

## Effects of Bases on the Stabilities of Nucleoside C4' Radicals

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**Abstract:** C4'-H bond dissociation enthalpies of nucleosides were predicted using theoretical methods to a precision of 1-2 Kcal/mol. It was found that the stability of the C4' nucleoside radical is slightly dependent on the base. The orders of stability are dA < dG < dT < dC for deoxynucleosides and U < G < A ≈ C for nucleosides.

**Keywords:** Nucleoside, homolysis, bond energy, DNA lesion, substituent effect.

Free radicals can cause damages to DNAs and RNAs<sup>1</sup>. They can react with the heterocyclic bases to alter their structures<sup>2</sup>, or attack the sugar-phosphate backbones and abstract hydrogen atoms to form sugar radicals<sup>3</sup>. Both damage pathways have been attracted considerable attention recently<sup>1</sup>.

For the lesions on the sugar backbone, not all the hydrogen atoms have equal probability of being abstracted. One reason for the different reactivity is the unequal conformation accessibility of each hydrogen atom toward oxidants. For instance, radicals that bind to the minor groove of DNA show the most oxidation at the 5'- and 4'-positions of deoxyribose in B-form DNA<sup>3</sup>. The other reason for the different reactivity is the unequal intrinsic stability of the sugar radical. This difference of stability would be easy to evaluate if one knows the homolytic dissociation enthalpy of each sugar-hydrogen bond (BDE). However, because of the relative complexity of nucleic acids, so far no experimental C-H BDE has been reported for them.

Fortunately, theoretical calculation provides an alternative approach to estimate these BDEs<sup>4-6</sup>. Recently, Osman and Boyd studied ribose and deoxyribose radicals in the absence of the bases. The radicals from heterocyclic bases in the absence of the sugar moiety were also studied<sup>6</sup>. Nonetheless, although in DNA/RNA chemistry the base, sugar, and phosphate moieties are usually considered as separate entities, the effects of the bases on the sugar properties are still noticeable. Recently, Crich *et al.* investigated the fragmentation of nucleotide C4' radicals. From concrete experimental data they showed that the rate of this fragmentation is a function of the base (T ~ G > A ~ C), and then that the stability of C4' radical of ribose or deoxyribose may also be dependent on the base was proposed<sup>7</sup>. This interesting proposal prompted us to investigate if the base really has any significant effect on the C4'-H BDE of nucleosides. Furthermore, we wish to obtain the BDEs of the C4'-H bond to a precision of 1-2 Kcal/mol. Since our

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approach to study the system relies on the theoretical calculation<sup>8</sup>, we at first need to know the accuracy of the theoretical methods for the system before investigating the nucleosides. Therefore, we calculated the C4'-H BDEs of a number of 1'-substituted tetrahydrofurans (THF) using different theoretical methods (**Table 1**).

**Table 1** Theoretical C<sub>4</sub>-H BDEs of 1-substituted tetrahydrofurans<sup>a</sup>. (Kcal/mol)

X	UB3LYP/ 6-31g(d) (Kcal/mol)	UB3LYP/ 6-311++g(d,p) (Kcal/mol)	RB3LYP/ 6-31g(d) (Kcal/mol)	RMP2/ 6-31g(d) (Kcal/mol)	CBS-Q (Kcal/mol)	G3 (Kcal/mol)
H	91.2	90.2	91.1	85.9	93.9	93.9
CH <sub>3</sub>	-0.4	-0.2	-0.2	-0.5	-0.3	-0.2
NH <sub>2</sub>	+0.0	+0.0	+0.1	-0.1	-0.1	-0.1
OH	+1.4	+1.4	+1.5	+1.4	+1.6	+1.4
F	+2.4	+2.6	+2.5	+2.2	+2.6	+2.4
NHCH <sub>3</sub>	-0.1	-0.2	+0.0	-0.2	-0.1	-0.1
OCH <sub>3</sub>	+1.3	+1.2	+1.4	+1.3	+1.5	+1.3
CN	+1.4	+1.5	+1.6	+1.4	+1.7	+1.5
<i>r</i> <sup>b</sup>	0.996	0.996	0.998	0.994	0.999	1.000
<i>sd</i> <sup>c</sup>	0.10	0.10	0.07	0.12	0.05	0.00

a. Except for G3 and CBS-Q, all the calculations were done using UB3LYP/6-31g(d) optimized geometry. BDE for unsubstituted THF is the absolute value. For the substitute cases, the relative BDE compared to unsubstituted THF was shown. b. Correlation coefficient of the regression between BDEs from one method and BDEs from G3. c. Standard deviation (Kcal/mol) of the correlation between BDEs from one method and BDEs from G3.

According to **Table 1**, CBS-Q and G3 predict the C4'-H BDE of unsubstituted THF to be 93.9 Kcal/mol. According to the previous studies, this prediction should have a precision of 1-2 Kcal/mol<sup>9</sup>. In comparison, all B3LYP and MP2 methods underestimate this BDE. For the substituted THF, the C4'-H BDEs are reported as the relative values compared to the unsubstituted case using the same theoretical method. According to **Table 1**, it is clear that all the methods give very similar results for the relative BDEs<sup>10</sup>. For CH<sub>3</sub> substitution, different methods predicted that the corresponding C4'-H BDE should be about 0.2-0.5 Kcal/mol, which is smaller than C4'-H BDE for the unsubstituted THF. Therefore, 1'-CH<sub>3</sub> substitution stabilizes 4'-THF radical by 0.2-0.5 Kcal/mol.

In comparison, NH<sub>2</sub> and NHCH<sub>3</sub> lower the C4'-H BDE by 0.0-0.2 Kcal/mol. Thus, NH<sub>2</sub> and NHCH<sub>3</sub> stabilize 4'-THF radical by 0.0-0.2 Kcal/mol. It appears that NHCH<sub>3</sub> shows slightly more stabilization effect than NH<sub>2</sub>.

OH and OCH<sub>3</sub> increase the C4'-H BDE by 1.2-1.6 Kcal/mol. Therefore, OH and OCH<sub>3</sub> destabilize 4'-THF radical by 1.2-1.6 Kcal/mol. It appears that OCH<sub>3</sub> shows slightly less destabilization effect than OH.

F increase C4'-H BDE by 2.2-2.6 Kcal/mol. Therefore, F destabilizes 4'-THF radical by 2.2-2.6 Kcal/mol. Finally, CN destabilizes 4'-THF radical by 1.4-1.7 Kcal/mol.

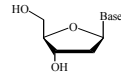
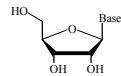
The good agreement between different theoretical methods in predicting relative BDEs indicates that we may be able to find good empirical correlation between BDEs calculated using relatively low level methods and BDEs calculated using CBS-Q and G3

for a group of THF-like compounds. In fact, plotting BDEs of 1'-substituted THF predicted by one method against BDEs predicted by G3, we obtained excellent correlation equations. The correlation coefficients are always larger than 0.99 (See *r* values in **Table 1**). The standard deviations of the correlation are about 0.1 Kcal/mol.

In **Table 1**, *r* and *sd* of UB3LYP/6-311++g(d,p) are not significantly better than that of UB3LYP/6-31g(d). RB3LYP provides slightly better correlation than UB3LYP. RMP2 gives the lowest correlation coefficient and largest standard deviation.

Considering the size of the system and the performance of the methods, we chose UB3LYP/6-31g(d) and RB3LYP/6-31g(d) methods to calculate the C4'-H BDEs of adenosine(A), guanosine(G), cytosine(C), uridine(U), deoxyadenosine(dA), deoxyguanosine (dG), deoxycytidine (dC), thymidine (dT), and their OH or NH<sub>2</sub> substituted models (**Table 2**). We used the empirical correlation, which was established on the basis of the results in **Table 1**, to convert the results of UB3LYP and RB3LYP to get the final values in **Table 2**. According to the above discussion, the values in **Table 2** should have good accuracy comparable to the G3 results, *i.e.* a precision of 1-2 Kcal/mol.

**Table 2** Theoretical C4'-H BDEs of nucleosides<sup>a</sup> (Kcal/mol)

Nucleoside	Base =		dA	dG	dC	dT
	OH	NH <sub>2</sub>				
	d-OH	d-NH <sub>2</sub>				
	r-OH	r-NH <sub>2</sub>	A	G	C	U
Nucleoside	UB3LYP/6-31g(d) <sup>b</sup> (Kcal/mol)		RB3LYP/6-31g(d) <sup>c</sup> (Kcal/mol)			
d-OH	93.0		93.3			
d-NH <sub>2</sub>	92.6		92.9			
dA	95.6		96.0			
dG	95.5		95.7			
dC	94.8		95.0			
dT	95.4		95.6			
r-OH	94.1		94.4			
r-NH <sub>2</sub>	93.9		94.1			
A	95.6		95.9			
G	96.0		96.3			
C	95.6		95.9			
U	96.5		96.9			

a. All the calculations were done using UB3LYP/6-31g(d) optimized geometry<sup>9</sup>. b. The values were converted from the original UB3LYP/6-31g(d) results using the correlation equation: BDE (predicted) = 0.99 × BDE (UB3LYP/6-31g(d)) + 3.9 Kcal/mol. c. The values were converted from the original RB3LYP/6-31g(d) results using the correlation equation: BDE (predicted) = 0.98 × BDE (RB3LYP/6-31g(d)) + 4.2 Kcal/mol.

According to **Table 2**, the C4'-H BDEs of all the nucleosides are about 95 Kcal/mol. This value is about 5 Kcal/mol larger than the previous estimation<sup>4</sup>. The C4'-H BDEs of the 1'-OH and 1'-NH<sub>2</sub> model compounds for nucleosides or deoxynucleosides are about 2-3 Kcal/mol smaller than those for the real nucleosides or deoxynucleosides. Therefore, in order to predict the properties of the nucleoside or deoxynucleoside radicals, the bases

must be taken into consideration.

Interestingly, there is a noticeable dependence of the C4'-H BDE on the base. For deoxynucleosides, the C4'-H BDEs decrease in the order dA > dG > dT > dC. Therefore, the stability of the C4' radicals increases in the order dA < dG < dT < dC. For RNA nucleosides, the C4'-H BDEs decrease in the order U > G > A ≈ C. So the order of the stability of the C4' radicals is U < G < A ≈ C.

It should be mentioned that the above orders are indicated by both UB3LYP/6-31g(d) and RB3LYP/6-31g(d) calculations. Therefore, we believe that the observed base dependence is real. In addition, the C4'-H BDE of dA is about 1 Kcal/mol larger than that of dC. It means that dC is five times more vulnerable than dA in the lesion at 4' position. Thus, although the base effect on BDE is small, it is not trivial.

In summary, using theoretical methods we obtained accurate C4'-H homolytic bond dissociation energies for all the nucleosides and deoxynucleosides. The results indicate that the stability of the nucleoside and deoxynucleoside radicals is slightly dependent on the bases, which provides concrete evidence for Crich's recent proposal. We are now trying to use the stability of the nucleoside/deoxynucleoside radicals to understand the reactivity and fate of the species observed in the biomedical chemistry of DNA and RNA lesion.

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### References and Notes

- (a) M. Dizdaroğlu, P. Jaruga, M. Birincioglu, H. Rodriguez, *Free Radical Biol. Med.*, **2002**, *32*, 1102. (b) S.S. Wallace, *Free Radical Biol. Med.*, **2002**, *33*, 1. (c) Q.H. Song, Q.X. Guo, *Progress in Chemistry*, **2001**, *13*, 428.
- C.J. Burrows, J.G. Muller, *Chem. Rev.*, **1998**, *98*, 1109.
- W. K. Pogozelski, T. D. Tullius, *Chem. Rev.*, **1998**, *98*, 1089.
- (a) K. Miaskiewicz, R. Osman, *J. Am. Chem. Soc.*, **1994**, *116*, 232. (b) N. Luo, A. Litvin, R. Osman, *J. Phys. Chem. A*, **1999**, *103*, 592.
- A.O. Colson, M. D. Sevilla, *J. Phys. Chem.*, **1995**, *99*, 3867.
- (a) S. D. Wetmore, R. J. Boyd, L. A. Eriksson, *J. Phys. Chem. B*, **1998**, *102*, 7674. (b) S. D. Wetmore, R. J. Boyd, L. A. Eriksson, *J. Phys. Chem. B*, **1998**, *102*, 10602.
- (a) D. Crich, X.S. Mo, *Tetrahedron Lett.*, **1997**, *38*, 8169. (b) D. Crich, Q. Yao, *Tetrahedron*, **1998**, *54*, 305. (c) D. Crich, W. Huang, *J. Am. Chem. Soc.*, **2001**, *123*, 9239. (d) D. Crich, D.H. Suk, X. Hao, *Tetrahedron*, **2002**, *58*, 5789.
- M. J. Frisch, G. W. Trucks, H. B. Schlegel *et al.*, *Gaussian 98*, Revision A.7, Gaussian, Inc., Pittsburgh, PA, **1998**.
- K.S. Song, Y.H. Cheng, Y. Fu *et al.*, *J. Phys. Chem. A*, **2002**, *106*, 6651.
- Similar insensitivity of the substituent effects towards theoretical methods was found for a number of systems before. See: (a) Y.H. Cheng, X. Zhao, K.S. Song *et al.*, *J. Org. Chem.*, **2002**, *67*, 6638. (b) Y. Fu, B.L. Lin, K.S. Song *et al.*, *J. Chem. Soc. Perkin Trans. 2*, **2002**, 1223. (c) Y.H. Cheng, Y. Fang, X. Zhao *et al.*, *Bull. Chem. Soc. Jpn.*, **2002**, *75*, 1715. (d) Y.H. Cheng, L. Liu, K.S. Song *et al.*, *J. Chem. Soc. Perkin Trans. 2*, **2002**, 1406.
- There are many stable conformations for each nucleoside. (See ref. 4, 6) To simplify the calculation and make the calculation results more relevant to biochemistry, we used the conformations documented in the database of Gauss View 2.1 as the starting point for geometry optimization for all the nucleosides.

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