Recognition of nucleic acid double helices by homopyrimidine 2′,5′-linked RNA

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ABSTRACT

We have studied the effect of a 2′,5′-RNA third strand backbone on the stability of triple helices with a ‘pyrimidine motif’ targeting the polypurine strand of duplex DNA, duplex RNA and DNA/RNA hybrids. Comparative experiments were run in parallel with DNA and the regioisomeric RNA as third strands adopting the experimental design of Roberts and Crothers. The results reveal that 2′,5′-RNA is indeed able to recognize double helical DNA (DD) and DNA (purine):RNA (pyrimidine) hybrids (DR). However, when the duplex purine strand is RNA and the duplex pyrimidine strand is DNA or RNA (i.e. RD or RR), triplex formation is not observed. These results exactly parallel what is observed for DNA third strands. Based on Tm data, the affinities of 2′,5′-RNA and DNA third strands towards DD and DR duplexes were similar. The RNA third strand formed triplexes with all four hairpins, as previously demonstrated. In analogy to the arabinose and 2′-deoxyribose third strands, the possible C2′-endo pucker of 2′,5′-linked riboses together with the lack of an α-2′-OH group are believed to be responsible for the selective binding of 2′,5′-RNA to DD and DR duplexes, over RR and RD duplexes. These studies indicate that the use of other oligonucleotide analogues will prove extremely useful in dissecting the contributions of backbone and/or sugar puckering to the recognition of nucleic acid duplexes.

INTRODUCTION

Nucleic acids based on 2′,5′-phosphodiester linkages instead of the predominant 3′,5′-linkage are extremely interesting molecules. 2′,5′-Linked ribonucleic acids (2′,5′-RNA) are produced during the template-directed non-enzymatic polymerization of ribonucleotides (1,2) and occur transiently during RNA splicing reactions (lariat RNA) (3) and in interferon-treated cells [i.e. 2′,5′-τ(Ap)4A; 4]. In addition, their study has become an area of great interest for their possible role in the prebiotic world (1,2,5,6) and potential use in the artificial regulation of gene expression (7,8). Much work has begun to focus on the formation of double and triple helices with 2′,5′-linked nucleic acids (9–28). Early studies on the interaction of RNA with small 2′,5′-RNA strands revealed the ability of 2′,5′-linked ribodeoxyanetides to form complexes in 1:2 stoichiometry with 3′,5′-linked poly(U) (9–13). Turner (14) and Damha (13,15) have described the self-association of 2′,5′-RNA, whereas Breslow and Switzer have explored the properties of 2′,5′-linked 3′-deoxyribonucleic acids (2′,5′-DNA) (16–18). The latter workers reported that 2′,5′-linked DNA containing adenine and thymine/uracil bases do not form stable duplexes, but rather favours triplex formation, and only at high salt concentrations. In contrast, 2′,5′-DNA based on cytosines and guanines associate into duplex structures at low ionic strength (19,20). Also intriguing is the recent finding that strands of 2′,5′-RNA and 2′,5′-DNA have the ability to discriminate between single-stranded RNA and DNA, forming duplexes only with RNA (12,13,15,21–23). Such RNA binding selectivity has also been observed with other 2′,5′-linked analogues (24), suggesting that this is a general property of oligonucleotides constructed with 2′,5′-linkages. More recently there has been considerable interest in the antisense properties of 2′,5′-DNA (25) and 2′,5′-RNA (15,26) which resist enzymatic hydrolysis and show less non-specific binding to plasma and cellular proteins in comparison with 3′,5′-linked analogues. Apart from a few theoretical studies (27,28), little is known about the recognition of 3′,5′-linked duplexes by 2′,5′-linked nucleic acids. The ability of 2′,5′-linked oligonucleotides to act as a ‘third strand’ in triplex formation can be extremely important not only for biomedical applications (‘antigene strategy’), but also for gaining a better understanding of the molecular forces that stabilize triple helices.

It is known that the presence of a 2′-OH group in third strand oligoribonucleotides (R) thermally stabilizes triplexes relative to triplexes containing the corresponding 2′-deoxy oligoribonucleotide third strand (D) (29–31). Importantly, an RNA pyrimidine third strand binds to all four possible duplex combinations with each of the two duplex strands composed of either DNA or RNA (i.e. DD, DR, RD and RR), whereas a DNA pyrimidine third strand binds only when the polypurine strand of the duplex is DNA (i.e. DD and DR). What are the properties of a 2′,5′-RNA third strand? In terms of duplex recognition, does 2′,5′-RNA ‘mimic’ a DNA third strand or the regioisomeric RNA third strand? These questions were examined by conducting a comparative study of triplexes formed by 2′,5′-RNA, RNA and DNA third strands using a combination of gel electrophoresis and spectroscopic techniques. Our results are reported below.

MATERIALS AND METHODS

Oligoribonucleotide R* (2′,5′-linked), whose sequence is shown in Figure 1, was synthesized using silyl-phosphoramidite chemistry as previously described (15). The oligonucleotide was purified by gel electrophoresis and desalted via size exclusion chromatography (Sephadex G-25; Pharmacia). The isolated product gave satisfactory MALDI-TOF mass spectra (calculated M+ 3602.4; found

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Figure 1. Hairpin duplexes and single strands selected for study. The DNA and 2′,5′-RNA sequences are shown in bold and italic, respectively.

The hairpin duplexes DR, RD and RR and the control strand R (Fig. 1) were synthesized according to Damha and Ogilvie (32). The DD hairpin and D sequence were obtained commercially.

Molar extinction coefficients for oligonucleotides were calculated from those of the mononucleotides and dinucleotides according to nearest neighbour approximations (33). The values for the hybrid hairpins were assumed to be the sum of their D plus R components: DD, 26.5; DR, 27.1; RD, 26.7; RR, 27.7. The molar extinction coefficient for the 2′,5′-RNA (R*) strand was assumed to be the same as for the normal RNA strand (9.6 × 10^4/M/cm). Complexes were prepared by mixing equimolar amounts of interacting strands, e.g. R* + hairpin DD, and lyophilizing the resulting mixture to dryness. The resulting pellet was then re-dissolved in the buffer. The solutions were 2 mM in each strand, 100 mM NaOAc, 1 mM EDTA (pH 5.5). The solutions were then heated to 80°C for 15 min, cooled slowly to room temperature and stored at 4°C overnight before measurements. Melting curves were determined using a Varian Cary Model 1 UV spectrophotometer equipped with a Peltier temperature programmer for automatically increasing the temperature at a rate of 0.5°C/min. Melting temperatures (Tm) were calculated from the first derivative of the melting curves. Gel electrophoresis (mobility shift assays) and CD measurements were carried out as described previously (34).

RESULTS

Experimental design

In order to examine the interaction of 2′,5′-RNA with duplexes the experimental design of Roberts and Crothers was adapted (29). The target duplexes are Pu/Py hairpins and contain the four possible combinations of DNA and RNA strands (designated DD, DR, RD and RR, where the first letter describes the 5′-homopyrimidine stem strand that binds to the third strand and the second letter the 3′-homopyrimidine sequence) (Fig. 1). The 2′,5′-linked oligoribopyrimidine strand, designated R*, was chemically synthesized to explore triple helix formation with the hairpin duplexes.

The equilibrium between single-, double- and triple-stranded species was first monitored by polyacrylamide gel electrophoresis. This method provides a convenient way to monitor triplex formation and serves as a qualitative check on the stoichiometry of interaction of the strands (35). The results in Figure 2 show that the 2′,5′-RNA strand, hairpins and triple helical complexes can be separated with excellent resolution under the experimental conditions (4°C, pH 5.5). The results also show that R* interacted with hairpins DD and DR in a 1:1 stoichiometry, as evidenced by the appearance of a new (triplex) band of reduced mobility. This is in contrast to the 3′,5′-RNA (R) strand that formed a stable triplex with all DD, DR, RD and RR duplexes (29–31,34). The results also show that R* bound to DD and DR, but not RD and RR, exactly parallels what was observed for D third strands (29–31,34). This is in clear contrast to the 3′,5′-RNA (R) strand that formed a stable triplex with all DD, DR, RD and RR duplexes (29–31,34).
The low temperature transition was assigned to the dissociation of the R* strand from the target DD and DR duplexes. Biphasic melting behaviour was also observed for the control triplexes D-DD and D-DR, R-DD and R-DR, in agreement with the results of Roberts and Crothers (29; Fig. 3). The \( T_m \) data given in Table 1 show that the low temperature transitions resulting from mixtures of \( R + DD \) or DR hairpins are considerably higher than \( T_m \) values for transitions from the corresponding mixtures containing \( R^* \) or \( D \) strands. For example, the \( T_m \) for the dissociation of \( R \) from the DD hairpin is 62°C, compared with 35 and 38°C for the dissociation of the \( R^* \) and \( D \) oligonucleotides, respectively. Biphasic behaviour (triplex formation) was observed for \( R + RD \) and \( R + RR \) mixtures, in agreement with the results of Roberts and Crothers (29). For the mixtures of \( R^* \) (or \( D \)) + hairpins RD or RR only the high temperature transition corresponding to the melting of the duplex was observed, confirming the gel experiments that \( R^* \)-RD and \( R^* \)-RR (and D-RD and D-RR triplexes) do not form under these conditions (Fig. 3 and Table 1).

**Table 1.** Thermal dissociation of complexes in 100 mM sodium acetate, 1 mM EDTA buffer (pH 5.5).  

<table>
<thead>
<tr>
<th>Duplex</th>
<th>Third strand</th>
<th>( T_m ) (°C)</th>
<th>1st</th>
<th>2nd</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>D</td>
<td>38</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>62</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2′5′ R</td>
<td>35</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>DR</td>
<td>D</td>
<td>45</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td></td>
<td>69a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2′5′ R</td>
<td>39</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>RD</td>
<td>D</td>
<td></td>
<td>84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>43</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2′5′ R</td>
<td></td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>D</td>
<td></td>
<td>84</td>
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</tr>
<tr>
<td></td>
<td>R</td>
<td>45</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2′5′ R</td>
<td></td>
<td>84</td>
<td></td>
</tr>
</tbody>
</table>

Oligonucleotide concentration is 2.0 μM in each strand.  

*a* Single transition for \( R \)-DR (triplex) \( \rightarrow \) \( R \) + helix DR (duplex) \( \rightarrow \) \( R \) + DR (coil) processes (29).  

As noted by Roberts and Crothers (29), the hairpin duplexes exhibited considerable differences in CD spectra, indicating the existence of different conformational families (Fig. 4B). The CD spectra of the DR hybrid is closer to that of the pure DD duplex, while the CD spectrum of RD resembles that of the pure RR duplex. The situation is different in the case of the triplexes, which exhibited appreciable spectral similarities (Fig. 4C–F). For example, the spectra of \( R^* \)-DR is strikingly similar to that of \( R \)-DR, being only slightly different to the \( D \)-DR spectra (Fig. 4D). These similarities can be ascribed to the conformation of the underlying duplex (DR) which dominates the CD spectrum. The CD spectra of a 1:1 mixture of \( R^* \) and RD or \( R^* \) and RR show reduced band intensities and did not differ significantly from the calculated average of the spectra of \( R^* + RD \) or \( R^* \) + RR, consistent with the lack of association of these compounds.

**DISCUSSION**

We have shown that single-stranded 2′,5′-RNA binds to duplex DNA (DD) and hybrid DNA (Pu):RNA (Py) (DR). When the duplex purine strand is RNA and the duplex pyrimidine strand is DNA or RNA (i.e. RD or RR), triplex formation is not observed. These results exactly parallel what is observed for DNA (29–31) and 3′,5′-linked arabinonucleic acid (ANA) strands (Fig. 5; 34). In fact, based on the \( T_m \) data the affinities of 2′,5′-RNA, DNA and ANA third strands towards DD and DR duplexes are also similar. This is in clear contrast to an RNA third strand which forms significantly more stable triplexes with all four hairpins (Table 1 and Figs 2 and 3). The following hypotheses can be used to explain these observations.

(i) 2′,5′-RNA, DNA and ANA third strands have similar conformations (e.g. adopt the same ring puckering) and differ from the RNA conformation. In agreement with this hypothesis, DNA favours the C2′-endo conformation, whereas RNA favours the C3′-endo conformation (36). This appears to also be the case when DNA or RNA third strands fit into a duplex major groove (37–39). For instance, Taillandier and co-workers have shown the existence of C2′-endo sugars in all three strands of the \( dT \)-dAdT (DD) triplex (37), whereas C3′-endo sugars are observed in the third strand of R-DD triplexes (38). In the case of 2′,5′-RNA, calculations predict a C2′-endo pucker for a pure 2′,5′-RNA duplex (27) and NMR studies of small 2′,5′-RNA oligomers revealed the existence of a predominant C2′-endo conformation (40,41). Also, NMR and X-ray diffraction investigations of a duplex DNA containing araC inserts have shown that the arabinose sugars take the C2′-like-endo form (42–44). Thus, a common sugar pucker in 2′,5′-RNA, DNA and ANA may explain, at least in part, the similar thermal stabilities of \( R^* \)-DNA and \( R^* \)-RNA strands.

(ii) Replacing the duplex DNA purine strand to an RNA purine strand (e.g. \( DD \) → \( RD \)) changes the sugar pucker from a C2′-endo to a C3′-endo form; this conformation would be compatible with the binding of a RNA third strand (C3′-endo), but not with the binding of 2′,5′-RNA, DNA and RNA strands (C2′-endo) (31). This would account for the observed selectivity of \( D \), \( R \) and \( A \) strands for DD and DR duplexes, over RD and RR duplexes. Furthermore, there is ample evidence that [ \( DD \) and \( RD \) or \( RR \) ] duplexes belong to different conformational families (45–49). The work of Gray and co-workers (45) and others (47,48) have shown dramatic CD differences in the spectra of RD...
Figure 4. Circular dichroism (CD) of (A) single strands, (B) hairpin duplexes, (C–F) mixtures of hairpin + single strands as indicated. Concentration is 2 µM in each strand and the buffer is 100 mM sodium acetate, 1 mM EDTA, pH 5.5.

(Pu/Py) and DR (Pu/Py) hybrids (for example the spectra of RD and DR, Fig. 4B). Lesnik and Freier (49) have also found that the electrophoretic mobilities of DD versus RD hybrids are different, indicating the existence of different classes of conformation. RR and RD hybrids are conformationally confined to the A-form helices with narrow and deep grooves, whereas DD and DR (Pu/Py) duplexes adopt B- and B/A-like forms, respectively (48,50). In the case of DD and DR duplexes, the cavity of the major groove is wider and thus there may be more room to optimize third strand interactions.

(iii) The sugars of an RNA third strand favour the formation of short contacts between the α-2′-OH groups and phosphate groups of the duplex purine strand (31). This proposed intermolecular contact may account for the observation that RNA (but not DNA, ANA and 2′,5′-RNA) recognizes RR and RD duplexes. Such a mechanism cannot occur with DNA and may not be possible for ANA (β-2′-OH) and 2′,5′-RNA (α-3′-OH) strands since their sugar hydroxyl groups are oriented differently.

On the basis of the available data, we cannot decide which of the above effects is more important, but we suspect that more than one of these are operating in an important way.
In summary, our results show that 2',5'-RNA is able to recognize double helical DNA and DNA (purine):RNA (pyrimidine) hybrids. With respect to the hybridization behaviour of 2',5'-RNA versus the regioisomeric RNA, the following principles apply. If the target nucleic acid is single-stranded DNA or a double helix with an RNA in the purine strand, only RNA will bind. If the target is single-stranded RNA or a double helix containing a DNA in the purine strand, RNA or 2',5'-RNA will bind. In all cases, the duplexes or triplexes formed by 2',5'-RNA are thermally less stable than those formed by 3',5'-RNA. Our results also suggest that triplex stability is governed not only by the chemical nature of the third strand (e.g. ribose versus arabino or 3'-linked RNA versus 2',5'-linked RNA), but more precisely by their backbone conformation. In analogy to the arabinose and 2'-deoxyribose third strands, the possible C2'-endo puckers together with DD and RR backbones are believed to be responsible for the selective binding of 2',5'-RNA to DD and DR duplexes, over RR and RD duplexes. Further work utilizing 2'-deoxy third strands with locked C2'-endo and C3'-endo puckers together with DD and RR duplexes, in order to uncover the relative importance of the proposed 2'-OH/phosphate contact mechanism (31) and the sugar conformation of the third strand, is in progress.

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