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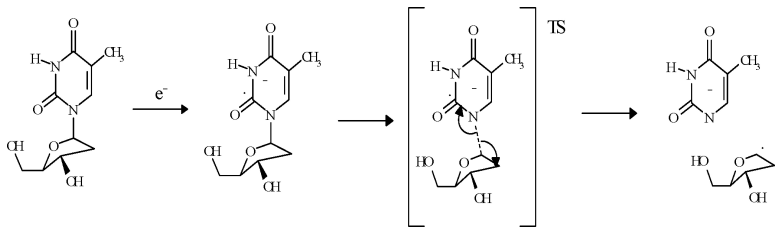

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Glycosidic Bond Cleavage of Pyrimidine Nucleosides by Low-Energy Electrons: A Theoretical Rationale

Jiande Gu,*,† Yaoming Xie,‡ and Henry F. Schaefer III*,‡

Contribution from the Drug Design & Discovery Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, CAS, Shanghai 201203 P. R. China, and Center for Computational Chemistry, University of Georgia, Athens, Georgia 30602-2525

Received April 7, 2004; E-mail: jiandegush@go.com (J.G.); hfs@uga.edu (H.F.S.)

Abstract: DNA damage by attachment of low-energy secondary electrons is a very interesting and important mechanism. Electron capture and subsequent base release are thought to be the elementary steps of this mechanism. The process of the N1-glycosidic bond breaking of anion radicals of pyrimidine nucleosides, specifically the 2′-deoxyribothymidine (dT) and 2′-deoxyribocytidine (dC) anions, has been investigated theoretically at the B3LYP/DZP++ level of theory. The release of nucleobases by the attachment of low-energy electrons depends on the formation of a stable anion radical of the nucleoside. The lower bond-breaking activation energy and the higher vertical electron detachment energy for dT enables the heterolytic cleavage of the N1-glycosidic bond. However, with the higher bond-breaking activation energy and the lower vertical electron detachment energy for dC, the release of cytosine might be impractical when the incident electrons have high kinetic energy. Furthermore, the release of cytosine would have a quantum yield much lower than that of dT when the incident electrons have lower kinetic energy. This study also demonstrates the importance of the proton at O5′ of 2′-deoxyribose in the base release process. Extending this investigation from dT to dC advances the insight into the mechanism of the N1-glycosidic bond-breaking process. The information from this extensive investigation should be valuable for further experimental studies of cytosine release in irradiated DNA.

I. Introduction

The release of nonmodified nucleic acid bases is an important pathway for DNA damage caused by ionizing radiation in the solid state.[1,2] This damage has been attributed to the oxidation of DNA involving either the deprotonation of base radical cations or the fragmentation of sugar phosphate radical cations.[2−5] Due to the effects of transfer of water radical cations to the sugar moiety, the oxidative-related base release processes are expected to be increased by hydration.[2,4,6]

The knowledge of the interaction between the incident electrons and individual compounds, such as nucleobases and nucleosides, is necessary for a detailed understanding of the mechanisms responsible for DNA damage through attack by low-energy electrons.[7] Recent experiments involving the bombardment of a nucleoside (thymidine) via low-energy electrons demonstrated[7] that (1) low-energy electrons effectively break the N-glycosidic bond of thymidine, and (2) the release of thymine is independent of the degree of hydration, which is expected to increase the extent of base release if oxidative process is involved. On the basis of their observations, Sanche et al. proposed a new mechanism of base release,[7] which is different from other well-studied pathways for DNA damage. Electrons with low kinetic energy were suggested to be attracted to the region of the antibonding orbital for the N1-glycosidic bond of the thymidine nucleoside. It was suggested that this causes the heterolytic cleavage of the N1-glycosidic bond, along with the formation of thymin-N1-yl anions and neutral 2-deoxyribose-C1(*H*)-yl radicals.

Since the mechanism of base release is crucial in the low-energy electron-induced damage process, theoretical investigations may be helpful in providing a rationale for the reaction. Specifically, a detailed understanding of this important DNA damage pathway requires information concerning both the structure of the transition state and the activation energy for the heterolytic cleavage of the N-glycosidic bond. No such theoretical study has been reported to our knowledge. An earlier study used the HF/6-31+G(d) approach to examine the breaking of the sugar−phosphate C−O bond of a nucleotide (2′-deoxycytidine-5′-monophosphate).[6] However, at the HF/6-31+G(d) level of theory, the electron affinity of deoxycytidine

† Shanghai Institutes for Biological Sciences.
‡ University of Georgia.

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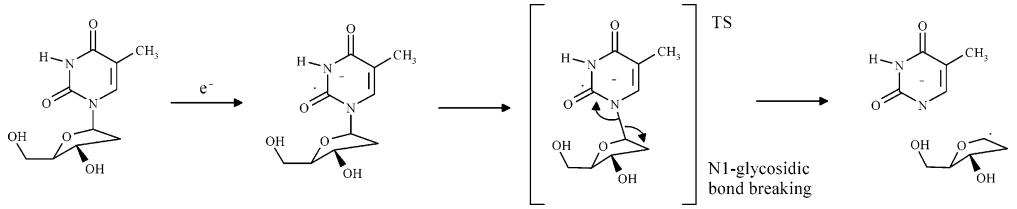
Scheme 1. Proposed Mechanism for N1-Glycosidic Bond Cleavage of Thymidine by Low-Energy Electrons[a]



A stable thymidine anion is formed in the first stage, and then the N1-glycosidic bond breaks to release the thymine anion and the 2-deoxyribose radical.

phosphate is predicted to be negative without the presence of hydration. The latter result appears to be inconsistent with the known experimental EA determination and with higher-level theoretical values.[8−13]

The reliable determination of the physical properties of radical anions depends on the theoretical methods chosen. Recent synergy between experiment and theory has resulted in the development of a comprehensive DFT bracketing technique which has been tested in conjunction with extensive experimental work for the determination of the physical properties of anions.[14] With this reliably calibrated B3LYP/DZP++ method, an accurate description of the newly proposed low-energy electron-induced DNA damage pathway is now possible. Here, we report theoretical investigations of the mechanism for the N1-glycosidic bond breaking of the anionic radical 2′-deoxyribothymidine (dT). We also extend this study to 2′-deoxyribocytidine (dC) anions, even though there are no similar experimental studies of dT.[7] The involvement of dC in the present investigation is based on the fact that the electron affinity of dC is significantly higher than those of the purines, but close to that of dT. In a previous study,[13] the adiabatic electron affinity (EA_{ad}) of dC was estimated to be 0.33 eV, comparable to the value 0.44 eV predicted for dT. A stable anion radical of dC is then expected to exist under conditions similar to that of dT. Extending this investigation from dT to dC should advance the understanding of the mechanism of the N1-glycosidic bond-breaking process. The information from this extended study should be valuable for future experimental studies of cytosine release in radiated DNA.

II. Theoretical Methods

In accord with previous work,[11−13] a DFT bracketing technique was employed in which five generalized gradient approximation (GGA) exchange−correlation density functionals were used, with reliable middle range values produced by the B3LYP functional, which is a combination of exchange from Becke's 3-parameter HF/DFT hybrid exchange functional (B3)[15] with the dynamical correlation functional of Lee, Yang, and Parr (LYP).[16] The GAUSSIAN 98 system[17] was used for all computations.

The present work was carried out using double-ζ quality basis sets with polarization and diffuse functions (denoted DZP++). The DZP basis sets were constructed by augmenting the Huzinage−Dunning[18,19] contracted Gaussian double-ζ functions with one set of p-type polarization functions for each H atom and one set of five d-type polarization functions for each C, N, and O atom [$\alpha_p(H) = 0.75$, $\alpha_d(C) = 0.75$, $\alpha_d(N) = 0.80$, $\alpha_d(O) = 0.85$]. To complete the DZP++ basis, one even-tempered diffuse s function was added to each H atom, while sets of even-tempered diffuse s and p functions were centered on each heavy atom. The even-tempered orbital exponents were determined according to the prescription of Lee and Schaefer:[20]

$$\alpha_{\text{diffuse}} = \frac{1}{2}\left(\frac{\alpha_1}{\alpha_2} + \frac{\alpha_2}{\alpha_3}\right)\alpha_1 \qquad (1)$$

where α_1, α_2, and α_3 are the three smallest Gaussian orbital exponents of the s- or p-type primitive functions for a given atom ($\alpha_1 < \alpha_2 < \alpha_3$). The final DZP++ set contains six functions per H atom (5s1p/3s1p) and nineteen functions per C, N, or O atom (10s6p1d/5s3p1d). This basis has a significant tactical advantage since it has been systematically examined in comprehensive calibrative studies[14] of a wide range of electron affinities. The present theoretical study does not attempt to incorporate solvent effects because the Sanche experiments[5] refer to unsolvated molecules. Furthermore, we would say that the deepest understanding of biochemistry will require first an understanding of the free molecules, then the effects of finite solvent clusters (microsolvation), and finally full macroscopic solvation.

III. Results

On the basis of the experiments of Sanche's group,[7] the N1-glycosidic bond breaking of 2′-deoxyribopyrimidines via low-energy electron attacking may be understood by following Scheme 1. That is, Scheme 1 displays our mechanism for the physical process under discussion. Our previous study indicated that the attachment of an electron to form a stable nucleoside anion in the first step is energy favored for the 2′-deoxypyrimidine nucleosides.[13] To explore the potential energy surface for the N1-glycosidic bond-breaking process, we have located the transition states for this process for both deoxythymidine and deoxycytidine anions. These transition states were characterized by single imaginary vibrational frequencies of 515i cm^{-1} for dT and 496i cm^{-1} for dC. The normal mode related to the imaginary frequency is found to correspond to the N1-glycosidic

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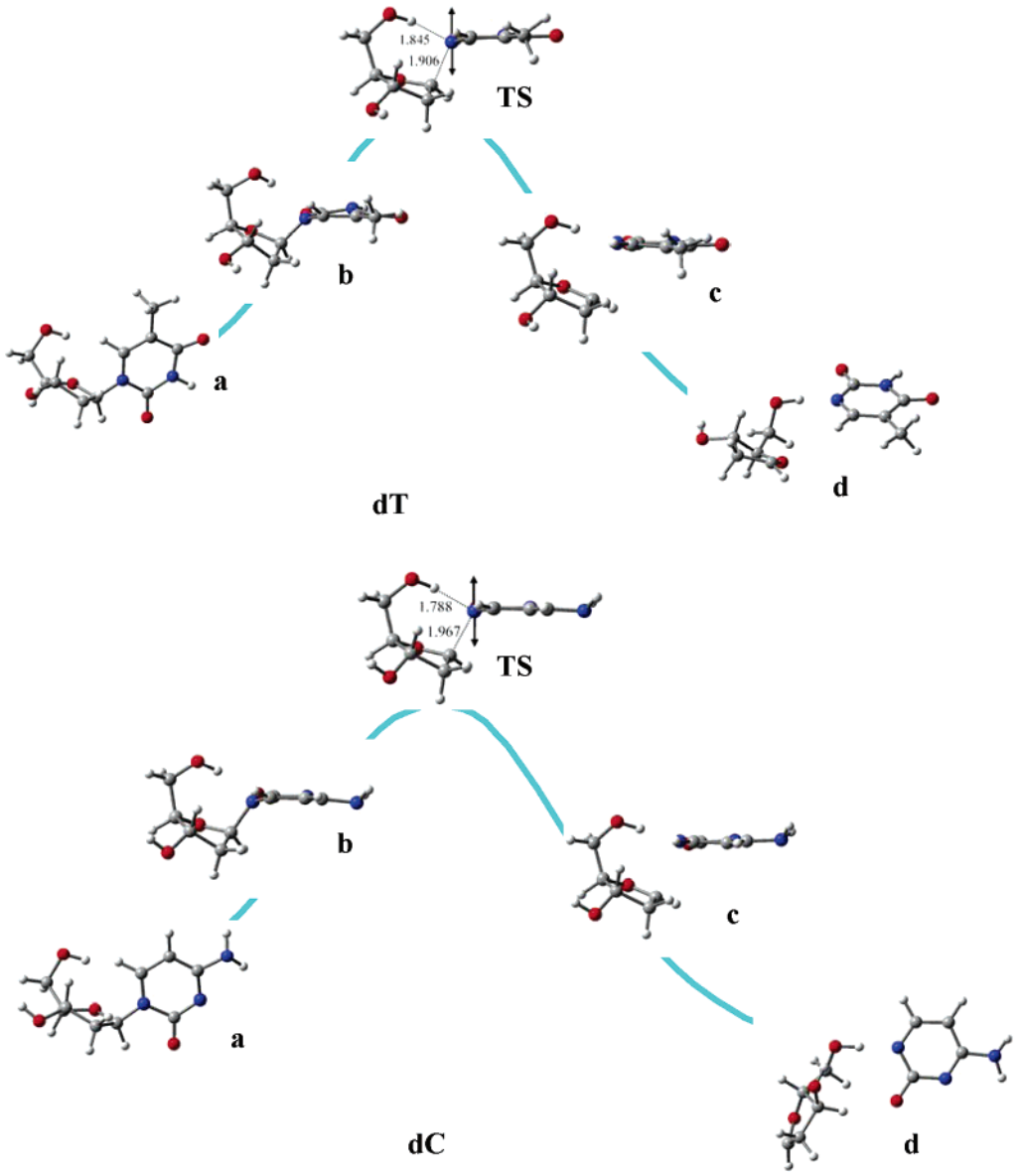

Figure 1. N1-glycosidic bond-breaking process. (**a**) Covalently bound anion radical; (**b**) turning of the base; (**TS**) transition state; (**c**) N1−C1′ bond broken; (**d**) N1···H(O5′) H-bonded sugar radical and base anion complex. Arrows in **TS** represent the vibrational mode corresponding to the imaginary frequency.

bond breaking (Figure 1). An intrinsic reaction coordinate (IRC) analysis confirmed the transition states to connect the covalently bound pyrimidine nucleoside anions and the N1-glycosidic bond-ruptured products (N1 deprotonated base anions and the sugar radical). It should be noted that the base anions and the sugar radical are attached through the N1···H(O5′) H-bond. Further separation of this complex necessitates about 8 kcal/mol of energy. The whole reaction profile is depicted in Figure 1. This process involves first the turning of the base under the influence of the proton from O5′ of 2′-deoxyribose (**b** in Figure 1). Subsequently, we see the formation of the N1···H(O5′) H-bond, which leads to the transition state corresponding to the N1-glycosidic bond break. Finally, the N1-glycosidic bond ruptures, forming the H-bonded base anion and sugar radical complexes.

Notice that the base plane in the transition state structure is perpendicular to the original position of the stable structure of the dT (or dC) anion. Therefore, the turning of the base in the primary stage of the reaction is of importance. The driving force for this turning may be seen from the electronic structure of the pyrimidine anions.[13] In a simple picture, the excess charge resides on the π^* orbital of the conjugated base in the thymine (or cytosine) anion, causing the C6 pyramidalization, which in turn, results in the pyramidalization of its neighboring atom, N1. Since the direction of the lone pair of N1 points to O5′ of the sugar moiety, the pyramidalization of N1 increases under the influence of the O5′ proton, leading to the turning of the base.

The most striking feature from the geometry of the transition states is that the proton attached to O5′ of the 2′-deoxyribose strongly interacts with atom N1 of the base anions. The N1···H(O5′) atomic distance is 1.84 Å in dT and 1.79 Å in dC, signifying the formation of normal hydrogen bonds. Meanwhile, the elongated N1−C1′ bond lengths (1.91 Å in dT and 1.97 Å in dC) characterize the N1-glycosidic bond rupturing under the influence of intramolecular H-bonding. This intramolecular H-bonding reduces the activation energy associated with the transition states by redistributing the excess charge between N1

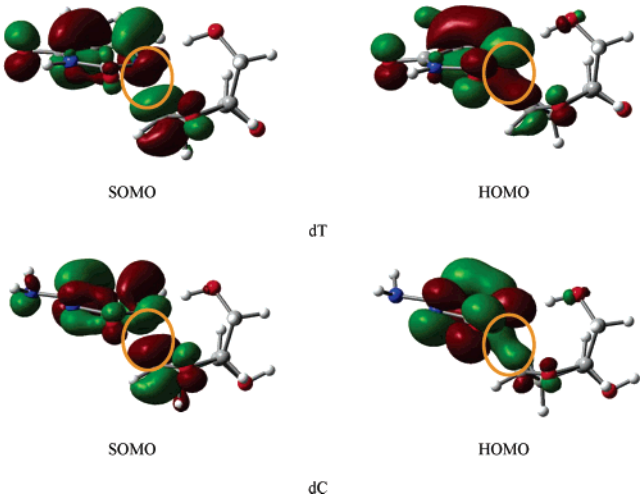

Figure 2. Highest doubly occupied molecular orbital (HDOMO) and the singly occupied molecular orbital (SOMO) for the transition states of the dT and dC radical anions. N1−C1′ bonding may be seen from the HOMO, while the corresponding antibonding interaction is exhibited in the SOMO (in the circled area).

of the base moiety and O5′ of the sugar. The singly occupied molecular orbital (SOMO) and the highest doubly occupied molecular orbital (HOMO) in the transition state for both dT and dC demonstrate the antibonding and bonding characters, respectively, of the N1-glycosidic bond (displayed in Figure 2). Due to the cancellation of the bonding (HOMO) and antibonding (SOMO) effects, atom N1 of the base and C1′ of the sugar are essentially nonbonded at this stage. The formation of a strong H-bond between N1 of the base and the O5′ proton of the sugar clearly increases the N1−C1′ separation.

The natural population analysis (NPA) reveals that, in the N1-glycosidic bond-broken products, the unpaired electron resides largely on atom C1′ (with a product spin density of 0.82 au for dT and 0.83 au for dC) of the sugar, forming a neutral 2-deoxyribose radical, consistent with the experimental suggestions. Also, the "excess" charge in the H-bonded products is found mainly on the nucleic acid bases thymine and cytosine (NPA = −0.89 au for cytosine-N1-yl and −0.88 au for thymine-N1-yl). These are examples of typical distonic character for radical anions.[21] Distonic radical cations have been studied thoroughly, and it has been stated that studies of their unique properties may lead to a better understanding of the biological consequences of ionizing irradiation.[22] For distonic radical anions, the studies are later and relatively few, but recently some studies have shown that they have similar properties to distonic radical cations and are biologically important.[23−25] The distonic character for dT^- and dC^- will increase their stabilities, and they should become standard examples of distonic radical anions in biochemistry.

The energetic properties of the covalently bound anions, the transition states, and the base anion−sugar radical complexes for the pyrimidine nucleosides are summarized in Table 1 along

Table 1. Energy Properties of the Anion Radicals, Transition States, and H-Bonded Base Anion and Sugar Radical Complexes of the Pyrimidine Nucleosides

	E (hartree)	ΔE^a	$\Delta E^{\circ\,a,b}$	$\Delta G^{\circ\,a,c}$	$EA_{ad}{}^d$	VDE[e]
			dT			
dT anion	−875.34106	0.0	0.0	0.0	0.44[f]	0.94[f]
TS	−875.31100	18.9	17.6	18.0		
$T^- + 2$-deoxyribose	−875.37406	−20.7				
			dC			
dC anion	−816.11789	0.0	0.00	0.0	0.33[f]	0.72[f]
TS	−816.08348	21.6	20.4	21.2		
$C^- + 2$-deoxyribose	−816.13474	−10.6				

[a] In kcal/mol. [b] ΔE° is the corrected zero-point energy. [c] ΔG° is the free energy difference at 298 K. [d] $EA_{ad} = E_{neutral} - E_{anion}$ (in eV). [e] VDE is the vertical detachment energy (in eV). [f] From ref 13.

with the electron affinities of the neutral pyrimidine nucleosides. The activation energy for the N1-glycosidic bond breaking in the dT anion is predicted to be 18 kcal/mol. Considering that the attachment of an electron on a dT neutral species releases about 0.44 eV (10 kcal/mol) of energy, this activation energy barrier could be overcome easily by the energy brought by the attached electron. On the other hand, the vertical detachment energy of 0.94 eV (22 kcal/mol)[13] suggests that the incident electron with higher kinetic energy might not be able to produce a stable dT anion, which has a lifetime long enough to process N1-glycosidic bond rupture. Instead, detachment of an electron may happen when the kinetic energy of the incident electron is high. Accordingly, a broad maximum is expected for the release of thymine as a function of the energy of incident electrons, as observed in the experiments.[7]

Relatively high activation energy (20 kcal/mol) was predicted for the dC anion. To effectively break the N1-glycosidic bond of dC, a higher kinetic energy of the incident electrons is needed. However, the lower vertical detachment energy of 0.72 eV (17 kcal/mol) implies that the formation of the dC anion by the incident electrons with high kinetic energy might be impractical. Therefore, the observation of the release of cytosine is expected under the bombardment of the electrons with lower kinetic energy and with a quantum yield much lower than that of dT.

In the earlier experiments,[3,4] base release was observed to be in favor of the pyrimidines. Our previous theoretical studies of nucleosides revealed that the N-glycosidic bonding and antibonding orbitals of the neutral purine and pyrimidine nucleosides are basically the same.[13] Therefore, direct occupation of an excess electron in their antibonding orbitals should not have given a preference for pyrimidines or purines. Therefore, it is unlikely that, as proposed by Sanche et al.,[7] the low-energy electron attaches directly on the antibonding orbitals of the bond being ruptured. In fact, the unpaired electron occupies the N1−C1′ antibonding orbital only at the transition state. Considering that the EA_{ad} of pyrimidines is much higher than that of purines, the bias of base release observed at the pyrimidines suggests that the formation of a relative stable anion radical by electron attachment to the conjugated π^* orbital of the base at the primary stage could be crucial.

In summary, our studies indicate that the release of nucleic bases by the attachment of low-energy electrons depends critically on the formation of a stable anion radical of the nucleoside. The relatively low bond-breaking activation energy and high vertical electron detachment energy of dT enables the heterolytic cleavage of the N1-glycosidic bond. Moreover, the

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observation of the release of cytosine is predicted to occur with incident electrons of lower kinetic energy and with a quantum yield much lower than that of dT. This study also demonstrates the importance of the proton at O5′ of 2′-deoxyribose in the base release process. It is especially interesting that in the transition state, the charge is localized in the bond to be broken. One referee has described this as "charge-induced dissociation."

It should be noted that because of the negative charge associated with the phosphate, electron binding might be different in the nucleotides. However, since the anion radicals of nucleosides could be products in the nascent stage of single strand breaks (SSBs) of DNA by low-energy electrons, our theoretical rationale advances the understanding of the base release processes in the SSBs. The above remarks notwithstanding, we should add a cautionary note concerning the application of our mechanism to DNA. Namely, the solvation of the nucleic acid base by the neighboring bases might be large. Note that charge resonance stabilizes a positive charge in GC by more than 0.5 eV in comparison to that of a single G. Then the charge might be trapped and be less able to concentrate, shift, and break the bond.

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