Computational and Experimental Studies of Ligand Binding in the Malachite Green RNA Aptamer

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Abstract

RNA aptamers are a central motif in many biological processes and is therefore an important target for drug development. In recent years, an increasing amount of attention has been paid to the use of RNA aptamers as potential drug candidates. Herein, we describe our studies of the complex structure of a ligand-RNA complex, the malachite green-binding RNA aptamer. We present the solution structure of an RNA aptamer that binds to triphenylphosphonium ion in complex with malachite green. The complex structure was obtained by an NMR solution structure determination approach using a combination of chemical shift restraints (CS) and distance constraints (DC) derived from a series of mixtures of ligand-RNA complexes. The results show that the ligand forms a stable complex with the RNA aptamer, which is characterized by a strong hydrogen bond interaction between the ligand and the RNA. The complex structure allows for a detailed discussion of structural changes that have been observed in the ligand in the context of the overall complex structure.

Results and Discussion

The complex structure of the RNA aptamer in the presence of the ligand was determined by a combination of NMR methods, including 2D NOESY experiments and a variety of distance constraints derived from a series of mixtures of ligand-RNA complexes. The results show that the ligand forms a stable complex with the RNA aptamer, which is characterized by a strong hydrogen bond interaction between the ligand and the RNA. The complex structure allows for a detailed discussion of structural changes that have been observed in the ligand in the context of the overall complex structure.

Electronic Changes in Ligand Upon Binding

Analyzing the NOESY spectra of RNA aptamer complexes clearly indicates that the two methyl groups of malachite green molecules bind to RNA, and that the ligand undergoes a conformational change upon binding. The two NOE-spectra are related and refer to A and B in the following discussion using (Figure 1): Ring A, as deeply buried inside the binding pocket, is strongly coupled to the RNA through hydrogen bonds and van der Waals contacts.

Table 1 shows the van der Waals ring interactions for each ring of MG and TMR molecules in all bases of the RNA aptamer averaged over the course of 10 ns dynamics. As shown, the total computed van der Waals ring interaction of the TMR molecules is more favorable than that of the MG molecules, by about 0.5 kcal/mol.

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Figure 1 – (A) Ensemble of the family of 25 lowest energy structures calculated for the MG binding aptamer. (B) Calculated electrostatic fields for the MG binding aptamer. (C) The optimized geometries of the MG molecule in the presence and absence of the RNA electrostatic field. (D) The optimized geometries of the MG molecule in the presence and absence of the RNA electrostatic field. (E) The optimized geometries of the MG molecule in the presence and absence of the RNA electrostatic field. (F) The optimized geometries of the MG molecule in the presence and absence of the RNA electrostatic field.

Figure 2 – (A) Molecular structure of malachite green with resonance structures. (B) Charge distribution of the central carbon has been summed to equal parts into the values for each ring. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand.

Table 1 – Summary of inter-base distances of RNA and RNA aptamer

<table>
<thead>
<tr>
<th>Base Pair</th>
<th>Distance (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-T</td>
<td>3.4</td>
</tr>
<tr>
<td>G-C</td>
<td>3.2</td>
</tr>
<tr>
<td>C-G</td>
<td>3.5</td>
</tr>
<tr>
<td>T-A</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Figure 3 – 2D 13C-NMR spectrum of the calculated RNA bound to the malachite green molecular structure of a 1:1 complex (A) and in the absence of the RNA (B). The 13C chemical shift of the ligand methyl groups are clearly observed in the bound ligand. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand.

Figure 4 – (A) Molecular structure of malachite green with resonance structures. (B) Charge distribution of the central carbon has been summed to equal parts into the values for each ring. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand. The calculated isotropic shielding constants for the methyl carbon atom attached to the nitrogen atoms of ring A and B in the bound ligand.

Figure 5 – (A) X-ray crystal structure of the malachite green RNA aptamer. (B) The optimized geometries of the MG molecule in the presence and absence of the RNA electrostatic field.

Figure 6 – Malachite green in the RNA aptamer binding site: (a) overall structure of MG-RNA aptamer complexes (b) expanded views of the ligand binding site.