Vibrational Analysis of Carbonyl Group of ^{18}O labeled and Unlabeled Guanine: Carbonyl Vibrational Frequency Including ^{18}O Isotopic Effect and ^{13}C-NMR Chemical Shift of Carbonyl Group in Guanine

Using Ab Initio (RHF, MP2) and DFT (B3LYP, BPW91) Calculations

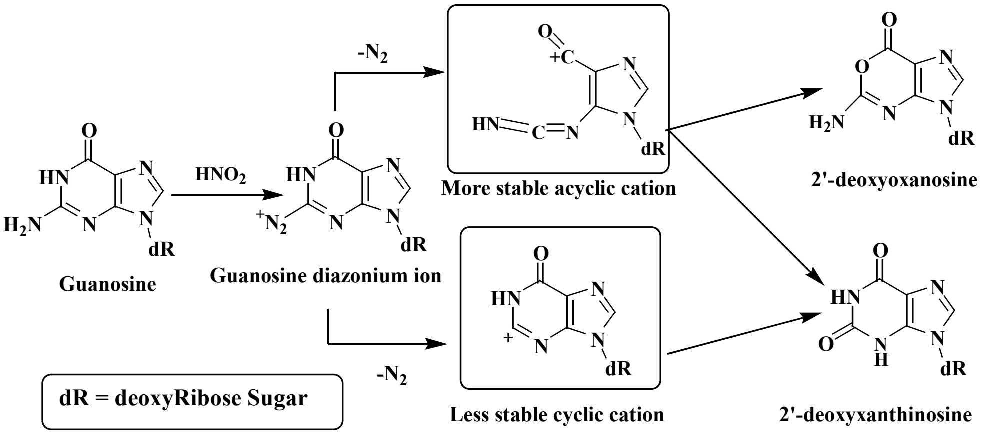
TAK-JHAL: Maria G Gomes & Papiya Majumdar

Introduction:

DNA base deamination and interstrand cross-linking due to reaction with HNO_2 or NO are likely to occur in human body resulting various kinds of disorders in people. The nucleobases adenine, cytosine, guanine contain one primary amino function that can undergo deamination and cross-linking via a diazonium ion intermediate due to reaction with HNO_2.[1]

It was commonly accepted for a long time that guanosine deamination follows a simple nucleophilic aromatic substitution path.[1] If water attacks the diazonium ion, the N_2 group is replaced by an OH group and subsequent tautomerization would yield the major product xanthinosine, with the pyrimidine ring staying intact at all times. But this mechanism can not explain the formation of novel product 2′-deoxyoxanosine reported by Suzuki et al. [2]

Scheme 1. Cyclic and Acyclic Structures of the Cation Derived by Dediazoniation of Guanosinediazonium ion and Formation of Oxanosine and Xanthinosine.



---

[1] Nair, V.; Richardson, S. G. *Tetrahedron Lett.* **1979,** 1181-1184; Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980**, *45*, 3969-3974;

[2] Suzuki, T.; Yamaoka, R.; Nishi, M.; Ide, H.; Makino, K. *J. Am. Chem. Soc.* **1996**, *118*, 2515; Suzuki, T.; Kanaori, K.; Tajima, K.; Makino, K. *Nucleic Acids Symp. Ser.* **1997**, *37*, 313; Suzuki, T.; Yamada, M.; Kanaori, K.; Tajima, K.; Makino, K. *Nucleic Acids Symp. Ser.* **1998**, *39*; Suzuki, T.; Ide, H.; Yamada, M.; Indo, N.; Kanaori, K.; Morii, T.; Makino, K. *Nucleic Acid Res.* **2000**, *28,* 544-551.

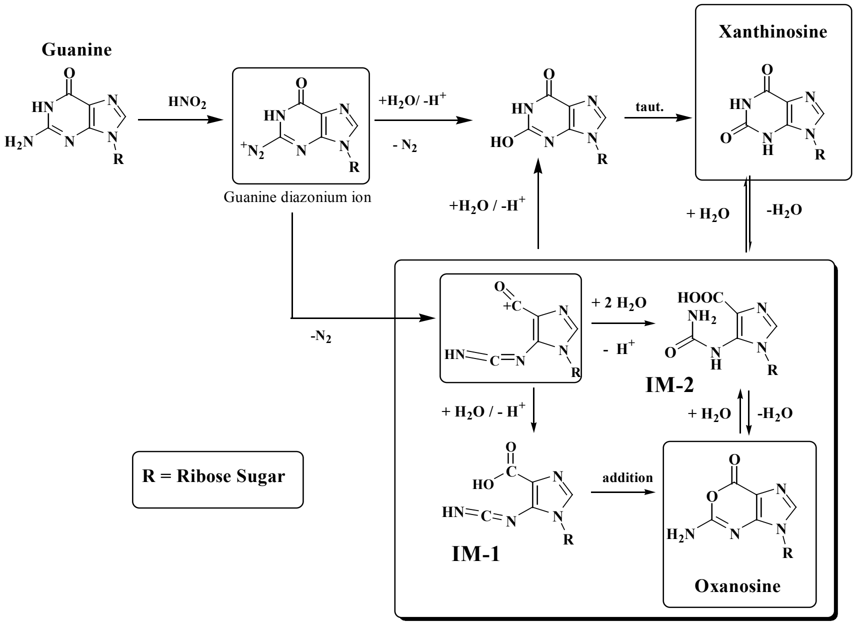
Recent theoretical study showed that these kinds of diazonium ions are very unstable, and easy to discompose to pyrimidine ring-opened intermediate. MP2(full)/6-31G** potential energy surface calculation showed that pyrimidine ring opened cation (acyclic structure, scheme 1) is 0.6 Kcal/mole more stable than the ring closed (cyclic structure, scheme 1) cation. Based on these calculations a new mechanistic hypothesis is suggested which involves a pyrimidine ring-opened cation as the key intermediate, which does account for all observed products, xanthinosine and oxanosine.[3]

To test this hypothesis that pyrimidine ring-opening occurs during the nitrosative deamination of guanosine, ^{18}O labeled guanosine will be synthesized and their deaminations will be studied in aqueous solution.

Goals and Aims:

The mechanism proposed by Glaser et al is presented in Scheme 2. According to the mechanism incorporation of ^{18}O in the products are expected and can be identified by NMR and IR spectroscopy. Significant changes are expected in the vibrational frequency and chemical shifts of carbonyl group of ^{18}O labeled and unlabeled guanosine, xanthinosine, and oxanosine.

Scheme 2. Proposed Mechanism for the formation of Oxanosine and Xanthinosine



Vibrational frequencies and chemical shifts can be calculated by computational approach. The goal of our project is to study carbonyl vibrational frequency including ^{18}O isotopic effect and ^{13}C-NMR chemical shift of carbonyl group in guanine using Ab Initio and DFT level Calculations.

[3] Glaser, R.; Son, M.-S. *J. Am. Chem. Soc.* **1996**, *118*, 10942; Glaser, R.; Rayat, S.; Lewis, M.; Son, M.-S.; Meyer, S. *J. Am. Chem. Soc.* **1999,** *121,* 6108

Proposed Research:

Choice of Methodology:

Molecular frequencies depend on the second derivative of the energy with respect to the nuclear positions. Analytic second derivatives are available for the ***Hartree-Fock, Second Order Moller-Plesset, and Density Functional*** Theoretical procedures. So, these methods can be applied to find the vibrational frequency of a molecule which is very important in IR spectroscopy.

In our study, we are interested in the isotopic substituted vibrational frequency analysis of carbonyl group in guanine. DFT has proven to be a powerful tool to calculate the vibrational frequency of small molecules as well as large molecules.[4] Since guanine is a large molecule with many heavy atoms, a medium sized basis size can be used. We know that ***HF method*** provides a reasonable model for a wide range of problems and molecular systems. However it has limitations because it does not include a full treatment of the effects of ***electron correlation. Second Order Moller-Plesset Perturbation method*** is one of the least expensive ways to improve on HF theory, because this adds ***higher excitations*** to HF theory as a ***non-iterative correction. Density Functional Theory*** with ***Gradient-corrected functionals*** involves both the values of the ***electron spin density*** and ***their gradients***. DFT methods, such as, B3LYP, BPW91 are also less expensive than traditional correlated methods.

We will apply these three methods for the optimization of the geometry of the guanine molecule. Vibrational frequency calculation will be performed on this optimized structure to obtain guanine carbonyl stretching frequency and the effect of ^{18}O substitution on this. In addition to this we will also compute the ^{13}C NMR chemical shift with this optimized structure including isotopic effect.

Scope of the Project:

1. Preliminary Results:

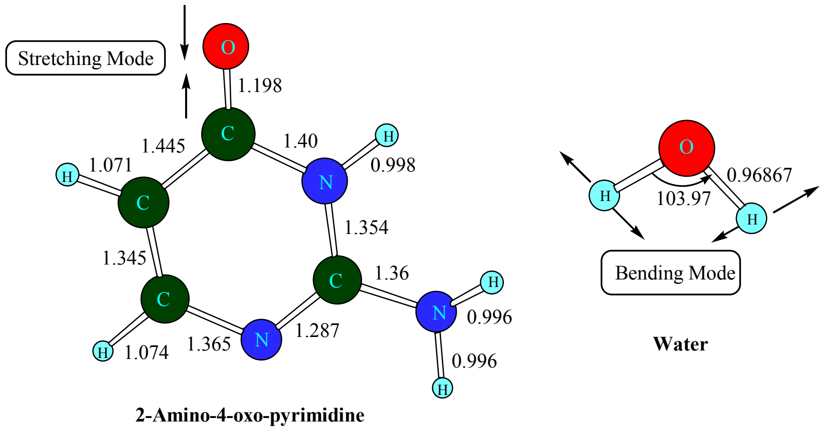
Before starting the main calculation, we performed some preliminary calculations on the simplest molecule, water. We optimized the geometry using a medium sized basis set, 6-31G* applying RHF, MP2 (Figure 1), B3LYP, and BPW91 methods. After that we computed the vibrational frequency on ^{18}O isotopically labeled and unlabeled water molecule. We used ***freq=(readisotopes,readfc)*** keyword and specified temperature (298.15K), pressure (1 atm), and ***exact atomic weight*** of all the atoms (according to z matrix) in our input file for ^{18}O substituted water.

The results are tabulated in Table 1. In all of these calculations, we found that, the vibrational frequency for ^{18}O substituted water was less than that of the unlabeled one. This can be explained as follows: ***The frequency,*** $\nu = ½\pi\sqrt{k}/\mu$. Since frequency (ν) is inversely proportional to the reduced mass (μ), it decreases with the increase of the mass of the molecule. In the ^{18}O substituted H_2O, the frequency is expected to be lower than the unlabeled water molecule.

Table 1. Optimized Geometry Parameters and Frequency values for Labeled ($^{18}OH_2$) and Unlabeled ($^{16}OH_2$) Water:

	*RHF/6-31G**	*MP2/6-31G**	*B3LYP/6-31G**	*BPW91/6-31G**	*Exp.*
O-H Distance/Å	**0.94734**	**0.969**	**0.969**	**0.976**	**0.958**
H-O-H (°)	**105.498**	**103.97**	**103.61**	**102.98**	**104.45**
Energy/Hartree	**-76.01075**	**-76.00980**	**-76.40895**	**-76.40081**	
Freq. (^{16}O)	**1827**	**1735**	**1713**	**1686**	
Freq. (^{18}O)	**1819**	**1728**	**1706**	**1539**	

Figure 1. Optimized Structure of 2-Amino-4-oxo-pyrimidine (RHF/6-31G*) and water (MP2/6-31G*) and their Stretching and Bending Mode respectively.



After studying water molecule, we also attempted to continue our calculation on 2-Amino-4-oxo-pyrimidine, which mimics the pyrimidine ring of guanine. We optimized the geometry using RHF/6-31G* method (Figure 1) and obtained frequency of labeled (^{18}O) and unlabeled (^{16}O) molecule with this optimized structure. As expected, we found the carbonyl vibrational frequency for ^{18}O substituted molecule was lower than that of the unlabeled one, as listed in Table 2. We also found that when we multiplied the calculated frequency with the scaled factor, the value became closer to the expected frequency (1705 - 1730 cm^{-1}, as listed in Table 2).

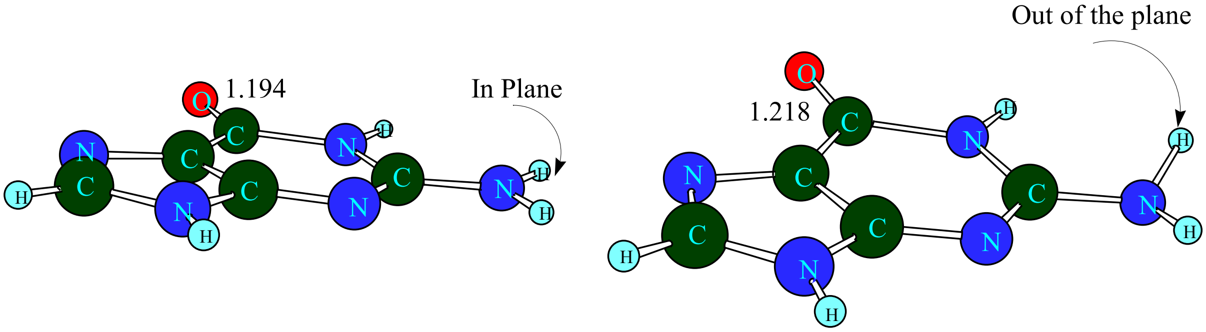
2. Our Present Work:

So far the frequency value that we obtained for the carbonyl group in Table 2, we can predict that similar calculation can be performed on “Guanine” and we should obtain the comparable values for carbonyl frequency. We are presently calculating the vibrational frequencies on the optimized guanine structure with RHF/6-31G*, MP2/6-31G*,B3LYP/6-31G*, and BPW91/6-31G*. We optimized the geometry on both the Cs and C1 symmetry structure of guanine and found that Cs symmetry contains an imaginary mode in frequency output file referring a higher energy state. So, we decided to proceed our frequency calculation on C1 symmetry.

Table 2. Optimized Geometry Parameters and Frequency values for Labeled ($C_4H_5N_3{}^{18}O$) and Unlabeled ($C_4H_5N_3{}^{16}O$) 2-Amino-4-oxo-pyrimidine:

	*RHF/6-31G**	*Multiplied by 0.8929 (HF Scale Factor)*
C=O Distance/ Å	**1.198**	
Energy/Hartree	**-392.61737**	
Freq. (^{16}O)	**1979**	**1768**
Freq. (^{18}O)	**1951**	**1742**

Figure 2. Optimized Structure of Guanine with RHF/6-31G*(Cs Symmetry) and BLYP/6-31G*(C1 Symmetry)



Cs Symmetry of Guanine

C1 Symmetry of Guanine

Interpretation and Proposal:

We are currently running more frequency calculation on isotopic substituted guanine on Darwin. We assume that the carbonyl frequencies of the ^{18}O labeled guanine will be lower than that of unlabeled guanine. After synthesizing ^{18}O labeled guanine, we will compare the calculated value of carbonyl frequency with the experimental one. Since the calculation is performed on ***gas phase molecule,*** we will always obtain an over-estimated value. But, we assume that the difference will be minimized in the case of DFT calculation.

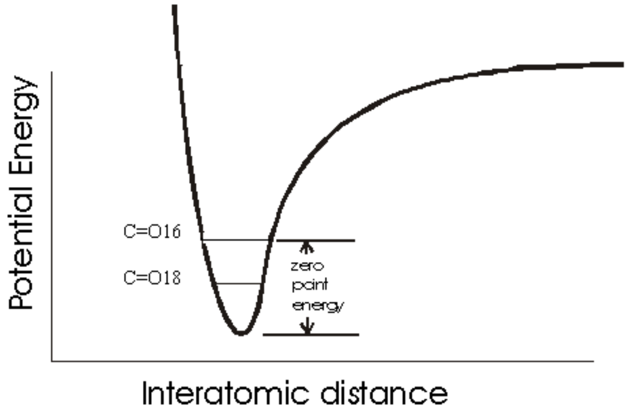
Table 3. Optimized Geometry Parameters and Frequency Values for Guanine

	*RHF/6-31G**	*MP2/6-31G**	*B3LYP/6-31G**	*BPW91/6-31G**	*Exp.*
C=O Distance/Å	**1.193**	**1.225**	**1.218**	**1.228**	
Energy/Hartree	**-539.39387**	**-539.38597**	**-542.55009**	**-542.51606**	
Freq. (^{16}O)	**1999**		**1832**	**1780**	**1699, 1676**
Freq.*Scale factor	**1785**				

^{13}C-NMR Chemical Shift Calculation:

As ^{18}O is heavier than ^{16}O, zero point vibrational energy of C=^{18}O will be lower than C=^{16}O, and also closer to minimum (Figure 3). Hence, C=^{18}O will be shorter, carbon will be more shielded than in C=^{16}O. And as a result, the chemical shift of carbonyl carbon atom will be slightly shifted to up-field.

Figure 3. Vibrational energy diagram of carbonyl stretching:



To predict the chemical shift we need to perform NMR calculation using HF/6-311+G(2d,p) method on optimized geometry calculated by B3LYP/6-31G* method. This is a recommended model for reliable NMR predictions by Cheeseman and Coworkers.[5]

We propose to perform the calculation of ^{13}C isotropic chemical shifts at HF, BLYP, and B3LYP/6-311+G(2d,p) levels of theory for the B3LYP/6-31G(d,p) optimized geometry of guanine.[6]

Facilities and Feasibility:

We performed preliminary and guanine frequency calculation on both Shiva and Darwin hardware with Gaussian 98 software. However, Shiva is not suitable for higher level calculation because it is expensive and also time consuming. So, we will perform all the proposed calculation on Darwin. But, one drawback of this hardware is that it can run only one job at a time.

Timeline:

The CPU time to run the BLYP/6-31G* geometry optimization of guanine was 20 min. 20 sec. on Darwin. We assume that our proposed work will take 3 months if we perform it on Darwin working 8 hours a day 5 days a week.

---

4. Cuevas, G.; Renugopalakrishnan, V.; Madrid, G.; Hagler, A. T. *Phys. Chem. Chem. Phys.*, **2002**, *4,* 1490-1499
5. Cheeseman, J. R.; Trucks, G. W.; Keith, T. A.; Frisch, M. J. *J. Chem. Phys*., **1996**, *104*, 5497
6. Vikic-Topic, D.; Pejov, L. *J. Chem. Inf. Comput. Sci.,* **2001**, *41,* 1478-1487