Inorganica Chimica Acta, 55 (1981) 147–152

Binding of *cis*- and *trans*-dichlorodiammineplatinum(II) to Nucleic Acids Studied by Raman Spectroscopy. Part. I. Salmon Sperm DNA

ALAIN J. P. ALIX, LUCIEN BERNARD, MICHEL MANFAIT*

Laboratoire de Recherches Optiques, UER Sciences Exactes et Naturelles, BP 347, 51062 Reims-Cedex France

and PRASANTA K. GANGULI and THEOPHILE THEOPHANIDES*

Département de Chimie, Université de Montréal, C.P. 6210, Succ. A. Montreal, Que., H3C 3V1, Canada

Received October 8, 1981

Raman spectra of salmon sperm DNA and its complexes with the antitumor drug cis-$Pt(NH_3)_2Cl_2$ *and its inactive* trans-*isomer at low Pt/P doses (r = 0.06) have been obtained and studied. The spectra have been recorded as water solutions at low concentrations (8.5 mg/ml). The use of computer-ware allowed us to subtract the water bands. The carbonyl regions show significant differences between the* cis- *and the* trans-*complexes. These changes are indicative of a perturbation of the guanine carbonyl band in the case of the antitumor drug* cis-*platinum. Other changes in the spectra are consistent with a premelting of DNA in its interaction with* cis-*platinum, whereas the* trans-*isomer disrupts the DNA structure more rapidly as shown by the large increase of intensity of the characteristic thymine bands at 1238 and 1662* cm^{-1}. *From changes in the 1628* cm^{-1} *band in the presence of* cis-*platinum it is evident that the drug reacts differently with the DNA from the inactive* trans-*isomer. In addition, the 684* cm^{-1} *band characteristic of guanine, exhibits intensity changes with the* cis-*platinum which are related to the stacking of the guanine bases. The* trans-*isomer does not show these changes. Furthermore, the present study confirms the fact that both the drug and its inactive* trans-*isomer are covalently bound to the* N_7-*guanine sites but the perturbation is different in the two cases.*

Introduction

The antitumor drug [1] *cis*-dichlorodiammineplatinum(II), *cis*-$Pt(NH_3)_2Cl_2$ (*cis*-platinum) was discovered by Rosenberg and his collaborators [2] in 1964. Since that time a great number of papers have been published in order to elucidate the heavy metal nucleoside, nucleotide and nucleic acid interaction and to determine the mechanism of antitumor activity of *cis*-platinum as compared to the *trans*-isomer which is inactive against tumors. It is widely believed that the primary intracellular target of the platinum drug is the DNA [3–6]. Many researchers have looked for differences in the DNA binding capacity of the two platinum isomers and came out with a number of hypotheses as for the type of interaction of these two isomers and other platinum salts with the cellular materials [7–14]. Several other studies [10, 15–18] have shown, for example, that the drug reacts preferentially with the DNA bases in particular with the G-C pairs of DNA and that interstrand cross-links are important.

Studies on the interaction of *cis*-platinum and its *trans*-isomer with nucleosides have shown that platinum blocks preferentially the N_7 site of guanine [19–22]. Similarly, reactions with nucleotides have shown again interaction of *cis*-platinum with the N_7 site of the guanine base [11, 21]. Reactions of the drug with DNA suggest from chemical evidence that the metal reacts preferentially with the N_7 and O_6 sites of guanine [7–9] forming covalent bonds. The interactions of *cis*-platinum and *trans*-$Pt(NH_3)_2Cl_2$ with the nucleic acids are particularly interesting and more data are needed in order to clarify the antitumor drug reaction. *Cis*-platinum binds to DNA molecules, however the antitumor mechanism needs still clarification.

Raman spectroscopic studies [11] on the interactions of the drug and its *trans*-isomer with DNA have shown that the drug binds covalently to the DNA at the N_7 site of guanine. However our preliminary Raman studies [23, 24] have shown that the drug reacts not exclusively with the N_7 site of gua-

*Authors to whom reprint requests and correspondence could be addressed.

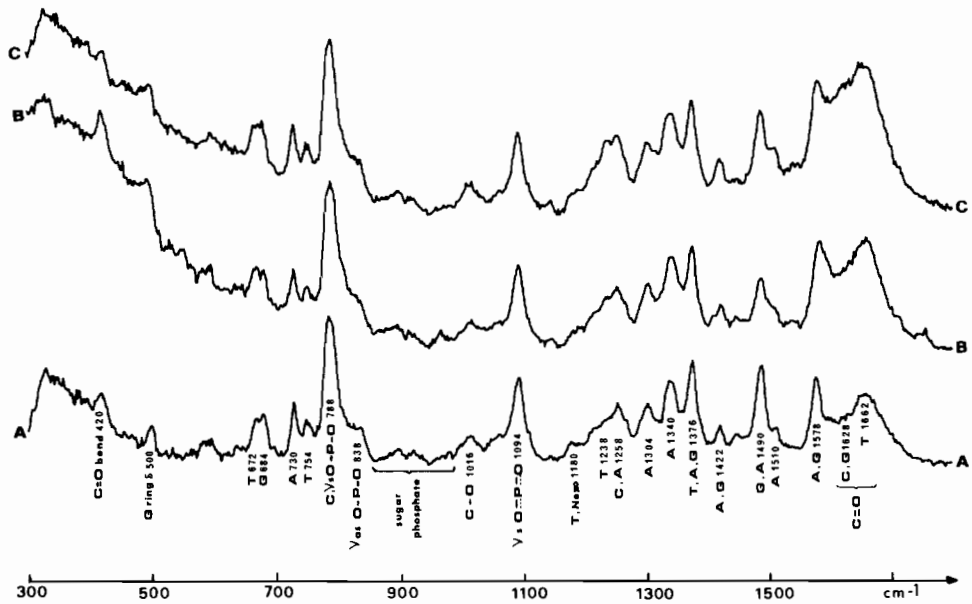

Fig. 1. Raman spectra of salmon sperm DNA (A), DNA + *cis*-platinum (B) and DNA + *trans*-isomer (C). The Raman spectra of the water was subtracted in all three cases. Concentration of DNA was 8.5 mg/ml in the presence of 8 m*M* NaCl. The Pt/P ratio was 0.06; pH = 6.5 at 25 °C. Excitation wavelength 488 nm. Slit width = 6 cm^{-1}.

nine. Following these preliminary Raman results we have started our Raman investigation by comparing the spectra of the drug and its isomer with DNA in water solutions. The purpose of this work was to investigate carefully the carbonyl region in both *cis*- and *trans*-reactions with DNA and to observe any differences in binding of DNA to the drug or to its inactive isomer. Earlier studies [7–9] suggested that there was an interaction of the carbonyl oxygen at O_6 of guanine with the *cis*-platinum but not with the inactive *trans*-isomer. To date no Raman spectra have been published on the drug or the *trans*-isomer complexes with DNA in which the water band at 1640 cm^{-1} has been subtracted in order to observe the modifications of the double bond vibrations of the bases due to their interaction with the drug.

We report in this paper difference spectra in which the solvent bands are subtracted showing the changes occurred in the carbonyl band by complexation with low doses of the drug. In addition, other important effects have been observed in the spectra which are characteristic of changes taking place in the secondary structure of DNA. Models of drug action are proposed to account for the Raman spectral changes.

The results of the Raman spectra have been reported only at a single Pt/DNA ratio (0.06). However, several attempts with ratios (0.02 to 0.2) have been examined before. The choice has been a compromise between lower values, which did not appreciably affect the spectrum of the starting DNA, and higher values, which gave spectra heavily affected by melting phenomena resulting in similar spectral perturbations for both isomers. This was also confirmed by circular dichroism spectra [28].

Experimental

Materials and Methods

Salmon sperm DNA

Salmon sperm DNA, Na-salt, highly polymerized, was purchased from Sigma Chemical Co., St. Louis, Mo, U.S.A. The DNA stock solution was prepared according to Jordan [25] as described in details earlier [10].

Dichlorodiammineplatinum(II)

Cis- and *trans*-$Pt(NH_3)_2Cl_2$ were prepared as described earlier [7]. Stock solutions of about 1 m*M* concentration in bidistilled water were prepared, filtered and stored in the dark at 24 °C. Concentrations of Pt in these stock solutions were determined by Inductively Coupled Plasma Emission Spectrometry (SMI Spectrospan III, Plasmatherm-2500).

Reactions of cis- *and* trans-$Pt(NH_3)_2Cl_2$ *with DNA*

Appropriate volumes of the stock solutions of DNA in 4 m*M* NaCl were mixed with those of *cis*- or *trans*-Pt-compounds in bidistilled water to achieve 0.06 Pt/P ratios. The reaction was allowed to proceed at 35 °C for at least 24 hours. UV absorption

TABLE I. Raman Band Frequencies (cm^{-1}) of Salmon Sperm DNA and Its Complexes with *cis*-Platinum and *trans*-Isomer.[a]

Salmon Sperm DNA	DNA + *cis*-platinum	DNA + *trans*-isomer	Assignments[a]
420	420 Increase in intensity	424	C=O bend
500	494 Shift to lower cm^{-1}	496	G ring bend
–	550, 532 New bands	Not visible	$Pt-NH_3$
672	672	672	T
684	684 Small decrease	682	G
730	730	730	A
754	752	754	T
788	788	788	C
838	834 Small decrease	834 Small decrease	OPO diester stretch
–	968 New band	–	OPO diester stretch
1016	1016	1016	C–O stretch
1094	1094	1094	PO_2^- dioxy symmetric stretch
1180	1182 Small decrease	1182	T, C–N stretch (external base)
1216	1216	1216	T
1238	1238 Small increase	1238 Increase	T
1258	1256	1256	C, A,
1304	1306	1304	A
1340	1340	1340	A
1376	1376 Small decrease	1376	T, A, G
–	1412 New band	–	G*
1422	1422	1422 Small increase	A, G
1490	1490 Large decrease	1490 Small decrease	G, A
1510	1510 Small increase	1510 Increase	A
–	1540 New band	~1540	Indicative of G platinated at N_7 and in keto form
1578	1584 Shift to higher cm^{-1}	1580	A, G
–	1596 New band	–	C=O stretch of G*
1628	– Shift to 1596 cm^{-1}	1626	C=O of G, C
1662	1662 Small increase	1658 Large increase	C=O of T
–	1716 New band	~1700	C=O of G from local melting
	1756 New band		C=O free

[a]A: Adenine, T: Thymine, C: Cytosine, G: Guanine, G*: platinated G.

spectra showing bathochromic and/or hyperchromic changes indicated the binding of Pt-compounds with DNA [10, 28]. The solutions (control and *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ treated) were then concentrated by slow evaporation to give a final concentration of 8.5 mg/ml for DNA and 8 m*M* for NaCl. The evaporation was carried out inside a partially evacuated dessiccator containing KOH pellets and kept in the dark at 35 °C for 7 days.

Instrumentation and Sample Handling

Raman spectra were recorded on a CODERG-PHO spectrometer in the wavelength region 250–1850 cm^{-1}. A Coherent Radiation Model 52B Ar^+ laser of 1500 mW power with the 488.8 nm was used with a small two-prism monochromator (ANASPEC 300 S) to remove the background plasma lines. A cool photomultiplier EMI 9558 QB was used as a detector. The signal pulse from the photomultiplier was amplified then counted digitally and stored in the computer. The computer system (ALCYANE, MBC, France) coordinates the scanning (in steps of 2 cm^{-1}) and the spectral data acquisition. The maximum counting time for each step was 2 sec. Data accumulation (time averaging) was used to obtain the spectra and to improve the signal to noise ratio. The computer enables one to carry out several operations on the stored spectra [26, 27].

The samples were centrifuged before each measurement and were placed in a rectangular quartz cell and thermostatted at 25 ± 0.5 °C. The slit widths were 6 cm^{-1}, the frequencies are accurate to ±2 cm^{-1} and the intensities were measured by their peak heights. The spectra were normalized to the same relative Raman intensity taking the water band as a reference. A careful comparison of the intensities of the spectra of the solvent and of the solutions shows that the region 1850 cm^{-1} can be considered as nearly 'zero Raman intensity' at least or the wavelengths below 1850 cm^{-1}. The solvent spectrum is subtracted from that of the solution with an appropriate coefficient in order to keep the horizontal base-line unchanged in the difference spectrum (see Fig. 1).

TABLE II. Modifications of Some Characteristic DNA Raman Bands of Alkylation, Melting and Platination with *cis*- and *trans*-$Pt(NH_3)_2Cl_2$.

DNA	Alkylated DNA [33]	Melted DNA [30]	DNA + *cis*-platinum	DNA + *trans*-isomer
684 (G)	Disappears	Decrease in intensity	Small decrease	No change
838 (OPO)	Shift to lower cm^{-1}	Shift to lower cm^{-1}	Shift to 834 cm^{-1}	Shift to 834 cm^{-1}
1238 (T)	Increase	Large increase	Small increase	Increase
1376 (T, A, G)	Increase 1400 Alkylated G	Moderate increase	Small decrease 1412 (G*)[a]	No change
1490 (G, A)	Decrease	Moderate increase	Large decrease	Small decrease and shift to 1510 cm^{-1} (shoulder)
	1530 Alkylated G		1540 (G*)	1540 (G*) Slightly visible
1578 (A, G)	Decrease	Large increase	Shift to 1584 cm^{-1}	No change
1628 (C=O of G, C)			Shift to 1596 cm^{-1}	No change
1662 (C=O of T)		Large increase and shift to lower cm^{-1}	Small increase	Large increase

[a]G*: platinated G.

Results and Discussion

The Raman spectra of salmon sperm DNA and its complexes with *cis*-platinum and the *trans*-isomer are shown in Fig. 1. The Raman bands of the DNA molecule and the complexes are given in Table I. The present Raman spectra have been obtained with low doses of Pt/P (r = 0.06) in order to differentiate the reactions with the two platinum salts. Indeed at high doses both *cis*- and *trans*-$Pt(NH_3)_2Cl_2$ disrupt DNA and it is difficult to differentiate them in their binding with DNA. At low Pt/P doses *cis*-platinum does not seen to disrupt greatly the DNA secondary structure compared to the *trans*-isomer which shows considerable changes in the secondary structure of DNA [28]. Comparison of the DNA Raman spectra with those of the platinum–DNA complexes shows characteristic changes in intensity in several parts of the spectra, particularly in the carbonyl region after subtraction of the water spectra. In Table I we have listed all the important bands and the band assignments of DNA together with the modifications in intensity and wavenumber of the bands that are affected on complexation with the drug *cis*-platinum and the *trans*-isomer. On the other hand, in Table II we have attempted to compare the Raman frequencies and modifications of the bands of DNA, on alkylation, melting and platination with the two platinum salts.

The Raman spectrum of DNA (Fig. 1A) is similar to that published [29, 30]. The spectra of DNA with the drug and the *trans*-isomer at low doses (r = 0.06) confirm the N_7-platinum binding in both cases from intensity decreases of the characteristic band at 1490 cm^{-1}. This band decreases in intensity more drastically with the *cis*-platinum (40%) than with the *trans*-isomer (10%) and shows a shoulder at 1510 cm^{-1}. This difference seems to suggest an intra-strand $(G)N_1-Pt-N_7(G)$ binding with the drug [31, 32], but not with the *trans*-isomer. In previous [22] Raman studies on defined complexes of *cis*- and *trans*-Pt-$(NH_3)_2Cl_2$ and other platinum compounds with guanosine the band near 1490 cm^{-1} disappeared completely. It has been shown by Raman spectroscopy that alkylation in DNA at the N_7 site of guanine also causes decrease [33] and even disappearance of the 1490 cm^{-1} band as in the cases of 7-methylated [33] or 7-protonated guanine [34] (see Table II). The new band at 1540 cm^{-1} appearing in the spectra of *cis*-platinum with DNA, shown very weakly in the case of *trans*-isomer with DNA, is also found in the alkylated G, alkylated poly(dG)-poly(dC), alkylated DNA and in 7-methyl G in the keto form [33], and is assigned to G* (platinated guanine at N_7). This behavior seems to indicate that platinum is bound to some extent in a similar way to the alkylated species. Similarly, the new band at 1412 cm^{-1} which is observed only with the *cis*-platinum, but not with the *trans*-isomer is attributed to the effect of platinum binding at the N_7 site of guanine. The blocking of the N_7 site of guanine with platinum causes a perturbation in the 684 cm^{-1} band of guanine decreasing its intensity in the case of *cis*-platinum, but not in the case of the *trans*-isomer. Furthermore, the band at 1578 cm^{-1} assigned to A, G shifts to 1584 cm^{-1} with the *cis*-platinum in accordance with the previous Raman results [11]. If one examines the Raman spectra reported by these authors [11] one will also notice the decrease in intensity of the 680 cm^{-1} band on complexation of DNA with the *cis*-platinum (see [11] Fig. 9 for r = 0.2 and r = 0.4).

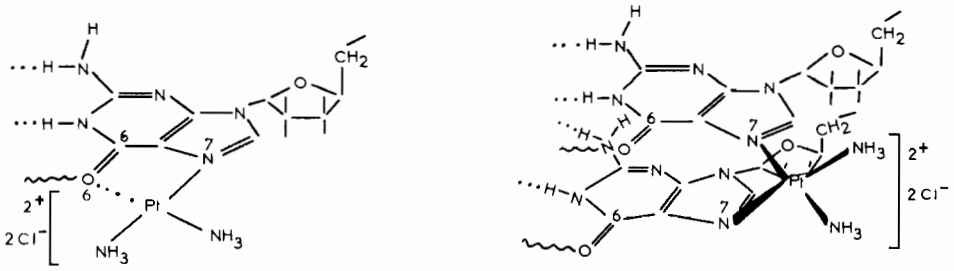

Fig. 2. Proposed model of binding of *cis*-platinum to guanine bases.

The weak sugar-phosphate band at 838 cm^{-1} (OPO diester antisymmetric stretch, see Table I) characteristic of the B conformation of DNA decreases in intensity and slightly shifts to 834 cm^{-1} both with the *cis*-platinum and the *trans*-isomer. Previous Raman studies [30] have shown that on complete melting this band disappears. The 1238 cm^{-1} and 1662 cm^{-1} bands assigned both to thymine in DNA, on complexation with the platinum compounds increase in intensity, however, much more with the *trans*-isomer than with the *cis*-platinum (see Table I and Fig. 1). It has been shown previously [30] that an increase in intensity of these bands is caused by a loss in secondary structure of DNA, which is characteristic of DNA melting. It appears therefore from our Raman results that the *trans*-isomer has a greater ability to break down the secondary structure of DNA causing a greater decrease in intensity of these two thymine bands at 1238 and 1662 cm^{-1}. Analogous results have been obtained on the interaction of poly C with *cis*- and *trans*-dichlorodiammineplatinum(II) [35]. The *cis*platinum on the other hand does not disrupt drastically the DNA structure at these low P/Pt doses. These Raman results are in total agreement with circular dichroism and UV results [28]. The *trans*-isomer is found at very low P/Pt ratios (~0.02) to cause a decrease in the secondary structure, whereas the *cis*-platinum at these low doses does not seem to disrupt the secondary structure even though it does bind more heavily to the bases of DNA than the *trans*-isomer. Under these conditions a premelting phenomenon exists with the *cis*-platinum and not with the *trans*- [28].

The carbonyl region of the DNA spectra after careful subtraction of the water band at 1640 cm^{-1} shows one weak band at 1628 cm^{-1} which seems to shift on complexation to 1596 cm^{-1} with *cis*-platinum, but not with the *trans*-isomer (see Tables I and II). This band has been observed in poly G poly C in H_2O at 1640 cm^{-1} and in D_2O at 1659 cm^{-1} [36, 37]. Changes in this band could be related to the platination of G at N_7 and to the perturbation of the carbonyl oxygen at O_6 either by direct/or indirect interaction with the platinum bound covalently at N_7 of guanine (see Fig. 2). The perturbation of the guanine carbonyl band is observed only with the *cis*-platinum, but not with the *trans*-isomer. This is significant and suggests that *cis*- and *trans*-platinum react differently with DNA. This could also be explained if the *cis*-platinum binds to two adjacent N_7 atoms of guanine, whereas the *trans* cannot bind to two adjacent guanines. This mode of action perturbs the guanine carbonyl differently in the two cases (see Fig. 2).

The Raman spectrum of poly G in heavy water [37] shows two bands in this region at 1659 and 1713 cm^{-1} corresponding to the carbonyl of guanine hydrogen bonded in the GC pair and C=O hydrogen bonded to D_2O, respectively. In our Raman spectra of DNA + *cis*-platinum and DNA + *trans*-isomer, weak bands are observed at higher frequencies at 1716 cm^{-1} and near 1700 cm^{-1} for the two platinum compounds respectively (see Tables I and II). These bands may be assigned similarly to free carbonyl bands of G (not hydrogen bonded) produced on melting partially the DNA, caused by the *cis*- and *trans*-platinum compounds. The increase in intensity of the thymine band at 1662 cm^{-1}, which also shifts slightly in the case of the *trans*-isomer is indicative of denaturation or local melting of DNA. This melting is greater in the case of the *trans*-isomer (see Tables I and II) than with the drug, *cis*-platinum. Finally in the low frequency region we find the ring deformation and carbonyl bending frequency at 420 cm^{-1} to increase in intensity with the *cis*-platinum, but not with *trans*-. This again indicates perturbation of the carbonyl and may be explained by unstacking of the guanine bases on formation of the $(G)N_7$–Pt–$N_7(G)$ type complex (see later). The new bands at 532 and 550 cm^{-1} are assigned to Pt–NH_3 stretchings. The Pt–N_7 guanine stretching in the Raman spectra of platinum–nucleoside complexes was found in the region 340–360 cm^{-1} [22], but it was not observed in the present platinum–DNA spectra. Probably it is very weak here.

It is well-known [38] that stacking of nucleic acid bases is related to the intensity changes of the Raman bands at 690 (G), 725 (A) and 785 cm^{-1} (C). Our data in Table I shows that the 684 cm^{-1} band decreases slightly in intensity after reaction with the *cis*-platinum, but not with the *trans*-isomer. This change may be due to the fact that *cis*-platinum binds to two N_7 atoms of guanine adjacent or not, but in

the same strand (intra-strand cross link). This type of binding obviously will have an effect on the stacking of the guanine bases which is observed (see Tables I and II). It is apparent from these Raman studies that *cis*- (the drug) and *trans*-platinum first bind mainly to the DNA strands, causing a local melting or unwinding of DNA, followed by a further reaction of the *cis*- with an adjacent guanine at N_7. This guanine, however may not be adjacent since *cis*-platinum can attract to itself another guanine further away in the same strand from the site of the first attack, which could cause some shrinking of DNA. Shortening of circular DNA on reaction with *cis*-platinum has been observed [13, 39]. An inter-cross linking occurs, if the second guanine molecule is on the other strand. The *trans*-isomer does not show any intensity changes in the band at 684 cm^{-1} and therefore even though it melts and disrupts the DNA secondary structure it does not seem to affect the stacking of the bases in DNA.

The *cis*-platinum, thus binds in a specific manner to DNA requiring adjacent guanines or guanines brought together after a local melting to form a specific $(G)N_7-Pt-N_7(G)$ binding in *cis*-geometry (see Fig. 2). This type of binding at low doses of Pt/P does not seem to cause a great disruption of the secondary structure of DNA. The *trans*-isomer, however, is not capable to give the above type of bonding. On the other hand *trans*- seems to melt and disrupt more effectively DNA. Thus, *cis*-platinum apparently affects stacking but not *trans*-. The guanine carbonyl band at 1628 cm^{-1} is modified with the *cis*-platinum, but not with the *trans*-isomer (see Tables I and II). This could be explained either by a direct weak interaction (see Fig. 2) of the *cis*-platinum with the carbonyl (Pt···O=C) after blocking one N_7 site of guanine or by an unstacking of the guanine bases, as it is shown from the intensity decrease of the Raman band of guanine at 684 cm^{-1} in the case of the *cis*-platinum by forming a PtG_2 type of binding with the *cis*-$Pt(NH_3)_2G_2$-geometry.

Acknowledgements

(AJPA) and (MM) wish to thank the Department of Chemistry of the Université de Montréal for the invitation and some assistance. The support of NSERC and the loan of platinum metals from Johnson Matthey and Mallory Ltd. is gratefully acknowledged. Many thanks also to Coopération France-Québec for making this work possible. We also wish to thank Professor W. L. Peticolas for sending us his work on poly C before publication.

References

1 B. Rosenberg, L. Van Camp, J. E. Trosko and V. H. Mansour, *Nature, London, 222,* 385 (1969).
2 B. Rosenberg, L. Van Camp and T. Krigas, *Nature, London, 205,* 698 (1965).
3 B. Rosenberg, *Platinum Met. Rev., 15,* 42–59 (1971).
4 B. Rosenberg, *Naturwissenschaften, 60,* 399 (1973).
5 H. C. Harder and B. Rosenberg, *Int. J. Cancer., 6,* 207 (1970).
6 P. Lecointe, J. P. Macquet, J. L. Butour and C. Paoletti, *Mutat. Res., 48,* 139 (1977).
7 J. P. Macquet and T. Theophanides, *Biopolymers, 14,* 781 (1975).
8 J. P. Macquet and T. Theophanides, *Bioinorg. Chem., 5,* 59 (1975).
9 M. M. Millard, J. P. Macquet and T. Theophanides, *Biochim. Biophys. Acta, 402,* 166 (1975).
10 P. K. Ganguli and T. Theophanides, *Eur. J. Biochem., 101,* 377 (1979).
11 S. Mansy, G. Y. H. Chu, R. E. Duncan, and R. S. Tobias, *J. Am. Chem. Soc., 100,* 607 (1978).
12 H. C. Harder, R. G. Smith, and A. F. LeRoy, *Cancer Res., 36,* 3821 (1976).
13 J. P. Macquet and J. L. Butour, *Biochimie, 60,* 901 (1978).
14 A. J. Thomson, *Platinum Met. Rev., 21,* 2 (1977).
15 L. L. Munchausen and R. O. Rahn, *Biochim. Biophys. Acta, 414,* 242 (1975).
16 P. J. Stone, A. D. Kelman, and F. M. Sinex, *Nature, 251,* 736 (1974).
17 J. J. Roberts and J. M. Pascoe, *Nature, London, 235,* 282 (1972).
18 P. Horacek and J. Drobnik, *Biochim. Biophys. Acta, 254,* 341 (1971).
19 S. Mansy, B. Rosenberg and A. J. Thomson, *J. Am. Chem. Soc., 95,* 1633 (1973).
20 P. C. Kong and T. Theophanides, *Inorg. Chem., 13,* 1167 (1974); *ibid., 13,* 1981 (1974).
21 P. C. Kong and T. Theophanides, *Bioinorg. Chem., 5,* 51 (1975).
22 T. Theophanides, N. Hadjiliadis, M. Berjot, M. Manfait, and L. Bernard, *J. Raman Spectrosc., 5,* 315 (1976).
23 L. Bernard, M. Manfait, M. Berjot, P. K. Ganguli and T. Theophanides, *Biochimie, 60,* 1039 (1978).
24 W. L. Peticolas and T. Theophanides, unpublished results.
25 D. O. Jordan, 'The Chemistry of Nucleic Acids', Butterworth, London, 178–276 (1960).
26 M. Manfait, J. L. Beaudoin and L. Bernard, 'Advances in Raman Spectroscopy', J. P. Mathieu ed., Heyden and Son, London, 76 (1972).
27 M. Manfait, J. Marx and E. Merienne, unpublished results.
28 P. K. Ganguli and T. Theophanides, *Inorg. Chim. Acta, 55,* L43 (1981).
29 E. W. Small and W. L. Peticolas, *Biopolymers, 10,* 1377 (1971).
30 S. C. Erfurth and W. L. Peticolas, *Biopolymers, 14,* 247 (1975).
31 R. E. Cramer, P. L. Dahlstrom, M. J. T. Seu, T. Norton, and M. Kashiwagi, *Inorg. Chem., 19,* 148 (1980).
32 L. G. Marzilli and P. Chalilpoyil, *J. Am. Chem. Soc., 102,* 873 (1980).
33 S. Mansy and W. L. Peticolas, *Biochemistry, 15,* 2650 (1976).
34 R. C. Lord and G. J. Thomas Jr., *Spectrochim. Acta, 23A,* 2551 (1967).
35 D. P. Strommen and W. L. Peticolas, submitted.
36 L. Lafleur, J. Rice and G. J. Thomas, Jr., *Biopolymers, 11,* 2423 (1972).
37 K. G. Brown, E. J. Kiser and W. L. Peticolas, *Biopolymers, 11,* 1855 (1972).
38 R. C. Lord, *Applied Spectroscopy, 31,* 187 (1977).
39 G. L. Cohen, W. R. Bauer, J. K. Barton and S. J. Lippard, *Science, 203,* 1014 (1979).