

Maria Brandl, Michael Meyer and Jürgen Sühnel  
Biocomputing, Institut für Molekulare Biotechnologie, Beutenbergstr. 11, D-07745 Jena, Germany

E-Mail: mbrandl@imb-jena.de; mmeyer@imb-jena.de; jsuehnel@imb-jena.de

Introduction

Recently, the first examples of water-mediated base pairs have been detected in two RNA structures<sup>1,2</sup>. So far, it is not clear whether the geometry of these complexes is due to their intrinsic properties or enforced by the nucleic acid environment. We have performed *ab initio* calculations on the smallest water mediated base pair known so far and report on the results obtained. Our model system is a uracil-cytosin mismatch base pair that occurs twice in the central region of a synthetic (GGACUUCGGUCC)<sub>2</sub>-duplex. Although they are mismatches, the four central UG and UC base pairs do not disturb the helical conformation of this structure. In the water mediated UC mismatch (WUC) base pair (Fig. 1) H42 of cytosin is hydrogen bonded to O4 of uracil. H3 of uracil and N3 of cytosin are linked by hydrogen bonds to a tightly associated water molecule (temperature factor: 11.7Å<sup>2</sup>).

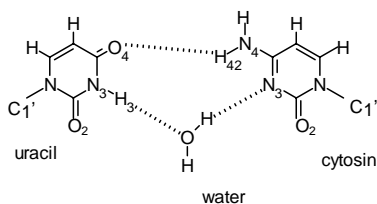


Fig. 1. The WUC base pair

Methods

The WUC base pair has been optimized at the HF/6-31G(d,p) level with a subsequent Hessian calculation. Interaction energies corrected for the basis set superposition error (BSSE) have been determined using the MP2/6-31G(d,p)//HF/6-31G(d,p) method. Cooperativity in the WUC complex has been checked by the method of Sponer et al.<sup>3</sup>. In order to investigate the effect of the structural water molecule, the complex has been optimized without the water molecule using the same method as described above.

Results

The positions of the heavy atoms in the optimized WUC complex are close to the ones observed in the crystal structure of the RNA dodecamer (Table 1, Fig. 2). The three hydrogen bonds of the crystal structure are conserved in the structure of the optimized complex. In the calculated structure we observe a buckle of approximately 18 degrees (Fig.3), which can be explained by the orientation of the free orbitals of the water molecule.

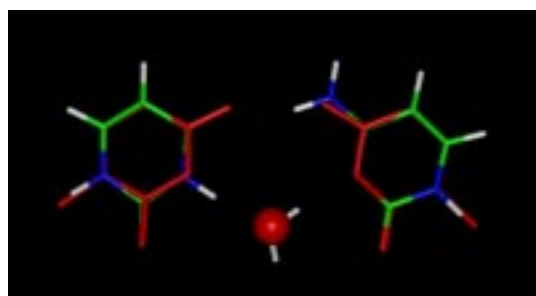


Fig. 2. Superposition of the WUC complex observed in the crystal structure (red) and the optimized WUC complex

For comparison we have minimized the structure of the UC mismatch base pair without water. The C1'-C1' distance of the direct UC base pair deviates more from the Watson-Crick cases than that of the WUC complex does. Repulsion between O2'(U) and O2'(C) causes a substantial propeller twist of about 25 degrees. The WUC complex and direct UC base pair differ considerably in shape (Fig. 3,4). The total interaction energy E<sub>0</sub> (-18.8 kcal/mol), which takes into account BSSE, electron correlation and deformation of the WUC complex, is substantially larger than for the direct UC base pair (-10.2 kcal/mol), Tab. 2. The three hydrogen bonds contribute in a similar order of magnitude to the total interaction energy.

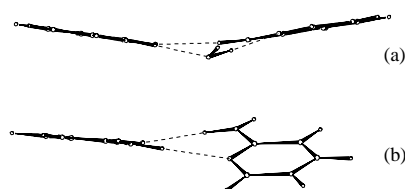


Fig. 3: Water-mediated (a) and direct (b) UC base pairs

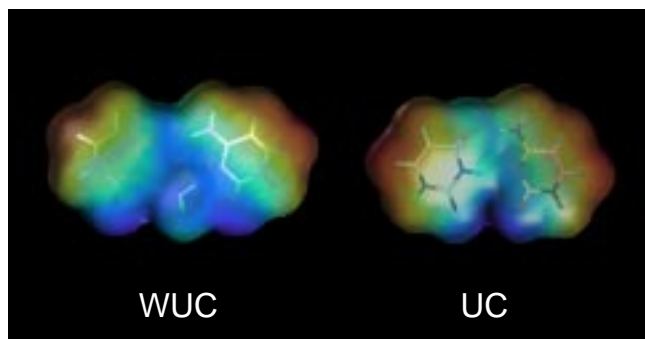


Fig. 4. Electrostatic potential at the Conolly surfaces of the direct and the water-mediated UC base pairs

Tab. 1. Structural parameters of the direct UC and WUC base pairs<sup>a)</sup>

	direct UC		WUC	
	HF <sup>b)</sup>	HF	exp. <sup>c)</sup>	
r(O4(U)...H4(C))	1.97	2.02	1.8	
r(H3(U)...O(H2O))	-	1.84	1.8	
r(H(H2O)...N3(C))	-	1.99	-	
r(O(H2O)...N3(C))	-	2.95	2.8	
r(H3(U)...N3(C))	-	2.14	-	
r(N3(U)...N3(C))	-	3.14	-	
r(C1'(U)...C1'(C))	8.79	11.69	11.7	
r(O2(U)...O2(C))	3.73	6.79	6.8	

a) distances in Å; b) 6-31G(d,p); c) Holbrook et al. 1991<sup>2)</sup>

Tab. 2. Interaction energies of UC and WUC<sup>a)</sup>

	direct UC	WUC
E <sup>MP2</sup>	-11.95	-22.79
E <sup>DEF</sup>	0.48	0.64
ZPE	1.26	3.32
E <sub>0</sub>	-10.22	-18.84

a) E<sup>MP2</sup> interaction energy (kcal/mol); E<sup>DEF</sup> deformation energy; ZPE zero point energy change; E<sub>0</sub> = E<sup>MP2</sup> + E<sup>DEF</sup> - ZPE total interaction energy.

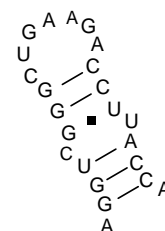


Fig. 5. UC in *Archaeoglobus fulgidus* 16S rRNA<sup>4)</sup>

UC mismatch base pairs occur in the secondary structure models of several ribosomal RNA molecules. Usually they are thought to break the helix, as indicated in Fig. 5. According to our results this is not necessarily the case. More investigations are needed to clarify the interactions between WUC and adjacent base pairs.

Summary

According to MP2/6-31G(d,p)//HF/6-31G(d,p) *ab initio* calculations insertion of water into a UC mismatch base pair leads to an intrinsically stable structure. The energy gain upon triplex formation is -18.8 kcal/mol, which is within the range of Watson-Crick base pairs. Cooperativity contributes -2.9 kcal/mol to the energy of stabilization. The overall shape of the triplex and in particular the C1'-C1' distance are more suitable for incorporation into helices than the respective properties of a direct UC base pair. The water mediated UC base pair is a likely candidate for the conformation of UC mismatches found in secondary structure models of rRNA. With a charge distribution that differs from each known canonical and non-canonical base pair it can serve as a highly specific recognition site.

Acknowledgement

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References

- Correll, C. C.; Freeborn, B.; Moore, P. B.; Steitz, T.A. *Cell* **1997**, 91, 705-712.
- Holbrook, S. R.; Cheong, C.; Tinoco, I. Jr.; Kim, S.H. *Nature* **1991**, 353, 579-581.
- Sponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem.* **1996**, 100, 1965-1974.
- Gutell, R.R. *Nucleic Acid Research*, **1994**, 22, 3502-3507.