II), Ca(II), Mn(II) and Co(II) Ternary Complexes with ATP and Dipyridylamine

R. CINI

Istituto di Chimica Generale, Università di Siena, 53100 Siena, Italy

M. SABAT, M. SUNDARALINGAM

Department of Biochemistry, University of Wisconsin, Madison, Wis. 53706, U.S.A.

M. C. BURLA, A. NUNZI, G. POLIDORI and P. F. ZANAZZI

Istituto di Mineralogia, Università di Perugia, 06100 Perugia, Italy

The enzyme-catalyzed reactions of transferring simple and substituted phosphoryl groups, such as nucleotidyl groups, are among the most fundamental biochemical processes. Enzymes which utilize one of the nucleotides as a cofactor or substrate usually require a specific complex of the nucleotide with metal ion for activity. The metal ions are involved in a number of mono-, bi- and tri-dentate coordination geometries and some of the forms may be preferred by specific enzymes over the others as substrates. X-ray studies of several metal-polyphosphate