Cytosine Iminyl Radical (cytN•) Formation via Electron Induced Debromination of 5-Bromocytosine: A DFT and Gaussian 4 study

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Abstract

Halogen-substituted pyrimidines, such as 5-bromouracil and 5-iodouracil, have been used as radio therapeutic (RT) agents in cancer treatment. The radiosensitizer activity of 5-bromouracil is attributed to its reaction with electron which produce the highly reactive uracil-5-yl radical by dissociating the C5-Br bond. Using density functional methods and highly accurate Gaussian 4 (G4) theory, herein, we show that 5-bromocytosine (5-Brcyt) after reaction with electron, also, leads to the formation of cytosine-5-yl radical. However, our results show that this species can subsequently undergo a base catalyzed tautomerization reaction to form the π-aminyl radical followed by a second tautomerization to the thermodynamically most stable σ-iminyl radical (cytN•) (see scheme 1). From the present theoretical calculations, we infer that the mechanism of the formation of cytN• by one-electron reduction of 5-Brcyt is straightforward and may take place in 5-BrCyt labeled DNA in competition with the usual reactions expected for the cytosine-5-yl radical such as abstraction and water addition.

Graphical abstract
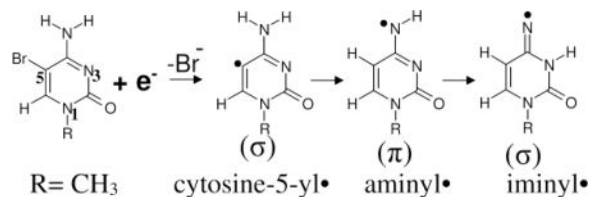
Introduction

Halogen substituted nucleosides have long been studied as radiosensitizers with the goal of radio therapeutic (RT) treatment of cancer.[1–9] Replacing thymine by 5-halouracil (5-XU; X= F, Cl, Br, and I) in cellular DNA leads to enhance the sensitivity to ionizing radiation (IR) and allows gene expression in unirradiated cells.[3,1,2,4–8] Most studied is 5-Bromo-2'-deoxyuridine (5-BrdU) as it has been found to sensitize hypoxic cancer cells to IR[5,6,10,11]

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Associated Content

The Supporting Information contains: (a) Optimized geometries of 5-brcytosine in neutral and anion radical state, (b) HOMO and LUMO of 5-bromocytosine, (c) Enlarged view of figures 1 -3, (d) Potential energy surfaces for proton transfer reactions and (e) complete Gaussian 09 reference. The Supporting Information is available free of charge on the ACS Publications website.

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and has been employed as a radiosensitizer in clinical trials, although, little survival advantage was reported.[12] 5-Bromo-2'-deoxycytidine has also been tested for its radiosensitization activity in cancer cell lines.[9]

The enhanced radiosensitization activity of 5-BrdU in DNA has been proposed to involve its reaction with radiation produced electrons. The 5-BrdU anion radical (π-type) formed converts to a weakly bond (σ-type) anion radical which subsequently dissociates into uracil-5-yl radical (σ-type) and bromide ion (Br^-).[4,5,13–18] The uracil-5-yl$^\bullet$ is highly reactive and is suggested to abstract a hydrogen atom from $C_{1'}$ or $C_{2'}$ site of the adjacent deoxyribose which ends up with DNA strand breaks.[4,5,15,18]. Li et al note that 5-BrdU in double stranded DNA is less electron affinic than in single stranded DNA which would make 5-BrdU selective for replicating DNA.[19] Electron spin resonance (ESR) work has shown that other reactions occur after electron addition to 5-BrdU, for example, the π-anion radical is shown to protonate at C6 to form a C5 radical (6, 6 dihydro-5-bromouracyl-5-yl$^\bullet$) under certain conditions.[20] Water addition to the uracil-5-yl$^\bullet$ at the C5 site has been found to form the 5-hydroxy-5-hydro-uracyl-6-yl radical.[13] Very recently, ESR and density functional theory (DFT) studies demonstrated that the reaction of uracil-5-yl$^\bullet$ with water may produce OH$^\bullet$ as an intermediate which may lead to strand breaks.[17] Interestingly, photochemical formation of uracil-5-yl$^\bullet$ in 5-XU containing DNA is used to probe the structure of DNA.[4]

Here, *ab initio* quantum chemical methods are employed to investigate the reaction of radiation produced electrons with 5-bromocytosine (5-Brcyt). These theoretical predictions clearly show that one-electron reduction of 5-Brcyt leads to the formation of π-type 5-Brcyt anion radical (I) which subsequently dissociates into a reactive σ-type cytosine-5-yl$^\bullet$ (IIa) and bromide ion. The σ-type cytosine-5-yl$^\bullet$ undergoes a tautomerization reaction which transfers a hydrogen from NH2 group to C5 atom of cytosine to produce a π-type aminyl radical (cytNH$^\bullet$) (III) which tautomerizes again by transferring hydrogen from NH to N3 of cytosine to form the more stable σ-type iminyl radical (cytN•) (IV) as shown in Scheme 1. The latter step (III to IV) has been shown by both pulse radiolysis and ESR experiments in which cytosine underwent one-electron oxidation.[21,22] This reaction is also supported by theory.[21,22] However, the chemistry induced by one-electron reduction of 5-Brcyt to form the iminyl radical (cytN•) by a double tautomerization mechanism is novel and predicted in this work.

Methods of Calculation

In the present study, we used B3LYP, ωb97X and M06-2X DFT methods with 6–31++G(d, p) basis set to calculate the relative stabilities of 5-Brcyt$^{\bullet-}$, cytosine-5-yl$^\bullet$, aminyl radical (cytNH$^\bullet$) and iminyl radical (cytN•), shown in Scheme 1, by calculating their free energies in aqueous phase via the integral equation formalism of the polarized continuum model (IEF-PCM) of Tomasi et al.[23] Geometry of each species was fully optimized by each of the DFT method mentioned above. Using optimized geometries, thermodynamics followed by frequency calculation was done to obtain the free energy (G) of each species. The vibrational frequencies of each species were found to be positive which ensure the occurrence of these species. All these DFT methods support the formation of the most stable iminyl radical (cytN•) via stepwise tautomerization of cytosine-5-yl$^\bullet$. The free energy barrier

height for reaction 5-Brcyt$^{\bullet-}$ → cytosine-5-yl$^{\bullet}$ + Br$^-$ in aqueous phase were also calculated by these DFT methods with 6–31++G(d,p) basis set.

These DFT predictions were further supported by high level *ab initio*-based Gaussian 4 (G4) level of calculation including IEF-PCM. The G4 theory is a combination of several electron correlated ab initio methods for accurate prediction of thermodynamic properties[24–26] of molecules developed by Curtiss et al.[24] The average absolute deviation in energies calculated at the G4 level including enthalpies, ionization energies, electron affinities, proton affinities, and hydrogen bond complexes for a data set of 454 experimental values is 0.83 kcal/mol and the root-mean-square deviation is 1.19 kcal/mol.[24] The details of the G4 theory is described elsewhere.[24] All calculations were performed using Gaussian 09 suites of program. [27] To mimic the effect of sugar moiety, methyl (CH$_3$) group is attached to the N1 site of the cytosine, see scheme 1.

Results and Discussion

Debromination reaction

The ESR studies at low temperature has shown that brominated-bases in DNA serves as efficient electron trap due to their high electron affinity in comparison to the canonical DNA bases.[14] Even in DNA the brominated-radical anions are found.[13] Our DFT calculations show that the adiabatic electron affinity (AEA) of 5-Brcyt predicted by the G4 level of theory and by the other DFT methods lie in the range 2.16 – 2.27 eV, see Table 1, which is in agreement with recent study by Chomicz et al.[28]. Theory and experiment have shown that C5-Br bond of the 5-bromouracil radical anion is quite weak and dissociates into Br$^-$ and uracil-5-yl$^{\bullet}$. [4,5,17,28,29]

In this study the reaction, 5-Brcyt$^{\bullet-}$ → cytosine-5-yl$^{\bullet}$ + Br$^-$, is studied using the B3LYP-PCM/6 31++G(d,p) method. The potential energy surface (PES) of C5-Br bond dissociation of 5-Brcyt$^{\bullet-}$ is shown in Figure 1. In Figure 1, the nature of the singly occupied molecular orbital (SOMO) is also shown at each step of the reaction. We found that in 5-Brcyt$^{\bullet-}$ (reactant), SOMO is delocalized on the cytosine ring as π-type. The transition state (TS) is a mixed σ-π state with the SOMO localized on the Br atom as the σ-state and delocalized on the cytosine base as the π-state as reported in earlier work on 5BrU by Sevilla and coworkers. [29] As the C-Br bond length increases a loosely bound metastable σ radical mainly localized to the C-Br bond (II) is formed (see Figure 1) (This species is observed in DNA containing Br-Cyt by ESR) [14]. On complete C5-Br bond dissociation (product, IIa), the radical formed shows the localization of spin density mainly on C5 atom (Figure 2) which characterizes it as σ-cytosine-5-yl radical (IIa). The free energy barrier height for C5-Br bond dissociation of 5-Brcyt$^{\bullet-}$ by the B3LYP-PCM/6–31++G(d,p) method is calculated as 1.8 kcal/mol having one imaginary frequency (−351 cm^{-1}) characterizing the transition state, see Figure 1. This is almost identical to the barrier found for 5-bromouracil of 1.9 kcal/mol found by Li et al in the gas phase using B3LYP/6–31+G(d) method.[19] On complete dissociation of the C5-Br bond of 5-Brcyt$^{\bullet-}$, the product (cytosine-5-yl$^{\bullet}$ and Br$^-$) is found to be thermodynamically more stable than the reactant (5-Brcyt$^{\bullet-}$) by 15 kcal/mol, see Figure 1. The present calculation shows that electron addition to 5-Brcyt leads to spontaneous C5-Br bond dissociation to produce cytosine-5-yl$^{\bullet}$ and bromide ion. In the gas phase, the

calculations performed by Li et al.[19] for 5-bromouracil anion radical show a large energy barrier (21 kcal/mol) for complete separation to "infinity" of the uracil-5-yl radical and bromide ion. This shows that solvation is the driving force for the full cleavage of the C-Br bond after electron attachment.

The potential energy surfaces (PES) for the tautomerizaton reactions from the NH_2 group of cytosine-5-yl[•] (IIa) to form the intermediate aminyl radical (III) and finally the iminyl radical (IV) were calculated using the B3LYP-PCM/6–31++G(d, p) level of theory in the presence of four water molecules including full aqueous phase via PCM. The complete results are presented in the supporting information. The barriers found for the tautomerization reactions from IIa to III and from III to IV were found to be high (24 kcal/mol and 18 kcal/mol, see Figure S7). These calculated barrier heights are large likely owing to the small size of the solvation layer considered. We note that the tautomerization of the aminyl radical (III) to produce the iminyl radical (IV) has been shown experimentally by ESR to occur even at low temperatures of 160 K (suggesting a barrier of < 3 kcal/mol), as well as, by pulse radiolysis experiments at room temperature.[21, 22] Thus the experimental barrier appears to be far lower than the calculated value of 18 kcal/mol. These experiments further showed that the tautomerization of aminyl radical (III) to produce iminyl radical (IV) was base catalyzed.[21, 22] We therefore investigated a model for the tautomerization from IIa to III in which an hydroxide anion was present in place of a water and solvated by two water molecules, see Figure S8 in the supporting information. In this case, the barrier was substantially reduced and proton transfer from NH_2 group of cytosine-5-yl[•] (IIa) to hydroxide anion takes place with negligible barrier (ca. 0.3 kcal/mol) by producing an intermediate surrounded by three water molecules, see Figure S8 in the supporting information. From this intermediate the proton transfer from a water molecule hydrogen-bonded to C5 to produce aminyl radical (III) proceeds via a barrier of ca. 5 kcal/mol. The present calculation shows that the tautomerization of cytosine-5-yl[•] (IIa) to form iminyl radical (IV) is favorable in an basic environment. The details for the transition state search are described in the supporting information.

G4 Calculations of the Tautomerization of cytosine-5-yl[•]

In contrast to the chemistry of 5-bromouracil radical anion which is well known to produce highly reactive uracil-5-yl[•], the present study shows that the chemistry of 5-bromocytosine radical anion does first lead to the formation of cytosine-5-yl[•], however, this species then tautomerizes to the thermodynamically more stable π-type aminyl radical (III) then tautomerizes to σ-type iminyl radical (IV) as shown in Scheme 1. Using the G4 level of theory and the chosen DFT methods, we calculated the relative stabilities of the different tautomers (III and IV) of cytosine-5-yl[•] (IIa) shown in Scheme 1 and Figure 2. In this work, we considered the different tautomers of cytosine-5-yl[•] by transferring a hydrogen atom of NH_2 group either to the C5 or the N3 sites of cytosine ring and calculated their stabilities by G4 and the other DFT methods with respect to cytosine-5-yl[•] (IIa), see Figure 2 and Table 1. We found that transfer of one hydrogen atom from the NH_2 group can be made to the C5 site or the N3 site to produce radicals III and IIIa shown in Figure 2. The G4 and DFT methods predicted structure III to be more stable than IIIa by over 30 kcal/mol, see Table 1. In addition, structure III is more stable than the cytosine-5-yl[•] (IIa) by ca. 9 kcal/mol at G4

level of theory (Table 1). Further, this aminyl radical (cytNH$^\bullet$ (III)) transfers the remaining hydrogen atom from NH to the open N3 site to produce σ-type iminyl radical (cytN$^\bullet$ (IV)) which is the most stable tautomer. The iminyl radical (cytN$^\bullet$ (IV)) is 13 kcal/mol (G4 level of theory) more stable that the parent cytosine-5-yl$^\bullet$ (IIa), see Table 1. The overall relative stability of 5-Brcyt after electron attachment and occurrence of different tautomers, calculated by G4 level of theory in aqueous phase, is shown in Figure 3. From Figure 3, it is evident that electron attachment to neutral 5-Brcyt leads to 5-Brcyt$^{\bullet-}$ formation (I) which reacts through a metastable σ-type intermediate (II) (not shown in Figure 3) which dissociates into cytosine-5-yl$^\bullet$ (IIa) and Br$^-$, the resulting cytosine-5-yl$^\bullet$ is further stabilized by tautomerization to III by N-H transfer to C5 and a second N-H tautomerization to N3 to form the σ-iminyl radical (cytN$^\bullet$ (IV)).

Conclusions

We report here a mechanism for the formation of the cytosine σ-iminyl radical IV (cytN$^\bullet$) by a reductive process starting with 5-bromocytosine. One-electron oxidation of cytosine was earlier investigated using EPR and pulse radiolysis experiments. [21,22,30,31] These studies report the formation of the π-aminyl radical (cytNH$^\bullet$ (III)) and its subsequent tautomerization to form the σ-iminyl radical IV (cytN•). Anderson et al.[31] proposed the formation of σ-iminyl radical IV (cytN$^\bullet$) by selective one-electron oxidation of cytosine with selenite radical in calf thymus DNA. This species (cytN$^\bullet$) then oxidized the hydrogen-bonded guanine in DNA. It is noted that in DNA, high energy radiation directly produces the guanine cation radical because ionizations at other bases undergo hole transfer to guanine which has the lowest IP of all the DNA bases. Thus, if the σ-type iminyl radical IV (cytN•) was formed in DNA by electron addition to 5Brcyt it would likely form one-electron oxidized guanine as proposed by Anderson et al.[31] Thus, 5-Brcyt labeling in DNA has an important implication as it can convert a relatively benign reducing agent, the solvated electron, to a series of reactive oxidizing radicals (IIA, III, IV) which ultimately would increase the formation of guanine cation radical. Thus, this mechanism has the potential to significantly increase oxidative damage in irradiated DNA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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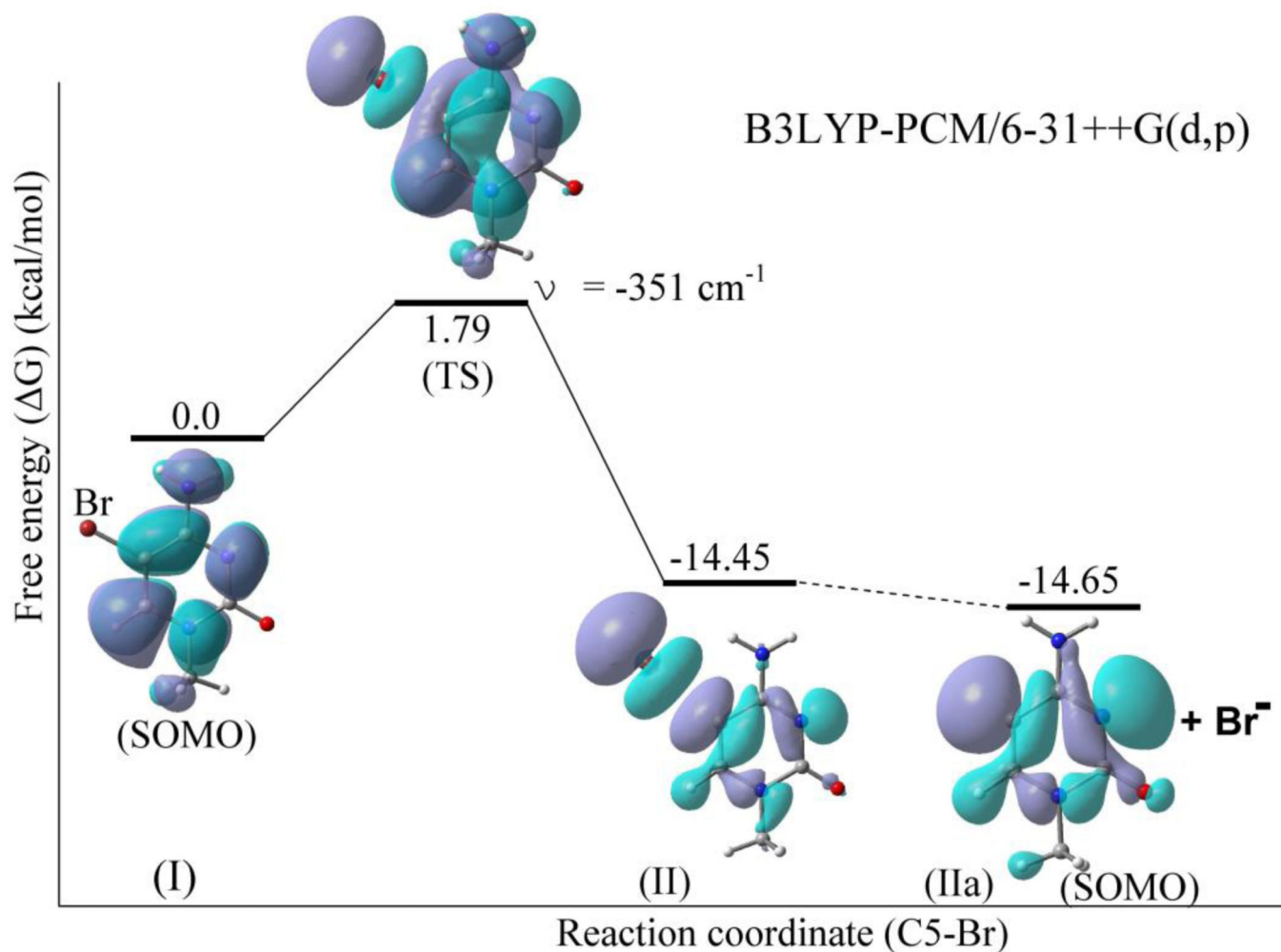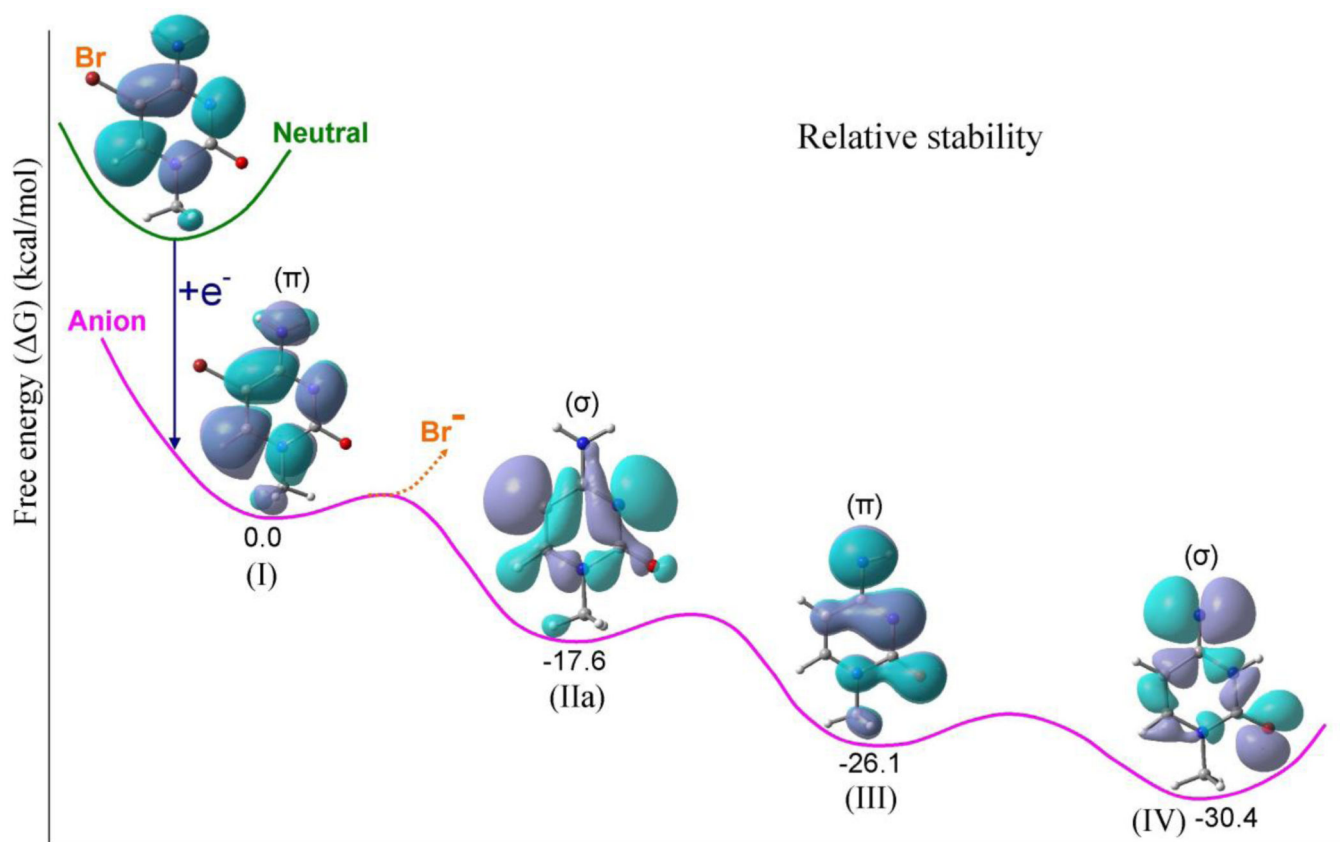
Figure 1.
Free energy (ΔG) reaction profile of 5-BrCyt•⁻ → Br⁻ + cytosine-5-yl• computed at the B3LYP/6–31++G(d,p) level in water using PCM solvation model. Energies are given in kcal/mol. TS = Transition state characterized by one negative frequency.
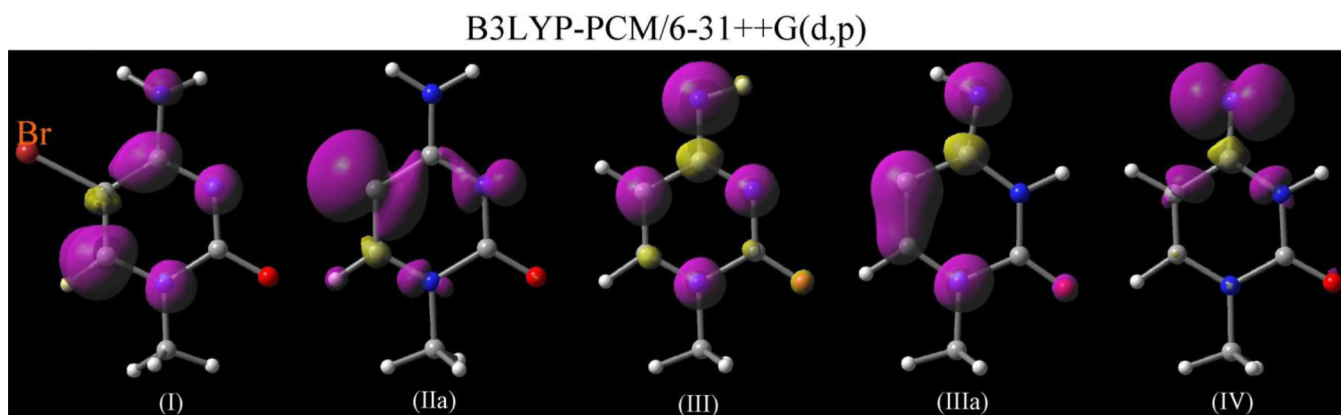
Figure 2.
Spin density plot of (I) 5-BrCyt•⁻ (π-type), (IIa) cytosine-5-yl• (σ-type), (III) aminyl radical (cytNH•, H-shift to C5) (π-type), (IIIa) aminyl radical (cytNH•, H-shift to N3) (π-type) and (IV) iminyl radical (cytN•) ((σ-type)). The spin density distributions are calculated by B3LYP-PCM/6–31++G(d,p) method. Radical (III) is over 30 kcal/mol more stable than its tautmer (IIIa) at G4, see Table 1. Species are numbered as shown in Scheme 1.

Figure 3.
Gaussian 4 calculated relative thermodynamic stabilities of radicals after an electron addition to 5-Brcyt. The G4(PCM) free energy of Br⁻ was added to the free energy of debrominated-radicals (IIa – IV) for the calculation of the relative stabilities with respect to 5-Brcyt radical anion. Relative free energies in kcal/mol are shown below each figure. For neutral 5-Brcyt LUMO (lowest unoccupied molecular orbital) is shown which is available for electron attachment. On anion surface, SOMO (singly occupied molecular orbital) is shown.
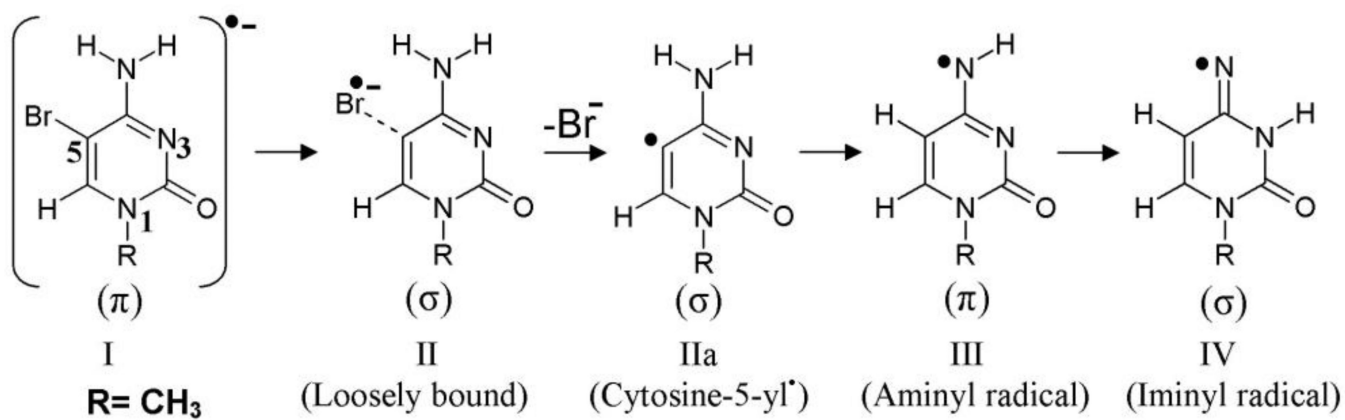
Scheme 1.
Proposed mechanism of iminyl radical (IV) formation by one-electron reduction of 5-Brcyt.

Table 1

Relative stabilities (ΔG) of radicals formed by electron attachment to 5-Brcyt in kcal/mol using different methods (all with PCM). The transition state (TS) for debromination of 5-Brcyt anion radical calculated by various DFT methods are given in kcal/mol.

System[a]	Relative stability ΔG (kcal/mol)			
	G4-theory	B3LYP[b]	ωb97x[b]	M06-2x[b]
5-brCyt (EA of Neutral)	2.27 eV[c]	2.25 eV[c]	2.23 eV[c]	2.16 eV[c]
(I) 5-brCyt (Anion radical)	0.0	0.0	0.0	0.0
(II)[d]		−14.45	−10.64	−11.43
(IIa)[e]	−17.58	−14.65	−7.48	−9.10
(III)[e]	−26.10	−25.00	−16.93	−16.88
(IIIa)[e]	7.22	9.11[d]	−0.21	−1.49
(IV)[e]	−30.35	−26.4	−21.07	−20.47
TS (ΔG#)	-	1.79	7.17	4.32

[a] See Scheme 1 and Figure 2 for species number.

[b] Calculated using 6–31++G(d,p) basis set using IE-PCM solvent model.

[c] Adiabatic electron affinity (AEA). AEA = Neutral – Anion

[d] Loosely bound complex. See Figure 1.

[e] ΔG of Br anion added to debrominated-radicals, structures shown in Figure 2, for calculation of the relative stabilities with respect to (I).