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Interaction of Copper Ion with Guanosine and Related Compounds*

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ABSTRACT: The complexing of copper(II) ion with guanosine, guanosine 5′-monophosphate, inosine, inosine 5′-monophosphate, and theophylline at pH 7.0 was studied with the aid of conductometric and potentiometric titrations, spectrophotometric titrations, and infrared absorption spectroscopy. Equimolar ratios of copper to the compounds tested were observed with no hydrogen ion release on complexing. No complex formation was observed for uridine, uridine 3′-(2′)-monophosphate, cytidine, cytidine 3′-(2′)-monophosphate, caffeine, and ribose using similar methods. Considerable diminution of the keto stretching vibration band was observed in the region of 1680–1720 cm^{-1} on complexing with copper ion. With these findings, it is proposed that the copper atom formed a pentacyclic complex involving enolic O at C-6 and N at 7.

Interaction of nucleic acids and polynucleotides with certain metal ions has been observed by a number of investigators. Yatsimirskii and Kris (1966) concluded that Fe^{3+}, Cu^{2+}, and Mn^{2+} attached chiefly to the phosphate group in DNA; however Cu^{2+} and Fe^{3+} also attached to DNA bases. Fishman *et al.* (1967) concluded that the magnesium ion bound only to the phosphate group in DNA. Coates *et al.* (1965) found cupric ion combining with heat-denatured DNA, thus suggesting that a copper complex was formed with some of the bases. However, Daune *et al.* (1966) proposed that silver ion is bound to the nitrogen atoms of purine and pyrimidine bases in polynucleotides.

The silver ion is believed to be attached only to purine and pyrimidine residues in DNA (Jensen and Davidson, 1966). Ropars (1966) postulated that guanine base was involved in copper–DNA complexes. Bryan and Frieden (1967) reported that the binding of cupric ion to DNA was sensitive to copper concentration. Schreiber and Daune (1967) investigated copper interactions with three different DNAs (*Escherichia coli*, *Micrococcus lysodeikticus*, and veal thymus); they found the same association constant regardless of DNA origin. This suggested that some definite group in DNA was involved in the complexing.

Eichhorn and Clark (1965) reported that copper ion helped in the unwinding of the double-helix DNA strands. Ivanov *et al.* (1967) found that the manner of copper binding to native and denatured DNA was different.

From these examples, it is clear that copper and other metal ions interact with nucleic acids; however, due to complexity and large molecular size, the exact location of the metal in the complex could not be identified. Metal complexes of such biologically important compounds cannot be elucidated precisely without fundamental knowledge regarding metal interaction with the components of nucleic acids. In a previous paper, we have reported the interaction of silver ion with guanosine and related compounds (Tu and Reinosa, 1966). In this investigation, the exact location of the silver atom was determined.

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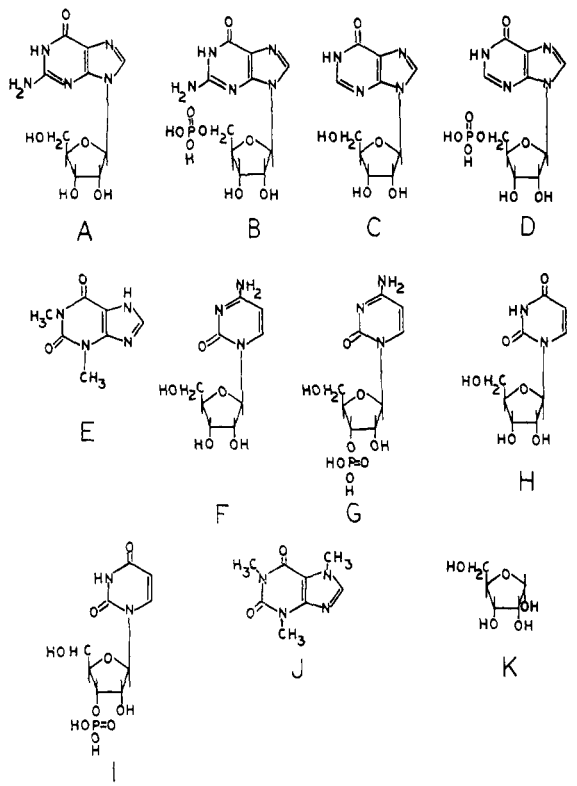


FIGURE 1: Structures of the compounds studied. (A) Guanosine, (B) GMP, (C) inosine, (D) IMP, (E) theophylline, (F) cytidine, (G) CMP, (H) uridine, (I) UMP, (J) caffeine, and (K) ribose.

In order to determine the location of copper in the complex, guanosine and GMP-5′, inosine, IMP-5′, and theophylline were studied. Uridine, UMP-3′(2′), cytidine, CMP-3′(2′), caffeine, and ribose were also used for further study.

Methods

Conductometric titration was done essentially by the method of Tu and Reinosa (1966). Samples containing 50–100 μmoles were dissolved in deionized water, adjusted to pH 7.0 with NaOH, and adjusted to a final volume of 50 ml. The solution was titrated 1 ml at a time with 0.01 M $CuCl_2$; the conductivity was measured at 25° using a conductivity meter, Radiometer CDM2 with a double platinum plate electrode. A water blank was run under the same conditions.

Potentiometric Titration. $CuCl_2 \cdot 2H_2O$ (1 ml of 1 mM) was titrated with 0.2 mM guanosine; the potential was measured using the cupric ion activity electrode, Model 92-29, manufactured by Orin Research Inc., Cambridge, Mass. A conventional calomel–KCl reference electrode was used in conjunction with the cupric ion activity electrode. A potentiometer, Model TTTlc Radiometer titrator with scale expander, type PHA 630 T, was used.

Spectrophotometric Titration. Absorption spectra of each sample at pH 7 was measured before and after complexing with cupric ion. The wavelength of maximum ultraviolet absorption was used for spectrophotometric titration; to obtain the desired information the absorbance change was plotted against the mole ratio of cupric ion to sample.

A Beckman DB-G ultraviolet spectrophotometer with recorder attachment was used for this study.

Hydrogen Ion Release. Each sample containing 50 μmoles was dissolved in deionized water, adjusted to pH 7, and made to a final volume of 25 ml. $CuCl_2$ (50 μmoles) was added and the mixture was incubated for 30 min at 25°; this was titrated with 0.01 N NaOH to the original pH of 7.0 using a Radiometer automatic pH titrator and autoburet, type ABUla. A water blank at pH 7.0 was also used.

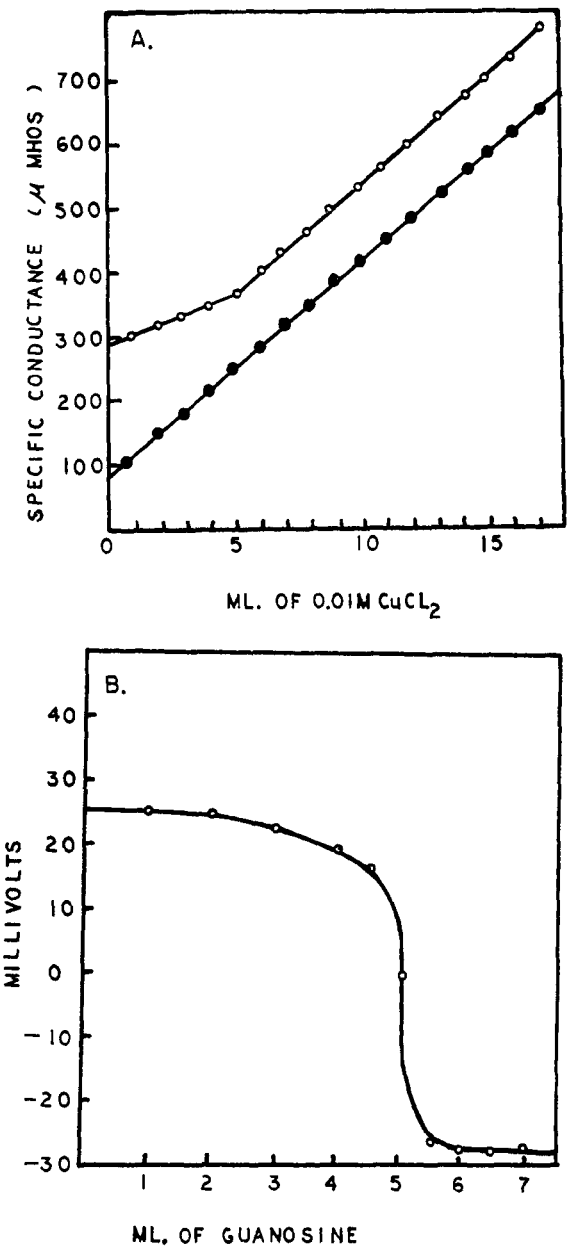


FIGURE 2: (A) Conductometric titration of GMP with $CuCl_2$. GMP (50 μmoles) in 50 ml of deionized water at pH 7.0 was titrated with 0.01 M $CuCl_2$. (-O-O-) GMP; (-●-●-) water blank at pH 7.0. (B) Potentiometric titration of $CuCl_2$ with guanosine. $CuCl_2$ (1 ml of 1 mM) was titrated with 0.20 mM guanosine.

TABLE I: Moles of Copper Ion Combined with the Compounds Tested by Conductometric Titration, Potentiometric Titration, and Spectrophotometric Titration.

Compounds	Mole Ratio[a] by Conductometric or Potentiometric Titration	Mole Ratio[b] by Spectrophotometric Titration	Nearest Integer
Guanosine	1.019[c]	1.070	1
GMP-5′	0.995 ± 0.005	1.037	1
Inosine	0.975 ± 0.014	0.984	1
IMP-5′	0.987 ± 0.062	0.969	1
Theophylline	0.966 ± 0.068	0.947	1
Cytidine	0.009 ± 0.003	0	0
CMP-3′(2′)	0.055 ± 0.010	0	0
Uridine	0.040 ± 0.050	0	0
UMP-3′(2′)	0.055 ± 0.060	0	0
Caffeine	0	0	0
Ribose	0	0	0

[a] Mole ratio was calculated based on ten separate determinations for each compound by conductometric titration. The result was expressed as mean ±95% confidence limit. [b] Three determinations were made for each compound at the wavelength of maximum absorption. Average mole ratio was calculated. [c] Three experiments were made by potentiometric titration using cupric ion activity electrode.

Infrared absorption spectra were made using 1% KBr pellets by the procedure of Tu and Reinosa (1966). Mineral oil (Nujol) was also used for infrared studies; a dry sample was mixed with Nujol and smeared on a transparent NaCl window. A Beckman Model IR-5 double-beam recording spectrophotometer was used for obtaining the spectra in the region from 625 to 5000 cm^{-1}. GMP or IMP was mixed with an equimolar amount of cupric ion and any excess ion was removed by dialysis at 3°. Water was removed by lyophilization and the solid copper complex was pulverized and thoroughly mixed with KBr. A thin transparent pellet was made by compressing. Unlike GMP and IMP, guanosine, inosine, and theophylline formed soluble complexes; therefore, these compounds were mixed with an equimolar amount of copper salt and the mixture was lyophilized. The dried samples were studied using the infrared spectra of the corresponding KBr pellet.

Results

Mole Ratio. The chemical structure of compounds tested is listed in Figure 1. The experiment was carried out at an initial pH of 7.0 and a final copper concentration of 10^{-3} M for conductometric titration and 10^{-5} M for spectrophotometric titration. Both conductometric titration and spectrophotometric titration

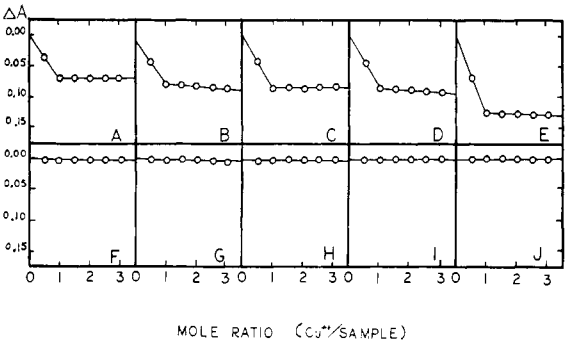


FIGURE 3: Spectrophotometric titration at the wavelength of maximum absorption. (A) Guanosine, (B) GMP, (C) inosine, (D) IMP, (E) theophylline, (F) cytidine, (G) CMP, (H) uridine, (I) UMP, and (J) caffeine.

indicated that 1 mole of copper ion combined with 1 mole each of GMP-5′, inosine, IMP-5′, and theophylline. No combination was observed for cytidine, CMP-3′(2′), uridine, UMP-3′(2′), caffeine, and ribose under these conditions. However, at an initial pH of 7.0 and higher final copper concentration of 0.1 M, cytidine and CMP combined with copper. A sharp deflection point in the conductometric titration was not observed for guanosine; therefore potentiometric titration using a cupric ion activity electrode was employed. A unimolar ratio was also obtained for guanosine either using potentiometric titration or spectrophotometric titration.

Examples of conductometric titration using GMP-5′ and potentiometric titration with guanosine are illustrated in Figure 2A,B. It can be seen from these figures that sharp deflection points which correspond to a unimolar ratio were obtained.

When the ultraviolet absorption spectra was examined, it was noted that the compounds which combined with copper ion (Table I) gave a pronounced hypochromic effect at the wavelength of maximum absorption. By utilizing this property of hypochromicity the mole ratio was verified; these are shown in Figure 3. Guanosine, GMP-5′, inosine, IMP-5′, and theophylline gave distinct deflection points which are equivalent to the value obtained by the conductometric method. Uridine, UMP-3′(2′), cytidine, CMP-3′(2′), and caffeine did not give such deflection points (Figure 3).

Ribose does not have absorption at 260 mμ; thus spectrophotometric titration could not be used.

Hydrogen Ion Release. Equimolar amounts of Cu(II) ion were mixed with each compound at pH 7.0 and allowed to stand 30 min. At the end of 30 min, the mixture was back-titrated with 0.10 N standard NaOH for possible hydrogen ion release. No hydrogen ion release was observed for the compounds tested, as the same titers of standard NaOH were used in the $CuCl_2$ blank at pH 7.0. The amounts of standard NaOH used were 1.54 ml for guanosine, 1.39 ml of GMP, 1.49 ml for inosine, 1.43 ml for IMP, 1.58 ml for theophylline, 1.51 ml for uridine, 1.47 ml for UMP, 1.40 ml for cytidine, 1.47 ml for CMP, 1.47 ml for caffeine, 1.42 ml for ribose, and 1.50 ml for $CuCl_2$ solution.

This clearly indicated that no hydrogen ion was released.

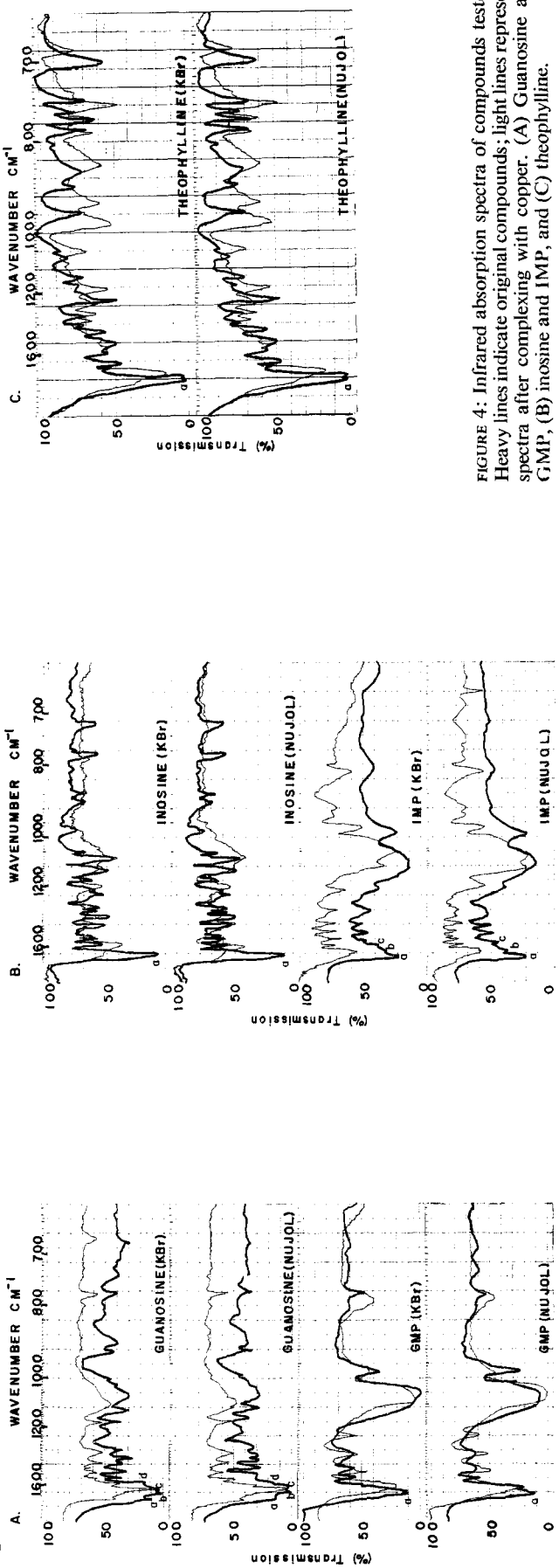


FIGURE 4: Infrared absorption spectra of compounds tested. Heavy lines indicate original compounds; light lines represent spectra after complexing with copper. (A) Guanosine and GMP, (B) inosine and IMP, and (C) theophylline.

Infrared Spectra. In previous investigations it was found that keto groups in purine and pyridimine bases gave strong stretching vibration bands in the region of 1680–1720 cm^{-1} (Blout and Fields, 1950; Tu and Reinosa, 1966).

It is generally recognized that the keto group in aromatic rings gives an intense stretching vibration band in the region of 1680–1700 cm^{-1} (Silverstein and Bassler, 1966). Aromatic C=C and C=N stretching bands in purine rings usually appeared in the 1555–1630-cm^{-1} region (Blout and Fields, 1950; Tu and Reinosa, 1966) (Figure 4).

The KBr pellet was made by compressing sample and KBr under high pressure. The spectra was also taken in a thin film using mineral oil. This was done to confirm that the infrared spectra is not affected artificially under high pressure. In each case, practically comparable spectra were obtained using either KBr or mineral oil.

When guanosine combined with cupric ion the C=O bond stretching bands in guanosine diminished and gave only a small peak and small shoulder at the same wave number (1725 cm^{-1} at a, 1690 cm^{-1} at b). Intense bands at 1610 cm^{-1} (c) and a smaller peak at 1590 cm^{-1} (d) were observed for Cu^{2+}–guanosine. The wave numbers at these two bands corresponded exactly to the original guanosine. Similarly, a band at 1690 cm^{-1} in GMP disappeared almost completely leaving only a small shoulder after complexing. The intense peak of inosine at 1680 cm^{-1} diminished considerably on complexing either using KBr pellet or mineral oil. When IMP-5′ combined with cupric ion, the band at 1700 cm^{-1} (a) disappeared and a prominent band at 1660 cm^{-1} was formed. The 1680-cm^{-1} peak of theophylline diminished on complexing and gave only a trace of the peak at the same wave number; an intense band at 1600 cm^{-1} was formed after combining with copper(II) ion. The wave number of this new peak is within the region of C=C and C=N bond stretching bands of purine compounds.

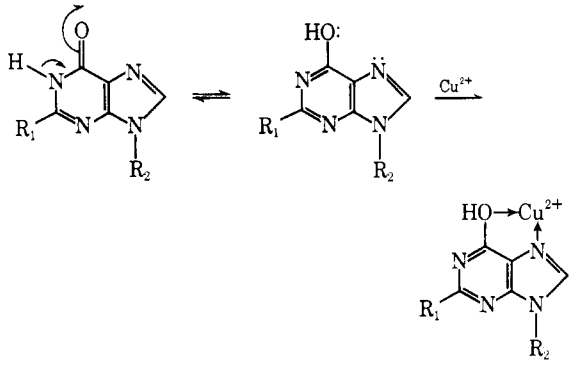
Discussion

Both conductometric (or potentiometric) titration and spectrophotometric titration indicated 1 mole of cupric ion combined with 1 mole each of guanosine, GMP-5′, inosine, IMP-5′, and theophylline. In contrast, all of the pyrimidine compounds tested (cytidine, CMP-3′(2′), uridine, and UMP-3′(2′)) gave no complexing at low copper concentration. These facts indicate that the ring structure involving C-4, C-5, N-7, C-8, and N-9 is essential for complexing with cupric ion.

Fiskin and Beer (1965) observed that cytidine at an initial pH of 4 in 1 M $NaNO_3$ formed a complex at a 0.1 M copper concentration. Eichhorn *et al.* (1966) also found that dCMP formed a complex at a concentration of 0.1 M copper nitrate and 0.1 M dCMP.

In our experiment we found no complex formation up to 1×10^{-3} M copper and cytidine concentration. When the experiment was done at the concentration of 0.1 M copper and cytidine, complex formation was observed.

SCHEME I



It is of particular interest to note that the cupric ion combined with theophylline but not with caffeine. Theophylline and caffeine are similar in structure except the former has methyl groups at N-1 and N-3 leaving N-7 free while the latter has three methyl groups occupying all three positions at N-1, N-3, and N-7.

From this result, it is indicated that N-7 and the keto group at C-6 are involved in complexing.

This was also the case for silver ion. Tu and Reinosa (1966) reported that silver ion complexed with guanosine, GMP-3′(2′), inosine, IMP-5′, and theophylline in a unimolar ratio, but negative results were obtained for uridine, UMP-3′(2′), caffeine, and ribose. In contrast to the case of silver ion, no hydrogen ion was released from the compounds tested after complexing with cupric ion; therefore, cupric ion did not displace hydrogen atom on complexing. The lack of hydrogen ion release by copper nucleosides was also shown by Eichhorn *et al.* (1966).

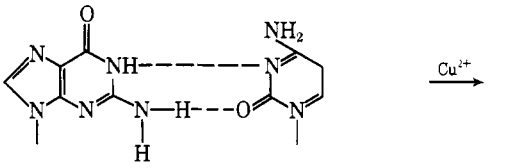
Interaction of cupric ion with DNA has been studied by a number of workers, and they proposed that guanine base is involved in complexing. The importance of N-7 of guanine base in copper–DNA complex was stressed by Eichhorn *et al.* (1966) and Minchenkova and Ivanov (1967).

Our conductometric study also indicated that the N-7 is involved; moreover, the infrared spectra indicated that the oxygen atom at C-6 was involved in complexing. In each case the C=O stretching vibration band diminished on complexing with cupric ion. This suggested that the C-6 lost keto group character on complexing; therefore, the most probable mechanism is proposed and shown in Scheme I.[1]

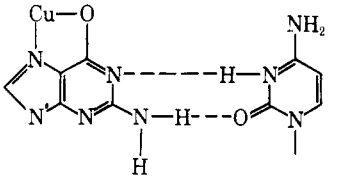
Ropars and Viovy (1965) found that cupric ion interacted with calf thymus DNA. They suggested that the copper atom is located between the oxygen at C-6 and N-7 in a structure comparable with ours.

The presence of a hydrogen atom after complexing with copper is in agreement with the finding of Drozdov-Tikhomirov and Kikoin (1967). They found the presence of enol forms owing to hydrogen in a copper–guanosine complex.

A somewhat different mechanism for Cu^{2+}–DNA formation was proposed by Minchenkova and Ivanov.



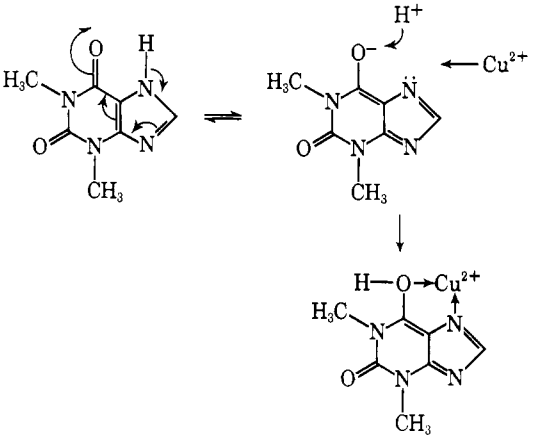
guanine–cytosine complementary pair in DNA



Cu–DNA complex

In this proposed copper–DNA complex, it is suggested that proton transfer from the N-1 of guanine to the N-1 of cytosine occurred on complexing.

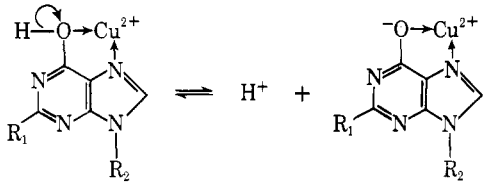
The proposed mechanism for copper–theophylline formation is illustrated here.



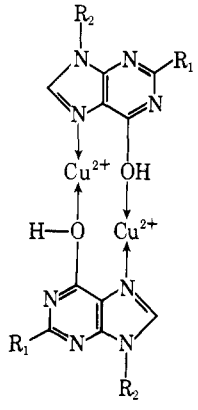
The evidence shown in the infrared spectra indicates that the metal attached both to the oxygen at C-6 and to N-7. Hypochromicity of GMP and IMP at maximum absorption after complexing also suggested that copper atom is likely in the bases rather than phosphate group. Guanosine, inosine, and theophylline, which do not have phosphate groups, also reacted with cupric ion similar to the corresponding nucleotide. Eichhorn *et al.* (1966) studied the interaction of dAMP and dTMP with copper ion using ^{31}P nuclear magnetic resonance spectra. They concluded that the metal interacts with phosphate group as well as with the bases as there were changes in the ^{31}P nuclear magnetic resonance spectra. Sigel (1968) determined the acidity constants of the N-1 proton in the nucleotides ITP and GTP and found them to be 9.2 and 9.6, respectively. The acidity constants of copper complexes were more acidic than the original compounds.

[1] $R_1 = NH_2$, R_2 = ribosyl group, for guanosine; $R_1 = NH_2$, R_2 = ribosyl 5′-phosphate, for GMP; R_1 = H, R_2 = ribosyl group, for inosine; R_1 = H, R_2 = ribosyl 5′-phosphate, for IMP.

Hydrogen atom in enolic form shown below would be more acidic than the hydrogen atom at N-1.



The proposed structure in Scheme I is reasonable; however, the alternative structure involving 2 moles each of the compounds and cupric ion cannot be excluded as it gives a unimolar ratio.



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