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ARTICLE

Proposition of a New Role for Cytarabine in Addition to Anti-Cancer Feature: A Theoretical Study

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Abstract: Density functional theory (DFT) was utilized to study intermolecular interactions between caffeine (**CAF**) and cytarabine (**CYT**) using B3LYP, PBEKCIS and MPWPW91 methods. Also, the mentioned interactions were investigated using MP2 method. Effects of various factors on stability of the most stable conformers of binary complex **CAF-CYT** were examined. Results indicate that competition between hydrogen bond and CH…N bond interactions influences the binding energy values of the conformers of binary complex **CAF-CYT**. Indeed, results of electronic charge densities, population analyses and charge transfer studies were considered. **CAF** is the analogue base of adenine (**A**) and may pair with thymine (**T**) to form **CAF-T** instead of **A-T** base pair and so has mutagenic effects. It seems that the intermolecular interactions between **CAF** and **CYT** play an interference role on mutagenic effects of this molecule. A new aspect was proposed for **CYT** in addition to traditional anti-cancer quality of this drug.
Keywords: DFT, Hydrogen bond, CAF, CYT, Base pair.

Introduction

Cytarabine or cytosine arabinoside is a chemotherapy agent that is mainly used in the treatment of cancers of white blood cells[1] and kills cancer cells by interfering with DNA synthesis. Effect of cytarabine on the NMR structure of a model Okazaki fragment, which intermediately occurs during lagging strand DNA replication, has been previously studied[2]. Reported results indicate that increased helical bending of cytarabine-substituted Okazaki fragments may influence the tendency of cytarabine to inhibit elongation of the lagging strand during DNA replication. Also, cytarabine antiviral activity can function by inhibiting deoxycytidine[3]. However, it is not a useful antiviral agent in humans because of its toxicity profile[4].

Development of cytarabine was firstly inspired by a series of compounds isolated from the Caribbean sponge *Cryptotheca crypta*. Also, gemcitabine, a fluorinated derivative of cytarabine, has been recently synthesized and found to have major activity in patients with bad tumors[5,6]. Moreover, cytarabine is used for synthesis of new prodrugs. For instance, Yuxia Luan and coworkers synthesized a fatty acyl di-cytarabine prodrug. They reported that this new prodrug has a lower IC_{50} and a higher cell inhibition rate compared to pure cytarabine. Thus, it has good efficiency for treatment of leukemic cells [7].

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Pure cytarabine has hydrophilic nature and isn't selective to tumor cells. Thus, stable lipid drug conjugate (LDC) of cytarabine with stearic acid has been previously synthesized for treatment of leukemia[8]. Also, two cytarabine lipid prodrugs; namely hexadecyloxyproppyl cytarabine 5'-monophosphate and hexadecyloxyproppyl cytarabine 3', 5'-cyclic monophosphate, have been synthesized from cytarabine and have shown limited aqueous solubility[9]. Furthermore, squalene acid has been conjugated to cytarabine to form the squalenoyl-cytarabine prodrug in order to improve the lipophilicity of the drug. This prodrug shows good affinity towards the environment of biological membranes[10].

A multistep protection/deprotection method has been formerly developed to enhance the ability of cytarabine through conjugation with PAMA dendrimer and linear PEG[11]. Also, the effect of cytarabine and gemcitabine on the activity of specialized polymerase β has been previously considered[12].

Binding of cytarabine to calf thymus DNA has been recently studied using spectroscopic techniques and molecular modeling simulations[13]. Results indicated tha drug makes noticeable changes for DNA interaction. Indeed, productive and non-productive binding modes of cytarabine and some anti-cancer drugs to Cytochrome P450 have been predicted by molecular dynamic and molecular docking simulations[14]. Furthermore, the major drug-binding residues in Cytochrome P450 have been recognized[14]. Also, interactions of cytarabine with lipid membranes have been investigated using molecular dynamics simulations[15].

Cristina Nerin and Marta Colon studied molecular interactions between caffeine and catechins in green tea [16]. Results proposed that these two components of green tea form complexes through intermolecular interactions in neutral media. However, these interactions are broken in acidic media. Also, intermolecular interactions between Eosin Y and caffeine have been studied using 1H-NMR spectroscopy [17].

Caffeine (**CAF**) has a mutagenic effect because it is the analogue base of adenine (**A**) and may pair with thymine (**T**) to form **CAF**-**T** instead of **A**-**T** base pair. Moreover, structure of anti-cancer drug Cytarabine (**CYT**) is similar to Cytosine and this molecule is capable of interaction with **CYT**. Thus, **CAF** and **CYT** can form binary complex **CAF-CYT** through intermolecular interactions. Formation of binary complex **CAF-CYT** may prevent the formation of **CAF**-**T** that leads to mutagenic effect of **CAF**. In this study, stability and properties of most important conformers of binary complex **CAF-CYT** were investigated using computational quantum chemistry methods. Results propose a new role for **CYT** in addition to traditional anti-cancer aspect of this drug.

Computational Methods

All geometries were optimized at the B3LYP/6-311++g(d,p) level of theory with Gaussian09 program package [18]. Interaction energy (ΔE) of each binary complex **CAF-CYT** is defined as:

$$\Delta E = E_{\mathbf{CAF\text{-}CYT}} - (E_{\mathbf{CAF}} + E_{\mathbf{CYT}}) \quad (1)$$

A satisfactory interaction between monomers **CAF** and **CYT** in the binary complex **CAF-CYT** leads to $\Delta E < 0$. Binding energy ($-\Delta E$) has an adverse sign in comparison to interaction energy and has a positive value for a favorable interaction. The binding energies of all complexes were calculated with correction for the basis set superposition error (BSSE) using the Boys-Bernardi counterpoise technique[19].

Also, binding energies of the complexes were calculated using PBEKCIS, MPWPW91 and MP2 methods. The topological properties of electronic charge densities have been calculated by the atoms in molecules (AIM) method on the wave functions obtained at the B3LYP/6-311++g(d,p) level of theory using AIM2000[20] program. The population analysis has been performed by the natural bond orbital (NBO) method[21] at the MP2/6-311++g(d,p) level using NBO program implemented under Gaussian09 program package. Atomic net charges were calculated using ChelpG scheme[22] on the optimized geometries obtained at the B3LYP/6-311++g(d,p) level of theory.

Results and Discussion

Energy Data and Structural Parameters

All geometries were optimized at the B3LYP/6-311++g(d,p) level of theory. Caffeine (**CAF**) is the analogue base of adenine (**A**) and may pair with thymine (**T**) to form **CAF-T** instead of **A-T** base pair. This mispairing is the basis of the mutagenic effect of **CAF**. Therefore, it seems that the interaction of **CAF** and **CYT** may prevent the mutagenic effect of **CAF**. Thus, possibility of formation of binary complex **CAF-CYT** was investigated in this study. The optimized structures of the most stable conformers of the **CAF-CYT** complex are shown in Fig. 1. Results indicate that the order of binding energies (in kcal mol^{-1}) of these conformers calculated at the B3LYP/6-311++g(d,p) level of theory is: **d** (7.85) < **c** (7.86) < **b** (8.46) < **a** (11.87). Basis set superposition error (BSSE) corrections were performed on the optimized complexes. Results reveal that the order of BSSE corrected binding energies is: **d** (5.08) < **c** (5.39) < **b** (5.89) < **a** (8.82). Also, the order of binding energies at the PBEKCIS/6-311++g(d,p) level is: **d** (6.71) < **c** (6.81) < **b** (7.38) < **a** (10.36). Moreover, binding energies of the complexes at the MPWPW91/6-311++g(d,p) level have the following order: **c** (5.93) < **d** (6.04) < **b** (6.46) < **a** (9.39). Furthermore, the order of binding energies at the MP2/6-311++g(d,p) level is: **c** (8.99) < **d** (9.21) < **b** (9.91) < **a** (11.99).

Structural parameters presented in Table 1 indicate that binding energies of all conformers are increased by decrease of hydrogen bond lengths with the exception of conformer **a** (which has the largest hydrogen bond length). Instead, the smallest CH…N distance corresponds to conformer **a**. In fact, there is no methyl steric hindrance in this conformer and **CAF** is close to atom N_2 of **CYT** from C_{39}-H_{46} moiety (see Figure. 1). On the other hand, a decrease of the CH…N distance is accompanied by an increase of binding energies of the conformers. Therefore, competition between hydrogen bond and CH…N bond interactions influences the binding energy values of the conformers of binary complex **CAF-CYT**.

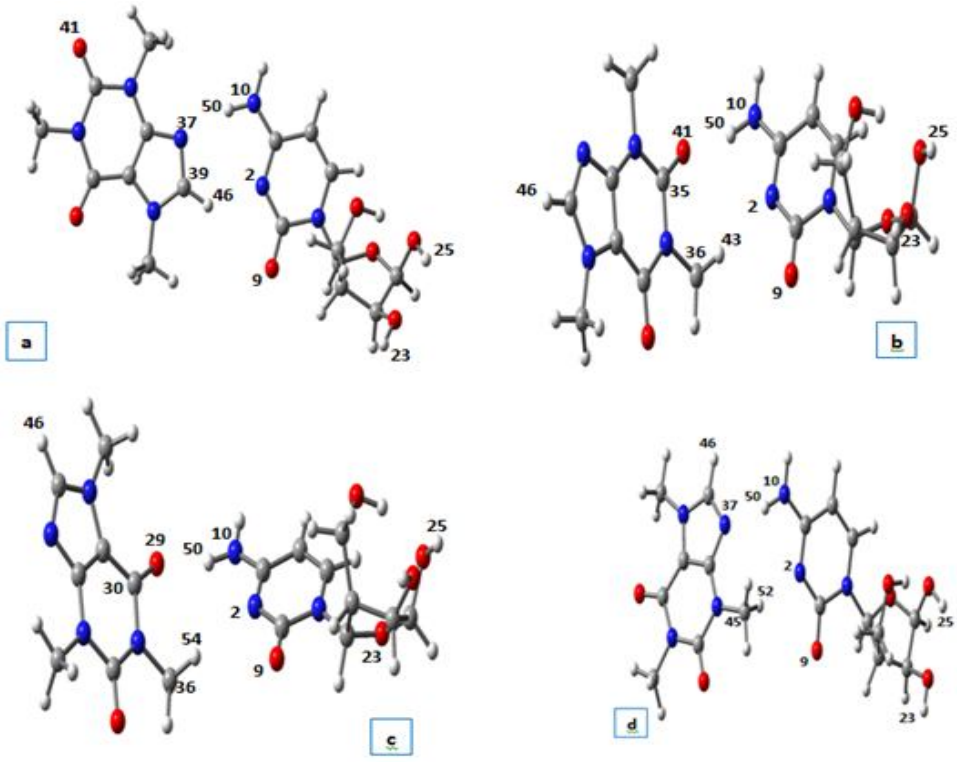

Figure 1. The optimized structures and atom numbering of the four conformers of the **CAF-CYT** complex.

Table 1. The most important structural parameters (in Å) and electron charge density properties (in au) of the optimized conformers of the CAF-CYT complex calculated at the B3LYP/6-311++G(d,p) level of theory.

Conformer	Hydrogen bond length	CH-N distance	$\rho_{BCP,\ H\ bond}\times10^2$	$\rho_{BCP,\ CH\text{-}N}\times10^2$	$\rho_{RCP,central}\times10^3$
a (N…H)	2.058	2.222	2.237	1.824	5.014
b (O…H)	1.929	2.445	2.340	1.165	3.128
c (O…H)	1.936	2.441	2.335	1.180	3.317
d (N…H)	2.051	2.426	2.314	1.214	3.177

AIM Analysis

The topological properties of electronic charge densities have been calculated by the AIM method to find the relationship between calculated electronic charge density values and binding energies of the conformers. Typical molecular graphs of the most stable conformers of the **CAF-CYT** complex are shown in Figure 2. Bond critical points (BCPs), ring critical points (RCPs) and paths of bonds are observed at the above mentioned molecular graphs. Results of BCPs presented in the Table 1 confirm the hydrogen bond lengths and CH…N distances of the conformers. As displayed in Figure 2, the arrangement of atoms of **CAF** and **CYT** in the four conformers of binary complex **CAF-CYT** builds a central ring at each molecular graph named as **A**, **B**, **C** and **D** in conformers **a**, **b**, **c** and **d**, respectively. The values of RCPs at these central rings are presented in the Table 1 denoted as $\rho_{RCP,\ central}$. Results indicate that conformer **a** has the largest $\rho_{RCP,\ central}$ value among other conformers and the order of $\rho_{RCP,\ central}$ values is in harmony with the order of $\rho_{BCP,\ CH\text{-}N}$ values in the above mentioned conformers. Actually, CH-N bond is more important than hydrogen bond in conformer **a**. This conformer has the largest $\rho_{BCP,\ CH\text{-}N}$ value and binding energy among other conformers. However, trends of $\rho_{BCP,\ H\ bond}$ values and binding energies are similar in other conformers. Therefore, hydrogen bonds are more important than CH…N bonds in the latter conformers.

NBO Studies

The population analysis has been performed by the NBO method on the optimized structures of the four conformers of binary complex **CAF-CYT** to better understand the role of donor-acceptor interaction energies (E^2 values) on stability of the above mentioned complexes. The most important donor-acceptor interaction energies obtained from the NBO analysis are presented in Table 2. As can be seen, the order of E^2 values (in kcal mol^{-1}) of the Lp $N_2 \rightarrow BD^*$ C-H interaction in the above mentioned conformers is: **d** (2.26) < **c** (2.35) < **b** (2.39) < **a** (4.85) which is consistent with the order of binding energies of these conformers. Thus, the Lp $N_2 \rightarrow BD^*$ C-H interaction plays an important role in the stability of the conformers of binary complex **CAF-CYT**.

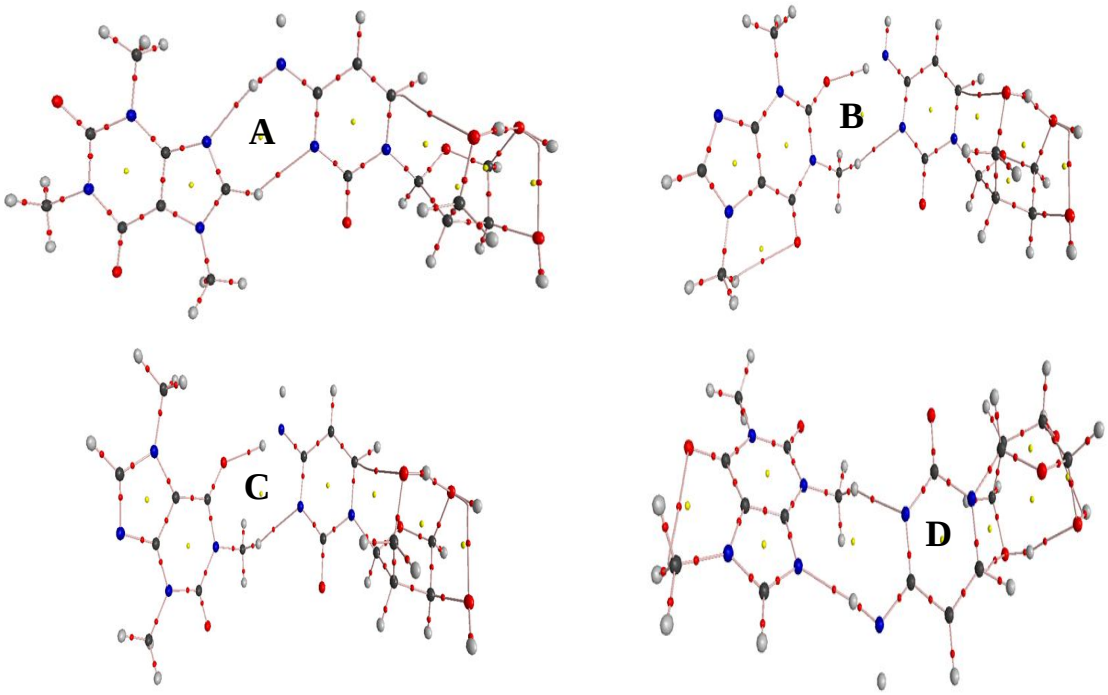

Figure 2. Typical molecular graphs of the most stable conformers of the **CAF-CYT** complex.

Table 2. The most important donor-acceptor interaction energies (in kcal mol^{-1}) obtained from the NBO analysis at the MP2/6-311++G(d,p) level of theory for conformers of the CAF-CYT complex.

Donor-acceptor interaction	E^2	Donor-acceptor interaction	E^2
a		**b**	
BD N_{10}-H_{50} → RY* N_{37}	0.46	BD N_{10}-H_{50} → RY* O_{41}	0.36
Lp N_{37} → BD* N_{10}- H_{50}	9.95	Lp(1) O_{41} → BD* N_{10}-H_{50}	6.23
BD C_{39}-H_{46} → RY* N_2	1.16	Lp(2) O_{41} → BD* N_{10}-H_{50}	1.50
Lp N_2 → RY* C_{39}	0.11	BD C_{35}-O_{41} → BD* N_{10}-H_{50}	1.77
Lp N_2 → RY* H_{46}	0.21	BD* C_{35}-O_{41} → RY* H_{50}	0.71
Lp N_2 → BD* C_{39}-H_{46}	4.85	Lp N_2 → BD* C_{36}-H_{43}	2.39
c		**d**	
BD N_{10}-H_{50} → RY* O_{29}	0.37	BD N_{10}-H_{50} → RY* N_{37}	0.53
BD C_{30}-O_{29} → BD* N_{10}-H_{50}	0.87	Lp N_{37} → BD* N_{10}-H_{50}	10.71
Lp(1) O_{29} → BD* N_{10}- H_{50}	5.97	Lp N_{37} → RY* H_{50}	0.42
Lp(2) O_{29} → BD* N_{10}- H_{50}	2.58	BD C_{45}-H_{52} → RY* N_2	0.33
Lp(1) N_2 → BD* C_{36}-H_{54}	2.35	Lp N_2 → BD* C_{45}-H_{52}	2.26
BD C_{36}-H_{54} → RY* N_2	0.43	Lp N_2 → RY* C_{45}	0.11

It should be noted that there is no meaningful relationship between E^2 interaction energies of Lp X→ BD* Y-H interactions and binding energies of these conformers (where X = N and O and Y = N). In fact, X = N in two conformers (**a**, **d**) and X = O in two conformers (**b**, **c**). Conformer **d** has the highest E^2 interaction energy for the Lp N_{37}→ BD* N_{10}-H_{50} interaction. Also, the most stable conformer (**a**) has a notable E^2 interaction energy for the Lp N_{37}→ BD* N_{10}-

H_{50} interaction. However, oxygen atoms have two lone pair electrons in two conformers (**b**, **c**) and there are two Lp X→ BD^* Y-H interactions in these conformers. Therefore, the sum of two interactions would be considered. Consequently, E^2 interaction energies of the Lp X→ BD^* Y-H interactions don't present a criterion for interpretation of the stability of conformers.

The atomic charges were calculated using ChelpG scheme to study the charge transfer which occurred between **CAF** and **CYT** in the conformers of binary complex **CAF-CYT** and to understand the relationship between properties of atomic charges and stability of these conformers. Results indicate that the order of atomic charges (in e) on carbon atoms which are existing in the CH…N bonds in the above mentioned conformers is: **d** (-0.2118) < **c** (-0.2069) < **b** (-0.2050) < **a** (0.2987) which is in agreement with the order of binding energies of these conformers. As can be seen, atomic charge on carbon atom of CH…N bond is positive in conformer **a**. In fact, this carbon atom exists in a five-member ring. However, carbon atoms of CH…N bonds correspond to methyl groups in other conformers and have negative charges. On the other hand, the order of atomic charges (in e) on hydrogen atoms which are involved in the CH…N bonds in these conformers is: **a** (0.1855) < **d** (0.1907) < **c** (0.1912) < **b** (0.1918). As can be observed, conformers with larger binding energies have higher positive charges on hydrogen atoms which are related to the methyl groups (with the exception of conformer **a**). Conversely, the hydrogen atom of CH…N bond is part of a five-member ring in conformer **a** and atomic charges on atoms of this ring are affected directly by the circulation of electrons which leads to a good CH…N bond in this conformer (C and H atoms have positive charges and N atom has negative charge). On the other hand, C and N atoms of the CH…N bonds have negative charges and H atoms of these bonds have positive charges in the other conformers. Really, hydrogen bond interactions have more contribution to stability of the latter conformers than CH…N bonds.

Results show that some of atomic charges on **CAF** in the conformers of binary complex **CAF-CYT** are negative and charge transfer direction in these conformers is: **CYT → CAF**. As can be seen in Fig. 3, decreasing of the amount of the mentioned charge transfer is accompanied by increasing of the binding energies.

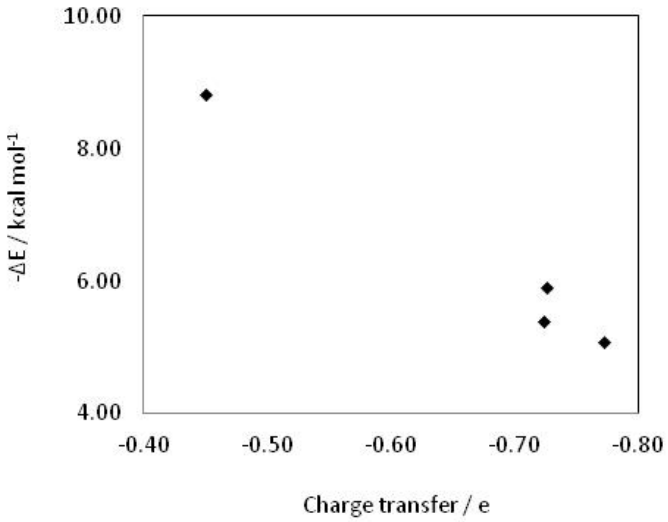

Figure 3. The binding energies *versus* magnitude of charge transfer in the conformers of the **CAF-CYT** complex.

Conclusions

Results indicate that the structure of **CYT** is capable of formation of intermolecular interactions with **CAF** which may prevent the formation of **CAF-T** in the DNA replication process. Competition between hydrogen bond and CH…N bond interactions influences on the binding energy values of the conformers. The order of E^2 interaction energies of the Lp $N_2 \rightarrow BD^*$ C-H interaction in the conformers is consistent with the order of binding energies. Results show that charge transfer direction in the conformers is: **CYT** → **CAF**. Also, decreasing of the amount of the charge transfer is accompanied by increasing of the binding energies. A new role was proposed for **CYT** in addition to the traditional anti-cancer feature of this drug.

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