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Raman Study of Metal–Guanosine-5′-Monophosphate Aqueous Solutions

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The Raman spectra of Guanosine-5′-Monophosphate (GMP) in aqueous solution and in the presence of a variety of metals have been obtained and analysed. The metal–GMP interactions and associations have been studied quantitatively from intensity measurements of several vibrational bands of GMP. The interactions of base pairing and base staking of GMP molecules in the presence and absence of metal ions are modified as it is indicated from the intensity measurement of the characteristic base or phosphate bands of the mono nucleotide. It was found that the relative intensities of the guanine base bands at 1488, 1366, 1323 and 672 cm^{-1} changed in the order Pt > Hg > Ni > Co > Mn > Mg > Ca in freshly studied aqueous solutions of GMP in the presence of the chloride salts of the above metals. In addition, the relative intensity of the phosphodiester bond (O–P–O) and the PO_3^{2-} at 810 and 978 cm^{-1} respectively do change in the presence of the above metal chlorides indicating conformational changes about the phosphodiester bond and some interaction with the PO_3^{2-} phosphate end of the molecule with most of the metals as a function of metal concentration in solution.

Introduction

The binding of pre-transition and post-transition metals to nucleic acids or their constitutents has been very important for the understanding of a large number of biological effects [1–5]. However, due to the complexity of the nucleic acids it is difficult to investigate the above interaction directly. Therefore, it is necessary to study the interaction of each purine base with these metals, and the interaction of the nucleic acids with the metals and then draw conclusions about the mechanism of metal–nucleic acid interactions.

Guanosine is a purine nucleoside with an O at the 6th position of the purine skeleton (See Fig. 1). The binding of this nucleoside in acidic, neutral and basic aqueous solutions with platinum metals has been studied [6–9]. It has been found that guanosine interacts strongly with platinum mainly at the nitrogen atom N(7). The coordinative positions N(1) and O(6) have also been proposed as active sites of bonding in aqueous solutions with metals depending on pH [6–12]. Furthermore, it is very important to examine the binding of guanosine with the rest of transition metals. In this work we report a systematic study of the interaction of guanosine-5′-monophosphate (GMP) a purine nucleotide with Ca, Mg and some transition metals in dilute aqueous solution using Raman spectroscopy. The phosphate derivative was used in the present study because it is very soluble in water and therefore suited for Raman spectroscopy.

Experimental

The Raman measurements were performed with a Jobin Yvon Ramanor HG.2S spectrometer using as a source an argon ion laser (Spectra Physics) tuned to the frequencies 4579 Å, 4880 Å and 5145 Å, and at power levels in the range of 50–500 mW. The liquid samples had a volume of one ml. The scattered light was collected at 90° to the incident collimated beam. In the Raman spectra the position of Raman lines and their intensities relative to H_2O or D_2O were observed and recorded. The intensity of the bands was measured by their peak heights. Guanosine-5′-monophosphate disodium salt (GMP) was obtained from ICN Pharmaceuticals, Inc., K_2PtCl_4 from Johnson Matthey and Mallory Limited, the halide salts, $MgCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $HgCl_2$ from Merck, and $CaCl_2 2H_2O$, $MnCl_2 \cdot 4H_2O$, $FeCl_3 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$ from Mallinckrodt. The polarization of the lines was determined by taking spectra

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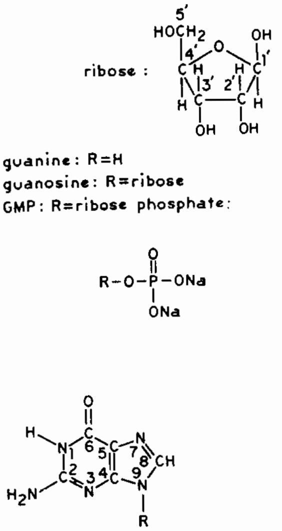


Fig. 1. The GMP molecule and the numbering of the purine ring.

with the incident polarization both parallel and perpendicular to the scattering light wave vector.

Most data were obtained with a concentration of GMP in H_2O of 0.02 M. The concentration of the metal in the solution was the same 0.02 M, but lower concentrations down to 10^{-4} M were also examined. Possible GMP degradation was of interest in some cases. The GMP was essentially in the non-protonated form as free base in the neutral water solution under examination. The Raman spectra were recorded with various frequencies, but the frequency of 5145 Å was taken as the most appropriate.

Results and Discussion

The Raman spectra of GMP in aqueous solutions were taken with alkaline-earth or different transition metals added in the solution. The introduction of the metals, Ca, Mg, Mn, Co, Ni, Hg and Pt in the solution as divalent chloride salts resulted in changes in position and intensity of some characteristic Raman bands of GMP. The pH of all the solutions was 7. The changes in position of some bands of GMP with the metals Mg, Ca, Mn, Co, Ni, Hg and Pt are presented in Table I. For the 1323, 1366, 1488 and 1577 cm^{-1} bands of GMP there is a significant displacement for the heavier metals, Co, Ni, Pt, Hg and a small displacement for the lighter metals Mg, Ca and Mn. For the 978 cm^{-1}, symmetric stretching frequency [6] of PO_3^{2-} there was observed a displacement for the heavy metals.

TABLE I.[a] Frequency Changes of Some Characteristic Raman Bands (cm^{-1}) of GMP in the Presence of Metal Halides.

Metal Bands Halide	978	1323	1366	1488	1577
Mg	−2	5	0	0	0
Ca	0	−3	0	−2	−2
Mn	0	2	4	0	0
Co	3	−2	−6	9	6
Ni	8	7	−3	9	6
Pt	20	5	−8	17	11
Hg	9	5	−10	4	21

[a] The perturbation is strongest for the heavier metals and least for the lighter ones (Mg, Ca, Mn).

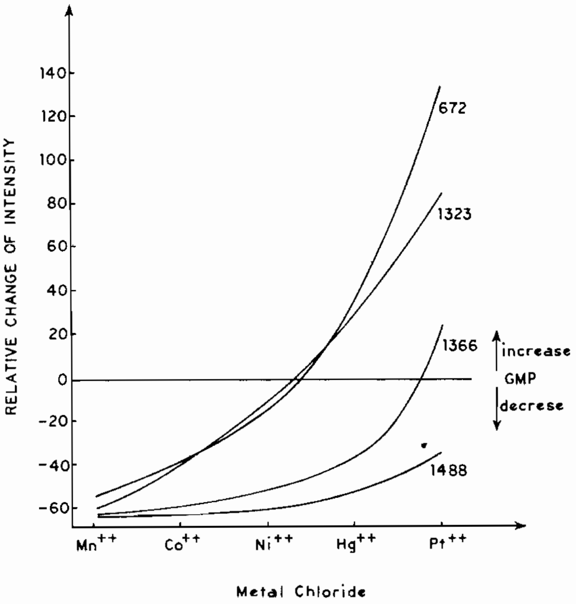


Fig. 2. Relative intensities of some Raman bands of GMP with respect to the intensities of GMP taken as zero (the horizontal line).

The relative changes in intensity of the characteristic bands of GMP with the metals Mn, Co, Ni, Hg and Pt are presented in Fig. 2. The relative change in intensity of each band was taken with reference to the 1640 cm^{-1} and 1200 cm^{-1} bands of water and D_2O and the 978 cm^{-1} band of PO_3^{2-}. Figure 2 shows that the intensity of the bands 1488, 1366, 1323 and 672 cm^{-1} of GMP changes with the introduction of the metal in the order Mn < Co < Ni < Hg < Pt. The intensities of the bands were calculated with respect to the free GMP which was taken as zero for GMP in Fig. 2 and changes in intensity were recorded as decreases and increases of relative intensities for the different bands in the presence of the various metals.

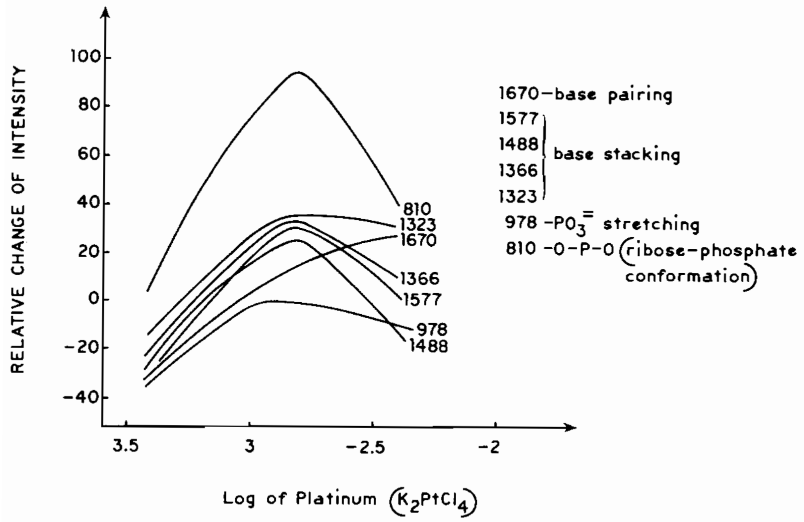


Fig. 3. Relative intensities of some Raman bands of GMP as a function of K_2PtCl_4 concentration.

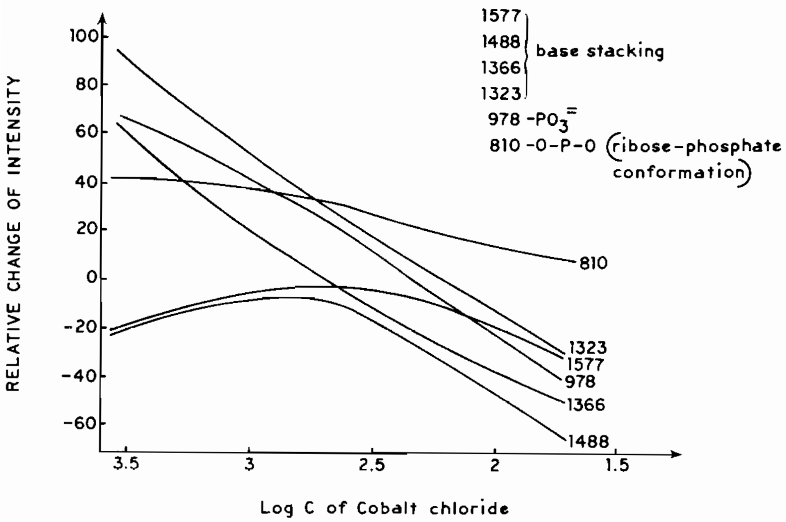


Fig. 4. Relative intensities of some Raman bands of GMP as a function of cobalt chloride concentration.

The Raman spectra of GMP in aqueous solutions with the metals Pt, Co and Mn were taken as a function of metal concentration. The molar concentration of the metals was in the range from 3 X 10^{-4} *M* to 2 X 10^{-2} *M*. The change in metal concentration resulted in displacements and significant changes in the intensity of some of the characteristic bands of GMP. The displacements of the characteristic bands of GMP with the metals Pt, Co and Mn in the concentration range 3 X 10^{-4} *M* to 2 X 10^{-2} *M* are presented in Table II. With the Pt metal there are significant displacements at all concentrations. With Co and Mn the displacements are smaller .

The relative change in intensity of the bands 1670, 1577, 1488, 1366, 1323, 978 and 810 cm^{-1} was taken as a function of metal concentration in the range 3 X 10^{-4} *M* to 2 X 10^{-2} *M*, with Pt, Co and Mn metals (See Figs. 3, 4 and 5). It is interesting to see that some of these changes in intensity in Figs. 3, 4 and 5 resemble the effect of Na^+ ion concentration on the tRNA conformation in solution [13]. For example, Fig. 3 shows that the band at 1670 cm^{-1} increases in intensity with higher concentrations of Pt in solution, which would indicate an increase in base pairing of GMP molecules and a species with O_6–N_7 binding, in addition to N_7 binding alone, which is the most preferential binding of platinum. The relative intensity of the bands at 1577, 1488, 1366 and 1323 cm^{-1} goes through a maximum. Base stacking arrangement and cooperative effects seems to be favoured with interaction between this nucleotide and the platinum compound and then destab-

TABLE II. Frequency Changes of Some Raman Bands (cm^{-1}) of GMP as a Function of Metal Concentration.

	Increasing conc. of the metal	672	978	1323	1366	1488	1577	1670
Mn	1×10^{-3} M	0	0	0	2	3	0	–
	2×10^{-2} M	0	3	2	4	–3	0	–
Co	2.8×10^{-4} *M*	0	0	–2	–3	0	0	–
	2.5×10^{-3} *M*	0	0	–2	–4	–2	–2	–
	2×10^{-2} *M*	–3	3	–2	–6	9	6	–
Pt	3.7×10^{-4} *M*	–7	–5	2	–2	–3	0	0
	1.5×10^{-3} *M*	0	–3	–2	3	–4	0	0
	4×10^{-3} *M*	–2	8	–3	–2	–5	0	0
	2×10^{-2} *M*	13	0	10	–19	15	7	18

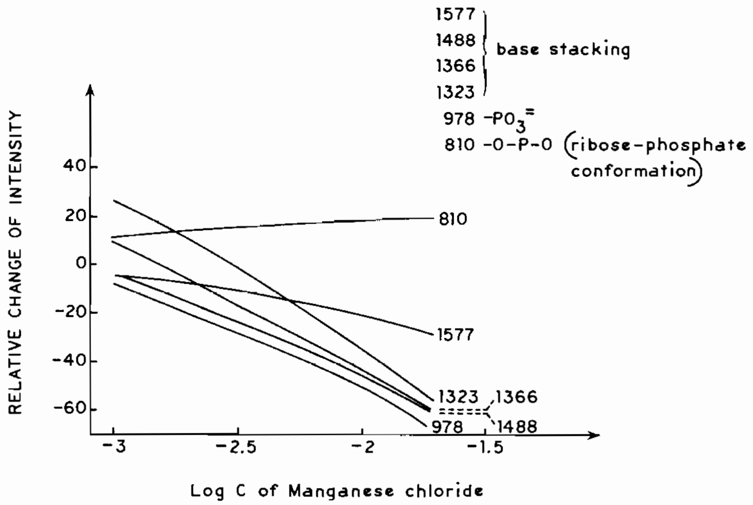


Fig. 5. Relative intensities of some Raman bands of GMP as a function of manganese chloride concentration.

ilized as a function of concentration. The relative intensity of the bands at 810 cm^{-1} and 978 cm^{-1} also goes through a maximum which indicates conformational changes around the phosphodiester bond of GMP due most likely to a direct platinum interaction with the phosphate groups (PO_3^{2-}). Figures 4 and 5 show that all bands decrease in intensity with higher concentrations of metal in solution, which indicates a decrease in base stacking and also phosphodiester conformational changes to metal interaction with the phosphate groups. It is evident from these Figures that platinum behaves differently from Co and Mn metals.

In Fig. 3 the 810 cm^{-1} band of GMP which involves the rebose-phosphate-diester linkage (OPO) in the presence of Pt in the range of concentrations 2.81×10^{-3} *M* to 2.4×10^{-3} *M* decreases in intensity as a function of concentration of Pt, whereas in Fig. 4 with cobalt the rate of decrease in intensity of the same band is slower and finally in Fig. 5 with manganese the intensity of this band remains constant throughout all the concentrations used.

The Raman spectra of GMP were also taken as a function of laser wave-length at 5145 Å, 4880 Å and 4579 Å. The relative intensity of some Raman bands was found to increase as the frequency of the laser light increased. The relative intensities of the bands were taken with respect to the 978 cm^{-1} band for the symmetric stretching of PO_3^{2-} or the 1200 cm^{-1} band of heavy water. The bands at 1323, 1488 and 1577 cm^{-1} were found to increase in intensity as the frequency of the laser light increased; their increase was of the same order of magnitude of about 40%. This was due to a pre-resonance effect with

TABLE III. Relative Intensities of Some Raman Bands (cm^{-1}) of GMP as a Function of the Exciting Line and in the Presence of the Chlorides of Magnesium, Nickel and Platinum Salts.

	1323		1488		1577		Observation
	r	R	r	R	r	R	
5145 A	0.550	4.6	1.560	1.3	2.572	2.1	GMP, pre-resonance
4880 A	0.753	4.8	1.966	1.3	3.514	2.2	
4579 A	0.957	4.3	2.510	1.1	4.574	2.0	
5145 A	0.586	4.8	1.871	1.5	3.155	2.6	GMP with $MgCl_2$; pre-resonance constant
4880 A	0.780	5.0	1.933	1.2	3.285	2.2	
4579 A	1.208	5.4	2.792	1.2	4.458	2.0	
5145 A	1.222	10.1	2.237	1.8	3.792	3.1	GMP with $NiCl_2$. The metal changes the condition of pre-resonance
4880 A	1.430	9.2	3.967	2.5	4.925	3.1	
4579 A	1.818	8.2	4.863	2.2	5.227	2.3	
5145 A	0.703	5.8	1.683	1.4	2.970	2.5	GMP with K_2PtCl_4. The metal changes the condition or pre-resonance
4880 A	0.786	5.0	2.095	1.3	3.488	2.2	
4579 A	0.926	4.2	2.383	1.1	3.827	1.7	

TABLE IV. Polarization Ratios of Some Raman Bands (cm^{-1}) of GMP and GMP–K_2PtCl_4 in D_2O Solutions.[a]

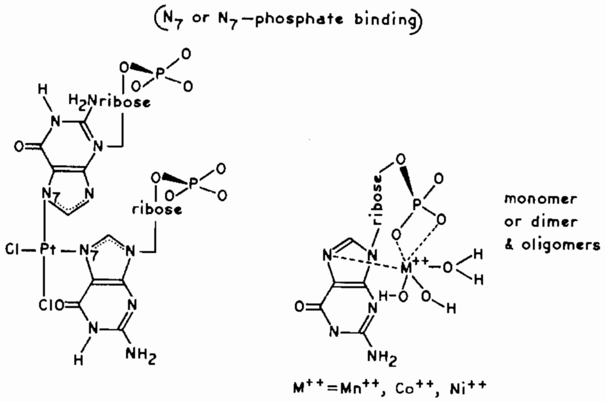
Raman bands	(GMP) pρ	GMP–K_2PtCl_4 ρ	Assignment
672	0.250 pp	0.400 p	G, breathing mode
978	0.356 pp	0.302 pp	$-PO_3^{2-}$, sym. str.
1200	0.517 p	0.518 p	ribose, C–O
1323	0.559 p	0.299 pp	C–N, str. & Amide III
1360	0.511 p	0.571 p	C–C, str. & Amide III
1480	0.328 pp	0.347 pp	C_8N_7, wave-like mode
1579	0.436 p	0.433 p	C=C, and C=N str.
1670	0.557 p	0.339 pp	C=O, str.

[a] p = polarized, pp = strongly polarized; $\rho = \perp/\parallel$; G = Guanine.

the lowest UV absorption band at 280 nm (35710 cm^{-1}) of GMP. Table III shows the relative intensities $r(\nu_o)$ and the ratio $R(\nu_o, \nu_e) = r(\nu_o)/(\nu_e - \nu_o)^4$ of the 1323, 1488 and 1577 cm^{-1} bands at the wavelengths 5145 Å, 4880 Å and 4579 Å. The ratio R is almost constant for all three frequencies which indicates that there is pre-resonance of these bands with the ν_e = 35710 cm^{-1} band of GMP [14].

The pre-resonance of the 1323, 1488 and 1577 cm^{-1} bands of GMP was also examined in the presence of Mg, Ni and Pt metals in solution. In Table III are given the relative intensities $r(\nu_o)$ and the ratios $(R(\nu_o, \nu_e)$ of the above bands at three wavelengths for Mg, Ni and Pt salts, the metal halide concentration in the solution was 0.02 *M*. The pre-resonance of all three bands remains constant in the presence of $MgCl_2$. However, in the presence of $NiCl_2$ or K_2PtCl_4 salts there is only the pre-resonance of the 1488 cm^{-1} band with the 35710 cm^{-1} band of GMP. Therefore, there is a substantial change in the pre-resonance of the 1323 cm^{-1} and 1588 cm^{-1} bands in the presence of Ni and Pt salts. The Ni and Pt salts exhibit an absorption band in the interval between the laser frequency and the UV absorption band frequency of GMP, while the Mg metal does not exhibit such an absorption band in the same interval.

The polarizations of most Raman bands of GMP were also examined in the presence of the platinum compound. Table IV shows the polarization ratios of most Raman bands of GMP in aqueous solutions and in the presence of K_2PtCl_4 with concentration 0.02 *M*. The 1323 cm^{-1} and 1670 cm^{-1} bands became strongly polarized in the presence of the platinum compound, while the other bands did not change polarization. The change in polarization could be due to the polarizing effect of the platinum



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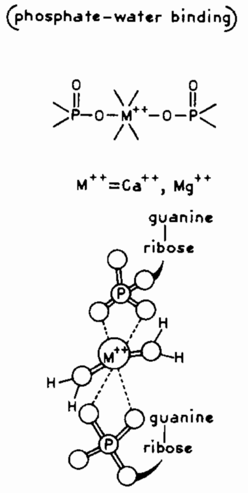

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coordination at the N(7) position in the imidazole ring of GMP suggesting the formation of a bisubstituted complex, $PtCl_2(GMP)_2$. This results in structural electronic changes at the N_7–C_8 and C_8–N_9 bonds, causing a local electronic perturbation in the molecule [16].

In the structures of metal–purine complexes it has been found that the predominant mode of metal binding takes place at the N(7) atoms of the imidazole ring [15]. Other studies in solution [17, 18] showed binding to the phosphate group of the nucleotide rather than to the purine base as the primary metal binding site. The solution geometry of the metal complexes of GMP in this study may be a little more complicated with the presence of several species in which each metal atom is bound to two or more nucleotide ligands and *vice versa* or to N(7) of one purine ring and to the phosphate oxygen of another nucleotide (See Chart). The bulk of crystal structures of metal complexes of guanosine monophosphate (5′-GMP) show the general formula $[M(5'\text{-}GMP)(H_2O)_5]$ with the nucleotide bound to the purine through N(7) and the octahedral sphere around the metal is completed with the 5 water molecules [15]. From this work it is suggested that the metal binds to N(7) of GMP in the cases of the metal salts, K_2PtCl_4, $CoCl_2 \cdot 6H_2O$, $NiCl \cdot 6H_2O$ and $MnCl_2 \cdot 6H_2O$. This is supported from the intensity changes of some Raman bands associated with changes in the pyrrole ring. However, in addition to perturbations in the bands of the base the phosphate band at 810 cm^{-1} is also perturbed (See Figs. 3, 4 and 5) suggesting that the GMP ligand is also interacting through the phosphate group and modifies the conformation around the –OPO–bond of the molecule.

The metals calcium ($CaCl_2 \cdot 6H_2O$) and magnesium ($MgCl_2 \cdot 6H_2O$) do not seem to perturb the position and the intensity of the base Raman bands substantially and are bound to the GMP most probably through weak metal–phosphate oxygen interactions of the ion–ion type and hydrogen bonding. There is a slight perturbation of the 810 and 980 cm^{-1} phosphate bands with these metals. This weak interaction of the metal with the phosphate oxygens may be direct or through a water molecule [6]. The aqueous mercury chloride solution when added in the aqueous GMP solution produced remarkable changes in the spectra not anywhere similar to the other heavy metals. The changes can be interpreted if it is assumed that the mercury is bound to the purine base. However, the mercury seems to be bound to both rings of the purine base most likely with two metals through the N(7) and N(1) sites following deprotonation at N(1). Precipitation occurred with this metal with time as it was also observed with some of the heavier transition metals Ni and Pt on longer standing. All the spectra were taken before precipitation had occurred. However, the precipitation was faster with mercury chloride than with any of the other metals.

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References

1 R. Philips, *Chem. Rev., 66,* 501 (1966), and references therein.
2 R. M. Izatt, J. J. Christensen and J. H. Rytting, *Chem. Rev., 71,* 439 (1971).
3 G. L. Eichhorn (ed.), 'Inorganic Biochemistry', Vol. 2, Elsevier, New York, 1973, chaps. 33 and 34.
4 M. Daune, Interactions of metal ions with nucleic acids, in 'Metal Ions in Biological Systems', Vol. 3 (H. Sigel, ed.), Marcel Dekker, New York, 1973, p. 1.

5 R. B. Martin and Y. H. Mariam, The Interaction between nucleotides and metal ions in solution, chap, 2, in 'Metal Ions in Biological Systems', Vol. 8 (H. Sigel, ed.), Marcel Dekker, New York, (1979).

6 T. Theophanides, Interaction of metal ions with nucleic acids, in 'Infrared and Raman Spectroscopy of Biological Molecules' (T. Theophanides, ed.), R. Reidel Publishing Co. Dordrecht, Holland 1979, p. 187, and p. 205.

7 N. Hadjiliadis and T. Theophanides, *Inorg. Chim. Acta, 16,* 77 (1976); P. C. Kong and T. Theophanides, *Inorg. Chem., 13,* 1167, 1981 (1974); *ibid., Bioinorg. Chem., 5,* 51 (1975); M. M. Millard, J. P. Macquet, and T. Theophanides, *Biochim. Biophys. Acta, 402,* 166 (1975).

8 B. Rosenberg, *Platinum Metals Revs., 15,* 42 (1971).

9 G. Y. H. Chu and R. Stuart Tobias, *J. Am. Chem. Soc., 98,* 2641 (1976); *ibid., 100,* 593 (1978).

10 N. A. Berger and G. L. Eichhorn, *J. Am. Chem. Soc., 93,* 7062 (1971).

11 C. F. Neumann, B. Prijs and H. Sigel, *Eur. J. Biochem., 41,* 209 (1974).

12 M. Sundaralingam, *Biopolymers, 7,* 821 (1969).

13 A. Dobek, A. Patkowski, D. Labuda, and J. Augustiniak, *J. Raman Spectros., 5,* 45 (1975).

14 M. Pezolet, T. J. Yu, and W. L. Peticolas, *J. Raman Spectrosc., 3,* 55 (1975).

15 R. W. Gellert and R. Bau, in 'Metal Ions in Biological Systems', Vol. 8 (ed.) Helmut Sigel, Marcel Dekker, Inc. New York, Chap. 1, p. 1 (1979).

16 T. Theophanides, M. Berjot, and L. Bernard, *J. Raman Spectrosc., 6,* 1109 (1977).

17 H. Sigel, *Angew. Chem., Int. Edn., 14,* 394 (1975); *Angew.Chem., 87,* 391 (1975); H. Sigel and D. B. McCormick, *Accounts Chem. Res., 3,* 201 (1979).

18 H. Sigel, *J. Inorg. Nucl. Chem., 39,* 1903 (1977).