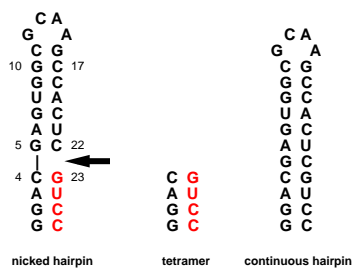


A Molecular Dynamics Analysis of Coaxial Stacking in RNA

Christoph Schneider and Jürgen Sühnel
E-Mail: csc@imb-jena.de; jsuehnel@imb-jena.de

Introduction

One of the structural motifs found in multibranch loops of RNA is coaxial stacking of helical stems. It occurs in structures of tRNA, pseudoknots, and the hammerhead ribozyme (Holland et al., 1999). Coaxial stacking interactions are usually not included in free-energy minimization algorithms for RNA secondary structure prediction. Recently, however, model systems have been studied to estimate the thermodynamic contribution of this motif to the total free energy of RNA folding (Walter et al., 1994). The model system consisted of an oligomer binding to a hairpin stem with a four-nucleotide overhang.



In these studies it has been shown that the oligomer binds approximately up to 1000-fold more tightly than predicted for a free tetramer duplex. Therefore, the authors have concluded that coaxial stacking provides large, favorable free energy contributions to RNA stability. Thus far, no structural information on the RNA model systems investigated in the thermodynamic studies is available. Moreover, the coaxial stacking motif in tRNA and pseudoknots is incorporated into a larger nucleic acid environment and may thus differ from the structure of the model systems. Here, we present the results of unrestrained molecular dynamics (MD) simulations of a tetramer binding to a 4-nucleotide overhang at the 5'-end of a hairpin (nicked structure) and of the corresponding continuous hairpin with Na⁺ as counterions and simulation times ranging between 2 and 3 ns.

Computational approach

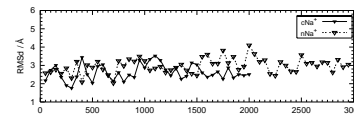
An initial hairpin structure was built from 2 subunits, loop and stem. The GCAA loop was taken from an NMR tetraloop structure (Protein Data Bank entry: 1zih; Jucker et al., 1996;). The stem structure was built de novo with SYBYL (Tripos Ass., Inc.) in a canonical A-RNA conformation and merged with the loop. A nicked structure (in the following denoted as n) was generated by removing the phosphodiester linkage between C22 and G23 from the continuous backbone hairpin (in the following denoted as c). These model structures were solvated with ~2800 TIP3P water molecules. A coulombic potential on a grid was calculated and the counterions were placed according to this potential with xLEaP from the AMBER package to neutralize the system. The concentration of Na⁺/Cl⁻ ions was 0.51/0.07 M. All simulations were run using the Sander module of AMBER 5 (Case et al., 1997) with the Cornell forcefield (Cornell et al., 1995). The nonbonded pair list was updated every 10 steps and a 10 Å cutoff was applied to the Lennard-Jones interactions. All structures were minimized for 1000 steps, afterwards water molecules and ions were relaxed, while the solute was kept fixed. Then all atoms were allowed to move. The system was gradually heated up to 300 K and equilibrated for additional 110 ps. Then production runs were performed for 2 to 3 ns at constant pressure (1 atm) and constant temperature (300 K) with a 2 fs time step. The simulation times were 2 ns for cNa⁺, and 3 ns for nNa⁺. The particle-mesh Ewald method was used for calculating the electrostatic interactions with a grid spacing of approximately 1 Å. SHAKE was applied to all bonds involving hydrogen. The simulated systems had a density of 1.06 g/cm³.

References

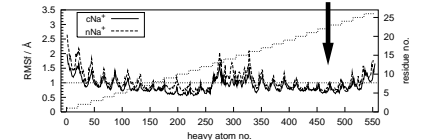
- Holland et al., *RNA* **1999**, 5, 257.
Walter et al., *PNAS* **1994**, 91, 9218.
Case et al., *AMBER* **5** **1997**.
Cornell et al., *JACS* **1995**, 117, 5179.

Results

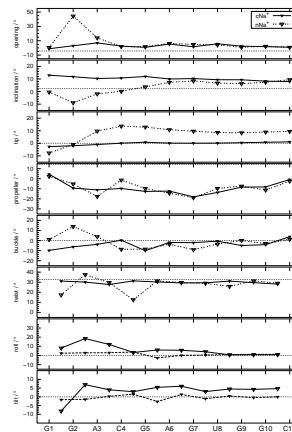
Root-mean-square deviation (RMSd)



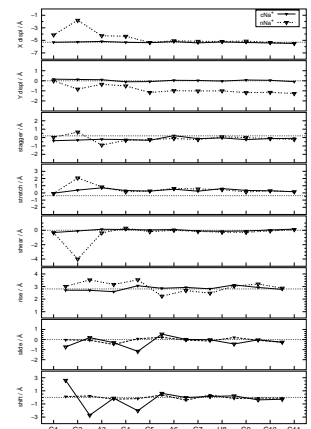
Root-mean-square fluctuation (RMSf)



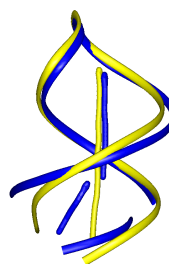
Global rotational helix parameters



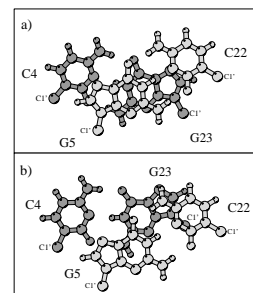
Global translational helix parameters



Superposition of continuous and nicked structures



Superposition of base pairs at the stacking interface (a. continuous, b. nicked)



Conclusions

- The simulation leads to a stable structure and generates a structural model for the nicked hairpin.
- The stacking interface is characterized by a reduced twist and shift and a slightly increased propeller twist. The rise is only slightly affected.
- This leads to an effective overlap between the amino group of C22 and the five-membered ring of G23 in the nicked structure.
- The local geometry changes around the stacking interface have a global structural effect on the helical axis. Whereas the continuous hairpin has an almost straight helical axis, the nicked structure exhibits a kink of about 40°.
- The stacking interface does not show an increased flexibility of base pairs as compared to the continuous hairpin structure.
- The comparison of continuous and nicked hairpin structures allows to speculate about the structural role of phosphate groups in RNA.