

Recognition of an Essential Adenine at a Protein–RNA Interface: Comparison of the Contributions of Hydrogen Bonds and a Stacking Interaction

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The energetic contribution of stacking interactions to the stabilization of protein–nucleic acid complexes is not well established.¹ Stacking interactions between proteins and nucleic acid helices are uncommon because the nucleic acid bases are already involved in this interaction in the helix.² However, bases in single-stranded regions of nucleic acids are able to stack with protein side chains without unfavorable conformational changes. Many RNA-binding proteins bind to single-stranded target sequences, and a number of structurally characterized RNA–protein complexes have displayed stacking interactions between RNA bases in single-stranded regions and amino acid side chains.³ We sought to compare the energetic contributions of stacking interactions and hydrogen bonds to the stability of the U1A–RNA complex by studying chemically modified systems. We show that (1) stacking interactions can rival hydrogen bonds in protein–RNA complexes and (2) the recognition of a single base by a combination of hydrogen bonds and stacking interactions is required for the stability of a high-affinity RNA–protein complex.

We have studied the RNA complex of a 101 amino acid peptide comprised of the N-terminal RNP domain of the protein U1A, called U1A101.⁴ The RNP domain is one of the best-characterized RNA-binding domains and binds single-stranded RNA in loops or linear sequences.⁵ In the RNP domain there are three highly conserved aromatic residues that are thought to be essential for RNA binding through stacking interactions. The N-terminal RNP domain of U1A contains two of these conserved aromatic amino acids, Phe56 and Tyr13, and both are observed to participate in stacking interactions with RNA bases in structures obtained by X-ray crystallography and NMR spectroscopy.^{3a,f}

Phe56 and Tyr13 participate in stacking interactions with A6 and C5 respectively of stem loop 2 of U1 snRNA (Figure 1). The hydroxyl group of Tyr13 hydrogen bonds to Gln54, while Phe56 does not participate in other interactions with the RNA or peptide.^{3a,f} Therefore, we have investigated the contribution of the stacking interaction between Phe56 and A6 to U1A101–RNA stability. To this end, we have mutated Phe56 to Ala, Leu, Tyr, Trp, and His.

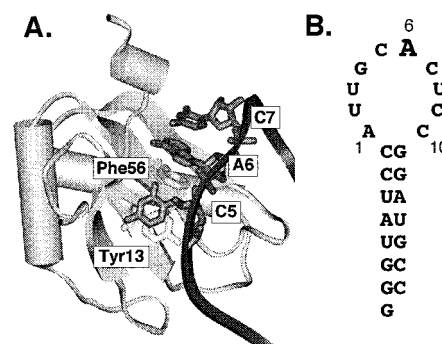


Figure 1. (A) Diagram of the complex formed between the N-terminal RNP domain of U1A and stem loop 2 from the X-ray cocrystal structure.^{3a} Only a portion of stem loop 2, in black, is shown. The stacking interactions between Tyr13 and C5 and between Phe56, A6, and C7 are displayed. (B) Stem loop 2 of U1 snRNA. The adenine that stacks with Phe56 is in bold.

Table 1. Binding Affinities of Mutant U1A101 Peptides

peptide	K_d (M)	ΔG (kcal/mol)
wild type	$5(\pm 2) \times 10^{-10}$	-12.7 ± 0.3
Phe56His	$1.6(\pm 0.3) \times 10^{-9}$	-12.0 ± 0.1
Phe56Trp	$1.4(\pm 0.4) \times 10^{-9}$	-12.1 ± 0.2
Phe56Tyr	$2.7(\pm 0.7) \times 10^{-8}$	-10.3 ± 0.2
Phe56Leu	$5(\pm 2) \times 10^{-7}$	-8.6 ± 0.3
Phe56Ala	$5(\pm 3) \times 10^{-6}$	-7.2 ± 0.3

To probe for potential structural changes in the mutants, we examined their secondary structure by CD spectroscopy. U1A101 and the mutant peptides displayed similar CD spectra, characteristic of peptides containing both α -helix and β -sheet structure.⁶ We measured the stability of the mutant peptides to guanidinium chloride denaturation by CD at 223 nm.⁷ The Trp and Tyr mutants are as stable to denaturation as U1A101 within the error of our experiments, $\Delta G = -8.9$ kcal/mol, while the remaining mutants are less stable: Leu, $\Delta G = -7.7$ kcal/mol; His, $\Delta G = -7.2$ kcal/mol; Ala, $\Delta G = -6.7$ kcal/mol. The error in the measurements is approximately 1 kcal/mol due to the long extrapolation back to zero guanidinium concentration. The order of conformational stability of the mutant peptides correlates with the β -sheet-forming propensities of the mutated amino acids,⁸ rather than the stabilities of the RNA–peptide complexes presented below. The conformational destabilization of the Ala and Leu mutants, 1–2 kcal/mol, is significantly less than the destabilization of their complexes with RNA. These results suggest that the low affinity of the Leu and Ala mutants for RNA is not due to a global change or destabilization of their conformations.

Peptide–RNA equilibrium dissociation constants were measured by gel mobility shift assays.⁹ Mutation of Phe56 to Ala results in a 5.5 kcal/mol loss in binding energy (Table 1). A portion of this energy, 1.4 kcal/mol, is regained by mutation to Leu, suggesting that aliphatic hydrophobic interactions can compensate, in part, for the loss of the aromatic ring. The destabilization of the Ala and Leu mutant–RNA complexes probably results from both the disruption of the stacking interaction and the disruption of other interactions that are cooperative with the stacking interaction. The Trp and His mutants bind with only 0.6 kcal/mol lower affinity to RNA than the wild type peptide, suggesting that the binding site is tolerant of significant

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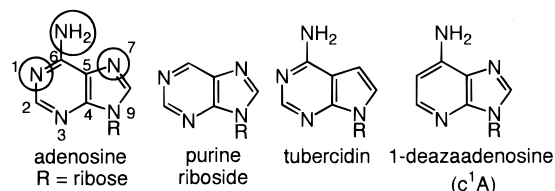


Figure 2. Modified bases used to probe the contribution of hydrogen bonds to U1A-RNA stability. The functional groups that hydrogen bond to U1A are circled in the adenine.

variation in the aromatic ring. In contrast, the RNA affinity of the Tyr mutant is substantially lower than the wild type peptide. Similar RNA affinities for the Tyr and Trp mutants have been reported recently by Kranz and Hall.¹⁰ They proposed that the RNA complex of the Tyr mutant may be destabilized by an alternative hydrogen bonding interaction formed by the Tyr hydroxyl group. The ability of the Trp and His mutants to bind RNA is surprising since Phe and Tyr are present in 70% of RNP proteins at this position, while Trp and His are rarely found.¹¹

We wished to probe whether the hydrogen bonds formed between A6 and U1A101 are as important as the stacking interaction between A6 and Phe56 for complex stability. In the structures of U1A-RNA complexes, N7, N1, and the 6-amino group of A6 participate in hydrogen bonds with the peptide.^{3a,f} We have eliminated each of these functional groups individually to give the modified adenosines, purine riboside, tubercidin, and 1-deazaadenosine,¹² shown in Figure 2. In addition to lacking the functional groups that participate in hydrogen bonds, these modified adenosines also vary in their ability to stack with C7 and Phe56. Since studies on stacking interactions have suggested that subtle modifications of base structure do not have a large impact on stacking energies,¹³ we expected that incorporation of these modified adenosines would primarily affect hydrogen bonding. All three hydrogen bonds formed between A6 and the peptide contribute to complex stability (Table 2). However, the removal of the aromatic amino acid side chain is significantly more destabilizing than any of these base modifications.

An analysis of whether the hydrogen bonding network involving A6 is still present in the Leu mutant-RNA complex would support our assertion based on CD data that the overall structure of the protein-RNA interface remains intact. We measured the ability of the Leu mutant to bind to the oligonucleotides containing

Table 2. Stability of Complexes of Wild Type U1A101 with RNA Target Sequences Containing A6 Modifications

RNA	K_d (M)	ΔG (kcal/mol)
wild type	$5(\pm 2) \times 10^{-10}$	-12.7 ± 0.3
A6tubercidin	$4(\pm 2) \times 10^{-9}$	-11.6 ± 0.3
A6c ¹ A	$3(\pm 2) \times 10^{-8}$	-10.4 ± 0.4
A6purine	$4(\pm 1) \times 10^{-8}$	-10.2 ± 0.2

Table 3. Stability of Complexes of Phe56Leu with RNA Target Sequences Containing A6 Modifications

RNA	K_d (M)	ΔG (kcal/mol)
wild type	$5(\pm 2) \times 10^{-7}$	-8.6 ± 0.3
A6tubercidin	$3(\pm 1) \times 10^{-6}$	-7.6 ± 0.3
A6c ¹ A	$6(\pm 1) \times 10^{-6}$	-7.2 ± 0.1
A6purine	$8(\pm 5) \times 10^{-6}$	-7.0 ± 0.3

purine riboside, tubercidin, and 1-deazaadenosine (Table 3). The destabilization of the Leu mutant-RNA complex upon elimination of each hydrogen bond indicates that these interactions do exist in this complex, implying that there has not been a large structural reorganization of the protein-RNA complex at this base, even though the Leu mutant binds RNA with dramatically reduced affinity. The Ala mutant also binds with reduced affinity to the modified oligonucleotides, but the association is too weak to measure accurate binding constants.

Our data demonstrate that replacing one aromatic functional group of a protein with hydrogen can have an energetic effect on an RNA-protein complex stability of 5.5 kcal/mol. In RNA-protein complexes studied previously, interactions between aromatic amino acids and nucleobases were estimated to have a much smaller value, 1 kcal/mol.¹⁴ It is unclear which stacking energy will be more common, but our work suggests that stacking interactions in cooperation with accompanying hydrogen bonds can be major contributors to RNA-protein complex stability. Thus, one should be able to alter biological function by targeting a single aromatic region of an RNA-protein interface.

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Supporting Information Available: Description of peptide and RNA syntheses and peptide-RNA binding reactions, a representative gel shift assay, and plots of the equilibrium dissociation of the RNA-peptide complexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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