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INTERACTION OF METAL IONS WITH NUCLEIC ACIDS. INTERACTION OF COPPER(II) WITH GUANOSINE AND ITS DERIVATIVES

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Interaction of copper(II) with guanosine, 2'-deoxyguanosine, 1-methylguanosine, 7-methylguanosine and GMP was studied with the use of spectroscopic and magneto-chemical methods.

The main site of copper(II) binding in guanosine is nitrogen N-7; participation of N-1 is not excluded. The involvement of carbonyl oxygen in copper binding or copper chelation to N-7 and O-6 is rather unlikely.

A crystalline complex of copper(II) with GMP [Cu(C_9H_13O_2N_3P)·(H_2O)_3] was obtained, and it was demonstrated that copper(II) is bound with N-7 and the phosphate group.

There are several sites in a guanosine molecule which may bind metal: N-1, N-3, NH_2 at C-2, N-7, and oxygen at C-6. From the possible metal binding sites, N-1 was taken into consideration only in the case of Ag(I) (Eichhorn et al., 1967) and CH_3Hg(I) (Mansy & Tobias, 1974). Eichhorn et al. (1966), Kotowycz & Suzuki (1973) and Fritzche et al. (1974) demonstrated by the ^1H and ^13C n.m.r. technique that binding of copper(II) to guanosine and GMP occurs in the neighbourhood of N-7. It is also assumed that, in guanosine and GMP, copper(II) is bound to N-7 and the C-6 oxygen forming a five-membered chelate ring (see Tu & Heller, 1974). So far, however, no direct evidence has been presented, and the participation of oxygen in the complex formation is postulated only on the basis of the copper(II)-induced changes in the intensity of the carbonyl band. In nucleotides in solution

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1 Abbreviations: n.m.r., nuclear magnetic resonance; e.p.r., electron paramagnetic resonance; DMSO-d_6, [U-^2H]dimethylsulphoxide; TMS, tetramethylsilane; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulphonate.
the binding of paramagnetic ions with phosphate has been demonstrated by the $^{31}$P n.m.r. technique (Cohn & Hughes, 1962; Sternlicht et al., 1965; Eichhorn et al., 1966; Missen et al., 1972; Lam et al., 1974) and it is assumed that ions of the bivalent metals belonging to the first series of transition metals (Mn - Cu) are bound by the phosphate group.

The present work was aimed at answering whether in guanosine simultaneous binding of copper to O-6 and N-7 occurs, and whether copper can bind to N-1 or N-3. An attempt was also undertaken to obtain a crystalline complex of copper(II) with GMP, in order to gain some information on the copper(II)-binding site, and to compare it with the data on copper binding with GMP in solution.

**MATERIALS AND METHODS**

*Reagents.* These were from the following sources: guanosine, British Drug Houses (Poole, Dorset, England); 1-methylguanosine and GMP disodium salt, Serva (Heidelberg, G.F.R.); 2'-deoxyguanosine, Calbiochem (San Diego, Calif., U.S.A.); $^2$HCl, NaO$^2$H and potassium bromide, spectrally pure, Merck (Darmstadt, G.F.R.); $^2$H$_2$O (99.75%) and DMSO-$d_6$ (99.5%), Institute for Nuclear Research (Swierk, Warszawa, Poland). Other reagents were analytical grade products provided by the Office for Distribution of Chemical Reagents (Gliwice, Poland).

*Cristalline copper(II)-GMP complex.* GMP-Na$_2$, 0.2 mmole, was dissolved in 40 ml of water, then 20 ml of 0.1 m-cupric nitrate was added. The whitish GMP-Cu precipitate formed was dissolved by dropwise addition of 0.2 m-HNO$_3$. At pH 3.4 a light-green, opalescent solution was obtained, which was clarified by 10-min heating at 50 - 60°C. After a few days and partial evaporation at room temperature, a crystalline light-green sediment was formed; it was dried in a desiccator over P$_2$O$_5$. Elementary analysis: found: C, 24.2; H, 3.6; N, 14.6; Cu, 13.1%; calculated for Cu(C$_{10}$H$_{12}$O$_4$N$_3$P)-(H$_2$O)$_3$: C, 25.1; H, 3.8; N, 14.6; Cu, 12.3%.

*Ir spectra* were recorded in spectrophotometer UR-20 (Carl Zeiss, Jena) or Unicam SP-1000. The solutions of the studied compounds with and without CuCl$_2$ contained the same ligand concentration; they were freeze-dried and 0.6% samples in KBr were prepared. In the case of the crystalline GMP-Cu(II) complex, 0.5% samples in KBr were used.

*Raman spectra* were measured on a JEOL-type JRS-S1B Raman spectrophotometer with argon (488 nm) laser excitation. Interpretation of the oscillation spectra was based on the data reported by Lord & Thomas (1967) and Tsuibo et al. (1973).

*E.p.r.* was measured in an ERP-X-3 instrument (Wroclaw Technological University, Poland) or in a RE 1301 spectrometer (U.S.S.R.). The e.p.r. spectra were interpreted using the molecular orbital theory elaborated for copper complexes by Maki & McGarvey (1958) and Kivelson & Neiman (1961).

*Magnetic susceptibility* was measured by the method of Faraday (cf Burger, 1973) over the temperature range 77 - 300 K (the sample was heated from the temperature of liquid nitrogen to room temperature).
N.m.r. spectra were recorded on 80 MHz Tesla unit or JEOL HR NMR JNM-PS-100 spectrometer operating in the continuous wave or Fourier-transform mode, and connected with a JAC-6 or EC-6 computer (Texas Instruments, U.S.A.).

The spectra of the studied compounds were measured, then an appropriate amount of CuCl₂ was added and the spectra were recorded again. The spectra for ¹³C were recorded at 25.15 MHz, for ³¹P at 40.48 MHz and for ¹H at 100 MHz (²H at 15.36 MHz).

Chemical shifts of ¹H, ¹³C and ³¹P were measured with DSS, TMS and 85% (w/w) H₃PO₄, respectively, as external standards.

The ¹H n.m.r. signals were assigned to individual protons as described in the literature (Gatlin & Davis, 1962; Schweizer et al., 1968; Townsend, 1973), and the ¹³C signals according to Dorman & Roberts (1970) and Mantsch & Smith (1972).

RESULTS

Guanine nucleosides

I.r. spectra. Of the three intense bands of guanosine at 1730, 1688 and 1633 cm⁻¹ (Fig. 1), the first two correspond to stretching vibrations of C(6)=O and C(4)=C(5), and the third to the scissoring vibrations of the NH₂ group. The bands at 1573, 1540 and 1488 cm⁻¹ correspond to vibrations of the purine ring.

![Infrared absorption spectrum](image_url)

Fig. 1. Infrared absorption spectrum, in KBr, of guanosine, and the effect of Cu(II). The CuCl₂:guanosine molar ratio is indicated in the Figure. The samples were freeze-dried from solutions of pH 5.5 - 5.7.

On gradual addition of copper up to the guanosine to Cu 1:1 ratio, the structure of the groups of overlapping bands (1730 - 1670 cm⁻¹) was broadened whereas the NH₂ band remained unchanged.
Fig. 2. Infrared absorption spectrum, in KBr, of 1-methylguanosine (——) and its equimolar mixture with CuCl₂ (···). The samples were freeze-dried from a solution of pH 5.6.

Fig. 3. Infrared absorption spectrum, in KBr, of 7-methylguanosine (——) and its equimolar mixture with CuCl₂ (···). The samples were freeze-dried from a solution of pH 7.0.

The i.r. spectrum of 1-methylguanosine after addition of copper showed considerable changes in the bands at 1650 - 1540 cm⁻¹ (Fig. 2), indicating the interaction of Cu(II) with the purine ring. The intensity of the band at 1700 cm⁻¹, corresponding to stretching vibrations of the carbonyl group, was unchanged. On the other hand, the interaction of copper with 7-methylguanosine resulted in decreased intensity of the carbonyl band at 1720 cm⁻¹ (Fig. 3).
The changes observed in the carbonyl bands could be due to interaction of copper with oxygen; enolization resulting from the copper-complex formation; or interaction of copper with N-1. They cannot be due to the interaction of copper with N-7, as in the case of 7-methylguanosine the intensity of this band was also decreased. At pH 7, corresponding to pK(N-1), a half of the 7-methylguanosine molecules are in the lactam form. Thus, the possibility of interaction between Cu(II) and deprotonated nitrogen N-1, which has strong electro-donor properties, is distinctly raised. However, it cannot be excluded that the lowered intensity of the 7-methylguanosine band at 1720 cm\(^{-1}\) is due to a shift in the lactam-lactim equilibrium. The interaction of copper with guanosine N-1 is supported by the fact that the intensity of 1-methylguanosine carbonyl band is not affected by the presence of copper. Thus, the observed broadening of the carbonyl band of guanosine is not necessarily due to enolization of the carbonyl oxygen but could result from copper interaction with N-1. This interaction seems to be more probable than the enolization suggested by Tu & Friederich (1968) and Tu & Heller (1974).

**N.m.r. spectra.** The observed effect of Cu(II) on the ¹H n.m.r. spectrum of 2′-deoxyguanosine in DMSO-\(d_6\) was closely similar to the spectrum reported by Eichhorn et al. (1966): the increase in Cu(II) concentration led to broadening of the proton H-8 signal, whereas the width of signals of NH\(_2\) and N-1-H protons was unchanged. This indicates coordination of copper in the vicinity of H-8, thus probably with N-7. When N-7 was blocked with the methyl group (7-methylguanosine), no broadening of any proton signals was observed (Fig. 4A); this indicates that, in guanosine, N-7 is the copper binding site.

The changes in the ¹H n.m.r. spectrum of 1-methylguanosine due to the presence of copper (Fig. 4B) were in agreement with expectations. The greatest broadening was observed for H-8. Also the ribose protons, especially H-1′, and protons of the methyl group underwent relaxation. It is not excluded that in 1-methylguanosine not only the region N(7)−C(5)−C(6)−O but also ribose was involved in the interaction with Cu(II).

It seems of interest that blocking of the carbonyl oxygen in 6-methoxyuridine riboside did not eliminate the region N(7)−C(5)−C(6)−O as the site for interaction with copper ions (the signal of H-8 was broadened; Maskos, 1978). Thus, in the case of guanosine, interaction with copper ion appears to be dependent on accessibility of N-7.

The theoretical calculations indicate that the carbonyl oxygen possesses a considerable negative charge (Jordan & Pullman, 1968; Pullman & Pullman, 1969); thus the metal-oxygen binding would be electronically favoured. However, formation of a five-membered chelate involving N-7 and O-6 seems sterically rather unlikely. The molecular model constructed for 13 guanine derivatives on the basis of the mean bond length and mean angles (Seshadri & Viswamitra, 1974) shows that, to bring the metal-to-oxygen distance to about 2 Å (i.e. the interatomic distance found in most of the compounds possessing the Cu−O bond; Orgel, 1965), the coordinating orbital of N-7 would have to be bent by 40° from its normal, symmetrical position (Scheme 1). On the other hand, assuming that the metal is located on the
Fig. 4. The effect of copper(II) on the $^1$H n.m.r. spectrum (100 MHz) of: A, 0.1 m-7-methylguanosine and B, 0.1 m-1-methylguanosine solutions in DMSO-$_d_6$. TMS was used as external standard. All spectra were recorded at the same amplitude and at 30°C. Sweep time 250 s, sweep width 1080 Hz. The top spectrum was recorded in the absence of copper. For the other spectra, the CuCl$_2$ concentration is indicated on the Figure.

bisecting line of the angle C(8)–N(7)–C(3) and is at a distance of 2 Å from N-7, the angle formed by the atoms C(9)–C(6)–O would have only 75° instead of 128°, the mean value calculated for the 13 guanine derivatives. Thus, copper ion cannot bind simultaneously to N-7 and oxygen at C-6.
E.p.r. spectra of Cu(II). A well-defined hyperfine structure was observed after freezing to 77 K aqueous solutions of CuSO₄ with guanosine and deoxyguanosine. The values of $\alpha^2$ and $\beta_1^2$, i.e. spin densities at orbitals $d_{x^2-y^2}$ and $d_{xy}$, respectively, and $\alpha^2$, the spin density of the unpaired electron on the ligand, were calculated from the determined magnetic parameters of the e.p.r. spectra and on the basis of the determined band of optical absorption $\Delta_1(=\Delta_2-E_{d_{x^2-y^2}})$. The values used for calculations and the spin densities obtained for guanosine and deoxyguanosine are presented in Table 1. No differences were found in the Cu(II) binding by these two compounds in aqueous solutions. The results indicate also that spin density of the unpaired electron is located partially on the metal ion, and partially delocalized on the ligand atoms. This conclusion is consistent with the results obtained by the same method for other nucleosides (Maskos, 1974). With the use of the e.p.r. method, Brun et al. (1975) found no differences in copper binding by ribonucleosides and deoxynucleosides dissolved in DMSO-$d_6$.

### Table 1

<table>
<thead>
<tr>
<th>Cu(II) complex</th>
<th>$g_| -2.0023$</th>
<th>$g_\perp -2.0023$</th>
<th>$A$ (cm$^{-1} \times 10^3$)</th>
<th>$d_1$ (cm$^{-1}$)</th>
<th>$\alpha^2$</th>
<th>$\alpha^2$</th>
<th>$\beta_1^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanosine</td>
<td>0.273</td>
<td>0.048</td>
<td>1.347</td>
<td>14 100</td>
<td>0.707</td>
<td>0.391</td>
<td>0.919</td>
</tr>
<tr>
<td>Deoxyguanosine</td>
<td>0.272</td>
<td>0.046</td>
<td>1.361</td>
<td>14 100</td>
<td>0.709</td>
<td>0.389</td>
<td>0.914</td>
</tr>
</tbody>
</table>

Guanosine 5'-phosphate

N.m.r. spectra. A nucleotide at p$^3$H 7.4 is present in the form of a doubly charged anion. Thus, in addition to nitrogen base, there appears a new ligand able to interact with copper ion. On addition of CuCl₂ to 0.08 mM concentration, the half-width of the phosphorus resonance signal changes from about 15 Hz to about 160 Hz.

The $^1$H n.m.r. spectrum of GMP at p$^3$H 7.4 changed after addition of copper (Fig. 5). Proton H-8, located in close vicinity of N-7, became relaxed, which supports participation of this nitrogen atom in complex formation. Similar changes in the dGMP spectrum after addition of copper were observed by Eichhorn et al. (1966).

In the $^{13}$C n.m.r. spectrum of GMP at p$^3$H 7.4 (Fig. 6), the broadening by copper of the signals was the same for C-8, C-5 and C-4 atoms, whereas the signal of C-2, C-6 and ribose carbons remained unchanged. Broadening of the C-8 and C-5 signals is ascribed to copper binding to N-7. In quasi-aromatic systems, such as purine bases, the effect of paramagnetic copper ion can be transmitted through a number of bonds. As demonstrated by the e.p.r. method (see above), spin density of the unpaired electron is only partially located on the metal ion, and partially delocalized on the ligand atoms. Thus, the interaction of copper(II) with N-7 may
Fig. 5. The effect of copper(II) on the $^1$H n.m.r. spectrum (100 MHz) of 0.1 M-GMP-Na$_2$ solution in $^2$H$_2$O (p$^3$H 7.4). The samples were freeze-dried three times with $^2$H$_2$O. DSS was used as external standard. The spectra were recorded as described for Fig. 4.

Fig. 6. The effect of copper(II) on the $^{13}$C n.m.r. spectrum (25.15 MHz) of GMP. A, The sample contained 500 µmoles of GMP-Na$_2$ in 1 ml of $^3$H$_2$O (p$^3$H 7.4); B, to the sample, 0.25 µmole of CuCl$_2$ was added. Repetition time 10 s, pulse width 10 µs, 800 accumulations, temperature 40°C. TMS was used as external standard.
shorten the relaxation time of the C-4 nucleus due to transmission through the \( N_{(7)}=C_{(5)}=C_{(6)} \) bonds of the spin density of the unpaired electron from the metal ion to the C-4 nucleus, with the resulting broadening of the C-4 signal.

Kotowycz & Suzuki (1973), who studied the interaction of Cu(II) with GMP by the \(^{13}\text{C} \) n.m.r. method, observed at higher copper concentrations a broadening of the C-6 resonance signal which, according to their opinion, supports participation of the carbonyl oxygen in copper complex formation. However, it is not excluded that broadening of the C-6 signal is due to transmission through the \( N_{(7)}=C_{(5)}=C_{(6)} \) bonds of the paramagnetic effect of the copper ion coordinating with N-7.

Fritzche et al. (1974), on the basis of broadening of the deoxyguanosine and dGMP \(^{13}\text{C} \) n.m.r. signals, also postulated copper binding only with N-7; however, they gave no explanation for the observed broadening of the C-4 and C-6 signals.

**Crystalline GMP-Cu(II) complex**

In the i.r. spectra of nucleotides, the region 900 - 1200 cm\(^{-1}\) corresponds to the phosphate residue. Symmetrical stretching vibration of \(-\text{PO}_3^{2-}\) (980 cm\(^{-1}\)) in GMP-Na\(_2\) was shifted to 1005 cm\(^{-1}\) for the crystalline GMP-Cu(II) complex (Fig. 7). The same changes were observed in the Raman spectra, and the shift was from 974 to 988 cm\(^{-1}\). These observations corroborate the known involvement of phosphate in the copper complex formation in solution.

![Graph](image)

**Fig. 7.** Infrared absorption spectrum, in KBr, of GMP-Na\(_2\) (—) and the crystalline GMP-Cu(II) complex (- - -).

The i.r. spectra show also the interaction of copper with purine base. The absorption profile of the complex over the region 1500 - 1700 cm\(^{-1}\) suggested overlapping of three bands. The band at 1610 cm\(^{-1}\) vanished, and a shoulder at 1625 cm\(^{-1}\) appeared. The moderately intense bands at 1540 and 1487 cm\(^{-1}\) disappeared, and
a shoulder at 1540 cm\(^{-1}\) and a weak, broad band at 1500 cm\(^{-1}\) appeared in the complex. Similar changes in the i.r. spectrum were observed with amorphous GMP-Cu(II) samples obtained by freeze-drying of an equimolar mixture of GMP and CuCl\(_2\).

The e.p.r. and magnetic susceptibility measurements indicate that the GMP-Cu(II) complex analysed is paramagnetic. The e.p.r. spectrum was asymmetrical and showed no hyperfine structure. The intensity of the spectrum increased with lowering of the temperature (\(g_{av} = 2.113\)); the reciprocal of the magnetic susceptibility was linearly dependent on temperature (\(\mu_{eff} = 1.84\) B.M. at 293\(^\circ\)K).

**DISCUSSION**

Copper may be expected to attack those atoms of a nitrogen base on which is localized the greatest negative charge due to \(\pi\)-electrons, i.e. the nitrogen atoms forming two bonds of the \(\delta\) type. N-3 and N-7 can be the sites of copper(II) coordination. Jordan & Pullman (1968) and Pullman & Pullman (1969) on the basis of quantum-mechanical calculations demonstrated that the carbonyl oxygen has the greatest negative charge of all guanosine atoms. Thus, it is probable that copper would attack the region of N-7 and C(6)=O, and this would result in changed relaxation time of the proton located in direct vicinity of these atoms, i.e. the proton bound to C-8. The observed broadening by copper of the n.m.r. signal of the H-8 proton of guanosine, 1-methylguanosine and GMP, and unchanged signals for 7-methylguanosine, are in agreement with this supposition.

Coordination of copper(II) with N-3 is theoretically possible, and it should lead to broadening of the signal of the amino group protons. The lack of the expected effect is inconsistent with this possibility. Moreover, Sundaralingam (1969), on the basis of conformational considerations, excluded the involvement of the amino group of nitrogen bases in complex formation with metals.

Direct involvement of the oxygen at C-6 in formation of the five-membered chelate should be treated with caution. From the steric data discussed in the present work it may be concluded that this is rather unlikely.

In the crystalline GMP-Cu(II) complex, as in the complex in solution, copper binds to N-7 and phosphate group. The results for the crystalline complex [Cu(C\(_{12}\)H\(_{12}\)O\(_8\)N\(_3\)P)-(H\(_2\)O)]\(_2\) are in agreement with the X-ray analysis of an analogous crystalline complex, reported by Aoki et al. (1976). However, their complex was found to be a polymer [Cu\(_5\)(C\(_{12}\)H\(_{12}\)O\(_8\)N\(_3\)P)\(_3\)·(H\(_2\)O)\(_8\)·4H\(_2\)O].

**REFERENCES**

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ODDZIAŁYWANIE JONÓW METALI Z KWASAMI NUKLEINOWYMI
INTERAKCJA MIEDZI(II) Z GUANOZYNĄ I JEJ POCHODNYMI

Streszczenie

Badano metodami spektroskopowymi oraz magnetochemicznymi interakcję miedzi(II) z guanozyną, 2'-dezoksyguanozyną, 1-metyloguanozyną, 7-metyloguanozyną i GMP.

W guanozynie głównym miejscem wiązania miedzi(II) jest azot N-7; nie wykluczony jest udział azotu N-1. Równoczesne wiązanie miedzi z azotem N-7 i tlenem przy C-6 jest mało prawdopodobne.

Otrzymano krystaliczny kompleks miedzi(II) z GMP o wzorze: Cu(C₉H₅O₄N₃P)_2·(H₂O)_₃. Wykazano, że miedzi(II) oddziaływuje z azotem N-7 i z grupą fosforanową nucleotydów.

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