Biochemistry

 Volume 33, Number 10 March 15, 1994

Accelerated Publications

Binding of an HIV Rev Peptide to Rev Responsive Element RNA Induces Formation of Purine–Purine Base Pairs†

John L. Battiste,‡ Ruoying Tan,§ Alan D. Frankel,§ and James R. Williamson*,‡

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, and Department of Biochemistry and Biophysics and Gladstone Institute of Virology and Immunology, University of California, San Francisco, San Francisco, California 94143

Received November 1, 1993; Revised Manuscript Received January 18, 1994®

ABSTRACT: The Rev responsive element (RRE) is an RNA secondary structural element within the *env* gene of HIV and is the binding site for the viral Rev protein. Formation of the Rev–RRE complex is involved in regulation of splicing and transport of mRNA from the nucleus. To understand the structural basis for the specific recognition of RRE by Rev, we have studied a model system for this interaction using NMR. We have obtained a specific 1:1 complex between an RNA derived from stem IIB of RRE, which contains the highest affinity Rev binding site, and a modified Rev_{34-50} peptide, which binds the RRE as an α-helix [Tan, R., et al. (1993) *Cell 73*, 1031–1040]. Binding of the peptide was accompanied by a conformational change in the RNA, which resulted in the formation of additional base pairs not present in the free RNA. Two of these induced base pairs are purine–purine pairs within the internal loop of RRE, which had been previously proposed on the basis of biochemical experiments [Bartel, D. P., et al. (1991) *Cell 67*, 529–536]. The formation of non-Watson–Crick base pairs, interactions in the major groove, and protein-induced conformational changes may prove to be common characteristics of RNA recognition of proteins.

Phylogenetic studies have shown that conserved regions of biologically important RNAs often reside in loops, bulges, and internal loops (Gutell et al., 1985). Structural studies on a variety of RNAs have revealed that these regions are often stabilized by non-Watson–Crick (mismatch) base pairs. Some examples include the GNRA and UUCG tetraloops prevalent in ribosomal RNA, both of which include base pairs between the first and last nucleotides of the loop (Cheong et al., 1990; Heus & Pardi, 1991). The sarcin/ricin and E loops of ribosomal RNA were also shown by NMR to have extensive mismatch base pairing (Szewczak et al., 1993; Wimberly et al., 1993). In addition to providing a role in RNA structure, non-Watson–Crick base pairs may also be important for RNA–protein recognition. Biochemical and genetic identification of protein binding sites in RNAs has shown that they often include important non-Watson–Crick regions (Cook et al., 1991; Gutell et al., 1985; Roy et al., 1990).

Another prevalent feature of RNA–protein interactions seems to be the conformational flexibility of the RNA. For example, in HIV TAR,[1] the essential base triple is not present in the free RNA but only forms as TAR changes its conformation upon binding of arginine or arginine-containing peptides (Puglisi et al., 1992). Comparison of the crystal structures of $tRNA^{Gln}$ free and bound to its cognate synthetase

† This work was supported by Grant GM-39589 to A.D.F. and J.R.W. from the National Institutes of Health and by a grant from the Searle Scholars Program of the Chicago Community Trust to J.R.W.

* To whom correspondence should be addressed.
‡ Massachusetts Institute of Technology.
§ University of California, San Francisco.

® Abstract published in *Advance ACS Abstracts*, February 15, 1994.

[1] Abbreviations: RRE, Rev responsive element; NMR, nuclear magnetic resonance; RNA, ribonucleic acid; HIV, human immunodeficiency virus; EDTA, ethylenediaminetetraacetic acid; TAR, transactivating region; NTP, nucleoside triphosphate; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; DQF-COSY, double-quantum-filtered correlation spectroscopy; TOCSY, total correlation spectroscopy; HSQC, heteronuclear single-quantum coherence.

0006-2960/94/0433-2741$04.50/0

also reveals conformational differences in the tRNA. Upon binding of the synthetase, the U_1-A_{72} base pair closing the acceptor stem is broken, and the acceptor arm folds back to form an hydrogen bond between the G_{73} amino group and the phosphate of A_{72} (Rould et al., 1989).

To extend our knowledge of how proteins bind to specific RNA sites, we have studied an RNA–peptide interaction which is a model of the HIV RRE–Rev interaction. Rev is essential for viral replication and functions by binding to a complex ~250-nt structure in the *env* gene of the mRNA called the Rev responsive element (RRE). Binding of Rev to RRE inhibits mRNA splicing and/or facilitates mRNA transport and is necessary for expression of the structural genes *gag*, *pol*, and *env*, as well as for production of genomic RNA (Cullen & Malim, 1991). As many as eight Rev protein molecules are thought to bind to the entire RRE; however, mutational studies have identified a high-affinity binding site localized to a relatively small stem–loop structure (Cook et al., 1991; Kjems et al., 1991a; Tiley et al., 1992). Rev, as well as peptides from the arginine-rich RNA binding domain of Rev, specifically binds to an RNA derived from this stem–loop (RRE IIB) (Kjems et al., 1992). Further studies have identified mismatch base pairs in the internal loop region of RRE which are critical for the interaction with Rev (Bartel et al., 1991; Iwai et al., 1992).

We have used NMR to study the RNA structure in this simplified model of the RRE–Rev interaction. Upon binding of the Rev peptide, the RRE RNA undergoes a conformational change which results in formation of two purine–purine base pairs in an internal loop. The structures of these base pairs are consistent with previous mutational and biochemical studies of the RRE–Rev interaction which suggest major-groove interactions. The results further illustrate the importance of non-Watson–Crick base pairs and conformational flexibility in RNA–protein recognition.

MATERIALS AND METHODS

RNA Synthesis. All RNAs were prepared by transcription from synthetic DNA templates using T7 RNA polymerase (Wyatt et al., 1991). Milligram quantities of T7 RNA polymerase were overexpressed and purified as described (Grodberg & Dunn, 1988). The RNA was purified on preparative (45 × 35 × 0.3 cm) 20% (19:1) polyacrylamide gels containing 8 M urea. The product band was cut from the gel, recovered by electroelution in an Elutrap (Schleicher & Schuell), and ethanol precipitated. The RNA was resuspended in 250 μL of H_2O and dialyzed overnight in a microdialysis chamber (Bethesda Research Laboratories) against 500 mL of NMR buffer (10 mM sodium phosphate, pH 6.5, 50 mM sodium chloride, and 0.1 mM EDTA). An 80-mL transcription reaction yielded 0.5 μmol of purified RNA.

Uniformly ^{13}C-enriched RNA was synthesized using ^{13}C-labeled NTPs, isolated from *Methylophilus methylotrophus* bacteria grown on 99.9% [^{13}C]methanol as previously described (Batey et al., 1992). ^{13}C-Labeled RNA was transcribed and purified as described above. A 50-mL transcription reaction yielded 0.25 μmol of purified ^{13}C RNA.

Peptide Binding Assay. The suc-Rev_{34-50}AAAAR-am peptide (TRQARRNRRRRWRERQRAAAAR) used in this study was synthesized and purified as described (Tan et al., 1993). To measure binding specificity for the modified RRE IIB RNA, increasing amounts of peptide were added to ^{32}P-labeled RNAs (wild type or modified) under NMR binding conditions. The free and bound RNAs were resolved by native gel electrophoresis, and K_d's were determined as described (Tan et al., 1993).

Proton NMR. All NMR spectra were recorded on a Varian VXR 500-MHz instrument, and data were transferred to a Silicon Graphics Personal Iris for processing using FELIX software (Biosym, Inc.). Purified RREIIB-TR was placed in a final volume of 500 μL of NMR buffer in 10%/90% D_2O/H_2O (final concentration 1 mM). 1D spectra in H_2O were recorded using a 1331 binomial solvent suppression pulse sequence (Hore, 1983) with a sweep width of 12000 Hz, 8K complex data points, and 64 scans. The excitation maximum was set to the imino proton region of the spectrum (12–14 ppm). 1D NOE difference spectroscopy was performed at 25 °C with 512 scans per imino resonance taken in an interleaved fashion. Each imino resonance was irradiated for 0.6 s at the lowest power required to achieve 90% saturation.

NOESY, DQF-COSY, and TOCSY experiments were recorded in 99.996% D_2O for RREIIB-TR (Varani & Tinoco, 1991). Data sets with 2048 complex points in t_2 and 512 complex points in t_1 were acquired with 5000-Hz sweep widths in both dimensions and 32 scans per slice. Two NOESY spectra were acquired with mixing times of 50 and 400 ms and a recycle delay of 1.2 s. The TOCSY spectrum was recorded with a 125-ms MLEV spin lock pulse and a recycle delay of 1.8 s. The DQF-COSY was recorded with WALTZ decoupling of ^{31}P during acquisition and a recycle delay of 1.9 s. All spectra were processed with a combination of exponential and Gaussian weighting functions and zero-filled to 4K × 4K data points.

A 1:1 complex of RREIIB-TR and suc-Rev_{34-50}AAAAR-am peptide was formed by adding 25 μL of 20 mM peptide solution in 5-μL increments into the RNA NMR sample. Complex formation was monitored by 1D NMR at 25 °C. 1D NOE difference spectroscopy on the complex was performed as described above with 1536 scans per imino resonance.

NOESY experiments in 10%/90% D_2O/H_2O were acquired using a Z-Spec MID500–3 indirect detection 3-mm probe. The RNA–peptide sample was dried, resuspended in 100 μL of H_2O, redialyzed against 500 mL of NMR buffer, and diluted to final volume of 175 μL with NMR buffer. A NOESY experiment was acquired at 25 °C using a jump–return echo pulse sequence for water suppression with the excitation maximum set to the imino resonances (Sklenar et al., 1987). The spectrum was 2048 × 256 complex data points with a sweep width of 12000 Hz, a mixing time of 250 ms, a recycle delay of 1.7 s, and 128 scans per slice. Homospoil pulses of 10 ms were applied during the recycle delay and mixing time. Spectra were processed with either exponential weighting functions or a skewed, shifted sine-bell function to resolve overlapped imino protons. Nonexchangeable 2D NMR spectra of the complex in 99.996% D_2O were taken with a 5-mm probe as described above for the free RNA.

Heteronuclear NMR. A 1:1 complex of ^{13}C-labeled RREIIB-TR and Rev peptide was placed in a final volume of 175 μL of NMR buffer in 99.996% D_2O (final concentration 1.5 mM). Using the Z-Spec 3-mm indirect detection probe, a constant time heteronuclear single-quantum coherence (HSQC) spectrum was acquired with the carbon transmitter frequency set to the aromatic region of the RNA (Santoro & King, 1992). The one- and two-bond heteronuclear coupling constants used in the experiment were J_{CH} = 180 Hz and J_{CC} = 60 Hz, respectively. The data set was 1024 × 256 complex data points with sweep widths of 5500 and 10000 Hz in the proton and carbon dimensions, respectively. A total of 64

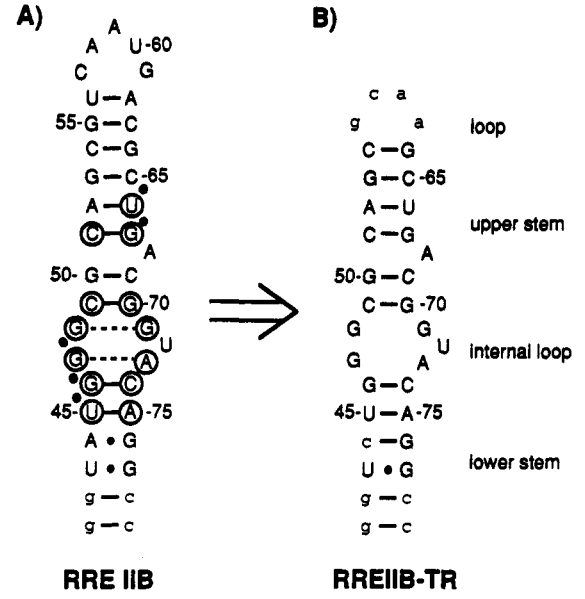

FIGURE 1: (A) Sequence of RRE IIB. Circles indicate important nucleotides identified by chemical modification interference and *in vitro* selection data, and filled-in circles indicate important phosphates from ethylation interference (Bartel et al., 1991; Kjems et al., 1992; Tiley et al., 1992). (B) Sequence of RREIIB-TR used for NMR studies. Lower-case letters indicate non-wild-type nucleotides. The numbering of RREIIB-TR is the same as wild-type RRE (tetraloop ignored).

scans were taken per t_1 slice with a recycle delay of 1.8 s. Carbon decoupling during acquisition was achieved using a WALTZ pulse scheme (Shaka et al., 1983).

RESULTS

Design of an RNA–Peptide Model System for NMR Study. To simplify the Rev peptide–RNA complex as much as possible, the previously characterized RRE IIB hairpin (Figure 1A) (Kjems et al., 1991b,1992) was modified as follows. Two base pairs were removed from the upper stem, the G_{77}-A_{43} mismatch was changed to a G-C pair, and the wild-type loop was replaced with a GNRA tetraloop to create RREIIB-TR (Figure 1B). The G_{77}-C_{43} pair substitution was designed to stabilize the lower stem, and the tetraloop sequence was chosen to prevent RNA dimer formation (data not shown). All of the sequence changes were made in regions of the RRE unimportant for specific binding of Rev and Rev peptides, as determined from previous studies (Bartel et al., 1991; Kjems et al., 1992), and gel shift assays confirmed that RREIIB-TR bound Rev peptides with the same specificity as wild-type RRE IIB (data not shown).

The Rev peptide used in this study, suc-Rev_{34-50}AAAAR-am (TRQARRNRRRRWRERQRAAAAR), has additional amino acids at the C-terminus of the Rev_{34-50} peptide and succinyl and amide blocking groups at the N- and C-termini, respectively. These modifications were shown to increase the α-helical content of the peptide with a concomitant increase in specificity of binding to RRE IIB (Tan et al., 1993).

NMR of Free RREIIB-TR. Analysis of the NMR spectra of RREIIB-TR in 10%/90% D_2O/H_2O indicated fewer imino proton resonances than expected from RNA secondary structure predictions. Imino protons were assigned using 1D NOEs, and assignments are given in Figure 2. Previous studies have suggested the presence of purine–purine base pairs in the internal loop region of RRE IIB (Bartel et al., 1991; Iwai et al., 1992); however, only formation of two stable helical regions from G_{41}-C_{79} to G_{46}-C_{74} and from C_{51}-G_{67} to C_{54}-G_{64}

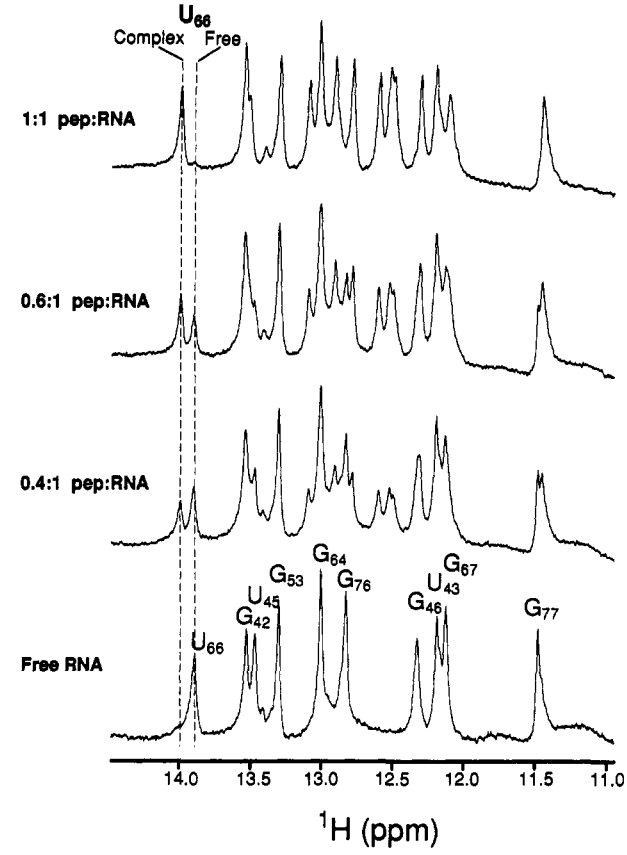

FIGURE 2: Titration of RREIIB-TR with Rev peptide resulting in formation of a specific complex. Molar ratios of peptide to RNA are shown to the left. The free and bound resonances of U_{66} are highlighted to show that the complex is in slow exchange with the free RNA. Assignments of the imino protons for the free RNA are shown.

were observed. No imino proton resonances were observed from the two proposed purine–purine base pairs in the internal loop. Surprisingly, two potential Watson–Crick base pairs between the bulge A_{68} and the internal loop also did not exhibit imino proton resonances.

Analysis of the nonexchangeable NMR spectra supported these observations. Sequential base to H1′ NOEs typical of A-form RNA were observed for the lower and upper stems, with an H8–H8 NOE placing the position of A_{68} stacked below G_{67}. However, assignments for the internal loop region, including the two potential Watson–Crick base pairs below the A bulge, were incomplete due to the lack of sequential NOEs. In addition, many of these resonances were broadened, suggesting intermediate exchange on the NMR time scale and possible multiple conformations.

Formation of the RREIIB-TR–Rev Peptide Complex. The formation of a specific complex was monitored by titration of RREIIB-TR with Rev peptide. New imino proton resonances were observed upon the addition of peptide, and some resonances from the free RNA disappeared. At substoichiometric peptide concentrations, two sets of resonances were observed, indicating slow exchange between free and bound forms of the RNA on the NMR time scale, as illustrated for the imino proton resonance of U_{66} in Figure 2. A similar titration with an unmodified Rev_{34-50} peptide produced quite different results. No additional imino protons were observed for complex formation, no changes in chemical shift were observed, and the line width of the resonances increased considerably (data not shown). These data suggest that the unmodified Rev peptide may bind in multiple conformations that are in intermediate exchange. However, the modified

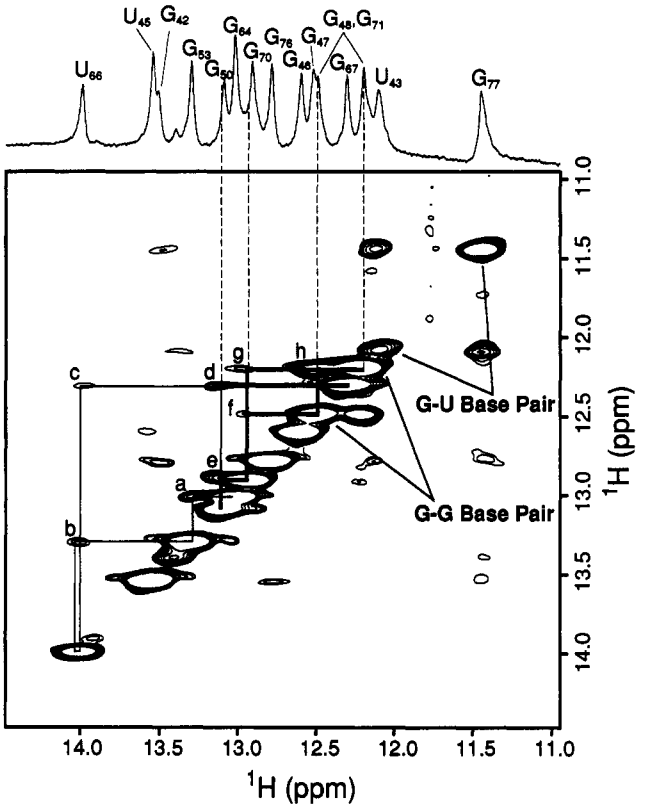

FIGURE 3: NOESY spectrum of the peptide–RNA complex at 25 °C in 10%/90% D_2O/H_2O. The imino–imino sequential NOE pathway for the upper stem is traced out. The bold lines are for the additional base pairs which form only in the complex. Imino–imino NOEs are (a) G_{64}-G_{53}, (b) G_{53}-U_{66}, (c) U_{66}-G_{67}, (d) G_{67}-G_{50}, (e) G_{50}-G_{70}, (f and g) G_{70}-G_{48}/G_{71}, and (h) G_{48}-G_{71}. Note that G_{48} and G_{71} imino protons were not individually assigned due to the symmetry of the base pair. The strong imino to imino NOE of G_{48}-G_{71} is characteristic of base pairs such as G-U which have two imino protons involved in hydrogen bonding.

Rev peptide forms a well-defined specific complex that is suitable for structural characterization by NMR.

Upon stoichiometric binding of the peptide, RREIIB-TR undergoes a conformational change resulting in five additional imino proton resonances that were partially assigned by sequential imino–imino NOEs. Two of the new imino protons observed in the bound form of RREIIB-TR were from the two Watson–Crick pairs below the A bulge. Part of the conformational change upon peptide binding is the stabilization of these upper stem base pairs that are not present in the free RNA.

Evidence for Formation of the G-G Base Pair. Two of the other new imino protons were assigned to a G-G base pair in the internal loop. Figure 3 shows the imino–imino region of the NOESY experiment at 25 °C. An NOE was observed from the G_{70} imino proton of the last Watson–Crick base pair in the upper stem to two more imino protons. These two imino protons had a very strong NOE to each other, which is consistent with the predicted formation of a symmetric G_{48}-G_{71} base pair with the H1 proton of one guanosine hydrogen bonding to the O6 carbonyl oxygen of the other.

Evidence for Formation of the G-A Base Pair. The fifth new imino proton observed in the complex was assigned to G_{47} base pairing with A_{73} in the internal loop on the basis of a strong NOE between an imino proton and an H2 proton. Two possible G-A pairs that are consistent with chemical modification data (Iwai et al., 1992) have been proposed where the Watson–Crick edge of G pairs with either the Watson–Crick edge or the Hoogsteen edge of A. The strong imino to

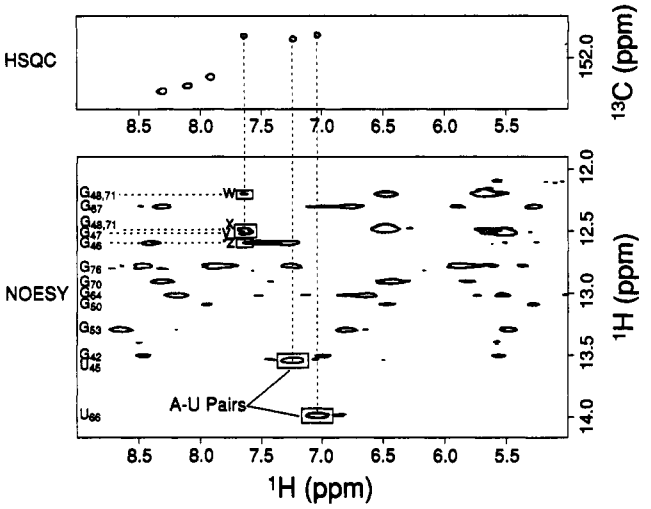

FIGURE 4: Imino–aromatic proton region of the same NOESY spectrum as in Figure 3 with the corresponding C2-H2 region of an HSQC spectrum of ^{13}C-labeled RREIIB-TR complexed with Rev peptide. The HSQC unambiguously identifies the position of the H2 proton resonances. Peaks w, x, and z are the sequential NOEs involving the A_{73}-H2 proton. The relatively weak intensity in this spectrum of peak y (intra-base-pair G-A NOE) compared to the A-U pairs is due to the effect of the sine-bell processing on the shorter T_2 of G_{47}-H1. Assignments of the imino protons are listed to the left of the NOESY spectrum.

H2 NOE rules out the latter possibility and suggests a base pair where the N1 of A is hydrogen bonded to the H1 of G and the HN6 of A is hydrogen bonded to the O6 of G (see Figure 5B). In this base pair, the imino–H2 distance is similar to that observed in a standard A-U pair (Gao & Patel, 1988). In 1D NOE and NOESY spectra, three imino protons exhibited strong NOEs to aromatic protons with line widths characteristic of adenosine H2's (data not shown). Two of the cross-peaks corresponded to the A-U pairs in the lower and upper stem with the third corresponding to the G-A base pair in the internal loop.

While no sequential imino–imino NOEs could be observed for the G-A pair in the internal loop due to resonance overlap, sequential assignment was obtained through the A-H2 proton of A_{73} to confirm the G-A pair. Figure 4 shows the imino–aromatic region of the NOESY spectrum in 10%/90% D_2O/H_2O as well as the corresponding C2-H2 region of an HSQC spectrum of the complex with uniformly ^{13}C-enriched RREIIB-TR in 99.996% D_2O. The HSQC spectrum unambiguously confirms the identities of the A-H2's, since the carbon chemical shift of C2 resonates in a region separate from all other RNA carbons with protons directly attached. The A-H2 at 7.63 ppm exhibits NOEs to both the imino protons in the G-G pair (peaks w and x) and the imino proton of G_{46} (peak z) in the Watson–Crick pair closing the lower stem. Peak z was more clearly resolved in a NOESY experiment performed at 30 °C (data not shown). In addition, NOESY spectra in 99.996% D_2O show that A_{73}-H2 exhibits an NOE to C_{74}-H1′ on its 3′ side, which is characteristic of helical adenosines (data not shown). Methodologies involving heteronuclear NMR are currently being implemented for full assignment of the RNA in the complex, and details of the three-dimensional structure will be presented elsewhere.

DISCUSSION

RNA Conformational Change. Upon specific binding of the Rev peptide to RREIIB-TR, the RNA undergoes a conformational change. Figure 5A summarizes the NMR

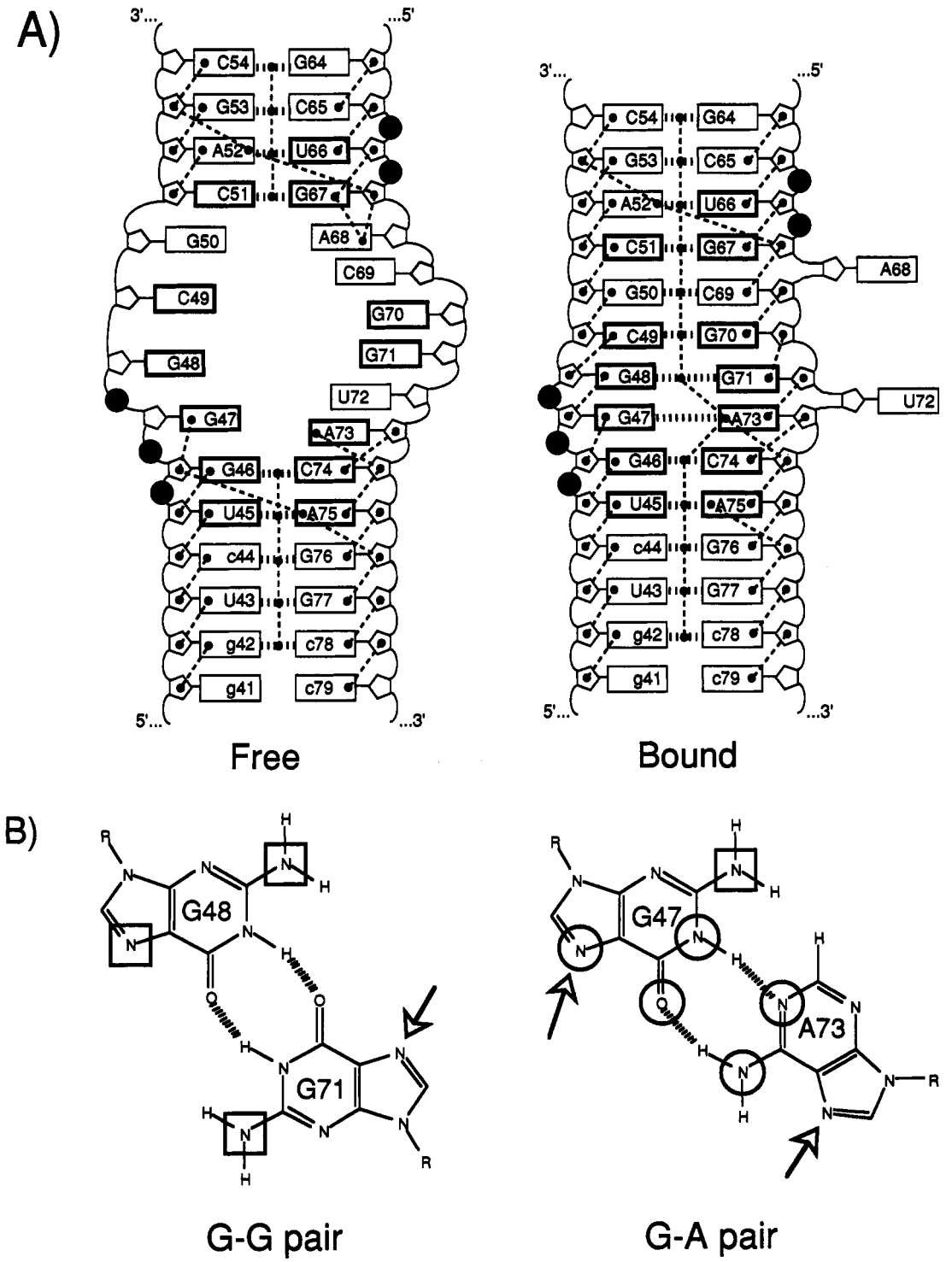

FIGURE 5: (A) Schematic diagram of the NMR data for free and bound RREIIB-TR. Thick dashed lines indicate observed imino protons in base pairs. Thin dashed lines indicate observed NOEs. Lines between the centers of the bases indicate imino–imino NOEs, and all other lines indicate base to sugar NOEs. Bold boxes indicate nucleotides important for binding of Rev, and dark circles indicate important phosphates for binding of Rev (Bartel et al., 1991; Kjems et al., 1992; Tiley et al., 1992). (B) Purine–purine base pairs proposed on the basis of the NMR data. Circles, squares, and arrows indicate functional groups important for binding from previous biochemical studies (Iwai et al., 1992; Kjems et al., 1992): circle, chemical substitution interfered with binding; square, chemical substitution did not interfere with binding; arrow, DEPC modification interfered with binding.

data for the secondary structure of RREIIB-TR in the free and bound form. In the free RNA A_{68} is stacked below the upper stem. Broadening of the internal loop nonexchangeable resonances indicates some conformational flexibility in the internal loop of the unbound RNA. The two Watson–Crick base pairs below the A bulge and the putative purine–purine pairs in the internal loop are destabilized relative to the bound conformation. It is possible that some of the additional base pairs seen in the complex are transiently formed in the free RNA; however, the lifetime of any particular structure must be relatively short. In the bound RNA structure, four additional base pairs are formed, including two purine–purine base pairs in the internal loop which were previously proposed to be critical for specific binding of the Rev protein (Bartel et al., 1991; Iwai et al., 1992). The purine–purine base pairs in the internal loop stack on each other while U_{72} appears to loop out into solution. In addition, A_{68}, which was stacked in the free RNA structure, is looped out into solution.

Conformational changes in RNA upon protein binding have been observed in TAR–arginine, $tRNA^{Gln}$–synthetase, and $tRNA^{Asp}$–synthetase complexes (Puglisi et al., 1992; Rould et al., 1989; Ruff et al., 1991). The conformational change in the RRE involves unstacking of the A_{68} bulge nucleotide and a stabilization of Watson–Crick and purine–purine base pairs in the internal loop. The α-helical peptide conformation is also presumably stabilized in the complex, since the free peptide is only ~50% helical (Tan et al., 1993). The simplest interpretation of these conformational changes would be that the affinity and specificity of binding are reduced compared to a completely preorganized binding site. Nevertheless, protein-induced conformational changes appear to be a common feature of formation of RNA–protein complexes.

Geometry of Purine–Purine Base Pairs in the Internal Loop. The geometries of the base pairs in the RNA internal loop of the complex are consistent with previous biochemical investigations of RRE. Figure 5B shows the proposed hydrogen-bonding pattern of the two purine–purine pairs, based on the NMR data, and the functional groups thought to be important for Rev binding based on biochemical data. For the G-G pair there is a strong imino–imino NOE which implies that the imino protons from each guanosine are very close in space. In addition, each imino proton in the G-G pair has a strong NOE to a proton with a chemical shift similar to freely rotating, non-hydrogen-bonded G-aminos. The only possible G-G base pair which satisfies these criteria is a symmetrical conformation in which each imino proton is hydrogen bonded to the carbonyl oxygen of the other guanosine. This type of base pair was predicted from the results of *in vitro* selection of RRE mutants that bind Rev. The prediction was based on the isolation of an A-A base pair which can form a base pair isosteric to the G-G base pair with hydrogen bonds between the amino and N1 positions of adenosine (Bartel et al., 1991). In addition, inosine substitution showed that removal of the G-amino group at both of these positions had no effect on Rev binding (Iwai et al., 1992). All of these data are consistent with the base-pair scheme shown in Figure 5B.

Interestingly, if G_{48} and G_{71} of RREIIB-TR are on antiparallel strands, as would be expected in a normal helix, one of the glycosidic torsion angles should be in a *syn* conformation in order to form the proposed base pair. However, neither G_{48} nor G_{71} has a strong H8–H1′ NOE characteristic of *syn* guanosines (data not shown). Therefore, it is likely that some aspect of phosphate backbone orientation is important for formation of this base pair without the presence of *syn* guanosines, and the resulting geometry might be critical for specific recognition by the peptide. Preliminary model building studies confirm that it is possible to build stereochemically reasonable structures consistent with the data. The presence of the bulged uridine in the internal loop appears to be necessary for the proper orientation of the G-G pair.

The geometry of the proposed G-A pair is also consistent with all previous biochemical data. The NMR data show a strong G-imino to A-H2 NOE and both nucleotides in an *anti* glycosidic conformation, supporting a non-Hoogsteen G-A base pair. Each functional group involved in a hydrogen bond in this base pair has been shown to be important for Rev binding (Iwai et al., 1992). The *anti–anti* orientation of the glycosidic torsions places both N7's in the major groove. Both of these N7's were shown to be important for binding by chemical interference studies, and it is thought that the peptide interacts with these functional groups in the major groove (Kjems et al., 1992; Tiley et al., 1992).

In addition to providing functional groups for hydrogen-bonding interactions with the peptide, the purine–purine base pairs may provide the proper structural environment for peptide recognition. Normal A-form RNA has a deep and narrow major groove, and distortions that open up the major groove are probably necessary for specific contacts with side chains (Weeks & Crothers, 1991, 1993). The two purine–purine base pairs in the RRE utilize the Watson–Crick face of both nucleotides in hydrogen bonding; therefore, the ribose to ribose distance across the helix is wider than in a purine–pyrimidine base pair. The bulge in TAR RNA serves a similar role, where a base triple involving a bulge uridine is necessary for widening of the major groove for contacts with arginine (Puglisi et al., 1992). The major groove of nucleic acids has a richer variety of functional groups available for protein contacts than the minor groove and is most often used for recognition of DNA. The non-Watson–Crick regions of RNA thus may have a general role of providing structural features that allow proteins to utilize the major groove of RNA for specific recognition.

ACKNOWLEDGMENT

We thank Robert Batey for assistance with the preparation of ^{13}C-labeled RNA and Jody Puglisi for critical review of the manuscript.

REFERENCES

Bartel, D. P., Zapp, M. L., Green, M. R., & Szostak, J. W. (1991) *Cell 67*, 529–536.
Batey, R. T., Inada, M., Kujawinski, E., Puglisi, J. D., & Williamson, J. R. (1992) *Nucleic Acids Res. 20*, 4515–4523.
Cheong, C., Varani, G., & Tinoco, I., Jr. (1990) *Nature 346*, 680–682.
Cook, K. S., Fisk, G. J., Hauber, J., Usman, N., Daly, T. J., & Rusche, J. R. (1991) *Nucleic Acids Res. 19*, 1577–1582.
Cullen, B. R., & Malim, M. H. (1991) *Trends Biochem. Sci. 16*, 346–350.
Gao, X., & Patel, D. J. (1988) *J. Am. Chem. Soc. 110*, 5178–5182.
Grodberg, J., & Dunn, J. J. (1988) *J. Bacteriol. 170*, 1245–1253.
Gutell, R. R., Weiser, B., Woese, C. R., & Noller, H. F. (1985) *Prog. Nucleic Acid Res. Mol. Biol. 32*, 155–216.
Heus, H. A., & Pardi, A. (1991) *Science 253*, 191–194.
Hore, P. J. (1983) *J. Magn. Reson. 55*, 283–300.
Iwai, S., Pritchard, C., Mann, D. A., Karn, J., & Gait, M. J. (1992) *Nucleic Acids Res. 20*, 6465–6472.
Kjems, J., Brown, M., Chang, D. D., & Sharp, P. A. (1991a) *Proc Natl. Acad. Sci. U.S.A. 88*, 683–687.
Kjems, J., Frankel, A. D., & Sharp, P. A. (1991b) *Cell 67*, 169–178.
Kjems, J., Calnan, B. J., Frankel, A. D., & Sharp, P. A. (1992) *EMBO J. 11*, 1119–1129.
Puglisi, J. D., Tan, R., Calnan, B. J., Frankel, A. D., & Williamson, J. R. (1992) *Science 257*, 76–80.
Rould, M. A., Perona, J. J., Soll, D., & Steitz, T. A. (1989) *Science 246*, 1135–1142.
Roy, S., Delling, U., Chen, C.-H., Rosen, C. A., & Sonenberg, N. (1990) *Genes Dev. 4*, 1365–1373.
Ruff, M., Krishnaswamy, S., Boeglin, M., Poterszman, A., Mitschler, A., Podjarny, A., Rees, B., Thierry, J. C., & Moras, D. (1991) *Science 252*, 1682–1689.
Santoro, J., & King, G. C. (1992) *J. Magn. Reson. 97*, 202–207.
Shaka, A. J., Keeler, J., & Freeman, R. (1983) *J. Magn. Reson. 53*, 313–340.
Sklenar, V., Brooks, B. R., Zon, G., & Bax, A. (1987) *FEBS Lett. 216*, 249–252.
Szewczak, A. A., Moore, P. B., Chan, Y.-L., & Wool, I. G. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 9581–9585.

Tan, R., Chen, L., Buettner, J. A., Hudson, D., & Frankel, A. D. (1993) *Cell 73*, 1031–1040.
Tiley, L. S., Malim, M. H., Tewary, H. K., Stockley, P. G., & Cullen, B. R. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 758–762.
Varani, G., & Tinoco, I., Jr. (1991) *Q. Rev. Biophys. 24*, 479–532.
Weeks, K. M., & Crothers, D. M. (1991) *Cell 66*, 577–588.
Weeks, K. M., & Crothers, D. M. (1993) *Science 261*, 1574–1577.
Wimberly, B., Varani, G., & Tinoco, I., Jr. (1993) *Biochemistry 32*, 1078–1087.
Wyatt, J. R., Chastain, M., & Puglisi, J. D. (1991) *BioTechniques 11*, 764–769.