The Role of 2′-Hydroxyl Groups in an RNA−Protein Interaction†

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ABSTRACT: The role of the 2′-hydroxyl group in RNA−protein interaction has been investigated using MS2 coat protein and its hairpin RNA operator as a model system. Derivatives of the MS2 translational operator were prepared where individual riboses were replaced by deoxyribose and their binding affinities to MS2 coat protein were determined. Only 1 (U_{-5}) out of 15 positions tested reduced protein affinity by 1.6 kcal/mol. A variety of other 2′-modifications were tested at this position to understand the role of this particular 2′-hydroxyl group. Normal binding of the U-NH_2 variant and weaker binding of the U-*O*-methyl variant are consistent with the ability of these functional groups to provide a hydrogen bond donor. This is also supported by recent crystallographic data which indicate a possible interaction between the 2′-hydroxyl of U_{-5} and the carboxylate group of glutamate 63 [Valegård et al. (1994) *Nature 371,* 623−626]. Complementary experiments introducing riboses into a DNA hairpin confirm the putative protein contact, and also identify a requirement for riboses in the two upper base pairs of the hairpin. Several arguments suggest these riboses are required to maintain an A-form helix in this region of the binding site. A minimum requirement of four 2′-hydroxyl groups for wild-type coat protein binding has been detemined, one of which is at the −5 position and other three in the upper stem in any combination. The data are analyzed in terms of the recently determined structure of the free RNA by NMR [Borer et al. (1995) *Biochemistry 34,* 6488−6503] and the cocrystal structure of the complex (Valegård et al., 1994.

The binding of R17 (and the close relative MS2) coat protein to its translational operator is one of the best biochemically characterized RNA−protein interactions [reviewed in Witherell et al. (1991)]. In this system, a dimer of the coat protein binds specifically to a single hairpin in the initiation region of the replicase gene, thereby blocking the initiation of translation. Synthetic oligonucleotides have been extensively used to identify RNA functional groups that interact with the protein (Carey & Uhlenbeck, 1983; Romaniuk et al., 1987; Wu & Uhlenbeck, 1987; Stockley et al., 1993). The coat protein recognizes a specific hairpin structure with a bulged A nucleotide and a four-residue loop with the consensus sequence ANYA (Y = U, C; N = A, U, C, G). It is also likely that the protein contacts the ribose−phosphate backbone of the hairpin, since substitution of phosphates at four specific positions with the phosphorothioates affected protein binding (Milligan & Uhlenbeck, 1989).

It is reasonable to expect that certain 2′-hydroxyl groups in the hairpin will also contribute significantly to its binding to coat protein. In both helical and nonhelical regions of known RNA structures, the 2′-hydroxyls often protrude into the solvent making them available for protein contact. Since 2′-hydroxyls can be both hydrogen bond donors and hydrogen bond acceptors, they can potentially interact with a variety of amino acid side chains as well as with the protein backbone. Indeed, both crystallographic (Rould et al., 1989; Cavarelli et al., 1993; Biou et al., 1994) and biochemical (Musier-Forsyth et al., 1991; Musier-Forsyth & Schimmel, 1992) analyses of several tRNA synthetases complexed with their cognate tRNAs indicate that these proteins bind certain 2′-hydroxyl groups and thereby contribute to substrate affinity. Earlier experiments with R17 or MS2 coat protein (Wu & Uhlenbeck, 1987; Milligan, 1988; Talbot et al., 1990; Stockley et al., 1993) have shown that substitution of a specific ribose with deoxyribose in the hairpin reduced affinity to coat protein while similar substitution at three other positions had no effect. In this paper, the role of 2′-hydroxyl groups in this RNA−protein interaction was examined in greater detail, and the data were interpreted using the recently determined crystal structure of the coat protein−hairpin complex (Valegård et al., 1994) and the structure of the free RNA determined by NMR (Borer et al., 1995).

MATERIALS AND METHODS

Preparation of RNAs. Most RNAs and mixed DNA−RNA polymers were chemically synthesized using phosphoramidite techniques (Usman et al., 1987). 2′-modified phosphoramidites [2′-*O*-methyl (Glen Research), 2′-*O*-allyl (Boehringer Mannheim), and 2′-*O*-propargyl (Chem Genes)] were procured commercially. 3′-*O*-Silyl 2′-phosphoramidite (Chem Genes) was used to produce RNA with a 2′−5′ linkage. RNAs which contained a 2′-fluoro or a 2′-amino at a single position were prepared by *in vitro* transcription from synthetic DNA templates using T7 RNA polymerase (Milligan et al., 1987) and 2′-fluoro-UTP or 2′-amino-UTP (generously donated by W. A. Pieken, NeXstar, Boulder, CO). RNAs were purified by electrophoresis on 20% polyacrylamide gels containing 7 M urea, visualized by UV shadowing, cut out, eluted, and desalted using a C-18 column. The purity of variants was confirmed by two independent methods. HPLC analysis of each gel-purified RNA displayed only one peak. Each RNA was digested with a mixture of ribonucleases and analyzed by two-dimensional thin-layer chromatography (Krug et al., 1982) to confirm the

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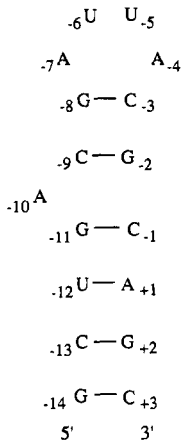

FIGURE 1: MS2 coat protein binding fragment. Subscripts refer to nucleotide position relative to the adenosine (+1) of the replicase initiation codon.

absence of base-protecting groups or protected 2′-hydroxyls.

RNAs were labeled at their 5′ terminus using [γ -^{32}P]ATP and T4 polynucleotide kinase. In the case of the *in vitro* transcribed RNAs, the 5′-triphosphate was enzymatically removed by calf intestinal alkaline phosphatase prior to radiolabeling.

Nitrocellulose Filter Binding. MS2 coat protein was overexpressed in *E. coli* strain BL21 (Studier et al., 1990) and purified by an acetic acid extraction procedure (Gott et al., 1991). Binding constants between coat protein and the RNA variants were determined with a nitrocellulose filter retention assay. Trace amounts (picomolar) of ^{32}P-labeled RNA were mixed in 150 μL reaction mixtures with a series of 23 coat protein concentrations ranging from 0.08 nM to 1 μM in 2 mM $MgCl_2$, 16 mM KCl, 80 μg/mL BSA, and 20 mM Tris-HCl (pH 8.5 at 4 °C). After incubation for 90 min at 4 °C, the reaction mixture was filtered under constant vacuum at 4 °C using 96-well dot-blot apparatus (Wong & Lohman, 1993) with nitrocellulose filter paper (Nitro ME, 0.45 μm, from Micron Separations, Inc., Westboro, MA) that had been soaked in the same buffer. In some cases, Multiscreen HA filtration plates (Millipore) were used. The amount of bound complex retained on the filter paper was quantified by a Molecular Dynamics Phosphorimager, and the K_D values were calculated according to Carey et al. (1983). K_Ds were measured in duplicate, and the values differed no more than 20%.

RESULTS

Replacement of 2′-Hydroxyls in the RNA Hairpin. A 17-nucleotide RNA that binds coat protein with high affinity was used as parent molecule (Figure 1). Fifteen different variants were prepared which contained a single deoxyribose in place of a ribose. A nitrocellulose filter binding assay was used to measure the affinity of each variant for coat protein (Table 1). All but one of the RNAs bound coat protein with an affinity similar to the all-RNA molecule. However, the dU-5 variant bound the coat protein about 25-fold less well, corresponding to an increase in ΔG from −10.9 to −9.3 kcal/mol. In order to further investigate this observation, we synthesized a series of variants with a 2′-modified sugar at position −5.

Using a template that produces an RNA with a single uridine residue at position −5 (Milligan & Uhlenbeck, 1989),

Table 1: Coat Protein Binding to Variants Containing a Single Deoxyribonucleotide

variants	K_D (nM)	variants	K_D (nM)
1 (all-RNA)	2.5	dU-6	3.1
dG+2	2.3	dA-7	2.7
dA+1	3.2	dG-8	2.9
dC-1	2.4	dC-9	2.5
dG-2	2.5	dA-10	2.6
dC-3	2.2	dG-11	2.9
dA-4	2.9	dU-12	3.2
dU-5	59	dC-13	2.4

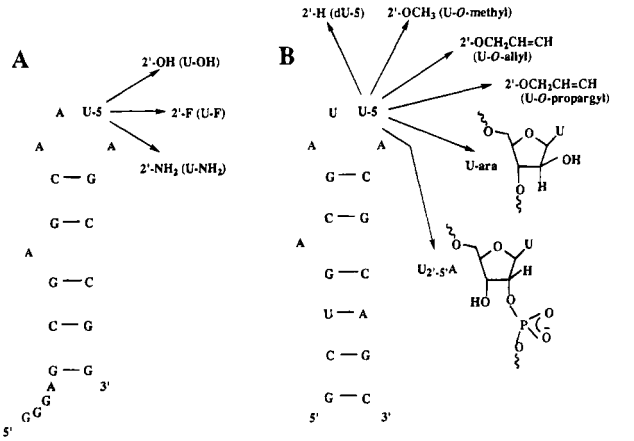

FIGURE 2: RNA variants containing 2′-modifications at position −5. (A) Variants made by *in vitro* transcription. (B) Variants made by chemical synthesis.

Table 2: Effect of Protein Binding Constant on 2′-Modification at Position −5 of the RNA Operator

variants	K_D (nM)
U-OH	2.5
U-F	2.6
U-NH_2	3.0
U-*O*-methyl	40
U-*O*-allyl	4.8
U-*O*-propargyl	9.8
U-ara	55
$U_{2'-5'}A$	62

in vitro transcription using 2′-fluoro-2′-deoxyuridine 5′-triphosphate or 2′-amino-2′-deoxyuridine 5′-triphosphate instead of UTP was performed to produce RNAs with 2′-fluoro or 2′-amino groups at that position (Figure 2A). While both these functional groups are similar in size, the 2′-fluoro group can only act as a weak H-bond acceptor (Withers et al., 1988) while the 2′-amino group can, like the 2′-hydroxyl, act both as a hydrogen bond donor and as a hydrogen bond acceptor. Both these variants bound coat protein with the same affinity as the corresponding all-RNA variant (Table 2).

Two variants were prepared which contained a 2′-hydroxyl group at position −5, but altered its orientation. The U-ara variant contains arabinouridine where the 2′-hydroxyl is *cis*-oriented relative to the glycosyl $C_{1'}$—N link. This change slightly alters furanose bond distances and angles, but does not influence sugar pucker (Sundaralingam, 1975). The $U_{2'-5'}A$ variant contains a 2′−5′ linkage between positions −5 and −4 instead of the 3′−5′ linkage. This change could alter the hydrogen-bonding properties of the 2′ and 3′ positions and may also distort the loop structure. The U-ara and $U_{2'-5'}A$ variants bind coat protein with K_Ds quite similar

Table 3: Coat Protein Binding of DNA Variants Containing a Variable Number of Ribose Sugars

variants	K_D (nM)
DR1 (all-DNA)	>2900
DR2 (ribose at −5)	52
four riboses in the stem	
DR3	45
DR4	19
DR5	2.5
three riboses in the upper stem	
DR6	3.5
DR7	3.7
DR8	6.1
DR9	6.3
two riboses in the upper stem	
DR10	12
DR11	12
DR12	12
DR13	15
no ribose at position −5	
DR14	57
DR15	230

to the deoxyribonucleotide substitution, suggesting that the positioning of the 2′-hydroxyl group at position −5 must be precise.

Variants containing U-*O*-methyl, U-*O*-allyl, and U-*O*-propargyl at position −5 (Figure 2B) were synthesized to test whether increasing steric factors will alter the interaction. The K_D for U-*O*-methyl is 40 nM which is in between the all-RNA and the dU-5 variants. On the other hand, the K_Ds of U-*O*-allyl and U-*O*-propargyl are lower than U-*O*-methyl, but still higher than that of the all-RNA molecule (Table 2).

Is One Ribose Sugar in the Hairpin Adequate for Protein Binding? Systematic deletion of single 2′-hydroxyls revealed only one position that affected protein binding. However, variant DR1, the all-DNA molecule with identical sequence, does not show detectable affinity for the coat protein (K_D > 2900 nM). This raises the question whether the introduction of a ribose at position −5 of DR1 would be sufficient to give normal protein binding. As shown in Table 3, variant DR2, which contains a single ribose at −5, binds coat protein much better than DR1, but still 20-fold weaker than the all-RNA molecule. While these data confirm that the ribose at position −5 contributes to protein binding, it is clear that other riboses must be introduced to give normal affinity.

The minimum number of ribonucleotides required to make DR1 bind normally was determined using a series of oligonucleotides containing an increasing number of ribonucleotides. Variants DR3−DR5 each contain the crucial ribose at position −5 and four additional riboses at different locations in the stem (Figure 3). DR5, which has four additional riboses in the upper stem, bound coat protein with an affinity similar to the all-RNA molecule. In contrast, DR3 and DR4, which have four additional riboses in the lower and middle regions of the stem, bound 18- and 8-fold less well, respectively (Table 3). Thus, tight protein binding requires the upper two base pairs of the stem to be RNA, but the lower helix can be DNA.

This requirement for the upper two RNA base pairs is independent of the ribose at −5. DR14, a variant that contains the four riboses in the upper base pairs but does not have a ribose at −5, also binds coat protein much better than the all-DNA molecule. Since the K_D of 57 nM for DR14 with coat protein is similar to the K_D of 59 nM for

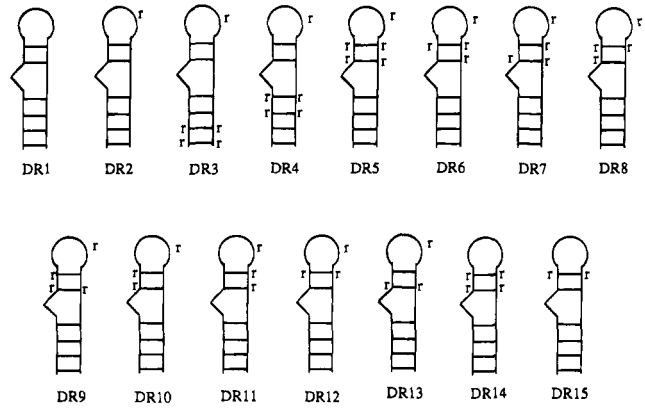

FIGURE 3: DNA molecules with sequence identical to Figure 1 and containing varying number of ribose sugars at positions indicated by "r".

dU-5, it appears that the thermodynamic contribution of the riboses in the upper two base pairs on protein binding is independent of the contribution of ribose at position −5. Thus, the ΔΔG for removing two riboses from the upper stem is nearly the same whether or not the DNA molecule contained ribose −5 (ΔG of DR5 − ΔG of DR12 = 0.86 kcal/mol) or did not (ΔG of DR14 − ΔG of DR15 = 0.77 kcal/mol).

In order to further dissect the ribose requirement for the upper two base pairs, the K_Ds for variants with a ribose at −5 and three (DR6−DR9) or two (DR10−DR13) ribose sugars in the upper stem were determined (Table 3). All four variants with three ribose sugars in the upper stem bound coat protein with the same high affinity while variants with two ribose sugars in several positions in the upper stem have a 5−6-fold weaker affinity for the protein. Thus, a minimum of three riboses in the upper stem is needed for tight binding, but their precise position does not matter. This suggests that these riboses act collectively to improve binding of the DNA hairpin, but no particular site is required. It is, therefore, possible that this ribose requirement for maximal protein binding reflects a need to maintain an A-type helical configuration in the upper part of the stem.

If the requirement for ribose sugars in the upper stem is simply to maintain an A-type helical configuration, then buffers containing organic solvents that induce DNA helices to adopt A-type conformations may be able to replace the requirement for these riboses (Brahms & Mommaerts, 1964; Ivanov et al., 1973). In Figure 4, the binding of several hairpins to coat protein is tested in a buffer containing sodium ion and various concentrations of ethanol. The K_Ds for the all-RNA and the weaker binding variants containing U-ara or $U_{2'\text{-}5'}A$ remain virtually constant as a function of ethanol concentration. Thus, the interaction of these molecules with the protein is not greatly affected by ethanol in the concentration range tested. In contrast, the K_D of DR2, the all-DNA molecule with a single ribose at position −5, shows a very different dependence on ethanol concentration. Without ethanol, the K_D of DR2 is about 21-fold greater than the all-RNA molecule, which is quite similar to the difference observed in the normal binding buffer. However, as the fraction of ethanol is increased, the binding of DR2 to coat protein becomes much tighter so that at 25% ethanol it binds nearly as well as the all-RNA hairpin. This indicates that ethanol in the solvent can replace the requirement for riboses in the upper stem thereby supporting the view that three

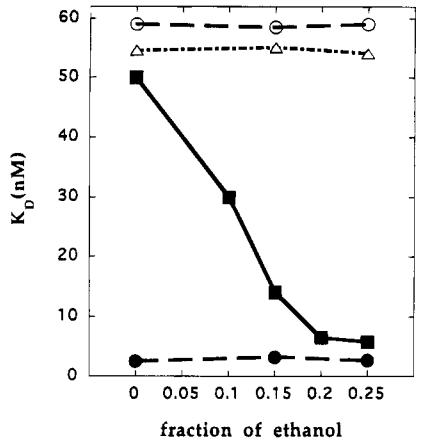

FIGURE 4: K_D of MS2 coat protein binding to all-RNA molecule (●), DR2 (■), $U_{2'\text{-}5'}A$ (○), and U-ara (△) in 20 mM Tris, pH 8.5, 20 mM NaCl, and varying v/v fractions of ethanol.

riboses in the upper stem, are required to maintain the A-type conformation in that region of the helix.

DISCUSSION

Systematic substitution of ribose by deoxyribose in the RNA binding site for MS2 coat protein revealed only one position where a 2′-hydroxyl is required. These experiments confirmed earlier experiments (Milligan, 1988; Stockley et al., 1993) identifying the ribose position −5 as important for protein binding and extended observations (Wu & Uhlenbeck, 1987; Talbot et al., 1990) that riboses at several other positions can be substituted for deoxyribose without any change in protein affinity.

Interpretation of the biochemical data prescribed here is greatly aided by two molecular structures recently determined in this system. Borer et al. (1995) have used proton NMR to determine the conformation of a 24-nucleotide variant of the RNA binding site. While the lower part of the hairpin has a clear A-type helical conformation with the A-10 residue stacked within the helix, the entire upper stem and loop appears quite flexible. Despite an average of 21 distance constraints per residue, at least 2 different loop conformations satisfy the data. Valegård et al. (1994) report a crystal structure of a 19-nucleotide RNA hairpin complexed to a MS2 capsid of 90 coat protein dimers. The structure of the protein in the complex is very similar to the structure of an empty capsid and to the structure of intact MS2 virus solved previously (Valegård et al., 1990). In contrast, the structure of the RNA in the complex is quite different from the structure of the RNA in solution determined by NMR. In contrast with the solution structure, A-10 and A-4 are unstacked and protrude into pockets formed by the same four amino acids in each subunit of the protein dimer. It is likely that the molecular details of the RNA−protein interaction observed in the crystal structure of the capsid are also present in the dimer−RNA complex since regions of interdimer contact in the crystal are well away from the RNA binding site and mutations which disrupt dimer−dimer interactions do not affect RNA binding (LeCuyer et al., 1995).

In order to evaluate the contribution of individual 2′-hydroxyl groups to the protein−RNA interaction, their structure in both the free and bound states must be considered. In the most common structure observed for the free RNA, all of the 2′-hydroxyls protrude into the solvent

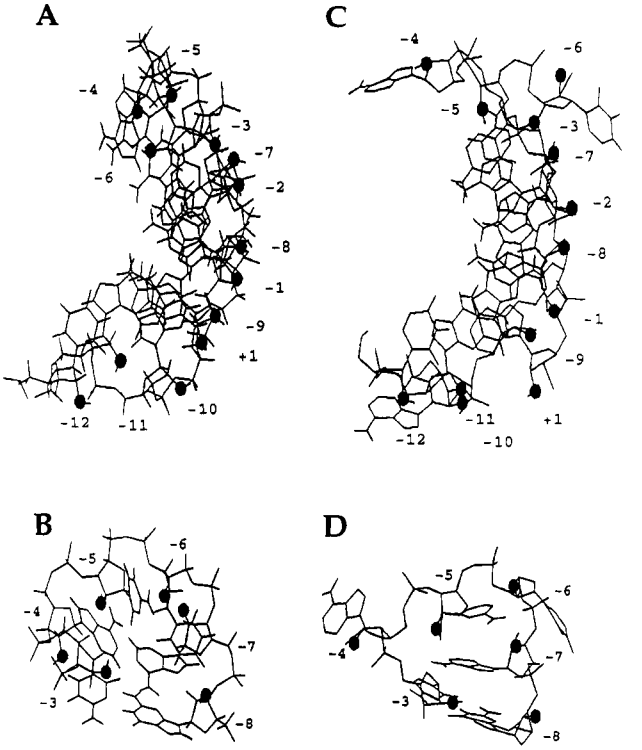

FIGURE 5: Three-dimensional structures of RNA residues −12 to +1 in the free form (panels A, B) determined by NMR (Borer et al., 1995) and in the bound form (panels C, D) determined by crystallography (Valegård et al., 1994). The sequences of several of the base pairs are different in the two molecules. Positions of the 2′-hydroxyls are numbered and indicated by solid circles (●). The four loop residues and closing base pair are shown for the two structures from a different angle in panels B and D to illustrate the environment around the 2′-hydroxyl at position −5. The sequences of these six residues are identical.

(Figure 5A). It can be deduced from the H1′−H2′ scalar coupling constraints that most of the riboses in the stem region are primarily in their favored 3′-endo(N) conformation. However, the riboses in the loop positions −4, −5, and −6 partially adopt the 2′-endo(S) conformation commonly found in DNA. In the most favored form of the loop structure, loop bases are directed into the major groove and the ribose at the −5 position faces the interior of the loop (Figure 5B). In the case of the protein−RNA complex, most of the 2′-hydroxyls are solvent-exposed (Figure 5C) and are more than 6 Å away from any protein functional group. One exception is the ribose at position −10, which, at the current resolution, is within 4.9 Å of the hydroxyl of serine 47, suggesting a possible water-mediated hydrogen bond (Valegård et al., 1994). In addition, the ribose at −5 differs from the other riboses in the molecule by being less exposed to the solvent because of its position within the structured loop (Figure 5D). While the published structure does not reveal a potential hydrogen bond donor or acceptor near ribose −5, a recent refinement of the structure revealed that the ribose at −5 is in the 2′-endo conformation and the 2′-hydroxyl can potentially form a hydrogen bond with the carboxylate of glutamate 63 which is approximately 2.7 Å away from the 2′-hydroxyl group (K. Valegård, personal communication).

The single-deoxynucleotide substitution data reported here are, for the most part, consistent with the structural data. Since most of the 2′-hydroxyl groups are solvent-exposed in both the bound and unbound structures, no change in the

free energy of protein binding is expected when they are changed to a deoxynucleotide. The absence of an effect on K_D for the dA-10 variant can be interpreted by the fact that the hydrogen bond to this hydroxyl is likely to be water-mediated and thus contribute little to the binding energy (Fersht et al., 1985; Withers et al., 1988). The potential hydrogen bond between the 2′-hydroxyl of ribose −5 and glutamate 63 observed in the refined structure appears to account for the 1.6 kcal/mol lower free energy of RNA binding of the d-5 variant. A value of 1.6 kcal/mol is within the range of the energetic contribution of a hydrogen bond to a charged amino acid (Fersht et al., 1985; Withers et al., 1988). Since the ribose conformation in both the free and bound states is primarily in the 2′-endo conformation, any conformational contribution by deoxynucleotide substitution would not be expected to make a large energetic contribution. It is possible that the different environment around the 2′-hydroxyl of ribose −5 between the bound and unbound states may also contribute to the weaker binding.

Protein binding to RNAs where the 2′-hydroxyl at ribose −5 is substituted by other functional groups is more difficult to interpret. Since glutamate 63 can only act as a hydrogen bond acceptor, one would expect that functional groups that can provide hydrogen bond donors could substitute for hydroxyls. In addition, different 2′-functional groups could potentially alter the sugar conformation in the free RNA. Although N and S ribose conformations are nearly isoenergetic as monomers (Saenger, 1984), they may not be so in the context of the hairpin. Thus, any energy needed to convert the conformation of sugar in the free RNA to the conformation present in the complex will be observed as a reduced K_D.

Only some of the binding data to 2′-ribose-modified RNAs can be easily reconciled with the structures. Normal binding of the 2′-amino RNA and weaker binding of the 2′-*O*-methyl derivative are consistent with the ability of these functional groups to provide a hydrogen bond donor. However, the relatively tight binding of the 2′-fluoro, 2′-*O*-allyl, and 2′-*O*-propargyl substituted RNAs is more surprising. In the case of the alkyl-substituted side chains, the loss of binding energy from the absence of a hydrogen bond donor may be offset by a new stabilizing interaction, perhaps as a result of the hydrophobic character of these groups. It is less easy to understand the tight binding of the 2′-fluoro-substituted RNA. Either it must make an unusually stable water-mediated hydrogen bond with glutamate 63 or its structure in the unbound state is more favorable for protein binding. Structural studies will be needed to resolve this issue.

A series of experiments with DNA hairpins containing a few riboses showed that in addition to the ribose at −5, at least three riboses must be present in the upper two base pairs of the hairpin in order for the DNA to bind as well as the RNA hairpin. Surprisingly, the position of the three riboses did not matter, and the single-deoxynucleotide substitution experiments did not reveal a ribose requirement in this region. A possible explanation for these results is a requirement for an A-type helical conformation in this region. In the crystal structure of the complex, both pairs are in the standard A-form configuration and lie between the critical A−4 and A−10 residues. In addition, the protein contacts two of the four phosphates of the pairs. Since the unbound RNA also adopts an A-type configuration, the two pairs will not need to change conformation upon protein binding. However, in a DNA molecule, these two base pairs are likely to be in a B-form helix when in the free form, but must adopt to the A form when bound. Although DNA base pairs can easily adopt the A-form helical configuration, the energy required to do this will be observed by a reduction in binding energy to the protein. While it is unlikely that the entire 1.7 kal/mol difference in binding energy between the all-RNA molecule and the DNA molecule containing a ribose at −5 can be solely attributed to converting the two pairs from B form to A form, this effect could account for at least some of the observed energy difference.

Experiments measuring the effect of ethanol on the affinity of the RNA−protein complex also support the view of a requirement for an A-type helical configuration for protein binding. In a low ionic strength buffer containing sodium ions, the affinity of the all-RNA hairpin or two of the position −5 variants to coat protein is not affected by the addition of up to 25% (v/v) ethanol. In contrast, the affinity of the coat protein to the DNA hairpin with a 2′-hydroxyl at position −5 (DR2) increases with increasing ethanol concentration until it equals that of the all-RNA molecule. Since this ethanol effect only occurs with DR2, it is unlikely to be explained by a change in some general property of the protein−hairpin interaction induced by ethanol, such as the change of the dielectric constant of the medium. Instead, we propose that the ethanol increases the propensity of DR2 to adopt the A-type helical conformation when it is not bound to protein. While the transition occurs at much lower ethanol concentrations than the 66−76% that has previously been reported for the B→A transition in high molecular weight DNA (Ivanov et al., 1973), shorter DNA oligomers require less ethanol to convert to the A form and the transition is also very sequence-dependent (Ivanov & Krylov, 1992). In addition, NMR studies of several short DNA fragments in low ionic strength buffers (James & Tinoco, 1993; Mooren et al., 1994) reveal that several of the deoxyriboses have a high fraction of the 3′-endo conformation, suggesting that they can be easily converted to A form. NMR experiments on DR2 as a function of ethanol concentration will be required to resolve this issue.

Chimeric DNA−RNA oligonucleotides have been used to investigate other RNA−protein interactions. The most extensive study involves the aminoacylation of short RNA duplexes with *E. coli* alanine−tRNA synthetase (Musier-Forsyth et al., 1991; Musier-Forsyth & Schimmel, 1992). In many respects, these data resemble those obtained with the MS2 system. Systematic deoxynucleotide substitution experiments revealed that in addition to the essential 3′ terminal adenosine, three specific riboses are important for full reactivity. Similar to the MS2 system, each single-deoxynucleotide substitution reduced the apparent affinity by 1−2 kcal/mol. A DNA molecule containing these crucial riboses was also unable to bind well until a number of additional riboses were inserted, presumably to convert the helical conformation to A form. Several other tRNA synthetases show some affinity to the corresponding DNA molecule (Khan & Roe, 1988; Paquette et al., 1990). The binding of chimeric DNA−RNA oligonucleotides to HIV Tat protein (Barnett et al., 1993) has also been reported. However, these experiments were not sufficiently extensive to distinguish between possible site-specific contacts and the more general requirement for A-type helical conformation.

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