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Effects of halogen substitution on Watson–Crick base pairing: A possible mechanism for radiosensitivity

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ABSTRACT

The halogen substituent effect on geometries and charge distributions of the A–T base pair derivatives was evaluated using density functional theory at B3LYP/6-31G* level. The results indicate that all of the substitutions affect geometries and charge distributions of the atoms contributing hydrogen bonds. These changes would be the reason of the radiosensitization of these compounds incorporating DNA.

Radiosensitizers are chemicals that make cells more sensitive to radiation by different mechanisms. Due to resistance of tumor cells to radiation, radiosensitizers are of great importance in cancer therapy. Drugs that affect nucleoside and nucleotide metabolism are among the most effective and most widely used agents to sensitize tumor cells to radiation treatment.[1,2]

Perhaps the most important class of these molecules is the 5-halouracils which are formed by a C5 replacement of hydrogen in uracil or the methyl group in thymine with a halogen.[3] The incorporation of halogenated pyrimidines into the DNA is known to increase the radiosensitivity of mammalian cells in vitro and in vivo. 5-Bromouracil (5-BrU) and 5-iodourail (5-IU) are already applied clinically to enhance locoregional effectiveness of radiotherapy.[4–6] These two compounds are readily incorporated into the DNA of mammalian cells. Incorporation of 5-flurouracil (5-FU) into DNA inhibits DNA replication and alters DNA stability by producing DNA single-strand breaks and DNA fragmentation. The level of radiosensitization by 5-halouracils has been shown to correlate with the degree of thymidine-replacement.[7–9] Cells that have incorporated 5-halouracils demonstrate an increase in the amount of radiation-induced DNA single and double strand breaks,[10,11] and chromosomal aberrations.[12,13]

So far studies have been performed to clarify the mechanisms of radiosensitivity by these compounds. Despite to understanding the cellular pathways, exact molecular mechanism underlying radiosensitization of these compounds has not been determined.[14]

It has been proposed that the physicochemical properties of altered DNA are influenced by thymine replacement. For example Incorporation of 5-BrU increases the forces that bind the strands of DNA together.[15]

Computational methods have been frequently used to study these cases. Wetmore et al. were studied these compounds computationally with emphasis on electron affinity and ionization potential. They concluded that decreasing ionization potential is responsible for enhancement sensitivity of this compound to ionizing radiation.[16]

Previous reports highlighted the importance of geometry, charge distribution and base pairing patterns in DNA. They are very important as they play important roles in the transcription and translation processes which are an integral component of the gene replication phenomena. The weak forces between the base pairs like the hydrogen bonding (H-bonding), plays the crucial role in the microscopic theory behind these biological processes.[17]

In the other hand, Guerra et al.[18] showed that halogen substitution at C8 position of purine and/or C6 position of pyrimidine affects Watson–Crick hydrogen bond geometry.

In this study, the effects of C5 substituents have been investigated computationally on adenine–thymine Watson–Crick base pairs where the methyl group C5 in the natural thymine was replaced with fluorine, chlorine, bromine, iodine and hydrogen substituents (Fig. 1). Although nucleoside form of these compounds (5-halo deoxyuridine) is used as drug, our computation performed on their base component (5-halo uridine). Geometry of thymine–adenine base pair and charge distributions were analyzed. Our computations were carried out on a personal computer (Pentium

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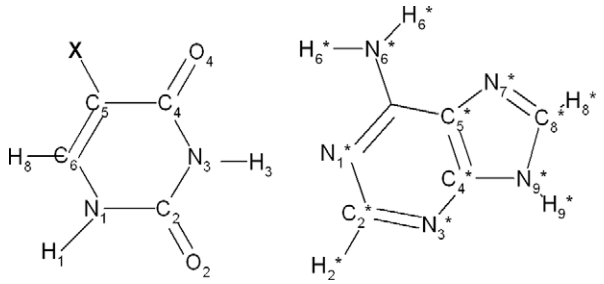

Figure 1. Watson–Creek T^{X5}-A base pair. (Thymine: X = CH3, Uracil: X = H and 5-HaloUracils: X = F, Cl, Br and I).

IV 3 GHz, IBM-compatible). The software used to construct the calculation was GAUSSIAN 98 program.

Density functional theory (DFT) method using 6-311G* basis set,[19,20] of GAUSSIAN program,[21] was employed for full geometry optimizations of thymine–adenine base pair structures and its substituents. For DFT calculations, the Becke's hybrid three-parameters functional combined with the Lee-Yang-Parr nonlocal correlation functional (B3LYP) were used. The calculations were done by LANL2DM basis set,[22] for 5-Iodo Uracil (5-IU), accompanying the 6-311G* basis set for nitrogen, carbon, hydrogen and other halogens atoms using 'Extrabasis' keyword.

Among various theoretical methods B3LYP was reported to be an appropriate method for studying the Watson–Crick base pairs for both the neutral and cationic systems.[23] This method has to be shown to save computer time as compared with other methods. Indeed, B3LYP is the most promising quantum mechanical method for modeling biomolecules.[24]

Global minima were specified on corresponding energy surfaces through relax scan and to confirm the nature of the stationary species, frequency calculations were performed.

Natural Bond Orbital (NBO) keyword was used to calculate charge distribution. NBO keyword requests a full Natural Bond Orbital analysis. These properties keywords control the molecular orbital and several types of population analysis and atomic charge assignments.

Meanwhile, the outputs of low level of theory, B3LYP/STO-3G are applied for inputs of higher level of theory, B3LYP/6-311G*.

Figure 1 illustrates the nomenclature we used. The letters A and T were used for natural adenine and thymine DNA bases and $T^{(X)}$ for 5-halouracils. Thus, $T^{(CH3)}$ represents the natural thymine (A) and $T^{(H)}$ represents the natural uracil (U) whereas $T^{(F)}$, for example, refers to 5-fluorouracil in which methyl group on the thymine C5 have replaced by fluorine atom. Normal letters were used for thymine and 5-halouracils atoms while asterisk letters (*) were used for adenine atoms.

The results of our B3LYP/6-311G* computations on the natural and halogen-substituted Watson–Crick AT base pairs are summarized in Table 1 (thymine bond lengths), Table 2 (thymine–adenine distance) Table 3 (thymine torsion angles) and Figure 2 (charge distribution). For better comparison, thymine–adenine distance and charge distribution on the atoms contributing to hydrogen bonds are showed graphically in Figures 3 and 4, respectively.

Table 1
Thymine bond lengths (Å)

Bonds	Substituents					
	F	Cl	Br	I	CH3	H
N1–C2	1.39356	1.39692	1.39730	1.39814	1.39253	1.39830
C2–N3	1.38444	1.38039	1.37953	1.37902	1.38039	1.37913
N3–C4	1.38927	1.39166	1.39309	1.39368	1.39001	1.39496
C4–C5	1.46060	1.46667	1.46663	1.46546	1.46444	1.45567
C5–C6	1.34355	1.34879	1.34924	1.34915	1.34999	1.34795
C6–N1	1.37585	1.36971	1.36955	1.36910	1.37500	1.37063
C5–X	1.34033	1.73678	1.89366	2.12354	1.49948	1.08004
C4–O4	1.22221	1.22054	1.22065	1.22032	1.22885	1.22646
N3–H3	1.04432	1.04485	1.04517	1.04541	1.04030	1.04081
C2–O2	1.21254	1.21187	1.21203	1.21167	1.21385	1.21285

Table 2
Thymine–adenine distance (Å)

Distance	Substituents					
	F	Cl	Br	I	CH3	H
N3–H3···N*1	2.87341	2.87357	2.86878	2.86996	2.89425	2.89098
O4···H*6–N*6	2.97448	2.97126	2.97722	2.96994	2.95853	2.95791
O2···H*2	2.84355	2.83618	2.81505	2.82028	2.89236	2.89219

As showed in Table 3 torsion angles remains essentially constant by substitutions, but bond lengths (Table 1) were affected more or less. The most significant change belongs to X-C5 lengths in which differ from 1.34033 Å in $T^{(F)}$ to 2.12354 Å in $T^{(I)}$ in compare with 1.49948 Å in natural T where the length of the other bonds have been changed too. The C4–O4 bond length has been decreased and H3–N3 bond length has been increased by all of the substitutions.

Substituting methyl group by halogen atoms (F, Cl, Br and I) in the Watson–Crick pair AT causes relatively small changes in hydrogen bond distances (see Table 2) if compared with the much larger effects that occur if associated atoms in hydrogen bond were replaced by halogen.[25] Illustrating in Figure 1, O4···H*6–N*6 distance is increased by all substitutions according to $T^{(F)} > T^{(Br)} > T(Cl) > T^{(I)} > T^{(CH3)} > T^{(H)}$ but N3–H3···N*1 distance is decreased: $T^{(CH3)} > T^{(H)} > T^{(Cl)} > T^{(F)} > T^{(I)} > T^{(Br)}$. Analysis of O2···H*2 distance showed a greater decrease for all substitutions (Table 2 and Fig. 3). These changes caused opening of $A^{(X)}$-T base pairing.

Figure 2 shows the charge distribution for all of the structures. As it shows, only thymine not adenine was affected in charge distribution changes due to halogen substitutions. Substituted halogens charges had great changes from −0.326 esu for fluorine to 0.0200 esu for iodine. All of the substituents have changed the charge distribution on the atoms contributing in hydrogen bonds; but this change is less than closer atoms to substituents.

Since 1960s it has been suggested that the DNA incorporation is the critical event for radiosensitization by BrdUrd and IdUrd.[26] However The exact mechanism underlying the enhanced response to radiation is not completely understood yet.

Once the monophosphates are formed, they readily convert to their corresponding triphosphates, which are the major cellular metabolites. Both Bromodeoxyuridine triphosphate (BrdUTP) and Iododeoxyuridine triphosphate (IdUTP) are excellent substrates for DNA replication and are readily incorporated into DNA instead of their endogenous competitor, thymidine 5′-triphosphate (dTTP).[27] The resulting incorporation of 5-bromodeoxyuridine-5′-monophosphate (BrdUMP) and 5-iododeoxyuridine-5′-monophosphate (IdUMP) into internal linkages in DNA leads to cytotoxicity.[26]

Several mechanisms have been suggested to explain for the radiosensitizing property of BrdUrd/IdUrd resulting from their incorporation into DNA.

Prusoff studies suggested that the effect of steric hindrance resulting from analogue incorporation into DNA was negligible; but the physicochemical properties of altered DNA were influenced by thymidine replacement. For example incorporation of BrUdR can alter DNA transcription and replication by altering the force of hydrogen bonds which it can increase sensitivity to radiation.[15] Also the large highly electronegative halogen atoms increase the cross-sectional area available for trapping radiation-produced electrons. In addition, migration of absorbed energy to a halogenated base has been demonstrated.[28] In the other hand, the affinity of chromosomal proteins for BrUdR- and IUdR-substituted DNA, asso-

Table 3
Thymine torsion angles (°)

Torsion angle	Substituents					
	F	Cl	Br	I	CH3	H
N1C2N3C4	000.000	−000.003	−000.006	−000.007	000.005	−000.004
C2N3C4O4	−180.000	−179.996	−179.996	−180.000	−180.000	−179.997
O4C4C5C6	180.000	179.997	180.000	−180.000	−179.996	180.000
C4C5C6N1	000.000	000.000	000.000	000.000	000.000	000.000
C5C6N1C2	000.000	000.000	000.000	000.000	−0.00004	000.000
C6N1C2N3	000.000	000.000	000.004	000.005	000.008	000.000
O2C2N1C6	−179.990	−179.996	−180.000	−179.997	−179.99	−180.000
O2C2N3C4	179.990	179.993	179.995	179.996	179.99	179.990
H6C6N1C2	−180.000	180.000	180.000	180.000	−180.000	180.000
H6C6C5C4	−180.000	−180.000	−180.000	180.000	179.994	180.000
H1N1C2N3	180.000	180.000	−180.000	180.000	179.994	180.000

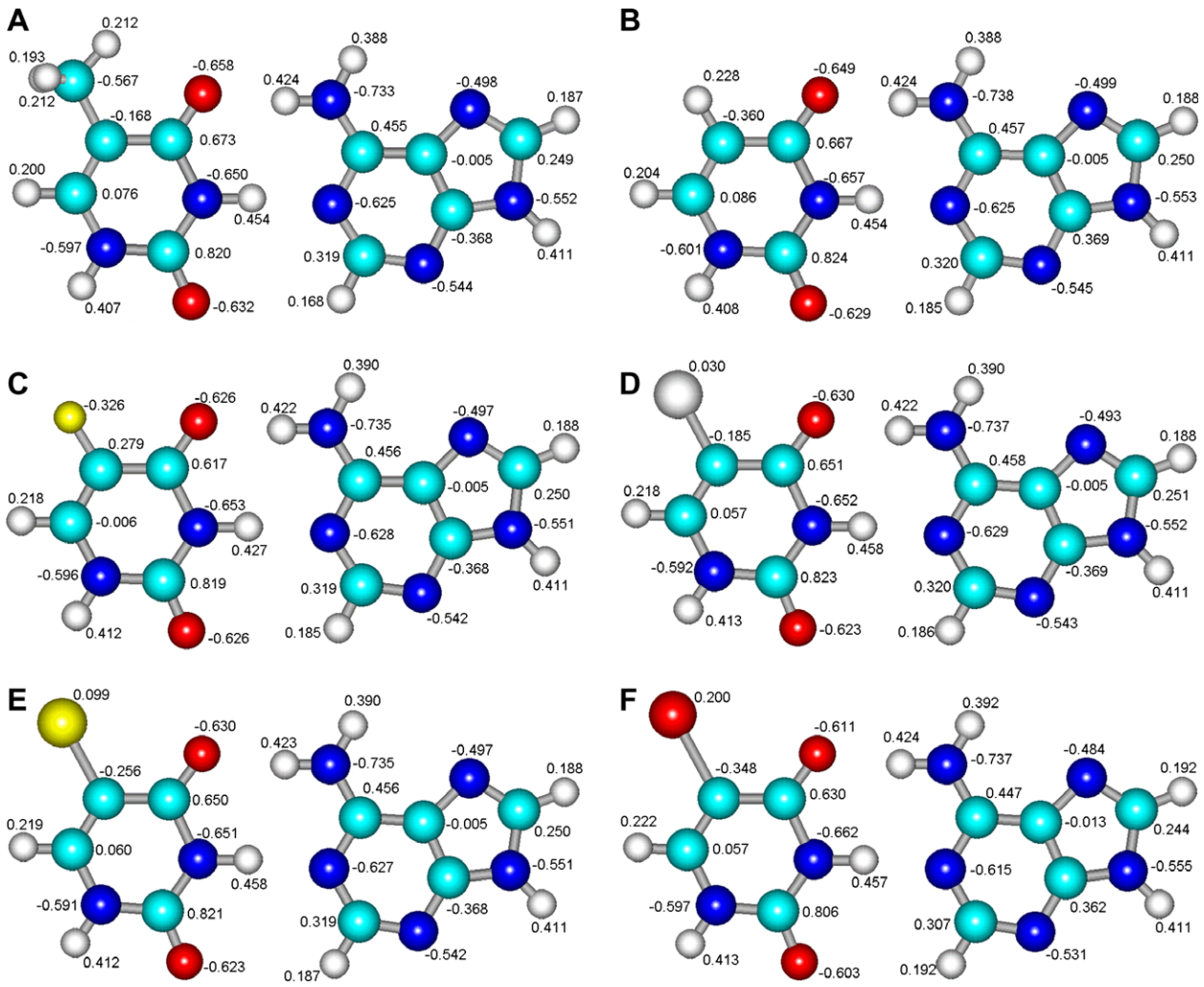

Figure 2. Charge distribution on A–$T^{(X)}$ Base pairs: (A) thymine; (B) uracile; (C) 5- fluorouracile; (D) 5-clorouracile; (E) 5-bromouracile; (F) 5-iodouracile.

ciated with the repression or induction of cellular proteins, receptors and growth factors, is increased.[29]

Wetmore et al. investigated these compounds computationally with emphasis on electron affinity and ionization potential.[16] They concluded that halogen substitution has a smaller effect on the ionization potential than the electron affinity. The electron affinities were calculated to increase according to $T^{(CH3)} < T^{(H)} < T^{(F)} < T^{(Cl)} < T^{(Br)}$.

In this study, the effect of different halogen substitutions on C5 position of thymidine base has been investigated computationally. It showed that these substitutions have lead to different changes in adenine–thymine Watson Crick base pairs.

Due to these substitutions, the geometry of thymine molecule, charge distribution on it and hydrogen bond lengths were altered. These parameters have a key role in cellular process such as the accuracy of DNA repair, replication and transcription.

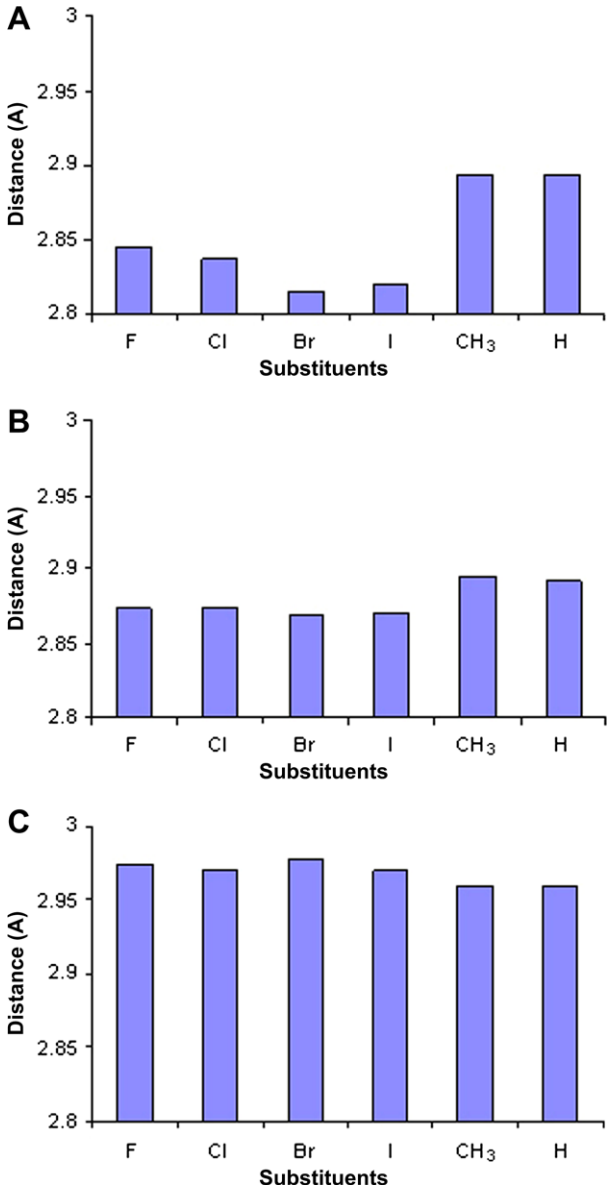

Figure 3. Thymine–adenine distance: (A) O2···H*2 distance, (B) N3–H3···N*1 distance and (C) O4···H*6–N*6 distance.

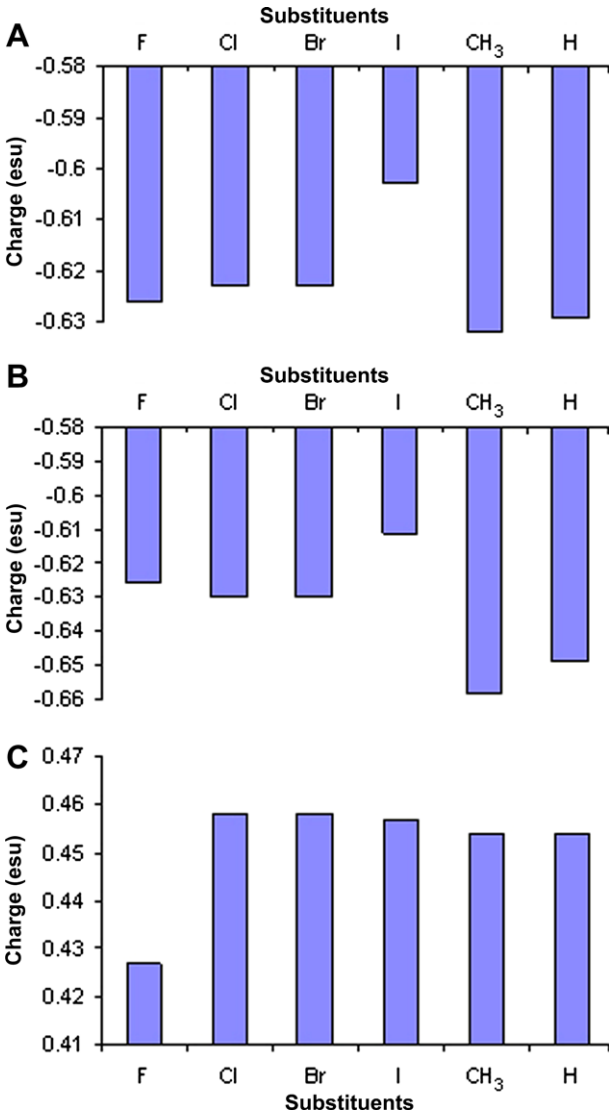

Figure 4. Charge distribution on the atoms contributing in hydrogen bonds: (A) O_2 charge; (B) O4 charge; (C) H3 charge.

Since this compounds show their effects even in the absence of radiation, it is therefore necessary to propose a mechanism to justify the fact. This mechanisms should represent more detailed cellular and molecular aspects, in addition to change in ionization potential and electron affinity, as suggested by Wetmore et al.[16] To get close to this idea we believed that physicochemical properties alteration of double strand DNA caused by halogens substitutions can be considered as alternative mechanism. Subsequent to induction of single and double strand breaks due to radiation exposure, incorporation of these thymine analogues in DNA is increased by DNA repair system.[7–9] As this study indicated, changes would occur in DNA conformation and charge distribution owing to such substitution, which eventually may lead to changes in the size of DNA major and minor grooves. Furthermore, it has been already confirmed that changes in DNA conformation specially in geometry of hydrogen bonds, play a key role in normal cellular processes such as replication and transcription.[30–32] Indeed, it is noticeable that such changes must be small enough in order that the nucleotide analogues would be admitted by cellular machinery in the repair process. Otherwise, incorporation of such analogues in DNA will not allow to be occurred.[33]

Further studies should be performed to investigate the effect of incorporation these analogues in DNA polynucleotide fragments to facilitate the analysis of additional DNA structure parameters such as base stacking and overall conformational changes.

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