Radicals formed in cytosine–hydrochloride(thiocytosine) single crystals: Insights from a DFT study

Vjeran Gomzi*

Faculty of Pharmacy and Biochemistry, A. Kovacica 1, Zagreb, Croatia

1. Introduction

From the early days of investigations of the DNA, oxygen–halogen interactions have been recognized for their potential importance in ligand binding and molecular folding processes. Recently, the understanding of the same kind of interactions has been exploited in design of supramolecular assemblies as well as in the light-controlled synthesis of oligonucleotides on DNA chips [1,2]. Chlorine-containing (photo)sensitizers are investigated as potential agents for photodynamic treatment and diagnostic purposes [3–5]. Furthermore, interest in intricacies of cytosine–halogen interactions is also spurred by the antitumour activity of cytosine–BCl3 and cytosine–BF3 compounds [6,7]. On the other hand, the potentially adverse effects of the excess chlorine and chlorine-containing compounds in the environment of organic molecules also warrant care. The reactivity of biomolecules, and more specifically nucleic-acid bases towards the aqueous chlorine has in several investigations found to be important for the structure as well as for the preservation of information contained in DNA [8,9]. The carcinogenicity and cytotoxicity of the byproducts formed by the chlorine-containing compounds used for the disinfection of drinking water have recently also raised some attention [10].

In many instances the charge transfer properties between the chlorine-containing moiety and the biomolecule are of special interest [11,12]. Following the results obtained from this work it will be possible to give comments on this kind of interactions but, as is well known and to be explained in detail later, special care has to be taken of the effects from the radical environment. This comes as a recursive notion from a series of works dealing with theoretical modeling of the radicals in systems of ordered nucleic-acid bases [13,14].

The radicals formed in cytosine–hydrochloride(thiocytosine) monocrystals have been investigated by DFT calculations of the g-tensor of sulfur centered radical. Taking account of the crystal environment of the radical site it has been shown that the main reason for difficulties in interpretation of spectroscopic as well as theoretical results in this model system is the considerable spin density spread on both chlorine and sulfur atoms. From comparison of the results for the proposed model structure with accessible spectroscopic data, the specific direction in which charge transfer in the investigated system may take place has been pointed out.

2. Calculation method

Properties of several three-molecular structures obtained by imposing different charge/multiplicity and geometry optimization
restrictions have been calculated using B3LYP density-functional method as implemented in Gaussian03 program [18]. The initial positions of atoms in all the examined structures have been obtained from the partially geometry optimized structure of six molecular cluster, formed by five nearest molecules surrounding arbitrary chosen site of oxygen to sulfur substitution. The initial geometry optimizations of the model structure have been performed in a series of steps: (1) Cytosine molecules and chloride atoms have initially been located at their crystal positions [17]; (2) In such a structure the oxygen to sulfur substitution has been made at a chosen cytosine molecule; (3) Optimizations of a neutral structure and the structure formed by electron extraction have been performed using HF level of theory and STO-3G, and 3-21G basis sets. In the geometry optimization process, only the modifications of atom positions of the thiocytosine–Cl center and some of the atoms of the molecules closest to it have been allowed. Atoms of the more distant molecules (>4 Å from either S or Cl atoms of the thiocytosine–Cl center) have been kept fixed at their crystal positions; (4) 6-31G(d) B3LYP DFT geometry optimization calculation has been performed for the cation radical structure obtained in step (3). The use of several calculations including neutral system and lower-level basis sets were required to obtain convergence of molecular orbitals. These are then used as an initial guess for the higher-level basis set calculations as well as the calculations of the cation radical model structure. From the results of such optimizations, the presumed thiacytosine cation radical site model structure including two nearest cytosine molecules as well as chlorine atoms has been chosen for further investigation. Partial geometry optimization calculations have been further performed on a chosen structure, keeping molecules surrounding thiacytosine fixed at their positions from the optimization calculation of the six molecular structure, while still allowing for the small geometry modifications by allowing the variations of positions of H atoms closest to the thiacytosine–Cl center. The thiacytosine molecule has been in a three-molecular model kept close to its optimized geometry by holding three of its ring atoms fixed during geometry optimization. The geometry optimized structure is shown in Fig. 1. The chlorine atom indicated by its chemical symbol in Fig. 1 has been explained elsewhere [19–21]. The principal directions for g-tensor have been obtained by diagonalization of the g-tensor matrix as given in the Gaussian output. For comparison with the experimental data, the following expression has been used

\[ A_{ii} = A_{ii}^{iso} + A_{ii}^{anis} \]

relating diagonal elements of total hyperfine coupling tensor \( A_{ii} \) to the isotropic \( A_{ii}^{iso} \) and anisotropic \( A_{ii}^{anis} \) coupling elements of the hyperfine coupling obtained from the output of the numerical calculation.

3. Results and discussion

Changes of geometry parameters of thiacytosine–chlorine radical structure formed in cytosine–hydrochloride crystal lattice obtained by the optimization process of a three-molecular cluster as explained above are shown in Table 1. In Table 2 are presented g- and A-tensor values obtained in this work by DFT calculation and compared to the available experimental data. Two sets of

![Fig. 1. Three-molecular cluster obtained by geometry optimization protocol explained in the text and used as a thiacytosine–Cl radical model for single-point DFT calculation.](image-url)
Comparison of calculated $g$ and $A$-tensor elements to experimental data

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<td>$&lt;A_{\text{max}}&gt;$ $\times 10^{-4}$</td>
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Experimental data in the table denoted by A and B refer to two radical structures assigned from experimental data. The angle (in degrees) between minimum $g$-tensor value ($g_{\text{min}}$) and maximum $A$-tensor value ($A_{\text{max}}$) directions and the direction of thiocytosine ring normal [$n$] have in the respective order been subsequently listed in the table. The theoretically predicted principal values of both $g$- and $A$-tensors in three-molecular model are reproducing respective experimental values reasonably well. Taking into account, the experimental uncertainty of the principal tensor directions of about $4^\circ$, the predicted directions of the largest principal $A$-tensor and the smallest principal $g$-tensor element deviate by 10–20$^\circ$ from the respective experimental directions.

Total spin density on chlorine (Cl atom indicated by the symbol without the prime in Fig. 1) obtained by this calculation (0.47) agrees quite well with the value of 0.46 obtained in previous work from both AM1 molecular orbital calculation of a single molecule thiocytosine radical cation and experimental findings (structure I in ref. [16]). The DFT predicted total spin densities on S2 (0.39) and C2 (0.03) atoms however, are much lower than the values of 1.1 and −0.47, respectively, previously published for single molecule radical model. Part of the reason for this discrepancy is larger spin density spread in the three-molecular model although this effect cannot account for so a large discordance. Much more probably the reason for this, as well as for the considerable deviation from the expected tensor directions, lies in the limitations of the calculation model. Despite all the efforts made and a large cluster of molecules included in the geometry optimization it is possible that some of the important interactions from neighboring molecules or ions have been neglected. It is also important to point out that in a situation in which spin density is spread over more than one center, the analysis of spectroscopic data presents some difficulty. Furthermore, previously calculated data and the thiocytosine–Cl radical geometry have been obtained in such a way so as to reproduce best the values for spin densities on atoms evaluated from the experimental data [16]. Here, no assumptions of the radical structure other than it is expected to be charged radical located on sulfur center in cytosine–hydrochloride crystal lattice have been made. The density-functional method is then applied and difference in comparison to the experimental values should then be attributed to the accuracy of the used model structure or the accuracy of the previous data. Based on considerable agreement of the DFT predicted and spectroscopic values certain conclusions of the radical center properties in this system may be posed. In other research dealing with the properties of S–Cl radical center, spin densities on S and Cl of, respectively, 0.44 and 0.56 [22] and 0.49 and 0.42 [23] have been reported. Distances of S and Cl atoms in S–Cl radical center of 2.40 Å [22] and 2.73 Å [23] have been found. From this comparison of spin densities on S and Cl atoms as well as the S–Cl bond length with the research of other systems showing the two-center three-electron σ/σ′–S–Cl bond character, it may be concluded that the S–Cl contact in thiocytosine–Cl radical is also of this type.

From the theoretical considerations presented here the site of S–Cl interaction in this system may also be elucidated and the structure of the thiocytosine–Cl radical proposed. In this calculation, the interaction of sulfur from the thiocytosine molecule is not predicted to take place with the chlorine ion close to N3–H (denoted as Cl$^*$ in Fig. 1), but rather with the one from the neighboring molecular plane (Cl in Fig. 1), as concluded from the predicted spin density distribution and the hyperfine coupling constants. Whereas this assignment might not agree entirely well.

Fig. 2. (a) Cluster of 7 molecules in cytosine–hydrochloride crystal shown in view close to $|x|$ crystal axis. The oxygen to sulfur substitution site has been shown modeling the incorporation of a small amount of thiocytosine. The array of Cl–O(S) atoms from different crystal planes is clearly seen. (b) The same structure shown in projection perpendicular to the $|y|$ – (z) plane normal.
with all the published spectroscopic data (the most important being the deviation of calculated tensor principal directions from the experimentally obtained), it certainly takes into account the crystal environment, although to an amount limited by the applied calculation model. The optimized geometry of the theoretical model obtained by optimization steps (1–4) described above predicts considerable geometry disturbance of the thioctosine–chlorine centered radical structure (Table 1). In the cytosine–HCl crystal structure chlorine atom is at the distance of 2.06 Å from the H3 atom of the neighboring cytosine molecule. During the optimization process, Cl close to the presumed radical site has moved closer to the S and further from H3 of the cytosine. In the optimized structure, chlorine from the thioctosine–Cl radical center is at the distance of 2.3 Å from H1 of the neighboring cytosine (Fig. 1). Significant interaction of thioctosine in generation of the radical form examined here is by the comparison of theoretical and spectroscopic data assigned to happen with the Cl site the initial (crystal) position of which is marked in Fig. 2. From inspection of the data in Table 1, it is seen that while the distance of chlorine atom from the S2 site has been predicted significantly shorter (2.71 Å in comparison to 4.71 Å as expected from the cytosine–HCl crystal lattice), the S2–Cl direction has not changed much. These findings implicate significant change of position of the chlorine atom to a position closer to S2 site along one of the special directions in the crystal. Such interaction is indicating the possible influence of interaction along the Cl–O(S) atom array existing in the crystal x-direction (Fig. 2a and b).

4. Concluding remarks

In a number of geometry optimization calculations of a three-molecular cluster imposing more loose restrictions (results not shown) a large disturbance of the molecular ordering has been predicted, not probable in the crystal. Thus only the optimization of a large molecular cluster surrounding the predicted place of radical formation/stabilization suffices to model the crystal environment to a reasonable degree. Subsequent geometry optimization with imposed strict restrictions followed by the single-point calculation of the radical immediate vicinity then yields results which compare well to experimental results. Furthermore, the spectroscopic parameters resulting from spin density spread on several centers, which is the case here, are difficult to model as well as to obtain experimentally with high accuracy. It is even more so in the case in which spin density distribution on one of these centers might be significantly influenced by the larger structure not feasible to be modeled. In such situation it seems plausible to consider the molecular ordering of the crystal structure also when posing assumptions of the radical center structure. Yet, to be considered definitive, these assumptions based on the available experimental data followed by theoretical investigation should be further verified by means of carefully designed charge transfer experiments in this system.

Acknowledgments

Valuable discussions with Dr. J.N. Herak are gratefully acknowledged.

This work was supported by Croatian Ministry of Science, Education and Sports Grant No. 006421.

References

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