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EPR SPECTROSCOPY OF SUPERHYPERFINE INTERACTION IN THE COPPER PARAMAGNETIC CENTER IN THE CRYSTAL OF CYTOSINE MONOHYDRATE

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Dedicated to the memory of Professor Vladimir Šips

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The planar complex of Cu(II) with two cytosine bases was formed by gamma irradiation of the cytosine monohydrate crystal, containing copper impurities. The paramagnetic complex was probably formed by trapping the radiation-induced migrating holes by Cu(I), that is originally present in the crystal lattice. The structure of the complex was analyzed from the superhyperfine pattern of its EPR spectra. The experimentally evaluated tensor elements of the three ^{14}N couplings and the observation of an additional proton coupling unequivocally confirm the structure. The *ab initio* B3LYP density functional method calculations are in good agreement with the experiments. Both experiments and the calculations demonstrate large spin delocalization in the cytosine ring plane.

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1. Introduction

Bonding of metal ions with DNA induces various physical and chemical changes, which might be of significant biological importance [1,2]. The type of ion, the place and the nature of bonding determine possible alteration of biological function of DNA [3]. Metal ions can be covalently bonded to the sugar-phosphate backbone or to the bases [2]. Alternatively, they could be incorporated between the pairs of the bases, thus replacing regular hydrogen bonds by much stronger coordination bonding.

The complexes of metal ions with the nucleic acid bases can be used as model systems for a more general investigation of the coordination bonding in DNA [4]. The bases with traces of the transition-metal ions could be grown in a suitable single crystal form. In such systems, the metal-ion complexes with the bases could be formed, analogous to those in natural nucleic acids. If the complexes are paramagnetic, being in single crystals, they could be analyzed in quite a detail with the use of the electron paramagnetic resonance (EPR) spectroscopy.

In our earlier paper [5], we described the planar complex associated with the copper ion in the crystal lattice of cytosine monohydrate. In the unperturbed crystal, two pairs of hydrogen bonds connect the ring atoms N_1 and O_2 of one molecule to N_3' and N_4' of the other molecule, as illustrated in Fig. 1. On the other hand, in the copper complex identified earlier [5], the hydrogen bonds are replaced by the copper coordination bonding to the four specified atoms. The EPR spectra of the paramagnetic center exhibit a superhyperfine structure, caused by the interaction of the unpaired electron, mainly located on copper, with the surrounding nuclei (mainly three ^{14}N). In already classical paper by Toriyama and Iwasaki [6], a copper center in cytosine monohydrate, exhibiting superhyperfine structure due to only one nitrogen coupling, has been described. In spite of the lack of evidence for the three nitrogen ligands, they assigned the spectra to the center shown in Fig. 1, which we identified on the basis of quite different spectroscopic data [5].

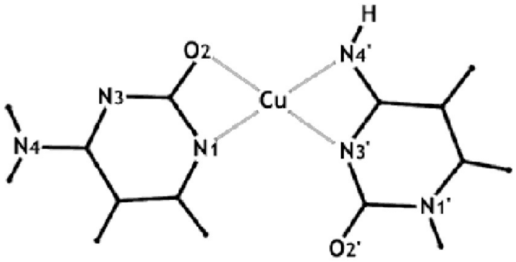

Fig. 1. Proposed structure of the copper complex formed in the crystals of cytosine monohydrate.

The difference in the EPR spectra attributed to the same copper complex with cytosine stimulated us to investigate this system in more detail. In the previous paper [5], we evaluated the leading terms in the spin Hamiltonian, and just noticed the presence of the smaller terms, originating from the hyperfine coupling of three nitrogen atoms (and a proton in non-deuterated crystals). The nitrogen couplings could not be analyzed primarily because of the complexity of the complete spectra. For the present study, crystals of better quality were prepared, resulting in better EPR spectra, which allowed the analysis of the superhyperfine structure for most crystal orientations. Thus, the subject of the present paper is the analysis of the nitrogen atoms coupling. The nitrogen coupling tensors give additional information on the positions of the nitrogen atoms in the complex and on the spreading of the unpaired spin over the complex.

2. Materials and methods

The cytosine monohydrate single crystals were grown by slow evaporation of the cytosine water solution to which small amount of metallic copper powder was added. Partly deuterated crystals were grown from heavy water in a similar manner. In the latter crystals, the hydrogen atoms bound either to oxygen or to nitrogen were replaced by deuterium. The crystal is monoclinic with a space group $P2_1/c$ [7]. There are four molecules in the unit cell, two of them being magnetically distinct. The bases form two systems of stacked bases and in that respect simulate the structure of the double-helix DNA. Untreated crystals did not show any EPR signals. The crystals irradiated with gamma rays exhibited well-defined EPR spectra. The irradiation was carried out at room temperature with a ^{60}Co source to a total dose of 30 kGy. The measurements were done with a Bruker X-band ELEXSYS 500 EPR spectrometer at the same temperature. The spectra were recorded every 10° as a crystal was rotated about a selected axis. For collecting a complete set of data, each crystal was measured in three mutually perpendicular planes, defined by the crystallographic axes $<\mathbf{a}^*>$, $<\mathbf{b}>$ and $<\mathbf{c}>$. In some cases, for better reconstruction of the complex spectra, the Win-EPR SimFonia Program (Bruker) was used.

The nitrogen coupling tensors were analyzed as a first-order perturbation to the dominating, the Zeeman term.

3. Results

3.1. EPR spectroscopy

The non-irradiated crystals did not give any detectable EPR signals. This is in contrast to the copper complex in cytosine monohydrate, described by Toriyama and Iwasaki [6]. After irradiation, we observed rather complex EPR spectra, as demonstrated in Fig. 2a. The spectrum was recorded for the magnetic field parallel to the $<\mathbf{c}>$ crystallographic axis. The deuterated crystals exhibited better-resolved spectra for the same orientation of the crystal in the magnetic field, as shown in Fig. 2b. An additional proton coupling of about $5 \cdot 10^{-4}$ cm^{-1} (≈ 0.5 mT) in the upper spectrum is responsible for the splitting of each line into doublets. That essentially isotropic coupling was not further analyzed. The absence of the extra proton coupling in the deuterated crystals proves that its origin is the remaining hydrogen atom of the cytosine amino group, the atom that is easily exchangeable upon deuteration. The deuteron coupling does not give rise to the resolved line splitting. The separated group of lines on the right side of the spectra in Fig. 2 belong to the cytosine radicals.

The spectrum associated with the copper complex consists of four groups of lines. Actually, there are two such quartets, coming from two copper isotopes ^{63}Cu and ^{65}Cu, with the natural abundance of 69 % and 31 %, each of spin $I = 3/2$. The ratio of the magnetic moments is $\mu(^{65}\mathrm{Cu})/\mu(^{63}\mathrm{Cu}) = 1.072$ and, therefore,

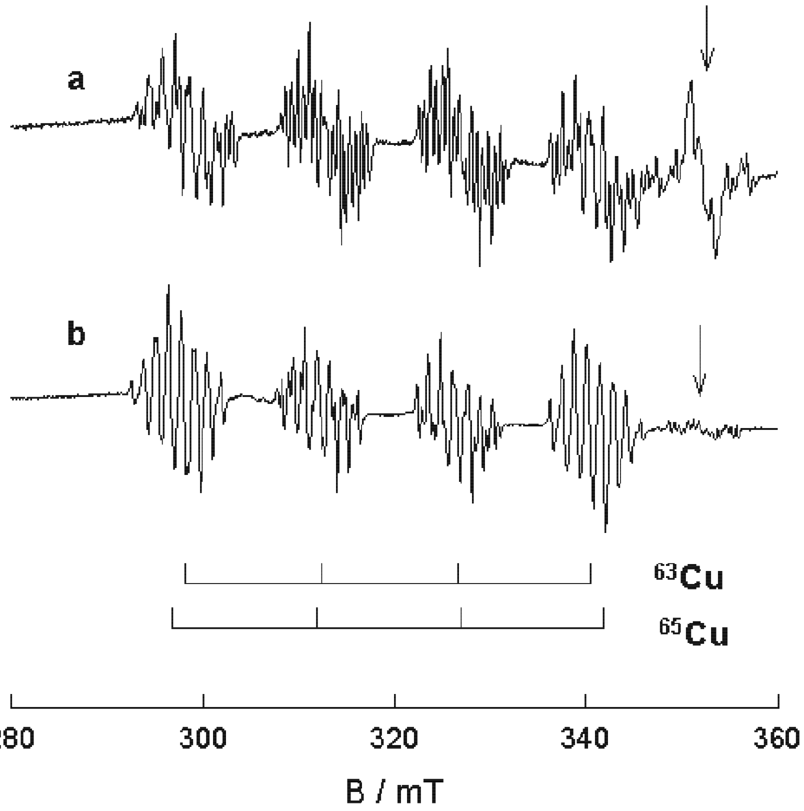

Fig. 2. EPR spectra of gamma-irradiated Cu*-doped single crystals of cytosine monohydrate grown (a) from regular and (b) from heavy water solutions. The spectra were recorded at room temperature for the magnetic field B in the direction of crystallographic axis* <**c**> *of the host crystal. The microwave frequency was 9.882* GHz, *and microwave power 10* mW. *The arrows indicate the position for* $g = 2.0036$.

the EPR patterns of the centers associated with the two isotopes overlap. In two crystal planes (**bc** and **a*b**), the spectra are even more complex due to the site splitting of the two magnetically distinct paramagnetic centers.

The stick diagram under the lower spectrum in Fig. 2 indicates the positions of the centers of four groups of lines for each copper isotope. The positions of these multiplets define the g tensor and the copper coupling tensor, A(Cu). Both of the tensors have already been evaluated [5]. Here we analyze the substructure of these groups. Each group contains the same information. Hence, for each crystal orientation, only the group with best-resolved lines of the deuterated sample is analyzed in more detail.

By careful analysis of the spectra, it is evident that the superhyperfine pattern is brought about by three nitrogen couplings, two of them being equal and the third one somewhat larger. Fig. 3a shows a more detailed EPR pattern of the second group from the left, for the magnetic field parallel to the crystallographic <**c**> axis. The spectrum is composed of 15 lines with the intensity ratios 1 : 2 : 1 : 3 : 2 : 1 : 2 : 3 : 2 : 1 : 2 : 3 : 1 : 2 : 1. The composite spectrum shown in Fig. 3a consists of two patterns, the bigger one associated with ^{63}Cu, and the smaller, shifted to the left, associated with ^{65}Cu. In order to further verify the proposed spectral pattern and assignment, the observed spectrum (Fig. 3a) was simulated with the use of the WinEPR SimFonia program. For the simulation, the line-width of 0.27 mT and the couplings for the three ^{14}N nuclei of 1.22 mT, 1.22 mT and 1.47 mT were used. The fit of the simulated spectrum (Fig. 3b) to the observed one is excellent.

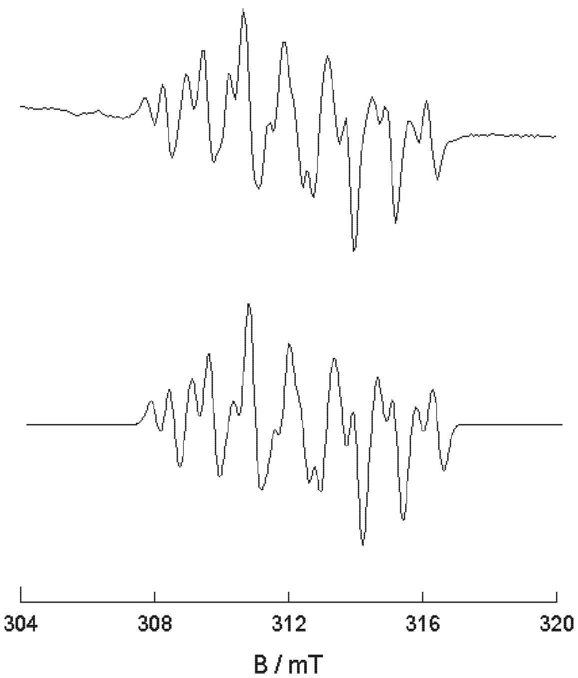

Fig. 3. Detailed structure of the second group of lines of the EPR spectrum shown in Fig. 2b (upper spectrum), and the corresponding simulated pattern (lower spectrum). The simulated spectrum is the superposition of the spectra for both Cu *isotopes with the appropriate intensity ratio and the relative shift of the patterns by 0.55* mT*. The Gaussian lines of 0.27* mT *width and two nitrogen couplings of 1.22* mT *and the third one of 1.47* mT *were used for the simulation.*

By measuring the nitrogen couplings in all the three crystal planes, it is observed that for all crystal orientations two couplings are the same, within the accuracy of the measurements, and the third coupling is somewhat larger. The angular variation of the nitrogen couplings, in the three crystal planes, is presented by full circles and squares in Fig. 4.

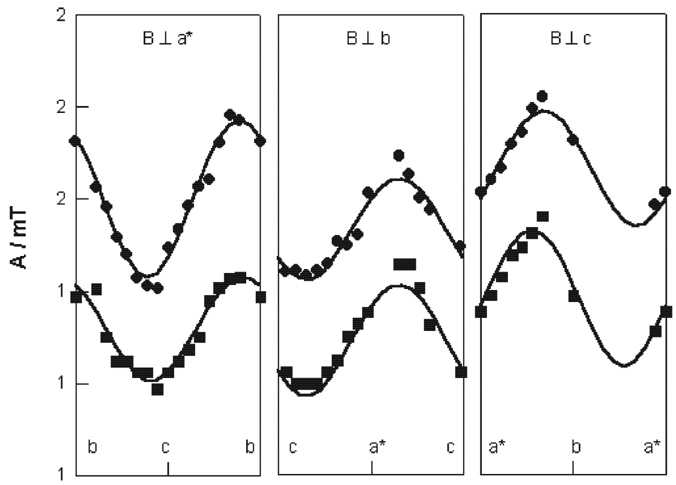

Fig. 4. The angular dependence of the nitrogen couplings in three crystal planes; the larger couplings are presented by full dots and the smaller by full squares. The solid lines are reproduced using the data presented in Table 1.

TABLE 1. Principal elements of the superhyperfine coupling tensors[a].

Atom	Principal values[b]		Direction cosines					
			<**a***>		<**b**>		<**c**>	
N_1	**11.88±1.0**	16.99	**0.7449**	-0.5848	**-0.6669**	0.7517	**0.0183**	0.3049
	12.44±1.0	17.27	**0.1846**	0.4833	**0.2324**	0.0210	**0.9549**	0.8752
	15.09±0.5	20.71	**0.6411**	0.6515	**0.7079**	0.6592	**-0.2962**	-0.3756
N'_4	**11.88±1.0**	13.89	**0.7449**	-0.5744	**-0.6669**	0.7415	**0.0183**	0.3468
	12.44±1.0	14.11	**0.1846**	0.5707	**0.2324**	0.0589	**0.9549**	0.8191
	15.09±0.5	16.91	**0.6411**	0.5869	**0.7079**	0.6684	**-0.2962**	-0.4570
N'_3	**14.16±1.0**	15.57	**0.5205**	0.8720	**-0.5060**	-0.2095	**-0.6878**	-0.4424
	15.83±1.0	15.75	**0.7510**	0.4672	**-0.1118**	-0.0866	**0.6507**	0.8799
	17.57±0.5	19.23	**0.4061**	0.1460	**0.8553**	0.9740	**-0.3218**	-0.1734
H'_4	≈ 5	6.21						
Normal to the ring plane, n[c]			0.4671		0.0244		0.8790	
$N_1-N'_4$ direction[c]			-0.8085		-0.3784		0.4508	
N'_3-O_2 direction[c]			0.1393		-0.9902		-0.0093	
H bond direction direction[c]			0.4254		0.8669		-0.2600	

[a]Experimental results are expressed in bold print and theoretical values are given in regular print.

[b]The couplings are expressed in units of 10^{-4} cm^{-1}.

[c]Data from the crystal structure [7].

The spin Hamiltonian representing the complex is

$$H = \beta B \cdot \vec{S} \cdot \hat{g} \cdot \vec{b} + \vec{I}_{\mathrm{Cu}} \cdot \hat{A}_{\mathrm{Cu}} \cdot \vec{S} + \sum_i \vec{I}_i \cdot \hat{A}_i \cdot \vec{S} + \sum_i \vec{I}_i \cdot \hat{P}_i \cdot \vec{I}_i - g_{\mathrm{N}} \beta_{\mathrm{N}} B \cdot \vec{I} \cdot \vec{b}\,, \quad (1)$$

where $\vec{b}$ is the unit vector of magnetic field direction, β is the Bohr magneton and $\hat{g}$, $\hat{A}$ and $\hat{P}$ are the g-tensor, the hyperfine tensor and the quadrupole tensor, respectively. g_{N} is the nuclear g-factor for copper and β_{N} is the nuclear magneton.

The first term describes the Zeeman interaction of unpaired electron with the external magnetic field, B. That term is much larger than the remaining terms of the Hamiltonian. The second term describes the interaction of the unpaired electron with the copper nucleus. The third term(s) represent the couplings of the neighboring nuclei (three nitrogens and a proton), giving rise to the so-called superhyperfine structure of the EPR spectra. The last two terms are the quadrupole and the nuclear Zeeman term, respectively.

In the previous paper all terms in the Hamiltonian, except the term responsible for the superhyperfine structure, were analyzed. As the nitrogen couplings are not much affected by other interactions, we calculated these coupling tensors independently, assuming the superhyperfine interactions to be a perturbation of a first order to the electronic Zeeman term

$$H = \beta \vec{S} \cdot \hat{g} \cdot \vec{B} + \sum_i \vec{I}_i \cdot \hat{A}_i \cdot \vec{S}\,. \quad (2)$$

The g tensor and, consequently, the Zeeman term have already been evaluated [5]. Each component of the perturbation is experimentally determined from the data in the three mutually perpendicular plains, as described in detail elsewhere [8]. Briefly, in each plain, the angular variation if A_i is defined by the relation

$$g(\theta)^2 A_i^2 = P_i \cdot \cos^2(\theta) + Q_i \cdot \sin^2(\theta) - 2R_i \cdot \sin(\theta) \cdot \cos(\theta)\,. \quad (3)$$

Since $g(\theta)$ is known, the fit of the above relation to the experimental data defines three parameters P_i, Q_i and R_i. From the measurements in the three plains, nine matrix elements (six of them independent) are determined. By diagonalizing the matrix $\hat{A}_i^2$, and hence $\hat{A}_i$ in the system of the principal axes, the principal values and direction cosines of nitrogen tensors are evaluated.

The experimental points in the three plains are shown in Fig. 4. The results of the evaluation are presented in Table 1. Solid curves in Fig. 4 are reproduced with the use of the coupling parameters specified in Table 1. For convenience, the direction of the normal to two adjacent cytosine bases and the directions of the relevant inter-atomic bonds, calculated from the crystal structure [7], are also shown in the table.

3.2. MO calculations

In order to find theoretical support for our proposed copper complex in cytosine, we performed the calculations of the cytosine dimer, bridged by a Cu(II) ion with the *a*b initio B3LYP density functional method with the Gaussian 98 package of programs [9]. For the geometry optimization, the 6-31G(d) basis set was used. The atom coordinates from the crystal structure [7] were used as the input values. The single point calculations were performed with the 6-311+G(2d,p) basis set. The calculations were done for the gas complex and for the complex related with the cytosine crystal lattice, by fixing the coordinates of three carbon atoms in each cytosine molecule coordinated with the metal ion. For comparison, the results of the calculations are presented in Table 1, together with the experimentally obtained values. As can be seen, the calculations confirm the existence of three nitrogen atoms in the complex, with the isotropic couplings close to the experimental values. The couplings with other nitrogen atoms are found to be negligible. The calculations predict different values for the three nitrogen hyperfine tensors, but not much different from the experimentally determined values. The axes of maximal principal elements are essentially in the ring plane, as expected for the complex presented in Fig. 1. In addition, the theoretical model predicts the amino-group proton coupling close to the observed value (Table 1).

Both the experiments and the calculations demonstrate large spin delocalization. The calculated spin distribution is illustrated in Fig. 5. As expected, most of the spin (unpaired electron) is located on the copper atom. However, the density on the nitrogen ligands is also large. Moreover, appreciable spin density is found even at the amino-proton, which is rather far from the copper atom.

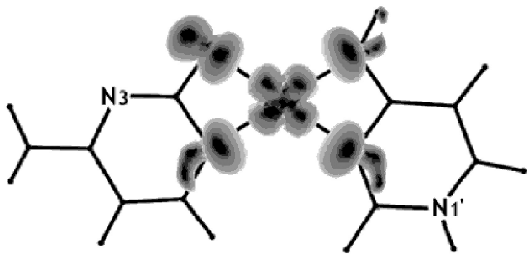

Fig. 5. Schematic picture of the spin density distribution for the isolated ("gaseous") complex, as calculated with the use of the Gaussian 98 package program.

4. Discussion

The experimental data described above undoubtedly prove the incorporation of the copper ions into the cytosine monohydrate crystal lattice. Since the untreated crystals were EPR silent, it is obvious that the incorporated copper ions are in a diamagnetic state, as Cu(I). Copper comes to the crystal lattice probably as a monodentate Cu-cytosine complex, with copper bound either to N_3 or O_2 of cytosine [2,10]. It is also known that in nucleic acids, copper ions preferentially bind to N_3 in cytosine, where they can coordinate with the carbonyl group [2,4]. Since the N_3 position of cytosine is involved in the Watson-Crick pairing, such a coordination bond might significantly impair regular biological functions of DNA.

Upon irradiation the paramagnetic Cu(II) ions are formed, probably by trapping migrating holes produced in the cytosine matrix. The partition of the copper-centered radicals in the total radical population by far exceeds the relative concentration of copper in the crystal of cytosine monohydrate, demonstrating the long-range migration of holes/electrons in this system. The migration of holes to large distances has already been proven in some other systems of stacked bases [11 – 14].

The assignment of the spectra to the copper complex shown in Fig. 1 is based on the possible copper sites in the lattice of cytosine monohydrate that could accommodate a complex with three nitrogen atoms as ligands. By carefully inspecting the crystal structure of the host crystal, no other place besides the selected one has been found. The superhyperfine couplings of the copper ion not only with three nitrogen nuclei but also with the additional proton of the amino group confirms the assignment.

In a planar complex, like that in Fig. 1, the maximal elements of the ^{14}N coupling tensors are expected to lie in the plain of the complex [6]. The data summarized in Table 1 are in agreement with the expectation. From the experimental data, the axes of maximum elements of two identical tensors make the angle of 94° and that of the third one 86° with the normal to the cytosine ring plain. The axes do not coincide with the copper coordination bonds in the complex plane, but are closer to the hydrogen bonds [7] in unperturbed crystal, as was also observed in Ref. [6]. The axes two of identical tensors make angles 16° and the third one 4° with the hydrogen bond direction. The axes of medium elements should be perpendicular to the complex plane but they make angles of about 70° with it. The theoretically predicted directions of the coupling tensor elements give essentially the same information. The calculations predict three different ^{14}N couplings, as expected for a complex lacking symmetry in the ligand plain. The predicted angles of the maximal coupling tensor element with the ring normal are 90°, 87° and 84°, for N_1, N'_3 and N'_4, respectively. The theoretically derived N_1 and N'_4 tensors are nearly coaxial, what suggested the assignments as presented in Table 1.

Both experimentally determined and calculated directions of the medium and smallest principal elements of the coupling tensors do not coincide with coordination bonding directions in the plain of the complex (as calculated from the crystal data). That could be partly attributed to the predominantly isotropic character of nitrogen tensors with relatively weak dipole-dipole interaction. On the other hand, that fact might indicate that the actual electronic structure of the Cu-center might deviate from the ideal structure assumed in the calculations, but accommodates the structure dictated by the constrains of the crystal lattice it is imbedded in.

Acknowledgements

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EPR SPEKTROSKOPIJA SUPRAHIPERFINOG MEĐUDJELOVANJA BAKARNOG PARAMAGNETSKOG CENTRA U KRISTALIMA CITOZIN MONOHIDRATA

Gama ozračivanjem kristala citozin monohidrata, koji je sadržavao bakarne nečistoće, pripremali smo planarni kompleks Cu(II) s dvije citozinske baze. Paramagnetski se kompleks vjerojatno stvarao vezanjem zračenjem proizvedenih šupljina i Cu(I) koji je prvotno bio u kristalnoj rešetci. Proučavali smo strukturu kompleksa analizirajući suprahiperfinu strukturu njegovog EPR spektra. Eksperimentalno određeni elementi tenzora triju vezanja ^{14}N i opažanje dodatnog protonskog vezanja pouzdano potvrđuju strukturu. *Ab initio* B3LYP računi metodom funkcionala gustoće u dobrom su slaganju s mjerenjima. Eksperimenti i računi pokazuju jaku delokalizaciju spina u ravnini citozinskog prstena.