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A NEW CYTOSINE-COPPER PARAMAGNETIC COMPLEX. SPECTROSCOPIC STUDY

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Two different copper complexes with cytosine molecules are formed in the process of crystal growth from the aqueous solution with traces of copper. One of them is diamagnetic, turning into paramagnetic upon ionizing irradiation (complex I). The other, the subject of the present study, is paramagnetic (complex II) as prepared. For complex II, EPR spectra demonstrate that the copper ion is coordinated with one nitrogen atom and three oxygen atoms. On the basis of the detailed EPR spectroscopic analysis and quantum-chemical calculations (in the DFT approach) the model of the complex has been proposed. Both experimental data and the theoretical results support the model with the copper atom, located between the two cytosine ribbons, ligated to a nitrogen and an oxygen atom from two opposing cytosine molecules and two oxygen atoms from water molecules. For complex II the Raman spectra demonstrated concerted restructuring of the hydrogen bonding in the cytosine crystal matrix upon insertion of copper ions.

Keywords: copper-cytosine complex, EPR, cytosine monohydrate

1. **Introduction**

It is well established that metal ions interact with various groups of nucleic acids [1, 2]. Electron paramagnetic resonance (EPR) has been used to study the complexes of nucleic acids, or their constituent parts, with paramagnetic ions. One of the earliest systems studied in such a way was cytosine-copper complex in the crystalline matrix of cytosine monohydrate [3]. From the careful analysis of the spectroscopic data, the authors, Toriyama and Iwasaki, could not identify the complex with certainty. The data did not fit well the complex of copper with two hydrogen-bonded cytosine molecules (cytosine-copper complex I), although an alternative structure was not feasible. Recently, we showed that indeed, the proposed copper complex (cytosine-copper complex I) with two hydrogen-bonded cytosines gives rise to completely different EPR spectra [4]. That fact stimulated us to further investigate the original system of Toriyama and Iwasaki (cytosine-copper complex II). The major difference between the two complexes is the ligand structure: complex I has three ligated nitrogen atoms and complex II only one.

We first reproduced the preparation and spectroscopic analysis of the system studied by Toriyama and Iwasaki (TI) and then searched for an alternative model of the cytosine-copper complex that would fit the experimental data. Here we report the results of our analysis and present the model for the cytosine-copper complex II, that is reasonably described by the observed experimental data and substantiated by the quantum-chemical calculations and the Raman spectra.

2. **Experimental methods**

Single crystals of cytosine monohydrate with copper impurities were grown by slow evaporation of the aqueous solution, to which a small amount of copper nitrate was added. Partly deuterated crystals, in which hydrogen atoms bound to nitrogen and oxygen were replaced by deuterium, were grown from the heavy water solution in the same way. The structure of the cytosine monohydrate crystals has been known for a long time [5]. The crystals as grown exhibited nice EPR signals, essentially the same as those reported [3]. EPR measurements were done with a Bruker X-band ELEXYS 500 EPR spectrometer at room temperature.

The **g** tensor, (and copper) and the copper coupling tensor, $\mathbf{A}^{\mathrm{Cu}}$, were evaluated with the use of the Computer Program EPR-NMR (Department of Chemistry, University of

Saskachewan, Canada) [6]. In the first step the positions of the EPR lines were used for the evaluation of **g** and $\mathbf{A}^{\mathrm{Cu}}$ in a second-order perturbation treatment of the spin Hamiltonian. The $\mathbf{A}^{\mathrm{N}}$ tensor was calculated separately in a first-order perturbation treatment.

Raman spectra of cytosine monohydrate single crystals were recorded using a Perkin Elmer Spectrum GX FT Raman spectrometer with 4 cm^{-1} resolution. The excitation was performed with a NdYaG laser, irradiating at 1064 nm, with power of 100 mW.

3. **Results**

The EPR spectrum recorded for the magnetic field parallel to the axis of the crystal symmetry is presented in Fig. 1. For a complete analysis of the spectroscopic properties the spectra were recorded upon step-wise rotation of the crystal about the three mutually perpendicular crystallographic axes, **<a*>**, **<b>** and **<c>** [5]. Generally, the EPR spectra of a Cu-complex consist of two sets of quartets, as a result of hyperfine interaction of the unpaired electron with two naturally occurring copper isotopes, ^{63}Cu and ^{65}Cu, having the same spin (I = 3/2) but slightly different magnetic moments [4]. Moreover, two magnetically distinct sites in the crystal give rise to two distinct sets of EPR spectra, making altogether four superimposed quartets, what makes the analysis more difficult. In the present study only the complex with naturally more abundant ^{65}Cu isotope was spectroscopically analyzed in more detail.

Angular variation of the EPR line positions and hence the derived spectroscopic parameters are essentially the same as those reported earlier [3]. A selection of the derived data is presented in Table 1. In both studies the **g** and $\mathbf{A}^{\mathrm{Cu}}$ tensors were found to be parallel, their maximum values deviating from the normal, **n,** to the cytosine ring plane by 17° in the earlier study and 14° in the present study, in good agreement with theoretically predicted value of 19° obtained from optimization of the model structure B.

Table 1
Comparison of the derived spectroscopic parameters for copper complex II

Source of data	g		A_{Cu}			A_{N}		
	Principal values	φ[a]	Principal values (×10^{-4} cm^{-1})	φ[a]	ψ[b]	Principal values (×10^{-4} cm^{-1})	φ[a]	ψ[b]
TI[c]	2.253	17	182.0	17	0	14.5	89	72
	2.057		17.5			10.7		
	2.053		−9.6			10.7		
Present (exper.)	2.259	14°	183.3	15°	1°	14.4	83°	96°
	2.057		12.7			11.7		
	2.055		9.0			11.9		
Theory A	2.282	29°	263.8	26°	3°	17.1	64°	90°
	2.057		38.6			12.2		
	2.055		27.8			11.9		
Theory B	2.237	28°	254.4	30°	2°	16.1	85°	81°
	2.097		49.0			11.6		
	2.065		28.2			11.2		

[a] Angle between maximum value of the tensor and n.
[b] Angle between maximum value of the tensor and g_{max}.
[c] Data from Ref. [3].

The significant deviation of $\mathbf{g}_{max}$ and $\mathbf{A}^{Cu}{}_{max}$ from the direction of **n** and the observation of only one nitrogen atom as a ligand suggests the positioning of the copper ion between two ribbons of the hydrogen-bonded cytosine molecules (See Fig. 2a).

From inspection of the crystal matrix we come to the only feasible model of copper complex II with the following ligands: the nitrogen atom from the cytosine amino group, the oxygen atom from opposing cytosine and two oxygen atoms from water molecules. The proposed complex could be formed either by displacement of one of the water molecules toward the copper ion, or by insertion of a water molecules into a cytosine vacancy in the crystal matrix.

The selected crystals of cytosine monohydrate with complex-I and complex-II were further investigated with the IR Raman spectroscopy to look for the possible structural differences in the two systems. Gamma-irradiated crystals gave the same spectra as the non-irradiated crystals.

The largest differences in the spectra of two crystals with different copper complexes appear at the high frequency region (Fig. 3). The spectra were recorded with the incident laser beam perpendicular to the *bc* crystal plane and the **<c>** axis held in upright position. As seen from the figure, the spectra of the two systems differ significantly in the broad complex band in the region from 2800 cm^{-1} to 2900 cm^{-1}. That region has not been analyzed so far [7], and could be assigned to the stretching and bending vibrations of water molecules in the hydrogen bonding network [8].

In order to further substantiate the model, quantum-chemical calculations of the spectroscopic parameters for the proposed copper complex were performed and the results compared with the experimental findings. The calculations were done with the use of the B3PW91 density functional method with Gaussian 03 package [9]. The geometry of the proposed complex was partly optimized using the 6-31G(d) basis set. For preservation of charge and satisfying multiplicity conditions, one of the water molecules participating in the bonding was deprotonized. The restrictions imposed by the crystal lattice to the flexibility of the copper complex were taken care of by keeping four ring atoms in each cytosine molecules (far from the anticipated copper ligands) fixed at their lattice positions during the optimization procedure. Two geometries of the paramagnetic centers were optimized: one with two cytosine molecules and two additional water molecules surrounding copper (structure A, Fig. 2b) and the other composed as above, with added three nearest cytosine molecules (structure B, not shown). The optimization of the structure was followed by single-point calculation of the electronic properties using the same density functional method and 6-311+G(2d,p) basis set. The theoretically derived data are shown in Table 1, together with the two independent sets of experimental values [3, 4].

4. Discussion

Two spectroscopically different paramagnetic copper centers found in the crystal matrix of cytosine monohydrate were assigned to the same structure by two groups of investigators [3, 4]. We have shown that the assignment, here named cytosine-copper complex I, is correct only for the crystals grown from the cytosine aqueous solution, with added small amount of powdered copper. The paramagnetic complex is formed only after ionizing irradiation [4], implying that copper is incorporated in the cytosine matrix as a monovalent cation, probably in a non-paramagnetic Cu-cytosine complex, transforming into a paramagnetic species upon irradiation by trapping a migrating hole formed in host matrix.

The other center, named cytosine-copper complex-II, gives EPR signals in the crystals as grown, meaning that the paramagnetic copper ion, Cu (II), is incorporated into the crystal matrix. The orientation of the spectroscopic tensor elements derived from EPR spectra and supported by DFT calculation demonstrate that the complex plane is not identical to the cytosine ring plane; these two planes differ in orientation by about 15^{o}. Another important spectroscopic feature of complex II is the coordination of copper: only one nitrogen atom and presumably three oxygen atoms as ligands. The available site for such positioning of the copper ion is between two adjacent ribbons of the cytosine molecules in the crystal matrix, as presented in Fig. 2a. The only alternative structure could be positioning of the copper ion into the planar hydrogen bonding network composed of water molecules and the cytosine rings. [10]. The possibility could be safely excluded because in that case the plane of the complex would be almost perpendicular to the cytosine rings, which is in contrast to the experimental results.

According to the crystal data, the four ligand atoms for the proposed complex-II are not all coplanar; the planarity could be achieved by a small shift of the ligated water molecules after incorporation of copper. The rings of the two opposite cytosine molecules involved in the complex are parallel, but being in two different ribbons they are slightly shifted one against the other. That fact could explain the orientational displacement of the complex plain from the cytosine ring plane.

In the process of the complex formation, due to the conservation of charge two hydrogen atoms must be released: one from the cytosine amino group and the other from one of water molecules, leading to the structure presented in Fig. 2b. The values of the spectroscopic parameters derived from the theoretical calculations are generally in a reasonable agreement with those derived from the experiments. The principal values of **g** are predicted in the range of the experimentally observed values, and the directions differ by about 10^o from the respective experimental values (Table 1). The fit of the hyperfine couplings to the experimentally derived values are generally better for nitrogen than for copper for both model structures, A and B. From our both experimental and theoretical data $\mathbf{A}^N_{max}$ is essentially in the plane perpendicular to $\mathbf{g}_{max}$, what is expected for a copper complex. Significant overestimation of the copper couplings using DFT is a known fact [11]. More complex structure B is more reliable, because it better mimicks the actual structure in the crystal, allowing for the displacement of the unpaired electron to the surroundings, as shown earlier [12, 13]. For both model structures the calculations predict additional proton coupling of about $6 \cdot 10^{-4}$ cm^{-1} that is not observed, indicating some rearrangement in the crystal matrix, not taken into account in the theoretically investigated model structures.

We tried to address the differences in structure between complex I and complex II by inspecting if there were some subtle differences in the hydrogen bonding network in the two respective crystals, possibly detected in the Raman spectra. We found the intensity of the broad band above 2800 cm^{-1} to differ significantly in the two systems (Fig. 3). Such a broad complex band has been observed in the IR and Raman spectra of a number of various molecular systems with hydrogen bonding network. The suggested number of underlying vibrations contributing to this band varies among the scientists [14]. There is no common agreement in what is important for the band structure: the number of hydrogen bonds, type of hydrogen bond, bending angles of water, nature of the formed or broken donor and acceptor hydrogen bonds and other. The profile and intensity of complex band depends on the number and ratio of individual absorptions. The higher intensity and better resolution of the lines in this band in the spectra of crystals with complex-II gives the evidence on the different vibrational dynamics of crystalline water in two the matrices.

It should be emphasized that the molar percentage of the copper atoms in the crystals with copper complexes ranges typically between 0.1 and 0.3 %. That means that a small concentration of point defects, introduced into the crystal lattice of cytosine monohydrate by incorporation of copper, induces severe changes in the hydrogen bonding network, easily detected in the Raman spectra. Apparently, the formation of point defects triggers concerted restructuring of the hydrogen bonding network; switching a proton to or from the copper center induces changes in the hydrogen bonding of the neighboring molecules, with subsequent changes in the next neighbors etc. It seems that, in contrast to the situation in the system with cytosine-copper center II, the deprotonation induced upon formation of cytosine-copper complex I does not bring about concerted restructuring of the hydrogen bonding.

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References

[1] W. Saenger, Principles of Nucleic Acid Structure, Springer, New York, 1984.

[2] A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems. Interaction of Metal Ions with Nucleotides, Nucleic Acids and their Constituents, Vol. 32, Marcel Dekker, New York, 1996.

[3] K. Toriyama, M. Iwasaki, J. Amer. Chem. Soc. 97 (1975) 6456-6460.

[4] D. Krilov, A. Lekić, E. Bešić, J.N. Herak, J. Inorg. Biochem. 99 (2005) 886-889.

[5] G.A. Jeffrey, Y. Kinoshita, Acta Cryst. 16 (1963) 20-28.

[6] Computer Program EPR-NMR v. 6.5, Department of Chemistry, University of Saskatchewan, Canada (accessible at URL: http://www.chem.queensu.ca/eprnmr/), Mar, 4 2003.

[7] J. Florian, V. Baumruk, J. Leszczynski, J. Phys. Chem. 100 (1996) 2278-5589.

[8] N.B. Colthup, L.H. Daly, S.E. Wiberley, Introduction to infrared and Raman spectroscopy, third ed., Academic Press, San Diego, 1990.

[9] M.J. Frisch et al., Gaussian 98 Revision A.11, Gaussian, Pittsburgh, PA (2001).

[10] G.A. Jeffrey, W. Saenger, Hydrogen bonding in biological structures, Springer, Heidelberg, 1991.

[11] E. Bešić, V. Gomzi, J.N. Herak, D. Krilov, Spectrochim. Acta A 61 (2005) 2803-2808.

[12] V. Gomzi, J.N. Herak, J. Molecul.Structure: THEOCHEM 629 (2003) 71-76

[13] V. Gomzi, J.N. Herak, Chemical Physics 333 (2007) 112-118

[14] M. Chaplin: Outline of methods for investigating water structure, in Water structure and behavior, http://www.lsbu.ac.uk/water/methods.html 2007.

Figures:

Fig. 1 EPR spectrum of single crystal of cytosine monohydrate grown from water solution with added traces of copper powder. Magnetic field ***B*** is along crystallographic axis <**b**>. The microwave frequency was 9.882 GHz and microwave power 10 mW. The stick diagrams under the spectrum indicate the positions of four groups of lines for two copper isotopes.

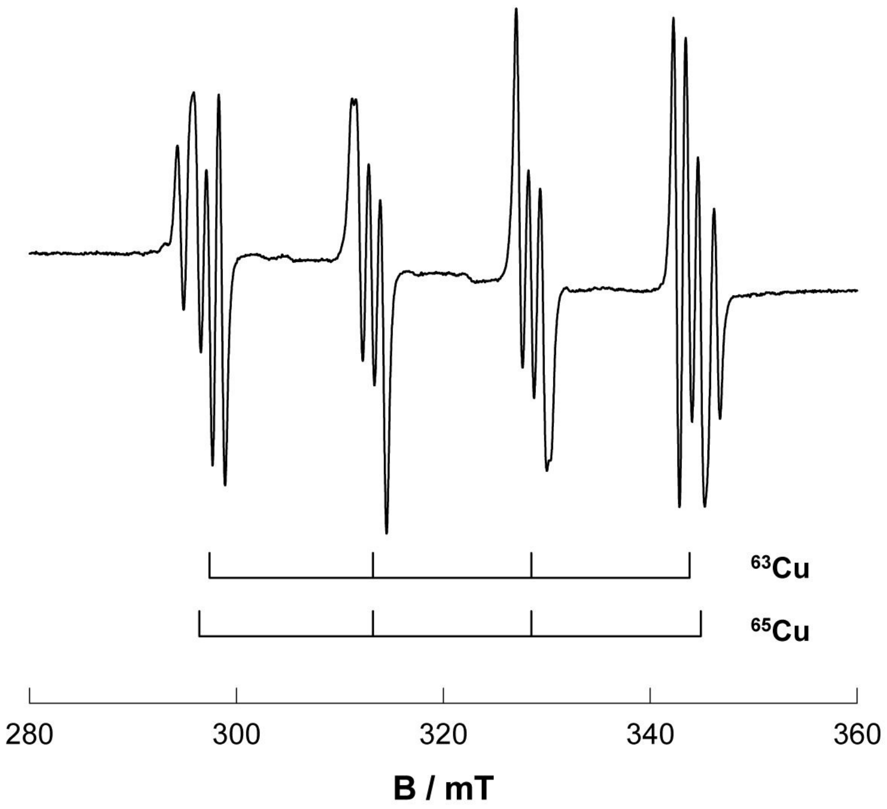

Fig. 2 (a) The proposed positioning of copper ion between the two ribbons of cytosine molecules, viewed in the *a*b* plane; (b) Geometry optimized model structures for a model with two cytosine molecules (structure A)

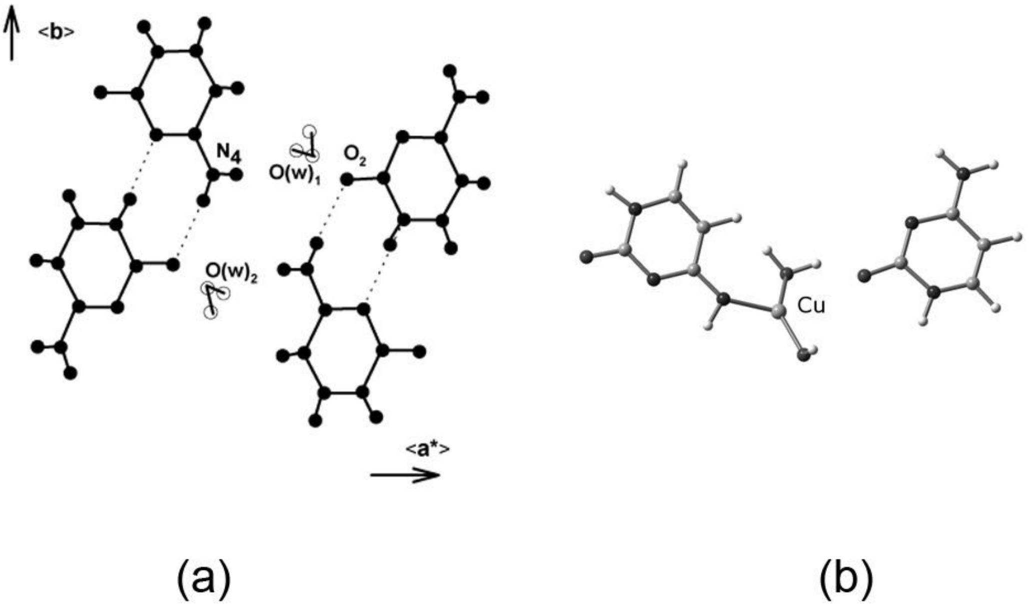

Fig. 3 IR Raman spectra (high frequency region) of single crystals of cytosine monohydrate with two different copper complexes. The crystal was positioned with the *bc* plane perpendicular to the incident laser beam. The spectra were recorded for the **<c>** axis in upright position. Spectrum (a) is from a crystal with complex-I and spectrum (b) from crystal with complex-II. The excitation was performed with NdYaG laser at 1064 nm, with power 100 mW, and resolution 4 cm^{-1}.