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Theoretical study of the pre- and post-translational effects of adenine and thymine tautomers and methyl derivatives

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Abstract The study of pre-translational effects (ionization, tautomerization) and post-translational effects (methylation) of adenine and thymine has only recently been the focus of some studies. These effects can potentially help regulate gene expression as well as potentially disrupt normal gene function. Because of this wide array of roles, greater insight into these effects in deoxyribonucleic acids (DNA) are paramount. There has been considerable research of each phenomenon (tautomerization, methylation and ionization) individually. In this work, we attempt to shed light upon the pre-translational effects and post translational effects of adenine and thymine by investigating the electron affinities (EAs) and ionization potentials (IPs) of the major and minor tautomers and their methyl derivatives. We performed all calculations using the density functional theory (DFT) B3LYP functional accompanied with 6-311G(d,p), 6-311+G(d,p) and 6-311++G(df,pd) basis sets. Our results reveal that the thymine tautomer has a higher EA and IP than the adenine tautomers. The higher EA suggests that an electron that attaches to the AT base pair would predominately attach to the thymine instead of adenine. The higher IP would suggest that an electron that is removed from the AT base pair would be predominately removed from the adenine within the base pair. Understanding how tautomerization, ionization and methylation differences change effects, discourages, or promotes one another is lacking. In this work, we begin the steps of integrating these effects with one another, to gain a greater understanding of molecular changes in DNA bases.

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Introduction

Understanding the fundamental processes that govern deoxyribose nucleic acid (DNA) has been a primary focus for many scientists [1–4]. DNA is the basic genetic heritage material that is passed from one generation to next. It is responsible for many biological functions, cellular replications, chemical regulations, and other processes critical to life [5]. Disruption of DNA can lead to other detrimental issues and diseases that threaten life. A complete understanding on the mechanism of DNA and how it regulates these processes, including the disruptions that lead to complex changes in its structure, has great intrinsic value.

A number of studies over the past 50 years have contributed valuable insight into the basic structure and function of DNA [3, 5, 6]. The structure of DNA (particularly nucleobase-pairing within the DNA structure), the role of enzymes in reading and regulating DNA expression and replication, the role that DNA plays in heredity, and the fidelity of the processes, have revolutionized both medical and scientific approaches to many areas from medical to agricultural fields [3, 7].

However, the study of post-translational effects (e.g., methylation) and pre-translational effects (e.g., ionization, tautomerization) has only recently been the focus of some research studies [8, 9]. These effects can potentially help regulate gene expression as well as potentially disrupt normal gene function. Because of its wide array of roles, greater insight into these effects of DNA is crucial.

Much work has focused on understanding each of these issues individually. The effect, of methylation is an

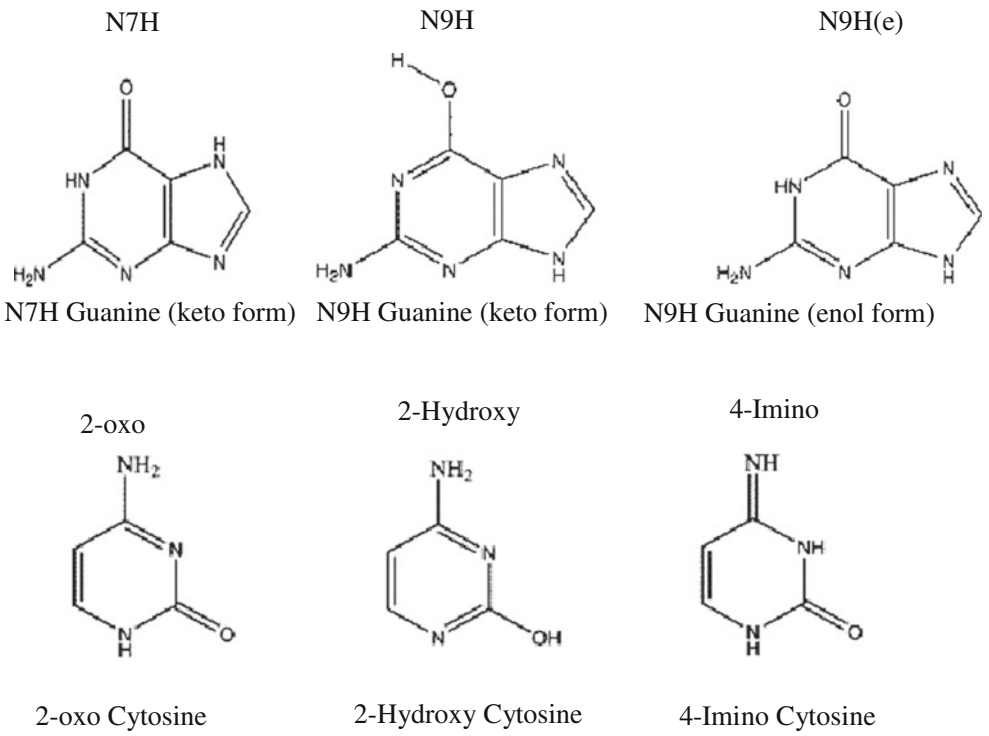

Fig. 1 Guanine and cytosine tautomers

epigenetic effect that can be natural to the organism for the purpose of regulating gene expression and DNA transcription. Methylation can also be inflicted by external chemicals (exogengous methylation) that can be misread by transcriptional processes, creating abnormal events. Methylation of DNA bases plays an imperative role in many biological processes [10].

Methylation of the adenine nucleobase has been shown to effect the interactions of regulatory proteins with DNA. Particularly, N3-methyladenine causes highly cytotoxic lesions, but low mutagenity [11]. By obstructing the interaction between DNA polymrase and adenine [1], unpaired methylated adenine acts as a lesion that blocks replication. Although research has identified tautomeric mutagenity, little has been done to understand the mutagenic mechanisms of tautomers and methyl derivatives. Yu et al. studied the effect of three methylating agents on the reaction probability of thymine using density functional theory methods. This study was used to determine the causes of mutagenesis, genotoxicity and carcinogenesis initiated by the carcinogenic methylating agents, idomethane (Mel), dimethyl sulfate (DMS) and methyl methanesulfonate (MMS) [12].

Similarly, tautomerization has long been the focus of computational studies, and limited experimental studies. Basic information of the tautomers of DNA is well-known and documented [13, 14]. These tautomers change from the enol to the keto form of each DNA base. This small change may seem miniscule, but for DNA, tautomerism causes point mutations to occur, which in-turn, causes mispairing, depurination or depyrimidation, and eventually molecular-based diseases [15]. Tautomeric interactions have played a paramount role in the disruption of complimentary base pairing of DNA. Although they are known to cause spontaneous mutations within DNA, little is known about the mechanisms of the tautomers [16–18]. Some researchers have reported types of tautomerization for each DNA base; nevertheless, the information gathered is not enough to understand the mechanisms of these structures.

Lastly, ionization is either caused by the oxidation of a DNA nucleobase by another nucleobase, or by the introduction of a radical electron via a free radical molecule, namely a hydroxy radical. It can lead to DNA damage and mutagenesis [19]. Ionizing radiation, a focus of this study, produces enough kinetic energy to remove an electron from (in our case) DNA. This lone electron can cause single strand and double strand breaks within the DNA helix. This type of radiation also causes cross-links and oxidative base modifications [2, 20].

There has been considerable research studying the individual effects of methylation, tautomerization and ionization of DNA nucleobases. However, there has been little work done to understand the interplay between phenomena. In this work, we begin the steps of integrating these effects to gain a greater

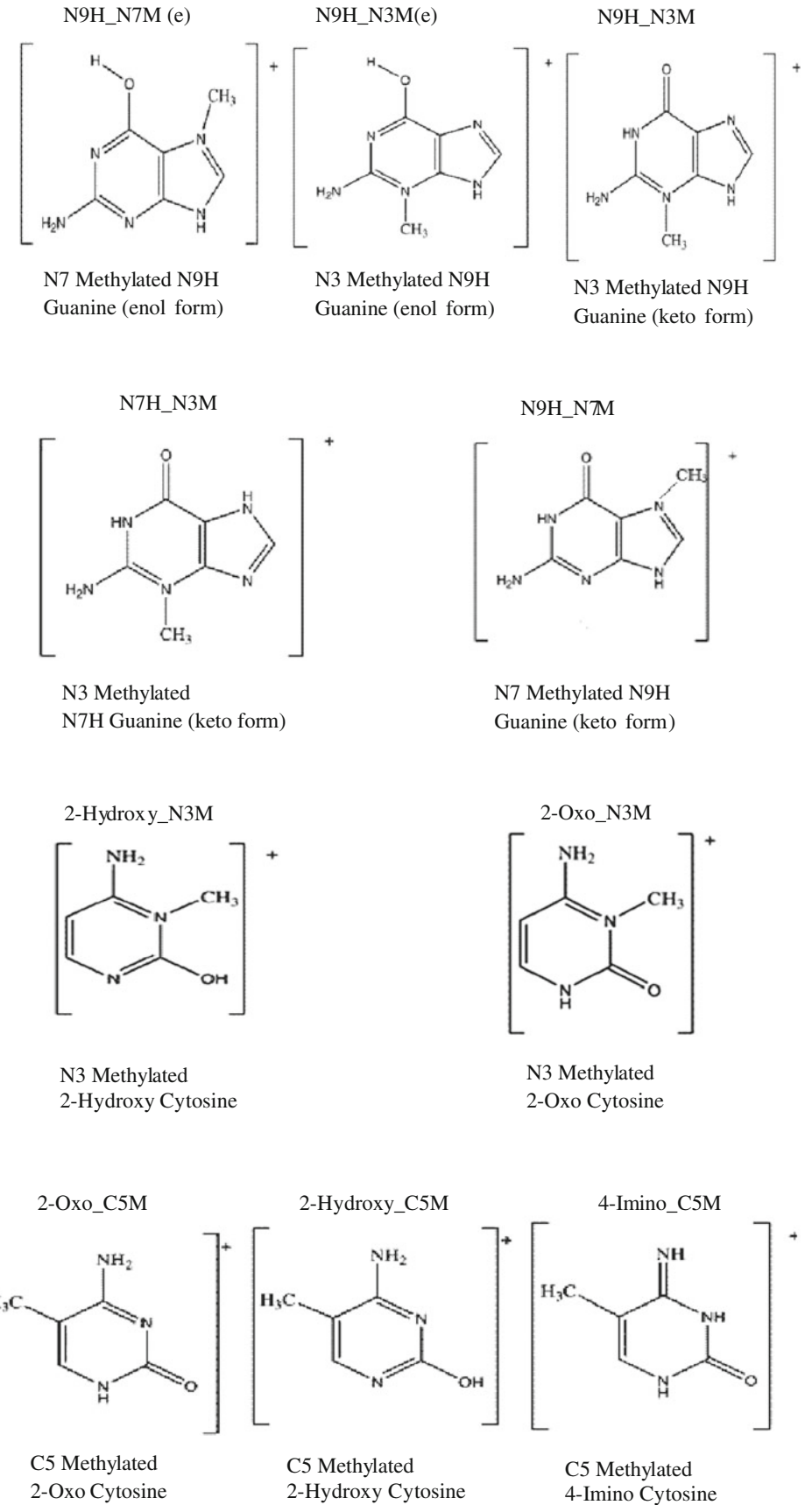

Fig. 2 Guanine and cytosine methyl derivatives

Table 1 Adiabatic and vertical electron affinities of adenine and thymine tautomers (eV)

Compound	B3LYP/6-311G(d,p)		B3LYP/6-311+G(d,p)		B3LYP/6-311++G(df,pd)		Experimental		Theory	
	EA_{ve}	EA_{ad}	EA_{ve}	EA_{ad}	EA_{ve}	EA_{ad}	EA_{ve}	EA_{ad}	EA_{ve}	EA_{ad}
N9H	−1.40	−0.86	−0.73	−0.48	−0.33	−0.33	−0.45[a]	–	−0.74[b]	−0.40[c]
N7H	−1.20	−0.51	−0.15	−0.19	−0.19	0.12	–	–	–	–
N3H	−0.59	−0.39	−0.17	0.01	−0.17	0.01	–	–	–	–
Thymine	−0.30	−0.69	−0.23	0.10	−0.20	0.09	−0.29[d]	0.12[e]	−0.32[b]	0.14[c]

[a] Ref [12]
[b] Ref [14]
[c] Ref [13]
[d] Ref [11]
[e] Ref [15]

insight of molecular changes that occur in DNA's adenine and thymine tautomeric and methylated bases.

Theoretical details

Full optimization of all geometric parameters of 12 model compounds were performed using the following level of theories (shown in Figs. 1 and 2): B3LYP/6-311G(d,p)> B3LYP/6-311+G(d,p)>B3LYP/6-311++G(df,pd). The B3LYP functional is in good agreement with the experimental value and has been shown to give accurate results by other researchers for determining electron affinities and ionization potential for DNA bases [12, 21]. Singlepoint energy calculations were carried out for the cation, anion, and neutral radicals of each optimization geometry. Adiabatic ionization potentials (IP_{ad}), electron affinities (EA_{ad}), vertical ionization potentials (IP_{ve}) and electron affinities (EA_{ve}) were calculated using Eqs. (1)–(4), respectively,

$$IP_{ad} = E(M_{n-1}\text{optimized}) - E(M_n\text{optimized}) \quad (1)$$

$$EA_{ad} = E(M_n\text{optimized}) - E(M_{n+1}\text{optimized}) \quad (2)$$

$$IP_{ve} = E(M_{n-1}) - E(M_n) \quad (3)$$

$$EA_{ve} = E(M_n) - E(M_{n+1}), \quad (4)$$

where E (M_n optimized) is the total energy of the optimized parent compound, E (M_{n-1} optimized) is the total energy of the optimized (n-l)-electron cation radical, E (M_{n+1} optimized) is the total energy of the optimized (n+l)-electron anion or neutral radical, E (M_n) is the total energy of the singlepoint energy calculation of the parent compound, E (M_{n-1}) is the total energy of the single point energy calculation of the (n-1)-electron cation radical and E (M_{n+1}) is the total energy of the single point energy calculation of (n+1)-electron anion or neutral radical. All density functional (DFT) quantum-chemical calculations were accomplished using the Gaussian-03 and 09 software package [22, 23].

Table 2 Adiabatic and vertical ionization potentials for adenine and thymine tautomers(eV)

Compound	B3LYP/6-311G(d,p)		B3LYP/6-311+G(d,p)		B3LYP/6-311++G(df,pd)		Experimental		Theory	
	IP_{ve}	IP_{ad}	IP_{ve}	IP_{ad}	IP_{ve}	IP_{ad}	IP_{ve}	IP_{ad}	IP_{ve}	IP_{ad}
N9H	8.21	7.95	8.37	8.11	8.37	8.11	8.44[a]	–	8.26[b]	–
N7H	8.49	8.15	8.67	8.32	8.67	8.32	–	–	–	–
N3H	8.34	7.87	8.52	8.04	8.52	8.04	–	–	–	–
Thymine	8.88	8.64	9.02	8.78	9.03	8.78	9.11[c]	–	9.01[a]	–

[a] Ref [18]
[b] Ref [16]
[c] Ref [17]

Table 3 Adiabatic and vertical electron affinities of the methyl derivatives of adenine and thymine (eV)

Compound	B3LYP/6-311+G(d,p)	
	EA_{ve}	EA_{ad}
N9H_N1M	4.34	3.83
N7H_N1M	4.74	4.04
N3H_N1M	4.96	4.86
N9H_N3M	4.33	4.05
N7H_N3M	4.55	4.13
N9H_N7M	4.72	4.19
N3H_N7M	4.58	4.20
T_O2M	4.94	4.69

Results and discussion

With an electron affinity of −0.33 eV, N9H, the canonical base tautomer of adenine, maintains the closest EA to the experimental value of −0.45 eV of the three tautomers. Both the N7H tautomer and the N3H tautomer have larger electron affinities, with the N3H tautomer EA being almost 0.5 eV larger than the canonical base. The 0.5 eV difference is significant enough, that N3H is the only tautomer that is predicted to add the electron because of a positive electron affinity (Table 1). This could explain the selection of the N9H tautomer over the other adenine base tautomers in the basic DNA makeup of many organisms. Thymine, the complementary base pair of adenine, was predicted to have a larger electron affinity than adenine. When allowed to relax in the presence of the electron, thymine becomes receptive to attaining the electron (Table 1). This would theorize that any electron that attaches to the AT base pair would predominately attach to the thymine base instead of the adenine base.

Table 2 presents the predicted values for the ionization potential of the adenine tautomers and thymine. All three basis sets, 6-311G(d,p), 6-311+G(d,p) and 6-311++G(df,pd), are in good agreement with existing experimental and theoretical values [14]. However, the basis sets with larger diffuse and polarized functions (6-311+G(d,p) and 6-311++G(df,pd)) gave very similar predictions. This, once again, suggests that the use of the larger basis set does not significantly add accuracy to calculating the ionization potential. In this work, 6-311+G(d,p) gave reasonable results consistent with experimental data [24]. When ranked in order of increasing electron affinity, adenine tautomers align in the following order; the highest ionization potential belongs to N7H followed by N3H, and, lastly N9H is the lowest. N7H is predicted to be less likely to lose an electron than the other tautomers.

The ionization potential for thymine is larger than the adenine tautomers (Table 2). The value is in agreement with current theoretical predicted values [25–27]. Again, thymine is predicted to more strongly resist removal of an electron, as compared to adenine. This would suggest that any electron removed from the AT base pair would be removed from the adenine base within the base pair.

Table 3 presents the predicted values for the electron affinities of the methyl derivatives of adenine and thymine. The addition of a methyl group does not change the order of the trend of EAs of the tautomers. The N9H methyl derivative EA values are the lowest as seen in the EA values of the N9H tautomer (Table 1). The N7H and N3H methyl derivatives continue to follow the trend. It should be noted that N1 methylated N3H methyl derivative (N3H_N1M) has the highest EA compared to the tautomers and methyl derivatives. This raises the question of what causes such a large difference between the N3H_N1M and the N3H_N7M structures. The N1 position seems to react more to the addition of the methyl group than the N7 position. More research should be performed to understand this phenomenon. Thymine (Table 3) deviates from the trend of the methylated DNA bases. The T_O2M methyl derivative has a lower EA than the adenine methyl derivatives. This suggests that an electron would attach to the methylated adenine instead of the methylated thymine in an AT base pair.

Table 4 depicts the ionization potential values of the methyl derivatives of adenine and thymine. The N7H_N1M methyl derivative was calculated to have the highest ionization potential compared to the other methyl derivatives while

Table 4 Adiabatic and vertical ionization potentials of the methyl derivatives of adenine and thymine (eV)

Compound	B3LYP/6-311G(d,p)		B3LYP/6-311+G(d,p)		B3LYP/6-311++G(df,pd)	
	IP_{ve}	IP_{ad}	IP_{ve}	IP_{ad}	IP_{ve}	IP_{ad}
N9H_N1M	13.02	12.74	13.08	12.80	13.08	12.81
N7H_N1M	13.45	13.02	13.52	13.08	13.52	13.10
N3H_N1M	12.90	12.44	12.98	12.51	12.97	12.52
N9H_N3M	13.04	12.70	13.10	12.76	13.11	12.77
N7H_N3M	13.35	12.93	13.42	13.00	13.42	13.01
N9H_N7M	12.84	12.62	12.91	12.69	12.91	12.70
N3H_N7M	13.43	12.94	13.49	13.00	13.49	13.01
T_O2M	13.59	13.39	13.64	13.44	13.64	13.38

N3H_N1M had the lowest ionization potential, implying that an electron would attach to it before attaching to the other methyl derivatives. This is a change in the trend compared to the adenine tautomers. The addition of a methyl group changes the trend for the ionization potential values of adenine. The thymine methyl derivative is comparably higher than the adenine methyl derivatives (which follows the trend of the thymine tautomer). This increase is due to the double bond between C2 and O2 causing the oxygen to be an ether oxygen. Since the N3 C2 bond is not double bonded, as it should be, a positive charge is located on N3. This positive charge explains the large IP_{vert} values in Table 4.

The difference between the electron affinity and ionization potentials of the various tautomers for the adenine is due to several factors; however, the amine group on the adenine base is the main focus. The amine group (NH_2) is considered to be an activating substituent on the ring at the para position. This activating group donates its lone pair of electrons into the ring or conjugated π system causing the π system to be more nucleophilic [28]. The substituent adjacent to the amine group (CH_3 and H, in this case) plays a distinct role as well, since size of substituents also affects the reactivity of the ring system [29]. It has been well documented that according to the location of protonation and/or methylation, aromaticity can increase or decrease depending on which ring (pyrmidine or imidazole) is protonated and/or methylated [30–32]. The amine group at the C6 position and proton located at the N7 position causes steric hindrance due to the character of the amine group. These findings suggest that the ionization potential of ring systems with an amine group and an adjacent substituent could decrease according to Table 2 and IV IP_{ad} values. For example, due to the location of the H at the N9 position the N9H adenine is not as affected by steric hindrance as N7H. Furthermore, the N3H and N9H (imino form) demonstrate the aforementioned characteristic as well. It is predicted that other DNA bases will exhibit this NH_2 trend.

Conclusions

A DFT study was performed to take steps toward bridging the gap of understanding between the pre-translational effects (tautomerization, ionization) and post-translational effects (methylation) of the tautomers and methyl derivatives of adenine and thymine. We conclude that;

1. As compared to other tautomers, the N3H tautomer had the highest electron affinity while the N9H tautomer had the lowest electron affinity. This adds to the explanation of why the N9H tautomer of adenine is considered to be the canonical structure. The N3H tautomer also had the highest ionization potential. Thymine had a higher electron affinity and ionization potential than the adenine tautomers. Therefore, the trend for the electron affinities and ionization potential from highest to lowest is as follows:

$$\text{Thymine} > \text{N3H} > \text{N7H} > \text{N9H}\,(\text{EA})$$

$$\text{Thymine} > \text{N7H} > \text{N3H} > \text{N9H}\,(\text{IP}).$$

2. As predicted, the addition of a methyl group does not affect the structure of the adenine and thymine, however, it does affect the ionization potential of adenine and thymine. The electron affinities of the methyl derivatives follow the same trend as seen above, however the ionization potentials of the methyl derivatives differ:

$$\text{Thymine} > \text{N7} > \text{N9} > \text{N3}\,(\text{IP}).$$

As stated earlier, the NH_2 plays a key role in the differences in the electron affinities and ionization potential of the various tautomers investigated in this theoretical study.

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