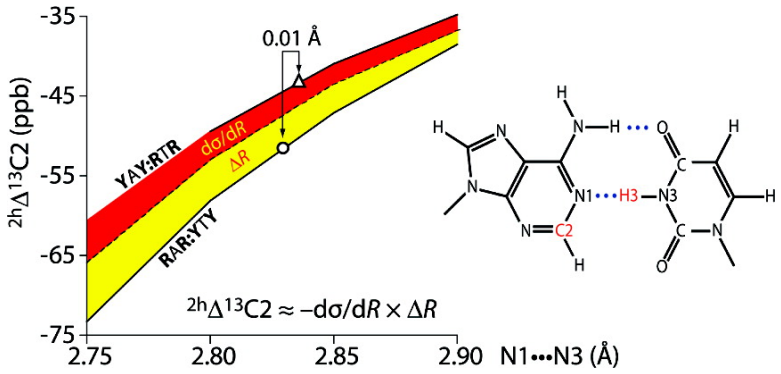
Communication

Hydrogen-Bonding and π–π Base-Stacking Interactions Are Coupled in DNA, As Suggested by Calculated and Experimental Trans-Hbond Deuterium Isotope Shifts

Marlon N. Manalo, Lisa M. Prez, and Andy LiWang

J. Am. Chem. Soc., **2007**, 129 (37), 11298-11299• DOI: 10.1021/ja0692940 • Publication Date (Web): 28 August 2007



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 08/28/2007

Hydrogen-Bonding and $\pi-\pi$ Base-Stacking Interactions Are Coupled in DNA, As Suggested by Calculated and Experimental Trans-Hbond Deuterium Isotope Shifts

Marlon N. Manalo,[†] Lisa M. Pérez,[‡] and Andy LiWang*,[†]

Department of Biochemistry & Biophysics and Laboratory for Molecular Simulation, Texas A&M University, College Station, Texas 77843-2128

Received December 26, 2006; E-mail: andy-liwang@tamu.edu

Hydrogen bonds (Hbonds) and aromatic base stacking in nucleic acids are usually assumed to be independent interactions. Although thermodynamic studies have tentatively suggested that Hbonds and base-stacking interactions are coupled in DNA,[1] direct evidence is lacking. Here, calculations and experimental measurements suggest that Hbond strengths of DNA base pairs are modulated by electronic interactions with adjacent bases to different extents depending on sequence context. It has been shown experimentally and computationally that the two-bond deuterium isotope shift, $^{2}\Delta^{13}C$, is a sensitive gauge of Hbond strength.[2,3] Recently, it was found that the through-space or trans-Hbond deuterium isotope shift of $^{13}C2$ of adenine in A:T base pairs ($^{2h}\Delta^{13}C2$) of DNA are more or less negative, respectively, in **R**A**R**:**Y**T**Y** or **Y**A**Y**:**R**T**R** contexts (**R**, purine; **Y**, pyrimidine).[4] This result suggests that Hbonds are shorter in **R**A**R**:**Y**T**Y** than in **Y**A**Y**:**R**T**R** tracts, which implies that consideration of the coupling between Hbonds and base stacking may be important to understanding sequence-dependent DNA elasticity. However, the notion that the sequence dependence of $^{2h}\Delta^{13}C2$ reflects variation of Hbond strengths in DNA is untested.

The adenine $^{13}C2$ nucleus experiences a frequency shift called the trans-Hbond deuterium isotope shift, $^{2h}\Delta^{13}C2 = \delta^{13}C2(^{1}H3) - \delta^{13}C2(^{2}H3)$, as a result of deuterium substitution at the imino hydrogen site (H3) of the base-paired thymine, and can be approximated by the equation $^{2h}\Delta^{13}C2 = -d\sigma/dR \times \Delta R$,[2,4] where σ is the $^{13}C2$ NMR shielding constant, and $\Delta R = R_{NH} - R_{ND}$ is the difference in the mean bond lengths of N3$-^{1}$H3 and N3$-^{2}$H3 of thymine. (For a more elaborate treatment of isotope effects, the reader is referred to Ruden and Ruud.[5]) As described previously, $d\sigma/dR$ is determined from the slope of σ calculated as a function of the thymine N3$-$H3 covalent-bond length, and ΔR is determined from a potential-energy surface scan along the N3$-$H3 bond.[4] ΔR is a measure of the anharmonicity of the N3$-$H3 vibrational potential, whereas $d\sigma/dR$ reflects the transmission of the isotope effect from the site of substitution to the site of the chemical shift measurement.[2,4] The sequence dependencies of $d\sigma/dR$ and ΔR of $^{2h}\Delta^{13}C2$ are calculated here in an effort to gain insight into the physicochemical basis of the experimentally observed sequence dependence of $^{2h}\Delta^{13}C2$.

Shown in Figure 1 are calculated values of $^{2h}\Delta^{13}C2$ of A:T base pairs in different sequence contexts as a function of the N1···N3 distance,[6] where it can be seen that $^{2h}\Delta^{13}C2$ (1) decreases with decreasing Hbond length and (2) depends on nearest-neighbor interactions. Consistent with experimental measurements, calculated $^{2h}\Delta^{13}C2$ is more negative in the **R**A**R**:**Y**T**Y** than **Y**A**Y**:**R**T**R** context. Previously measured $^{2h}\Delta^{13}C2$ values[4] placed on these calculated curves correlate with N1···N3 distances that are within one standard deviation of those observed in high-resolution X-ray crystal structures.[7]

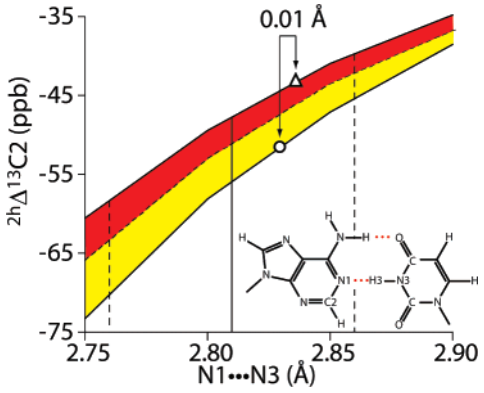


Figure 1. Sequence-dependent $^{2h}\Delta^{13}C2$ values of adenine of an A:T base pair[6] calculated as a function of the N1···N3 distance. The upper and lower solid curves are calculated for **Y**A**Y**:**R**T**R** and **R**A**R**:**Y**T**Y** contexts, respectively. The sequence dependent contribution of $d\sigma/dR$ to $^{2h}\Delta^{13}C2$ is given by the area colored red. The contribution of ΔR is given by the area colored yellow. The solid vertical line is the average distance determined from an analysis of high-resolution DNA X-ray crystal structures.[7] The standard deviation about this average distance is given by the dashed vertical lines. Computational details are provided in the Supporting Information. Experimental $^{2h}\Delta^{13}C2$ averages for the two different sequence contexts are shown as triangle (**Y**A**Y**:**R**T**R**) and circle (**R**A**R**:**Y**T**Y**).[4]

Figure 1 shows that at a given N1···N3 separation ΔR is larger in the **R**A**R**:**Y**T**Y** than **Y**A**Y**:**R**T**R** context. This calculation suggests that at the same N1···N3 distance an A:T Hbond is stronger in the **R**A**R**:**Y**T**Y** than **Y**A**Y**:**R**T**R** context, and that this difference in strengths is reported by $^{2h}\Delta^{13}C2$. Figure 1 also shows that **R**A**R**:**Y**T**Y** Hbonds are shorter than those of **Y**A**Y**:**R**T**R**, on average. Thus, these calculations suggest that the difference between experimental **R**A**R**:**Y**T**Y** and **Y**A**Y**:**R**T**R** $^{2h}\Delta^{13}C2$ values is due to stronger and shorter **R**A**R**:**Y**T**Y** Hbonds. In addition, Figure 1 shows that $d\sigma/dR$ of $^{2h}\Delta^{13}C2$ is steeper in the **R**A**R**:**Y**T**Y** than **Y**A**Y**:**R**T**R** context. This result suggests that the sensitivity of $^{13}C2$ shielding to changes in the N3$-$H3 covalent bond length of thymine depends in part on electronic interactions with adjacent bases as was also suggested earlier.[8] In contrast, the difference in $d\sigma/dR$ of $^{1h}\Delta^{15}N1$ between the **R**A**R**:**Y**T**Y** and **Y**A**Y**:**R**T**R** contexts at any given N1···N3 separation is proportionally much smaller (Table S7, Figure S4, Supporting Information). This comparison suggests that the sensitivity of $d\sigma/dR$ of $^{2h}\Delta^{13}C2$ to context is unrelated to Hbond strength. As a result, it appears that $^{1h}\Delta^{15}N1$ is a more accurate gauge of Hbond strength than is $^{2h}\Delta^{13}C2$, at least for the B-form conformation.

These calculations predict that the experimental dependence of $^{2h}\Delta^{13}C2$ on sequence[4] will be lost upon reducing base-stacking interactions. This prediction was tested by measuring $^{2h}\Delta^{13}C2$ in dilute ethanol$-$water mixtures, as ethanol can be used as a cosolvent to reduce hydrophobic aromatic stacking in DNA.[9] The sequence dependence of $^{2h}\Delta^{13}C2$ of DNA observed in water is indeed lost

[†] Department of Biochemistry & Biophysics.
[‡] Laboratory for Molecular Simulation.

10.1021/ja0692940 CCC: $37.00

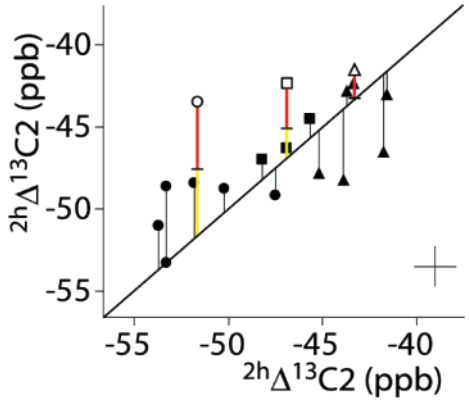


Figure 2. Correlation plot of experimental $^{2h}\Delta^{13}$C2 values of DNA in water (*x*-axis) and 5 mol % ethanol (*y*-axis). A total of five DNA sequences were examined here. Solid circles, squares, and triangles are used for **R**A**R**:**Y**T**Y**, **RAY**:**R**T**Y**, and **Y**A**Y**:**R**T**R** contexts, respectively. The open circle, square, and triangle are the calculated $^{2h}\Delta^{13}$C2 values of A:T base pairs in the **R**A**R**:**YTY**, **R**A**Y**:**R**T**Y**, and **Y**A**Y**:**R**T**R** (*x*-axis) and unstacked (*y*-axis) contexts. The yellow and red bars represent the contribution of ΔR and $d\sigma/dR$, respectively, to the change in $^{2h}\Delta^{13}$C2 from the stacked to unstacked environments. The small differences in the unstacked values of $^{2h}\Delta^{13}$C2 are due to the small differences in the N1···N3 distances as a result of geometry optimizations in the **R**A**R**:**Y**T**Y**, **R**A**Y**:**R**T**Y**, and **Y**A**Y**:**R**T**R** contexts. Computational details are given in the Supporting Information. The average uncertainties in the experimental values are shown in the lower right-hand corner of the plot.

upon the addition of 5 mol % ethanol (Figure 2). Interestingly, ethanol tends to increase and decrease $^{2h}\Delta^{13}$C2 values in the **R**A**R**:**Y**T**Y** and **Y**A**Y**:**R**T**R** contexts, respectively. Comparisons of chemical shifts (Table S13) and circular dichroism spectra show that these DNA samples retain their native structure in 5 mol % ethanol.[10]

It can be seen in Figure 2 that $^{2h}\Delta^{13}$C2 in the **Y**A**Y**:**R**T**R** context is predicted to be close to that of the unstacked value. Thus, reduced base stacking by ethanol should have little effect on **Y**A**Y**:**R**T**R** $^{2h}\Delta^{13}$C2. However, $^{2h}\Delta^{13}$C2 is also sensitive to Hbond length,[2,4] so any ethanol-induced changes in **Y**A**Y**:**R**T**R** $^{2h}\Delta^{13}$C2 should be due to a change in the N1···N3 separation. As Figure 2 shows, experimental **Y**A**Y**:**R**T**R** $^{2h}\Delta^{13}$C2 values tend to decrease with the addition of ethanol, which is consistent with Hbond shortening, and in line with studies that have shown that Hbond strengths increase in nonaqueous solvents for small molecules.[11] In contrast to the **Y**A**Y**:**R**T**R** context, the calculated stacking dependence of both $d\sigma/dR$ and ΔR in the **R**A**R**:**Y**T**Y** context is significant and predicts an increase in $^{2h}\Delta^{13}$C2 upon reducing base-stacking interactions (Figure 2). Other studies suggest that poly(A) is less hydrated than poly(AT).[12] Thus, there should be less shortening of Hbond lengths for **R**A**R**:**Y**T**Y** than **Y**A**Y**:**R**T**R** as a result of reduced water activity by ethanol. Together, these considerations forecast that ethanol-induced changes in **R**A**R**:**YTY** $^{2h}\Delta^{13}$C2 may be dominated by decreases in $d\sigma/dR$ and ΔR as a result of a reduction in $\pi-\pi$ stacking, and is in fact consistent with experimental observations (Figure 2). Thus, our calculations are able to rationalize the opposite effects of ethanol on the $^{2h}\Delta^{13}$C2 values of **R**A**R**:**Y**T**Y** and **Y**A**Y**:**R**T**R**.

Calculations by Barfield and co-workers suggest that $^1J_{NH}$ of imino groups in DNA is also sensitive to the N1···N3 Hbond length of Watson−Crick base pairs.[13] Yet, the correlation between $^1J_{NH}$ and $^{2h}\Delta^{13}$C2 is weak (Figure S3), which may now be explained at least in part by the sensitivity of $d\sigma/dR$ and ΔR terms of $^{2h}\Delta^{13}$C2 to base-stacking interactions.

Aromatic base stacking and DNA stiffness in general increase as TA < AT < AA.[14,15] Several studies have demonstrated a correlation between DNA elasticity or deformability and protein-DNA binding.[15−19] Generally, proteins bind more weakly to stiffer DNA. It appears that a better understanding of the sequence dependence of DNA elasticity may require consideration of the interdependence between Hbonds and aromatic base stacking. Our work predicts that proteins that reduce aromatic base stacking, by bending DNA for example, are expected to cause a greater decrease in Hbond strengths for poly(R) than poly(YR) tracts. Recent calculations by others have also suggested that $\pi-\pi$ interactions between aromatic heterocycles play a significant role in the Hbonding potential of an aromatic nitrogen base.[20]

Acknowledgment. We gratefully acknowledge the financial support provided by the Welch Foundation grant A-1471. The NMR instrumentation at Texas A&M University was supported by NSF grant DBI-9970232 and by the Texas Agriculture Experiment Station. We are also grateful to the Texas A&M Supercomputing Facility and the Laboratory for Molecular Simulation for computational resources. We would like to thank Dr. Michael Ramek at Technische Universität Graz for software used to fit our calculations to the Morse potential function.

Supporting Information Available: Computational details, table of $^{2h}\Delta^{13}$C2 values in water and 5 mol % ethanol, ^{1}H, ^{13}C HSQC-TROSY spectra of the DNA samples in water and 5 mol % ethanol, plots of $^{2h}\Delta^{13}$C2 vs imino ^{1}H shift and $^1J_{NH}$, calculated sequence dependence of $^{1h}\Delta^{15}$N, and examples of Gaussian 03 input files. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Moody, E. M.; Bevilacqua, P. C. *J. Am. Chem. Soc.* **2003**, *125*, 16285−16293.
(2) Abildgaard, J.; Bolvig, S.; Hansen, P. E. *J. Am. Chem. Soc.* **1998**, *120*, 9063−9069.
(3) Dziembowska, T.; Hansen, P. E.; Rozwadowski, Z. *Prog. Nucl. Magn. Reson. Spectrosc.* **2004**, *45*, 1−29.
(4) Kim, Y.-I.; Manalo, M. N.; Pérez, L. M.; LiWang, A. *J. Biomol. NMR* **2006**, *34*, 229−236.
(5) Ruden, T. A.; Ruud, K. Ro-vibrational corrections to NMR parameters. In *Calculations of NMR and EPR parameters*; Kaupp, M., Buhl, M., Malkin, V. G., Eds.; Wiley-VCH Verlag GmbH & Co.: Weinheim, Germany, 2004; p 153−173.
(6) Recently, we showed that the chemical difference between uracil and thymine has no measurable effect on $^{2h}\Delta^{13}$C2.[4] Thus, calculations were actually carried out on A:U base pairs to save computational time. For the sake of consistency, we refer to calculations on A:U base pairs as A:T.
(7) Dingley, A. J.; Masse, J. E.; Peterson, R. D.; Barfield, M.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 6019−6027.
(8) Vidossich, P.; Piana, S.; Miani, A.; Carloni, P. *J. Am. Chem. Soc.* **2006**, *128*, 7215−7221.
(9) Guckian, K. M.; Schweitzer, B. A.; Ren, R. X.-F.; Sheils, C. J.; Tahmassebi, D. C.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 2213−2222.
(10) Manalo, M. N.; Kong, X.; LiWang, A. *J. Biomol. NMR* **2007**, *37*, 257−263.
(11) Shan, S.-O.; Herschlag, D. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14474−14479.
(12) Elcock, A. H.; McCammon, J. A. *J. Am. Chem. Soc.* **1995**, *117*, 10161−10162.
(13) Barfield, M.; Dingley, A. J.; Feigon, J.; Grzesiek, S. *J. Am. Chem. Soc.* **2001**, *123*, 4014−4022.
(14) Dickerson, R. E. Nucleic Acids. In *International Tables for Crystallography*; Rossmann, M. G., Arnold, E., Eds.; Crystallography of Biological Macromolecules, Vol. F; Springer: Chester, U.K., 2006; p 588−622.
(15) Gromiha, M. M. *J. Biotechnol.* **2005**, *117*, 137−145.
(16) Hogan, M. E.; Austin, R. H. *Nature* **1987**, *329*, 263−266.
(17) Olson, W. K.; Gorin, A. A.; Lu, X.-J.; Hock, L. M.; Zhurkin, V. B. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11163−11168.
(18) Harrington, R. E.; Winicov, I. *Prog. Nucleic Acid Res. Mol. Biol.* **1994**, *47*, 195−270.
(19) Sarai, A.; Mazur, J.; Nussinov, R.; Jernigan, R. L. *Biochemistry* **1989**, *28*, 7842−7849.
(20) Mignon, P.; Loverix, S.; Steyaert, J.; Geerlings, P. *Nucleic Acids Res.* **2005**, *33*, 1779−1789.

JA0692940