Peptide-triggered conformational switch in HIV-1 RRE RNA complexes

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We have used NMR spectroscopy to determine the solution structure of a complex between an oligonucleotide derived from stem IIB of the Rev responsive element (RRE-IIB) of HIV-1 mRNA and an *in vivo* selected, high affinity binding Arg-rich peptide. The peptide binds in a partially α-helical conformation into a pocket within the RNA deep groove. Comparison with the structure of a complex between an α-helical Rev peptide and RRE-IIB reveals that the sequence of the bound peptide determines the local conformation of the RRE peptide binding site. A conformational switch of an unpaired uridine base was revealed; this points out into the solvent in the Rev peptide complex, but it is stabilized inside the RNA deep groove by stacking with an Arg side chain in the selected peptide complex. The conformational switch has been visualized by NMR chemical shift mapping of the uridine H5/H6 atoms during a competition experiment in which Rev peptide was displaced from RRE-IIB by the higher affinity binding selected peptide.

In RNA–protein complexes, intermolecular contacts between complementary surfaces of the components provide accurate molecular recognition. In most protein–RNA complexes investigated so far, preformed binding pockets and surfaces exist in the protein fold and the RNA components adapt to fit in these sites[1]. In peptide–RNA complexes, by contrast, conformational adaptation affects predominantly the peptide components[2–4]. Upon binding to RNA, the peptides, which are less structured when free in solution[3,5], assume ordered minimal elements of protein secondary structure[2,3].

Different RNA binding pockets may dictate distinct conformations of the same peptide. An Arg-rich peptide constituting the minimal RNA-binding domain of the HIV-1 Rev protein[5] forms an α-helix in complexes with its natural target, the HIV-1 Rev response element (RRE) RNA[6] and with an RRE-like RNA aptamer[7], while it adopts an extended conformation when bound to a second RNA aptamer[8]. Here, we have addressed the reverse question, namely, how do different peptides recognize the same RNA target?

We have determined the solution structure (Fig. 1) of an oligonucleotide (RRE-IIB) representing the stem IIB Rev-binding site of HIV-1 RRE in complex with an Arg-rich peptide (RSG-1.2) that had been evolved by selection against the RRE target and subsequent mutation[9,10]. RSG-1.2 binds the RRE with seven-fold higher affinity and 15-fold higher specificity than the Rev peptide[10]. In competition with Rev, the RSG-1.2 peptide completely displaces the intact protein from the RRE at low peptide concentrations[10]. Comparison of the three-dimensional structures of RRE-IIB in its complexes with RSG-1.2 and the Rev peptide[6] sheds light on the differences in binding affinity, specificity and conformational adaptation.

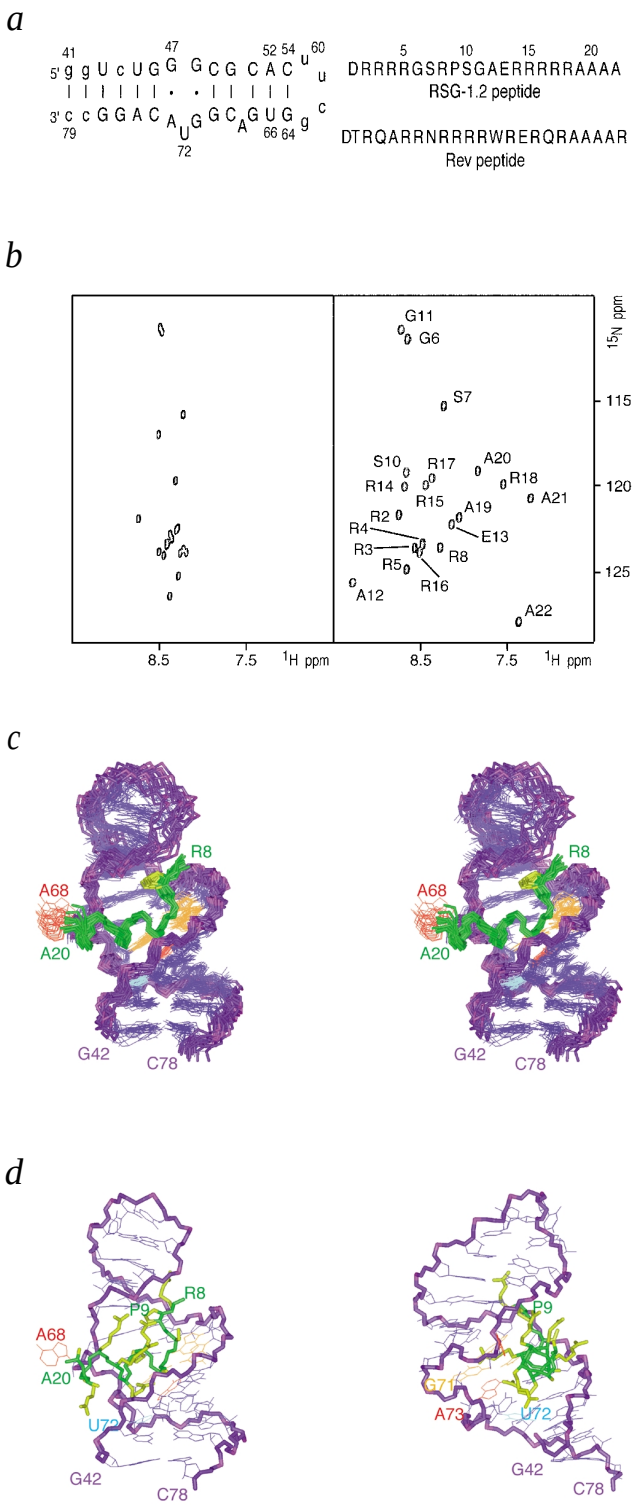

Fig. 1 The RSG-1.2–RRE-IIB complex. ***a***, Sequences of the RRE-IIB oligonucleotide representing the stem IIB Rev-binding site of HIV-1 RRE (non-wild type nucleotides are indicated in lowercase) and the evolved RSG-1.2 peptide[10] used in our structure analysis and the Rev peptide[6]. ***b***, ^{1}H-^{15}N HSQC spectra of the RSG-1.2 peptide free in solution (left) and in complex with the RRE-IIB RNA (right). ***c***, Stereo view of a superposition of 14 distance-refined structures and ***d***, two views of one representative structure of the RSG-1.2–RRE-IIB RNA complex. The superposition was performed on all heavy atoms of the well-defined core comprising the peptide backbone from Pro 9–Ala 21 and RNA residues U43–A52 and U66–G77.

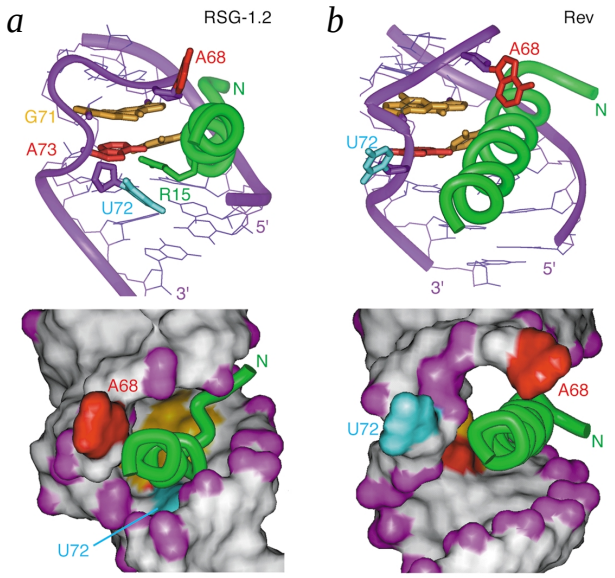

Fig. 2 The peptide binding sites in the RRE-IIB complexes. ***a***, The RSG-1.2 peptide (this study). ***b***, The Rev peptide[6]. The top views are aligned on the noncanonical G47-A73 and G48-G71 base pairs and in similar orientations. The bottom views, oriented with the peptide α-helices perpendicular to the image plane, emphasize the differences between the deeper binding pocket of the RSG-1.2 peptide and the groove binding mode of the Rev peptide. In the RSG-1.2–RRE-IIB complex, the unpaired U72 is stabilized in the RNA deep groove by stacking with the peptide Arg 15 side chain. U72 is flipped-out into the solvent in the Rev–RRE-IIB complex. In both complexes, the backbone of the 3′ strand in the RNA duplex adopts an S-turn conformation.

Characterization of the complex

In other structural studies, the stem IIB Rev-binding site of HIV-1 RRE has been modified to a variety of different oligonucleotide constructs[6,11–13]. The RRE-IIB sequence used in this work was identical to the RRE-IIB-TR oligonucleotide of the Rev peptide–RRE complex[6,11], except for substitution of the artificial GCAA tetraloop to UUCG and deletion of a G-C pair (G53-C65) adjacent to the loop that is not involved in peptide contacts[11]. Indeed, the modifications in the RRE-IIB oligonucleotide did not interfere with peptide binding as shown by the identical intermolecular NOE patterns of complexes between the RSG-1.2 peptide bound to RRE-IIB either with or without the G53-C65 base pair (data not shown).

Comparison of the fingerprint ^{1}H-^{15}N HSQC spectra of the free and RNA bound RSG-1.2 peptide (Fig. 1*b*) revealed a transition from the predominantly disordered free conformation, consistent with circular dichroism (CD) data[10], to extensive secondary structure formation upon binding to the RNA, indicated by significant peak dispersion in the proton dimension. Further analysis of peptide NMR spectra revealed that, in the complex, the six N-terminal peptide residues were disordered whereas amino acids 10–20 adopted an α-helical conformation as shown by their Cα, CO and Hα chemical shifts[14], three bond coupling constants ($^{3}J_{HNH\alpha}$ < 4.8 Hz for residues 10–20; ref. 15), characteristic interresidue NOEs between adjacent residues, and ^{1}H-^{15}N steady-state NOE values[16] (0.71–0.82 for residues 10–20, 0.02–0.53 for residues 2–7). The observation of a disordered peptide N-terminus is in accord with the finding that up to four N-terminal residues could be removed without decreasing the RNA binding affinity of RSG-1.2 (ref. 10).

In the bound RRE-IIB RNA, base pairings for all Watson-Crick pairs along with the noncanonical G47-A73 base pair were established by direct observation of NH···H hydrogen bonds in HNN-COSY experiments[17]. The observation of strong imino-imino NOEs identified the pairing alignments of the *cis* wobble G77-U43 and *trans* Watson-Crick G48-G71 base pairs.

Architecture of the complex

In the RSG-1.2 peptide–RRE-IIB RNA complex (Fig. 1*c*,*d*), the oligonucleotide forms a continuously stacked duplex capped by a standard UUCG loop[18]. The peptide binds in partially α-helical conformation in a pocket associated with the widened deep groove of the RNA. Large parts of the RSG-1.2 peptide binding site in RRE-IIB and the region responsible for Rev peptide recognition[6] are similar, including the hydrogen bonding arrangement in the noncanonical base pairs and an S-shaped distortion of the RNA backbone at residues G70–A73 induced by the *trans* G-G pair (Fig. 2). The binding pocket is further shaped by cross-strand stacking of G50 above G70, leaving C69 without a stacking partner base. The undertwisting of base pairs in the internal loop induced by the S-turn leads to an opening of the deep

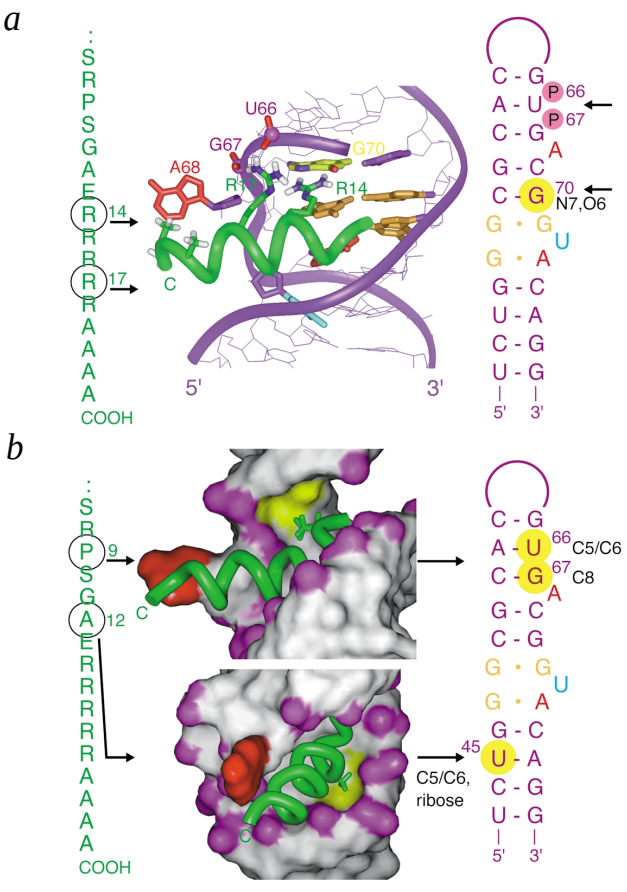

Fig. 3 Intermolecular contacts in the RSG-1.2–RRE-IIB complex. ***a***, The Arg 14 side chain approaches the Hoogsteen edge of G70 and the Arg 17 guanidinium group is braced between the two phosphate groups of U66 and G67. The guanidinium group of Arg 14 could form hydrogen bonds with both N7 and O6 of G70. The bulged-out A68 packs against the Ala-rich peptide C-terminus. ***b***, The ring of the Pro 9 side chain points at a nonpolar surface patch in the RNA produced by the C5-C6 edge of U66 and C8 of G67 (top). The methyl group of Ala 12 is directed towards a shallow hydrophobic pocket comprising the C5-C6 edge and ribose moiety of U45 in the RNA deep groove (bottom).

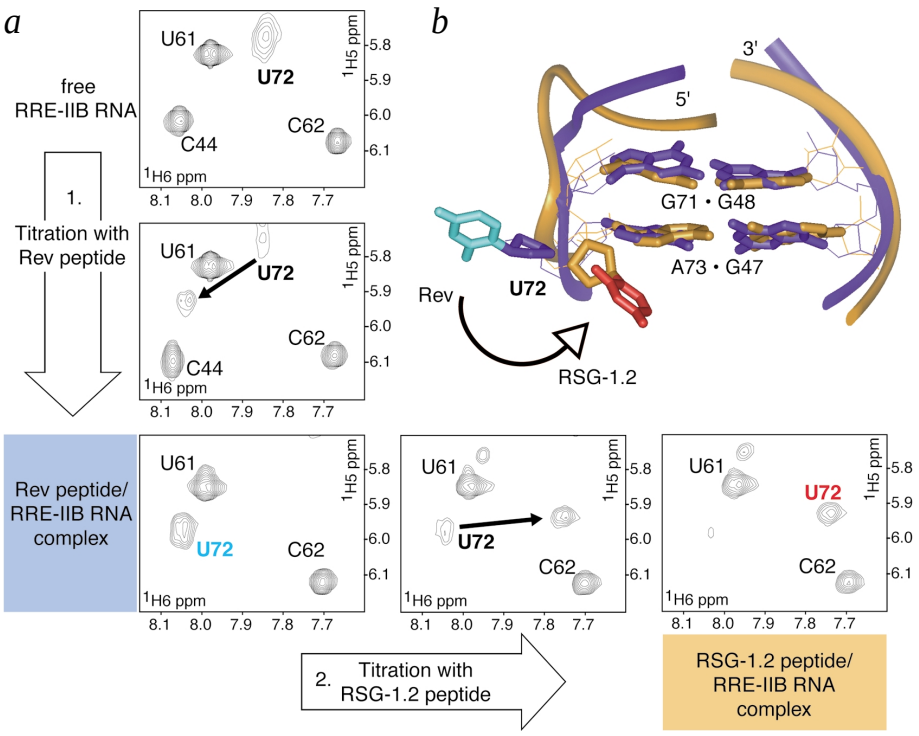

Fig. 4 Conformational transitions of U72. ***a***, Chemical shifts of pyrimidine H5/H6 proton crosspeaks were mapped in TOCSY NMR spectra during complex formation between the free RRE-IIB and Rev peptide (1. titration, vertical) and displacement of this peptide by the higher affinity binding RSG-1.2 peptide (2. titration, horizontal). The expanded TOCSY spectra show unchanged crosspeaks for the U61 and C62 tetraloop residues along with the shifting U72 crosspeak (in slow exchange), indicating that this nucleotide undergoes a conformational switch. ***b***, The U72 conformational switch is accommodated by localized changes in the RNA backbone without major disturbances in the flanking noncanonical base pairs.

groove at the peptide binding site lined by nucleotides between the A75-U45 and A52-U66 base pairs. The bulged A68 and U72 residues are extruded from the stacked duplex and participate in contacts with the peptide (Fig. 2*a*).

Whereas in the Rev peptide–RRE-IIB complex the α-helical peptide is inserted along the RNA deep groove, parallel to the sugar phosphate backbone[6] (Fig. 2*b*), the helical part of the RSG-1.2 peptide penetrates into a deep groove pocket, almost perpendicular to the helix axis of the RNA duplex (Fig. 2*a*). These distinct orientations of the Rev and RSG-1.2 peptides are likely to account for the different binding affinities and specificities of the two peptides, despite the fact that similar regions in the RNA are involved in the recognition of both peptides. The alignment of the RSG-1.2 peptide in the binding pocket allows an intimate contact between amino acids in the α-helical segment and base edges in the RNA deep groove, as shown by a large number of intermolecular NOEs.

Role of arginines in peptide recognition in the complex

Specific intermolecular contacts involving amino acids in the region between Pro 9 and Arg 18 anchor the RSG-1.2 peptide within the RNA pocket. Arg 14 approaches the Hoogsteen edge of G70 in an orientation that suggests that there are hydrogen bonds between the Arg guanidinium group and both N7 and O6 of the base (Fig. 3*a*). The C69 base stacks over the Arg 14 side chain that, in an 'arginine fork' alignment[19], could form a hydrogen bond with the C69 phosphate group. The recognition of a guanidinium group by simultaneous stacking and hydrogen bonding is a common mechanism in the ligand binding pockets of other natural RNA complexes[2,20] and aptamers[4].

A second hydrogen bonding and stacking motif in the RSG-1.2–RRE-IIB complex involves the side chain of Arg 15, which stacks on top of the bulged U72 base (Fig. 2*a*) and faces the Hoogsteen edge of A73, allowing hydrogen bonding between Arg 15 and N7 of A73. The A73 residue participates in the noncanonical G47-A73 base pair, which also plays a key role in peptide recognition in the Rev peptide–RRE-IIB complex, albeit as a docking site for an Asn side chain[6].

Three other Arg residues (Arg 16, Arg 17 and Arg 18) adopt conformations that suggest they make contacts with phosphate groups of the RNA backbone. Whereas the side chain orientations of Arg 16 and Arg 18 are not well-defined by NMR restraints, the guanidinium group of Arg 17 is consistently found interacting in a bridging fashion between the phosphate groups of U66 and G67 (Fig. 3*a*), which resembles an arginine fork alignment[19].

Role of hydrophobic contacts in peptide recognition

Polar groups dominate in RNA and hydrophobic pockets and surface patches are thus rare in RNA folds, rendering them highly specific recognition sites for surface-complementary contacts with ligand nonpolar groups[21]. In the RSG-1.2–RRE-IIB complex, a distinct hydrophobic interaction, well-defined by strong NOEs, is formed between Ala 12 and a nonpolar surface region within the deep groove of the lower stem RNA duplex (Fig. 3*b*). The Ala 12 methyl group rests on a hydrophobic surface patch formed by the C2′/C3′ edge of the U45 ribose along with the C5/C6 edge of the U45 base and the C8 proton of G46. Hydrophobic interactions involving the bulged-out A68 base, which packs against the nonpolar Ala-rich C-terminus of the RSG-1.2 peptide (Fig. 3*a*), explain earlier observations that deletion of the C-terminal Ala residues or their replacement with Gly in the RSG-1.2 peptide result in loss of binding affinity[10]. In addition to nonpolar interactions involving Ala residues, Pro 9 participates in hydrophobic contacts with the RNA. The Cγ/Cδ edge of the alicyclic Pro side chain is oriented towards a nonpolar region of the RNA comprising the C5/C6 edge of U66 along with C8 and C3′ of G67 (Fig. 3*b*).

The specific hydrophobic contacts between residues in the compact Pro 9–Ala 12 segment of RSG-1.2 and the RRE-IIB RNA explain the distinct binding mode of the selected peptide compared to the Rev peptide, both of which contain an Ala-rich C-terminus. Modeling of the Rev peptide sequence onto the

Table 1 Structural statistics for the RSG-1.2–RRE-IIB complex

NMR restraints in complex	
RRE-IIB RNA (G41–C79)	
Distance restraints	530
Torsion restraints (six per ribose for 29 sugars)	174
Hydrogen bond restraints	30
RSG-1.2 peptide (Arg 5–Ala 22)	
Distance restraints	330
Torsion restraints (ϕ angles)	14
Intermolecular distance restraints (G41–C79, Arg 5–Ala 22)	106
Structure statistics (14 conformers)	
NOE violations	
Number > 0.2 Å	6.4 ± 1.9
Maximum violations (Å)	0.31 ± 0.08
Deviations from ideal covalent geometry	
Bond lengths (Å)	0.013 ± 0.0003
Bond angles (°)	2.68 ± 0.09
Impropers (°)	2.03 ± 0.30
Pairwise r.m.s. deviations (Å) among the 14 refined structures	
All heavy atoms (G41–C79, Arg 5–Ala 22, side chains and backbone)	1.49 ± 0.38
Complex core (U43–A52, U66–G77, Pro 9–Ala 21 backbone)	1.14 ± 0.23

structure of RSG-1.2 bound to RRE-IIB would place the polar stretch of Arg 8–Arg 11 (Fig. 1*a*) at the N-terminus of the RSG-1.2 α-helix, abolishing all specific hydrophobic interactions with the RNA and leading to steric clashes involving the bulky Arg side chains within the deep peptide binding pocket.

Conformational switch in the RRE-IIB RNA

In the RSG-1.2–RRE-IIB complex, the U72 base is flipped inside the deep groove of the RNA duplex and appears to be predominantly stabilized by stacking interactions with Arg 15 (Fig. 2*a*). By contrast, in RRE-IIB bound to a Rev peptide, the unpaired U72 base is directed away from the RNA duplex and pointing into the solvent[6] (Fig. 2*b*). The conformational switch of U72 upon RSG-1.2 binding is accommodated by relatively minor changes in the RNA backbone (Fig. 4).

Since the identity of the base at position 72 does not affect Rev binding[22–24], it has been suggested that the looped-out U72 nucleotide acts as a flexible spacer involved in proper orientation of the flanking noncanonical base pairs in the Rev binding site[12]. Both NMR data[12] and a crystal structure[13] of free RRE-IIB suggest that U72 is the most mobile residue within a segment that might fluctuate between alternate conformations in the free RNA[12]. Comparison of the solution structures of RRE-IIB in complex with Rev peptide[6] and RSG-1.2 demonstrates that bound peptide locks U72 in one defined conformation that is determined by the peptide sequence. The conformational transitions of U72 induced by complex formation were followed by NMR chemical shift mapping of the pyrimidine H5/H6 atoms (Fig. 4). After a complex had been formed between RRE-IIB RNA and Rev peptide, RSG-1.2 peptide was added. In line with the finding that the RSG-1.2 peptide is able to completely displace intact Rev protein from the RRE[10], we observed displacement of the Rev peptide and formation of the RSG-1.2–RRE-IIB complex.

Whereas mutational data on the role of Arg 15 in the RSG-1.2 peptide is lacking, the solution structure of the RSG-1.2–RRE-IIB complex suggests that the side chain of this residue plays a major role in stabilizing the U72 base inside the RNA deep groove *via* stacking interactions. This intermolecular contact could contribute to the increased binding specificity of the RSG-1.2 peptide[10] compared to the Rev peptide, since RSG-1.2 employs an interaction with a nucleotide not involved in specific contacts in the Rev peptide–RRE-IIB complex.

Implications for targeting RNA folds with ligands

The solution structures of the RRE–peptide complexes represent a striking example of ligands determining the local conformation of an RNA binding site. Global features of the ligand binding site in RRE are conserved between the Rev peptide–RRE-IIB and RSG-1.2–RRE-IIB complexes. Differences in the interactions of the Rev and RSG-1.2 peptides with RRE-IIB include distinct alignments of the α-helical segments, which induce local conformational adaptation of the RNA. Remarkable is the conformational switch of the U72 base, which is mobile in free RRE-IIB but adopts defined conformations in the complexes that are determined by the sequence of the bound peptide.

These findings outline two principles that might constitute general strategies for targeting RNA structures with peptide and small molecule ligands, which would be especially important for exploiting RNA as a drug target[21]. First, recruitment of residues into the RNA target as interaction sites not used by the natural protein ligands might enhance the binding affinity of synthetic ligands. Second, the conformational locking of intrinsically flexible segments of an RNA fold by the bound ligand might both contribute additional binding specificity and provide a mechanism for interfering with the biological function of the RNA target.

Methods

Sample preparation. Unlabeled and uniformly $^{13}C/^{15}N$-labeled RNA or peptide samples were obtained by standard procedures as described[25]. All NMR samples were in buffer (pH 6.0) containing 10 mM sodium phosphate, 12.5 mM sodium acetate-d_4, 0.1 mM EDTA and 25 mM NaCl.

NMR spectroscopy. NMR spectra were recorded on Varian Inova 600 MHz spectrometers at 25 °C and 10 °C. Data were processed with NMRPipe[26] and analyzed with NMRView[27].

RNA base pair alignments were identified by HNN-COSY experiments[17]. Three-dimensional (3D) HCCH-COSY, HCCH-TOCSY[28] and HCCH-COSY-TOCSY[29] experiments were used to correlate ribose and pyrimidine H5-H6 spin systems. A combination of H8-(N3,N9) COSY and H1-(N3,N9) COSY experiments unambiguously correlated guanine H8 and imino protons. Uracil H5 and H3 protons were correlated through U-selective H5-(N3) COSY and H3-(N3) HSQC spectra. Cytidine amino H4 protons were correlated to the H5 proton through H5-(N4) COSY and H4-(N4) HSQC spectra. Through-bond connectivities between the aromatic protons and sugar H1′ protons were established by H1′ (N9)-H8 COSY spectra for G and A residues, H1′ (N1)-H6 COSY spectra for U and C residues[30], along with a set of pseudo-3D H1′C1′ (N9)-H8C8 and H1′C1′ (N1)-H6C6 COSY experiments, which used both N9/N1-editing and C1′ chemical shift dispersion.

Sequential and side chain assignments of the bound peptide were established using a set of standard triple resonance experiments[31]. All intermolecular NOEs were assigned using 3D ^{13}C-edited and ^{13}C-purged NOESY[32] experiments. Peak intensities were taken from ^{13}C-edited or ^{15}N-edited NOESY spectra and subjected to the same calibration criteria as intramolecular NOEs.

Restraint derivation. Peptide ϕ torsion angle restraints were extracted from the 3D HNHA spectrum[15]. Base pair alignments identified through direct observation of NH···H hydrogen bonds in HNN-COSY experiments were restrained by hydrogen bonds. RNA sugar puckers were restrained according to values of the $^{3}J_{H1'-H2'}$ coupling constants qualitatively estimated from the ^{1}H-^{1}H COSY and TOCSY spectra recorded at short mixing times. Nucleotides G47, U61, C62, G71 and U72 were restrained to C2′ *endo* sugar pucker based on their strong H1′-H2′ COSY peaks. Interproton distance restraints were calculated from peak intensities of ^{13}C-edited or ^{15}N-edited NOESY spectra at various mixing times and scaled using appropriate reference distances.

Structure calculations. The AMBER 4.1 package[33] was used for structure calculations starting from the peptide and RNA both in extended conformations placed 100 Å apart. Ninety folded structures were generated during 20 ps of molecular dynamics (MD) at 7000 K followed by a 25 ps cooling phase. Force constants for the covalent geometry, nonbonded terms and NMR restraints were scaled from 1% to full value over the initial 20 ps. Electrostatic interactions were switched off. Then the structures were subjected to MD simulation at 900 K for 10 ps with full interactions and NMR restraints along with electrostatic interactions gradually scaled from 10–50%. A cooling phase of 20 ps followed during which electrostatic interactions were scaled to full value, except for formally charged groups, which were kept at 50% of their regular charges. Fourteen final structures were chosen based on low energy values, low restraint violations and covalent geometry (Table 1).

Coordinates. The coordinates of the complex have been deposited in the Protein Data Bank (accession code 1G70).

Acknowledgments

This research was supported by a NIH grant to D.J.P. X.Ye was involved in the early stages of this project and S. Park provided technical assistance in the preparation of the labeled peptide.

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Received 18 August, 2000; accepted 3 November, 2000.

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