Stabilizing Molecular Orbital Interactions in the Anticodon of Transfer RNA

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During protein synthesis, tRNA anticodons are matched to mRNA codons and the correct amino acid is incorporated into the growing protein chain.
Proteins are Essential for Function

- Enzymatic - catalyze chemical reactions
- Structural - support
- Transport - vehicle for particle movement
- Receptor - cell recognition and particle reception
RNA and Nucleotides

- Three types – ribosomal (rRNA), messenger (mRNA), and transfer (tRNA)
- All formed by a nucleotide chain

Four common bases in RNA: A, G, C, and U
tRNA

- Translates mRNA information from codon into specific amino acids
- Carries amino acids to ribosomes to incorporate into polypeptide chain
- 73-93 nucleotides long

Five regions:
- D loop
- TΨC-loop
- Acceptor stem
- Variable arm
- Anticodon

tRNA from *S. cerevisiae* or Baker’s Yeast
Anticodon-Codon Recognition

- Anticodon nucleotides at positions 34-36 of tRNA molecule
- Three anticodon nucleotides must be in a stair-stepped conformation to read mRNA codon nucleotides in the context of the ribosome.

Anticodon bases of tRNA (red) reading codon bases on mRNA (green). Note the stair-stepped base stacking of the tRNA anticodon.
Anticodon Stair-Stepped Conformation

- Free tRNA anticodon exhibits stair-stepped conformation
- Stabilizing forces in anticodon conformation are unclear
Role of Modified Bases in tRNA

- Naturally occurring modified bases found in tRNA are required for correct recognition of mRNA.

- Base modification at the 37th position is required for stair-stepped conformation in tRNA$^{\text{Lys,3}}$. 

Human tRNA$^{\text{Lys,3}}$

- methylthio-N6-threonylcarbamoyl adenosine (ms$^{2}$t$^{6}$A)
- 5-methoxycarbonylmethyl-2-thiouridine (mcm$^{5}$s$^{2}$U)
Goals

- To understand the stabilization of the tRNA anticodon through the molecular orbital interactions.
- To quantify the specific interactions of the stair-stepped conformation.
- To determine how modified bases at the 37th position contribute additional stability.
Strategy

- Examine molecular orbitals and the interactions between bases in x-ray crystal structures of anticodons
- Extend analysis to systems with modified bases at the 37th position

Anticodon (34-36)

34-36 with Modified Base at 37
Methods

- Structures obtained from published tRNA structures
  - Edited to bases 34-36 (10 structures) or 34-37 (6 structures)
  - Addition of hydrogen atoms to the tRNA molecules
  - Hydrogen atom positions geometrically optimized

- Arnott standard A’-RNA trimers CUC and CAA were constructed for comparison with crystal structures
Calculations

- Density Functional: M05-2X
  - Accurate with non-bonded interactions
  - Mean unsigned error of 0.78 kcal/mol when calculating the binding energies for amino acid residue pairs

- Basis set: 6-31+G(d,p)
  - ~1400 functions for the anticodon
  - Diffuse functions accommodate lone pairs and overall negative charge

- Program: Gaussian 03 Revision E.01
Natural Bond Orbital Analysis

- Natural bond orbital (NBO) analysis calculates the contributions of traditional atomic and hybridized orbitals to the MO picture.

- Perturbation theory energy analysis identifies donor-acceptor MO pairs that stabilize the molecule.

The highest occupied molecular orbital of water as predicted by NBO analysis.
Results

- Most calculations have completed for the trimer systems, but for the tetramer systems they are incomplete.
- Dimers of bases 34-37 are also being evaluated to expedite results on modified bases.
  - Dimers containing a modified base in position 34 had a greater number of significant interactions.
- Numerous significant MO interactions are common in the crystal structures, for example:
  - A lone pair on an oxygen in the phosphate group of residue 34 interacting with an antibonding C-H orbital on the nucleotide
  - A lone pair on the cyclic oxygen in the ribose of residue 36 interacting with an antibonding molecular orbital on the 2’ C-H
34th Phosphate Oxygen & C8-H/C6-H

10.9 kcal/mol, PDB ID 2TRA
36th 4’ Oxygen & 35th 2’C-H

3.34 kcal/mol, PDB ID 2UUB
Conclusions

- Significant interactions found between separate nucleotides and between nucleotides and the sugar-phosphate backbone.
  - Lone pairs of electrons on the numerous oxygen atoms are often the donating orbital.
  - Antibonding orbitals are often the accepting orbital.
- Without standard RNA interactions for comparison, it is impossible to determine which stabilizing interactions are unique to the stair-stepped conformation in the anticodon.
Future Direction

- Further analysis and compilation of NBO output
- Completion of standard RNA calculations
- Investigation of NWChem as a Gaussian alternative for tetramer calculations using GridChem
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