Two 5-methyl-2-thiocytosine (5-MTC) radical species formed in 5-methylcytosine hemihydrate crystal lattice have been investigated by means of g-tensor and spin density distribution calculation using the B3LYP density functional. Theoretical values have been compared with experimental data for g-tensor eigenvectors. In model structures containing more molecules, significant difference of spin density localization on 5-MTC has been found for radical cation (radical I) in comparison to neutral radical of cationic origin (radical II). The structure of the radical centre as well as the mechanism of charge transfer and radical stabilization on 5-MTC in this system has been proposed.

Keywords: 5-methyl-2-thiocytosine; g-tensor; spin density; DFT calculation

1. Introduction

Cytosine methylation, which is present in DNA of nearly all organisms, has long been recognized as important for cellular signaling processes and proved to have a role in the aging of the cells [1,2]. In those processes the activity of certain cellular enzymes has been changed by cytosine methylation implying effects on various cellular response mechanisms [3,4]. Relationship of the cytosine methylation to B\rightarrow Z DNA transition probability as well as to the DNA melting temperature has been found [5–7]. Evidence of the role of cytosine methylation in the development of cancer from endogenous as well as exogenous mutagenic sources is also well founded and has already been used to propose possible ways of counteracting mutagenic effects [8–10]. Both methylated nucleobase analogues and nucleobase thioanalogues have with appreciable success been tested for antitumour and antiviral activity [11,12]. Specific properties of a cytosine thioanalogue 2-thiocytosine, such as proved localization of the electron spin in oxidation products formed in the cytosine crystal lattice might be of particular interest in prospective research in these and other related fields [13,14]. Although important and highly specific interactions with the environment must exist for cellular mechanisms to be effective, the complexity of physical interactions is not yet completely clear. Here, an investigation of a model system has been performed in order to help such an understanding.

The g-tensor data may in the EPR spectroscopy be used to estimate the spin density on sulphur. This is due to the large g-tensor of a radical center having significant spin density on sulphur in comparison to the free-electron value. This both enables one to distinguish such radicals rather easily in EPR measurements and also gives some insight into the spread of the spin density in such systems. In the work presented, the g-tensor of two 5-methyl-2-thiocytosine (5-MTC) radical species formed in 5-methylcytosine hemihydrate crystal lattice has been investigated in detail in order to establish or verify the assignments of the proposed radical structures based on previous experimental investigation, and to give some insight into the radical formation process as observed from the changes in spin density localization. Such acquired knowledge of distinct properties of a site with such substitution and its interactions with the environment may be of interest in the future development of prospective drugs that intervene to cellular signaling pathways.

2. Theoretical

Details of the numerical methods used for g-tensor calculations have already been explained elsewhere [15–17].
The standard procedure as implemented in the Gaussian 03 program [18] has been followed. This implementation uses the following form of the g-tensor, which includes four parts

\[ g_{rs} = g_s \delta_{rs} + \Delta g_{RMC} \delta_{rs} + \Delta g_{GC} + \Delta g_{RSOCS} \]  

(1)
of which the most important correction to the isotropic free-electron value \( g_s = 2.002319 \ldots \) usually comes from the last term presenting mixing of the orbital Zeeman and spin-orbit coupling matrix elements contributions and is written in the form

\[ \Delta g_{RSOCS} = -\frac{1}{\mu S} \sum_{\alpha=\beta} (-1)^{\delta_{\alpha\beta}} \sum_{i,a} \text{Im}(U_{ia}^{\sigma_i^r}) \times \text{Im}(\psi_i^{(0)} | \hat{H}_{SOC}^{\sigma_i^r} | \psi_a^{(0)}) \]  

(2)

Here, \( s \) and \( r \) refer to the respective components of the electron spin and the magnetic field vectors. This expression is obtained by expanding the one-electron spin-orbit Hamiltonian

\[ \hat{H}_{SOC}^{\sigma_i^r} = \frac{\alpha^2}{2} \sum_{\alpha i} \frac{Z_{eff}^\alpha}{(r_i - R_A)} l_i^{\sigma_i^r} s(i) \]  

(3)

where, \( s(i) \) is the \( i \)th electron spin operator, \( l_i^{\sigma_i^r} \) the \( i \)th electron angular momentum relative to the nucleus \( A \) on position \( R_A \) and \( Z_{eff}^\alpha \) is semiempirically chosen effective nuclear charge of atom \( A \), using perturbed orbitals of the form

\[ \psi_i^{(0)}(r) = \sum U_{ia}^\sigma \psi_a^{(0)}(r) \]  

(4)

Here, \( \psi_a^{(0)} \) and \( \psi_i^{(1)} \) are unperturbed and perturbed molecular orbitals of the respective spin and \( U_{ia}^\sigma \) are expansion coefficients. The second term in Equation (1) is a first-order relativistic mass correction, and the third term represents the gauge correction [16].

For comparison with experimental g-tensor principle values and eigenvector direction cosines it is of special interest to understand the importance of the two numerically distinct contributions in the \( \Delta g_{RSOCS} \) expression. Up to second order, the value of the correction is proportional to the couplings of ground-state and excited-state orbitals and inversely proportional to the differences in respective orbital energies.

3. Calculations

Properties of both types of the radical forms expected to develop on 5-MTC incorporated in the crystal lattice of 5-methylcytosine hemihydrate have been calculated in eight model structures (Figure 1). Both 5-methyl-2-thiocytosine radical cation (radical I) and 5-methyl-2-thiocytosine neutral radical of cationic origin (radical II) have been investigated in different molecular surroundings modelling the crystal conditions expected to be important for charge or energy transfer in the real system. Structures consisting of three molecules are chosen in such a way to describe the expected site of spin localization, 5-MTC sulphur atom in the best achievable way. Structures consisting of six molecules are, on the other hand, chosen in a way to allow for the possible effects of spin spread along the presumed \( \pi \)-orbital system formed in the structure of stacked 5-methylcytosine molecules. In the initial model structures all atoms have been located at positions determined by the crystal structure [19]. At a chosen 5-methylcytosine molecule in the model structure oxygen atom has been replaced by sulphur. Radical II model structures (denoted by the letter 'B' in the structure name) are obtained from radical I structures ('A' structures) by deprotonation of 5-MTC molecule at the N1 position, as shown in Figure 1 for 1A and 1B structures (isolated 5-MTC molecule).

Geometry of a given structure has been optimized using B3LYP density functional and the 6-31G(d) or 6-311G(2d,p) basis set, depending on the complexity of the structure. Electronic properties, spin density distribution and g-tensor values have been calculated for the optimized structures using the 6-311G(2d,p) basis set and the above mentioned density functional. During the calculations the initial coordinate system has been preserved to enable comparison of the calculated g-tensor principal directions with experimental data. Theoretically obtained g-tensor matrices have been diagonalized using the matrix diagonalization program available in [20].

The structures and calculations performed are summarized below:

1A Single 5-MTC molecule, radical I, full optimization B3LYP 6-311G(2d,p)//B3LYP 6-311G(2d,p)
1B Single 5-MTC molecule, radical I, single-point calculation only B3LYP 6-311G(2d,p)
2A Single 5-MTC molecule, radical I, single-point calculation only B3LYP 6-311G(2d,p)
2B Single 5-MTC molecule, radical II, single-point calculation only B3LYP 6-311G(2d,p)
3A Three molecules: 5-MTC molecule radical I, two neighbouring 5-methylcytosine molecules surrounded by three water molecules, partial optimization B3LYP 6-311G(2d,p)//B3LYP 6-31G(d)
3B Three molecules, 5-MTC molecule radical II, two neighbouring 5-methylcytosine molecules surrounded by three water molecules, partial
optimization B3LYP 6-31G(2d,p)//B3LYP 6-31G(d)

4A Six molecules: 5-MTC molecule radical I, 5 neighbouring 5-methylcytosine molecules surrounded by 6 water molecules, partial optimization B3LYP 6-311G(2d,p)//B3LYP 6-31G(d)

4B Six molecules: 5-MTC molecule radical II, 5 neighbouring 5-methylcytosine molecules surrounded by 6 water molecules, partial optimization B3LYP 6-311G(2d,p)//B3LYP 6-31G(d)

5A Six molecules: 5-MTC molecule radical I, 5 neighbouring 5-methylcytosine molecules surrounded by 6 water molecules, partial optimization B3LYP 6-311G(2d,p)//B3LYP 6-31G(d)

5B Six molecules: 5-MTC molecule radical II, 5 neighbouring 5-methylcytosine molecules surrounded by 6 water molecules, partial optimization B3LYP 6-311G(2d,p)//B3LYP 6-31G(d).

During the optimization of all the structures the heavy atoms of all the 5-methylcytosine molecules in the model have been kept fixed at their initial positions, as were the oxygen atoms of the more distant water molecules. In structures 3A and 3B, two water molecules closer than 4 Å to the sulphur atom were allowed to change position during the optimization as was the water molecule in structures 5A and 5B participating in N1(5-MTC)–H(w) hydrogen bond. To take account of the crystal constraints, the 5-MTC molecule has been held close to its presumed crystal position by keeping C5 atom fixed in the optimization process. Coordinates of all the protons and of the other 5-MTC molecule atoms have been allowed for optimization.

4. Results

From experimental data two types of 5-MTC radical have been found, initially proposed to be formed by one electron removal (radical I) and subsequent deprotonation on N1 site (radical II) [21].

Figure 1. Initial structures for the modelling of 5-MTC radical I and radical II species in 5-methylcytosine hemihydrate: single 5-MTC molecule of radical I (1A), single 5-MTC molecule of radical II (1B), 5-MTC molecule radical species I and two neighbouring cytosine molecules surrounded by three water molecules (3A), 5-MTC molecule radical species I and 5 cytosine molecules surrounded by six water molecules (4A), 5-MTC molecule radical species I and five cytosine molecules surrounded by six water molecules (5A). Structures 4A and 5A differ in the site of oxygen to sulphur substitution (marked on the structures). Model structures of radical II species (1B, 3B, 4B and 5B) are obtained from the respective radical I structures (‘A’ structures) by deprotonation of 5-MTC molecule at N1 position.
Comparison of calculated g-tensor principal values for different model structures with experimental data is given in Table 1. The eigenvalues (denoted as $g_{\text{max}}$, $g_{\text{mid}}$, and $g_{\text{min}}$ in the Table) have been ascribed so as to minimize the angle between theoretical eigenvectors and the respective experimental ones. In other words, the values for the angle between theoretical and experimental directions given in Table 2 are minimal when considering possible choices of which theoretical g-tensor eigenvectors should be compared to which experimental g-tensor eigenvectors. As seen from Table 1, the ordering of the eigenvalues and thus also directional angles for all the structures except 1B, 3B, and 4B follows the experimentally observed pattern. Whereas in the two above mentioned former structures $g_{\text{mid}}$ and $g_{\text{min}}$ have been exchanged in comparison with the experiment, in the latter $g_{\text{max}}$ and $g_{\text{mid}}$ have been predicted erroneously.

From the numerical data obtained from calculations and comparison of these results with g-tensor experimental values it was impossible to distinguish between the two radical forms, since the results of theoretical calculations are found to depend strongly on the choice of (a) molecules included in the model for calculation and (b) the crystal environment of the site of oxygen to sulphur substitution. This effect, encountered also in the previous theoretical research of 2-thiocytosine-based radical g-tensor in crystal matrix of cytosine monohydrate makes difficult the prediction of the expected accuracy of theoretical reproduction of experimental g-tensors in the general case [22]. The variance of the calculation results for different structures considered within the same modelled radical species is much larger than the difference between the two experimentally found g-tensors of two radical forms, although calculated principal direction cosines fit experimental data for the radical II somewhat better (Table 2).

From comparison of fit of the theoretical results for fully optimized single-molecule structures (1A and 1B) versus single-point calculations (2A and 2B) it is seen that g-tensor eigenvalues are much better reproduced in the latter case whereas the errors in eigenvector direction predictions are of comparable size. From this, it may be concluded that the gas-phase optimizations of a single 5-MTC radical molecule could not yield satisfactory results because of the complete neglect of radical crystalline environment. If both g-tensor direction cosines and eigenvalues are considered, best agreement with experimental data is obtained for calculations 2B, 5A and 5B. As expected, the best overall result has been obtained for the site of radical localization described most rigorously, including six molecules (5-MTC and 5 surrounding 5-methylcytosine molecules) in the model for optimization. Although maximum principal g-tensor values for both radical species are predicted to be larger than experimentally found, for the structure 5A (modelling radical species I) a smaller maximum principal g-tensor value has been predicted than for 5B structure (modelling radical species II). Furthermore, the difference between these values for two radical species is predicted to be the same as previously obtained from the experiment (Table 1). Theoretical maximum principal g-tensor directions for these two radical species also agree well with the previous findings (Table 2). The goodness of fit to the experimental data for the more complex model structures 5A and 5B thus

<table>
<thead>
<tr>
<th>Calculated structure</th>
<th>$g_{\text{max}}$</th>
<th>$g_{\text{mid}}$</th>
<th>$g_{\text{min}}$</th>
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</thead>
<tbody>
<tr>
<td>1A</td>
<td>2.4579</td>
<td>2.0119</td>
<td>2.0022</td>
</tr>
<tr>
<td>1B</td>
<td>2.8353</td>
<td>2.0022</td>
<td>2.0104</td>
</tr>
<tr>
<td>2A</td>
<td>2.2750</td>
<td>2.0089</td>
<td>2.0022</td>
</tr>
<tr>
<td>2B</td>
<td>2.0967</td>
<td>2.0084</td>
<td>2.0021</td>
</tr>
<tr>
<td>3A</td>
<td>2.1360</td>
<td>2.0082</td>
<td>2.0040</td>
</tr>
<tr>
<td>3B</td>
<td>2.2951</td>
<td>2.0022</td>
<td>2.0116</td>
</tr>
<tr>
<td>4A</td>
<td>2.0808</td>
<td>2.0063</td>
<td>2.0041</td>
</tr>
<tr>
<td>4B</td>
<td>2.0125</td>
<td>2.4308</td>
<td>2.0022</td>
</tr>
<tr>
<td>5A</td>
<td>2.1820</td>
<td>2.0078</td>
<td>2.0024</td>
</tr>
<tr>
<td>5B</td>
<td>2.2280</td>
<td>2.0114</td>
<td>2.0023</td>
</tr>
</tbody>
</table>

Table 1. Calculated g-tensor principal values comparison with experimental data for radical species I and II.

Note: aExperimental data from Ref. [21].
again confirms that important interactions of several molecules near the radical site must exist in the crystal. Furthermore, the additional molecules included are not located in the same crystal plane as defined by the 5-MTC ring but are in the same molecular stack. The comparison with the results obtained for structures \(3\text{AB}\) and \(4\text{AB}\) implies that interaction important for good modelling of spectroscopic parameters with these molecules must exist. This in turn suggests that this kind of electron orbital interaction over the molecule stack is present in the crystal environment.

For the estimate of the accuracy of the g-tensor values in model systems of radicals formed at specific impurity centres in monocrystals, Table 3 presents maximum g-tensor values \((g_{\text{max}})\) as well as the average g-tensor values \((g_{\text{av}})\) calculated as

\[
g_{\text{av}} = \frac{g_{\text{max}} + g_{\text{mid}} + g_{\text{min}}}{3}
\]

obtained from DFT calculations and compared with experiments. In comparison, only the data for the most successful model structures is used. From the table it may be seen that the calculations of the fairly large model structures tend to overestimate maximum g-tensor values by about \(0.02 \pm 0.04\), and the average g-tensor values by about \(0.01 \pm 0.02\). Significant error is mostly due to the strong dependence of the calculated g-tensor values on the radical environment included in the model, as reported previously [23].

Figures 2–5 show spin isodensity surfaces of 0.004. An important conclusion may already be drawn from observation of surfaces for \(1\text{A}\) and \(2\text{A}\) structures: for fully optimized radical I structure spin density has much more of the \(\sigma\)-orbital character than is the case for the orbitals obtained by single-point calculation from 5-methylcytosine crystal structure. Similar conclusions may be drawn for the radical II structures \(1\text{B}\) and \(2\text{B}\) (not shown). Also, structures consisting of three-molecular clusters (5-MTC radical and two neighbouring cytosine molecules) for which reproduction of experimental eigenvector directions is quite poor have the largest \(\sigma\)-orbital spin density character predicted (Figure 3).

From comparison of data in Tables 1 and 2 and spin densities for structures \(4\text{A}, 4\text{B}\) and \(5\text{A}, 5\text{B}\) shown on Figures 4 and 5 it is seen that the best accordance with the experiment is obtained in the model structure in which 5-MTC radical environment is carefully chosen. In such structures the mixing of \(\sigma\)- and \(\pi\)-orbital contributions to spin density is still present, but the \(\sigma\)-orbital character is significantly less pronounced than in structures yielding less correct results.

From comparison of g-tensor eigenvalues for all structures, larger g-tensors have been obtained for structures modelling radical species II in all calculations except single molecule single-point calculations. This is indicating more spin density localization on sulphur predicted for these structures supporting also the experimental findings. The difference between radical I and radical II species becomes even more transparent when examining spin density surfaces. In the six-molecule structures much spin delocalization

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Table 3. Comparison of calculated and experimental g-tensor maximum and average values.

<table>
<thead>
<tr>
<th>Source</th>
<th>(g_{\text{max}})</th>
<th>(g_{\text{av}})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFT ((\text{radical I}))</td>
<td>Experimental ((\text{radical I}))</td>
</tr>
<tr>
<td>Ref. [24]a</td>
<td>2.0388</td>
<td>2.0380</td>
</tr>
<tr>
<td>Ref. [23]b</td>
<td>2.0890</td>
<td>2.1320</td>
</tr>
<tr>
<td>Ref. [22]</td>
<td>2.1810</td>
<td>2.1440</td>
</tr>
<tr>
<td>This work ((\text{radical I}))</td>
<td>2.1820</td>
<td>2.1229</td>
</tr>
<tr>
<td>This work ((\text{radical II}))</td>
<td>2.2280</td>
<td>2.1693</td>
</tr>
</tbody>
</table>

Notes: aExperimental values are averaged values for radical species A and B from [26]. bDFT values are averaged values obtained for two (d) structures in [22].

---

Figure 2. Spin density distribution for full optimization (\(1\text{A}\)) and single point calculation only (\(2\text{A}\)) of a single 5-MTC radical molecule of species I.

Figure 3. Spin density distribution for three-molecular structures \(3\text{A}\) and \(3\text{B}\). Spin density is predominantly localized on sulphur atom.
is present for charged radical species (radical I), whereas spin density localization on 5-MTC takes place for neutral radical species (radical II). The model of type II radical formation by deprotonation on N1 of the 5-MTC molecule is thereby supported. Also, the localization of the spin on 5-MTC sulphur atom for both radical species is confirmed, in analogy to the observations in related cytosine-monohydrate (2-thiocytosine) system [25]. Localization of spin on 5-MTC radical I species has not been found only in 4A structure, probably because of significant spin spread on neighbouring 5-methylcytosine molecular stack.

Interestingly enough, it seems that neither the localization nor the radical species play a crucial role when modelling g-tensors of 5-methyl-2-thiocytosine-centered radicals in crystal lattice of 5-methylcytosine hemihydrate. (Compare the spin density distributions where applicable and table data for structures 2B, 4A, 5A and 5B.) The apparent discordance between the quality of fit for the g-tensor eigenvector directions and principal values (for structures 1A, 1B and 3A for example) raises the question of different influences affecting theoretically predicted g-tensor values, which is subject of further investigation.

5. Conclusions

Theoretical results for model structures best describing 5-MTC radical environment (5A and 5B structures) were able to reproduce both g-tensor principal values and directions of two radical forms with reasonable accuracy. Localization of spin density on the 5-MTC radical has been found for neutral radical (species II)
formed from radical cation (species I) by N1 deprotonation. The success of six molecular models, in which additional molecules are located in crystal molecular stack supports the assumption of the charge transfer mechanism over the system of stacked molecules and its subsequent localization on thioanalogues by deprotonation previously established. Significant mixing of $\sigma$- and $\pi$-character of the spin density has been found for the 5-MTC radical in this system, yet the structures showing much of the $\sigma$-orbital influence to the spin density distribution are yielding results in larger discordance to the experimental data.

Generally the structures for which calculations are best reproducing $g$-tensor principal values are not the same as those for which calculations of $g$-tensor directions are best reproducing the respective experimental data. That this effect is not exclusively system-dependent is seen from the fact that the same has been found in recent research of the $g$-tensor of a 2-thiocytosine-based radical in crystal matrix of cytosine monohydrate [22]. More theoretical investigation is required to find the means to solve this problem.

Nevertheless, if relative values are considered, models consisting of several molecules in the 5-MTC radical stack are able to reproduce the eigenvalues and $g$-tensor principal directions of both observed radical forms with reasonable accuracy.

Acknowledgement

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References

[18] M.J. Frisch, et al., Gaussian 03, Revision C.02 (Gaussian, Inc., Wallingford, CT, 2004).