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Modeling, Synthesis, and Hybridization Properties of (L)-Ribonucleic Acid

Gary W. Ashley

Contribution from the Department of Chemistry, Northwestern University,
Evanston, Illinois 60208. Received March 9, 1992

Abstract: Calculations suggest that nucleic acids based on (L)-ribose may hybridize with natural configuration DNA and RNA if a loosely wound, parallel-stranded A-like formation can be adopted. The tendency of RNA to adopt A-form helices suggest that (L)-nucleic acids may bind more tightly to natural RNA than to natural DNA. Oligomers of (L)-dU and (L)-rU have been synthesized to test these models. Neither [(L)-dU]₁₂ nor [(L)-rU]₁₂ mixed with natural poly(dA) shows hyperchromicity indicative of hybridization, nor does a mixture of [(L)-dU]₁₂ with poly(rA). Hybridization is observed between [(L)-dU]₂₀ and poly(rA) ($T_m = 31^\circ\text{C}$ in 10 mM Mg^{2+}), although this is substantially weaker than that observed between [(D)-dU]₂₀ and poly(rA) ($T_m = 42^\circ\text{C}$ in 10 mM Mg^{2+}). Mixtures of [(L)-rU]₁₂ and poly(rA) show strong hybridization ($T_m = 38^\circ\text{C}$ in 1.0 M NaCl), comparable to that observed between [(D)-rU]₁₂ and poly(rA) ($T_m = 40^\circ\text{C}$ in 1.0 M NaCl). Both RNA:RNA systems form triplex structures. (L)-RNA is resistant to both purified ribonuclease A and total cell extracts of L-cells.

The great potential of antisense oligonucleotides as antiviral and anticancer agents is compromised by their instability toward cellular nucleases. Of the many attempts to develop modified oligomers for use as antisense agents, no completely satisfactory approach has been discovered.¹ Alteration of DNA stereochemistry is one potential route for increasing metabolic stability, yet attempts to do this while maintaining high affinity for hybridization have met with mixed success. Inversion of the anomeric center, producing "α-DNA," results in strong parallel-stranded hybrids with natural-configuration DNA and RNA.² Duplexes between α-DNA and natural RNA are resistant to attack by

ribonuclease H, so that they do not appear to be effective antisense agents. A strategy based on the completely enantiomeric (L)-DNA, containing nucleosides based on 2'-deoxy-(L)-ribose, appears unlikely to be successful due to the relatively poor hybridization of (L)-DNA with natural RNA.³ We report here the ability of oligo-(L)-ribonucleotides, (L)-RNA, to recognize natural RNA with high affinity. (L)-RNA is resistant to ribonucleases, and so may provide a useful means of targeting cellular and viral RNA sequences in antisense strategies.

Experimental Section

Modeling. Molecular modeling calculations were performed on a Silicon Graphics 4D/25 workstation using BioGraf (Polygen/Molecular Simulations, Inc.) with a united-atom implementation of the AMBER force field. Electrostatic energies were calculated using a distance-dependent dielectric $\epsilon = \epsilon_0 r_{ij}$, with $\epsilon_0 = 1$.⁴ Qualitatively similar results were obtained using the all-atom Dreiding force field.⁵ The template

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Table I. Model Energies

complex	total	bond	angle	torsn	inv	vdW	Q	H bond
B-form Duplexes								
(D)-dA:(D)-dU	-508.8	8.6	119.8	323.2	2.9	-275.7	-476.0	-211.6
(D)-dA:(L)-dU	-426.5	13.7	181.0	336.5	6.2	-252.7	-496.2	-215.1
A-form Duplexes								
(D)-dA:(D)-dU	-322.5	10.5	144.8	320.8	3.3	-260.9	-327.1	-213.9
(D)-dA:(L)-dU	-340.4	11.5	162.9	338.6	3.9	-244.6	-397.2	-215.4
Triplexes^a								
(D)-dA:(D)-dU:(D)-dU	-771.8	23.2	191.3	578.6	7.8	-471.9	-687.6	-413.2
(D)-dA:(L)-dU:(L)-dU	-769.6	21.7	238.4	562.9	7.8	-413.3	-766.3	-420.8
(D)-dA:(D)-dU:(L)-dU	-714.1	22.0	235.7	524.9	6.3	-432.2	-637.5	-433.3

^aStrands in triplexes are listed in order of Watson:Crick:Hoogsteen.

strand [(D)-dA]₁₂ was built using standard coordinates for either A-form or B-form. The enantiomeric strand [(L)-dU]₁₂ was built in natural configuration and then inverted. To construct the double helix, the template strand was held fixed while the enantiomeric strand was docked so as to form standard Watson-Crick base pairs. The enantiomeric strand was subjected to energy minimization while constraining the base pairs. A period of annealing molecular dynamics (heating to 500 K followed by slow cooling to 0 K) was then used to shake the system into a lower energy conformation. Finally, both strands were allowed to move during energy minimization without constraints. Triplex structures were constructed in a similar manner.

5'-O-(4,4'-Dimethoxytrityl)-2'-deoxy-(L)-uridine 3'-(O-Cyanoethyl N,N-Diisopropylphosphoramidite). 2'-Deoxy-(L)-uridine⁶ was converted to 5'-O-DMT-2'-deoxy-(L)-uridine⁷ and subsequently to the O-cyanoethyl N,N-diisopropylphosphoramidite.⁸ The product was purified by flash chromatography on SiO₂ using ethyl acetate. This material is identical in all respects to the natural-configuration material (Sigma Chemical Co.) except for optical rotation.

Synthesis of [(L)-dU]₁₂. Long-chain alkylamine-controlled pore glass (500 Å, Sigma Chemical Co.) was derivatized with 5'-O-(4,4'-dimethoxytrityl)-(L)-2'-deoxyuridine through an oxalyl linker.⁹ Oligomer synthesis using the syringe technique¹⁰ consisted of (1) detritylation using 3% CHCl₂COOH/CH₂Cl₂; (2) neutralization using 3:1 CH₃CN/pyridine; (3) washing with CH₃CN; (4) coupling with equal volumes of 0.1 M phosphoramidite in CH₃CN and 0.4 M tetrazole in CH₃CN for 5 min; (5) washing with CH₃CN; (6) oxidation and capping with 0.1 M I₂ in 9:1 pyridine/acetic acid; and (7) washing with CH₃CN. Trityl yields were measured after each coupling and averaged 98%. After removal of the final DMT group, the glass beads were suspended in 1 mL of 14 M NH₃ and kept at 55 °C for 6 h. The product was purified by anion exchange on Sepharose QFF (0.1–1.0 M NaCl gradient in 10 mM NaOH) followed by neutralization (KH₂PO₄) and desalting by C18 reversed-phase HPLC. The purified product was analyzed for purity using reversed-phase HPLC, polyacrylamide gel electrophoresis, and ¹H-NMR.

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(tert-butylidimethylsilyl)-(L)-uridine 3'-(O-Cyanoethyl N,N-Diisopropylphosphoramidite). (L)-Uridine¹¹ was converted to 5'-O-DMT-(L)-uridine⁷ and then silylated.¹² The 2'-silylated derivative was isolated by flash chromatography on a short SiO₂ column. Conversion to the phosphoramidite⁸ followed by flash chromatography using ether gave the product as a white foam, spectroscopically identical with natural-configuration material (OmniChem, Wheaton, IL). ³¹P-NMR (CDCl₃): δ 147.5, 147.0 ppm.

Synthesis of [(L)-rU]₁₂. 5'-O-DMT-2'-O-TBDMS-(L)-rU was attached to long-chain alkylamine-controlled pore glass through an oxalyl linker, yielding 25 μmol of DMT-nucleoside per gram of glass bead.⁹ RNA oligomerization used the same protocol as that for the DNA synthesis above except that the coupling time was extended to 60 min. After the NH₃ treatment, the silylated RNA was carefully dried in vacuo and then

dissolved in 1 mL of 1.0 M Bu₄N⁺F⁻ in tetrahydrofuran (Aldrich Chemical Co.) for every 100 OD₂₆₀ units of RNA. After 48 h, the mixture was diluted with 1 mL of 0.1 M triethylammonium acetate, pH 6.5, and extracted three times with ether. The water phase was chromatographed on Sepharose QFF (0.1–1.0 M NaCl gradient in 10 mM phosphate, pH 6.5). The purified RNA was desalted by C18 reversed-phase HPLC. The product migrated as a single peak by reversed-phase HPLC.

Synthesis of [(D)-rU]₁₂. The natural-configuration RNA was synthesized as described above using commercially available reagents (OmniChem). The initial nucleotide was attached to CPG via a succinate linker. All aqueous solutions used after treatment with Bu₄N⁺F⁻ were treated with 0.1% diethyl pyrocarbonate for 12 h followed by autoclaving. The product migrated as a single peak by reversed-phase HPLC.

Nuclease Resistance. Reaction mixtures containing 1 OD₂₆₀ unit of either [(D)-rU]₁₂ or [(L)-rU]₁₂ and total soluble L-cell extract (30 μg total protein) in 50 μL of 50 mM Tris-HCl, pH 8.0, 10 mM MgCl₂, 1 mM EDTA, and 40 mM KCl were kept at 37 °C. Aliquots of 10 μL were removed periodically and injected onto a reversed-phase HPLC column equilibrated in 5% CH₃CN in 0.1 M Et₃N-HOAc, pH 6. After washing for 5 min, a gradient to 50% CH₃CN was run over 45 min. Peaks were detected by UV absorbance at 260 nm. Reactions using purified ribonuclease A (Sigma Chemical Co.) were run similarly.

¹H-NMR of Imino Protons. Samples were mixed in 0.5 mL of 90:10 v/v H₂O/D₂O containing 10 mM phosphate, pH 6.8, 0.1 mM EDTA, 100 mM NaCl, and 5 mM MgCl₂ and then annealed by heating in boiling H₂O followed by slow cooling to ambient temperature. Spectra were recorded at 600 MHz using a 1–1 excitation pulse set to give maximal excitation at 12 ppm.

Thermal Denaturation Profiles. Samples were dissolved in 3.0 mL of 10 mM phosphate, pH 6.8, containing the desired concentration of either NaCl or MgCl₂, in a stoppered quartz cuvette. The cell temperature was set using a Hewlett-Packard 89090A Peltier effect programmer controlled by Tempco software (Hewlett-Packard). The absorbance at 260 nm was measured every 0.5 °C, with a temperature ramp of approximately 0.2 °C/min. Melting temperatures were calculated from first derivative plots of absorbance versus temperature.

Results

Modeling. DNA modeling calculations were used to investigate the hybridization between two nucleic acids of opposing chirality. Given the very large number of possible conformations for the mixed-enantiomer complexes as well as potential inaccuracies in the simplified calculations, the study was limited to a direct comparison of mixed-enantiomer complexes and natural-configuration complexes in which the natural "template" strand was built in a standard conformation. Such a comparison should point out specific difficulties and advantages with mixed-enantiomer hybridization which might then be addressed through suitable molecular redesign. Complexes will be abbreviated according to their stereochemistry in the order (dA)₁₂:(dU)₁₂, such that the [(D)-dA]₁₂:[(L)-dU]₁₂ complex is abbreviated DL.

The complementary [(L)-rU]₁₂ strand was hybridized with the fixed template using standard Watson-Crick base pairs. This necessitated a parallel orientation of the strands, in agreement with previous suggestions.¹³

The B-like DL duplex was substantially higher in energy than the natural DD B-form duplex (Table I), primarily due to a large

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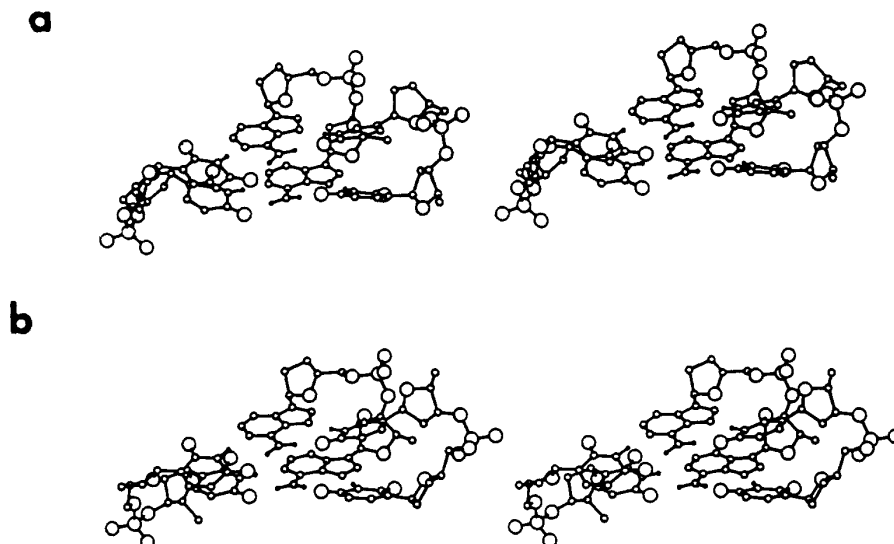


Figure 1. Representative base triplets showing orientation and stacking in natural and mixed-enantiomer triplex models: (a) (dA):2[(D)-dU] model (DDD) built according to 11-fold symmetric U-A-U triplex; (b) (dA):2[(L)-dU] model (DLL). Only two base triplets from each model are shown as stereo drawings for clarity.

increase in angle strain resulting from reversed helical winding of the (L)-strand. This is partially offset by a decrease in electrostatic repulsion. The reversed winding of the (L)-strand results in an extended conformation ($\gamma = 180^\circ$) about the phosphates, leading to increased intrastrand phosphate-phosphate distances and lowered anion-anion repulsion. Neutralization of the phosphate charges on the (L)-strand in the DL duplex and the cognate (D)-strand in the DD duplex resulted in equivalent electrostatic energies for the two complexes, in agreement with this analysis. Contributions from interstrand anion repulsion are minimal due to screening by the distance-dependent dielectric constant used in these calculations.

The A-like mixed duplex was comparable in energy to the natural A-form duplex. The increase in radius of curvature in going from the tightly wound B-form to the loose A-form conformation lessens the strain incurred by bending the (L)-strand backwards against its normal direction of helicity. Near this point, the more favorable electrostatic term is able to compensate for the unfavorable angle strain. While such compensation appears to lie at the heart of mixed-enantiomer hybridization, it should be noted that the electrostatic energies are particularly simplistic in these models,⁴ making it impossible to predict the exact point of compensation.

While it is not possible to predict solution-phase stabilities based on this data, the modeling results suggest that DL hybrids are more likely to form in systems predisposed toward more loosely wound, A-like conformations. Such mixed-enantiomer duplexes have previously been modeled as high-energy, essentially nonhelical sheets,¹³ or as B-like structures of unspecified energy.¹⁴ The present calculations suggest that a helical rather than sheet-like conformation may be preferred, as some base stacking interactions are preserved, and also make some predictions as to hybridization properties. It has been observed that natural DNA:DNA duplexes adopt the B-form in solution, while DNA:RNA and RNA:RNA duplexes adopt A-type conformations.¹⁵ Thus, it is expected that (L)-DNA and (L)-RNA may form more stable complexes with natural RNA than with natural DNA.

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Table II. Melting Temperatures for dU and rU Complexes

system	salt	T_m , °C
[(D)-dU] ₁₁ :poly(dA)	1.0 M NaCl	32
[(D)-dU] ₁₁ :poly(rA)	1.0 M NaCl	32
[(L)-dU] ₁₂ :poly(dA)	1.0 M NaCl	nto ^a
[(L)-dU] ₁₂ :poly(rA)	1.0 M NaCl	nto
[(D)-dU] ₂₀ :poly(dA)	5 mM MgCl ₂	46
[(D)-dU] ₂₀ :poly(rA)	5 mM MgCl ₂	41
[(L)-dU] ₂₀ :poly(dA)	5 mM MgCl ₂	nto
[(L)-dU] ₂₀ :poly(rA)	5 mM MgCl ₂	32
2[(D)-rU] ₁₂ :poly(dA)	1.0 M NaCl	30
2[(D)-rU] ₁₂ :poly(rA)	1.0 M NaCl	40
2[(L)-rU] ₁₂ :poly(dA)	1.0 M NaCl	nto
2[(L)-rU] ₁₂ :poly(rA)	1.0 M NaCl	38

^aNo hyperchromic transition observed = nto.

Triplex structures consisting of one dA₁₂ strand and two dU₁₂ strands were modeled similarly starting from the 11-fold symmetric U-A-U model¹⁶ (Table I). Such triplexes will be abbreviated by their stereochemistry in the order of (A):Watson-Crick-(U):Hoogsteen-(U). Again, inversion of the pyrimidine strands required hybridization with opposite strand polarity from the natural case (Figure 1). While the DLL triplex was of comparable energy to the natural DDD triplex, inversion of the Hoogsteen strand only to give a DDL triplex resulted in a highly unfavorable energy. The models suggest a complex basis for this result. Each strand inversion is accompanied by a decrease in electrostatic repulsion and an increase in angle strain, as discussed above. Inversion of the Hoogsteen strand is also accompanied by an increase in electrostatic repulsion due to the closer distance between the A- and Hoogsteen-U-backbones. For the DLL triplex, the effect of two strand inversions is sufficient to counterbalance the added

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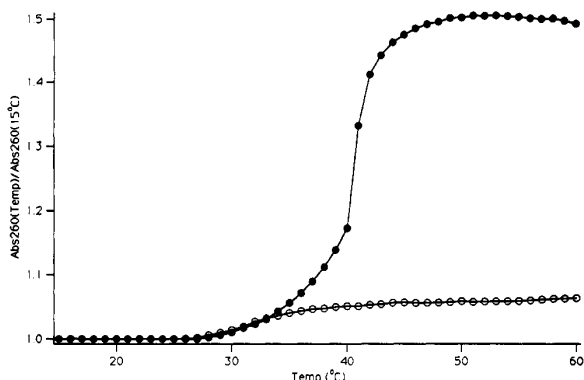


Figure 2. Normalized melting profiles for 1:1 mixtures of dU_n oligomers and poly(rA) in 10 mM MgCl₂, 0.01 M phosphate (pH 6.8), total nucleotide concentration 0.13 mM: (●) [(D)-dU]₂₀; (○) [(L)-dU]₂₀. Absorbances are normalized to their values at 15 °C.

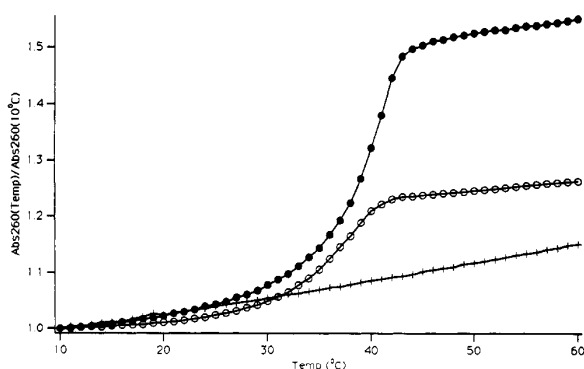


Figure 3. Normalized melting profiles for 2:1 mixtures of rU₁₂ with poly(rA) in 1.0 M NaCl, 0.01 M phosphate (pH 6.8), total nucleotide concentration 0.1 mM: (●) [(D)-rU]₁₂; (○) [(L)-rU]₁₂; (+) [(L)-rU]₁₂ with poly(dA).

cross-strand repulsion, but the gain from the single strand inversion in DDL is not. The electrostatic energy term for DDL is thus substantially more positive than that for DLL. It thus seems possible that two (L)-strands may pair with a natural strand to form a DLL triplex, yet the (L)-strand is unlikely to act as the Hoogsteen strand in a DDL triplex with a natural duplex structure under typical ionic strengths.

Synthesis. In order to test the results of the modeling calculations, oligomers of (L)-dU, (D)-dU, (L)-rU, and (D)-rU were prepared by solid-phase synthesis. The (L)-nucleosides were prepared according to Holy^{6,11} and derivatized using standard procedures.^{7,8} The 242-MHz ³¹P-NMR spectrum of the (L)-rU phosphoramidite revealed only two peaks, indicating either that migration of the silyl group had not occurred during synthesis or that the undesired 3'-silyl isomer had been removed by chromatography.²³ Oligomerization using standard protocols¹⁰ produced the desired materials as determined by reversed-phase HPLC and ¹H-NMR analysis.

Hybridization Properties. Hybridization of the dU oligomers with poly(rA) or poly(dA) was investigated by measurement of thermal denaturation profiles.¹⁸ In either 1.0 M NaCl or 10 mM MgCl₂, no hyperchromic transitions indicative of hybridization could be observed for mixtures of [(L)-dU]₁₂ with either poly(rA) or poly(dA) (Table II). This is in agreement with earlier observations on [(L)-dT]₈ but differs from the behavior of the complementary [(L)-dA]₆:poly(rU) system.³ Comparison with [(D)-dU]₁₁ indicates that the *T*_m for any complex between [(L)-dU]₁₂ and poly(dA) or poly(rA) must be at least 20 °C lower

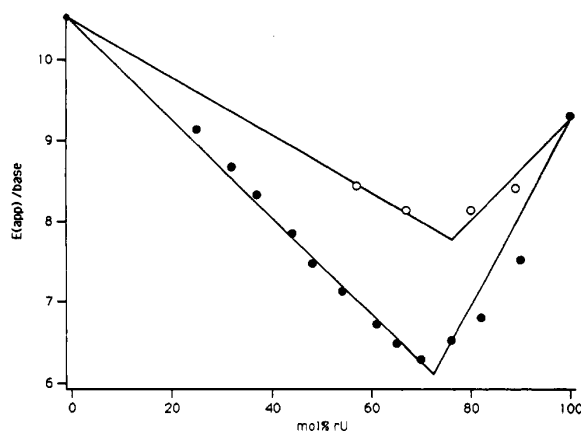


Figure 4. Analysis of complex stoichiometry for mixtures of either [(D)-rU]₁₂ (●) or [(L)-rU]₁₂ (○) with poly(rA) in 1.0 M NaCl. Aliquots or poly(rA) were added to a cuvette containing the rU oligomer thermostated at 10 °C.

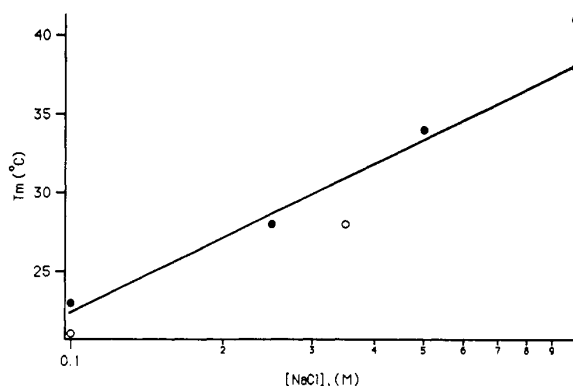


Figure 5. Variation of *T*_m with [Na⁺] for 2:1 mixtures of either [(L)-rU]₁₂ (●) or [(D)-rU]₁₂ (○) with poly(rA). The two complexes show essentially identical behavior.

than the corresponding natural complex. Weak hybridization was observed with a mixture of [(L)-dU]₂₀ and poly(rA) in 10 mM MgCl₂, although both the *T*_m and hyperchromicity are substantially lower than that of the natural system (Figure 2). No transition was observed for the complementary mixture of [(L)-rU]₁₂ and poly(dA) under either ionic strength condition (Figure 3). In contrast, a mixture of [(L)-rU]₁₂ and poly(rA) in 1.0 M NaCl shows a sharp hyperchromic transition indicative of hybridization (Figure 3). A single transition was observed with both 1:1 and 2:1 mixtures of [(L)-rU]₁₂ and poly(rA). This is similar to the behavior shown by the corresponding all-(D) system under the same conditions. No evidence of triplex formation was observed with mixtures of [(L)-rU]₁₂ and poly(dA):poly(T) in either 1.0 M NaCl or 10 mM MgCl₂.

The method of continuous variations¹⁹ was used to estimate the stoichiometry of the (L)-RNA complexes in 1 M NaCl. This indicated triplex formation for both [(D)-rU]₁₂ and [(L)-rU]₁₂ when mixed with poly(rA) (Figure 4). This is in agreement with previous results on the natural-configuration homopolymeric systems.²⁰

The dependence of *T*_m on [Na⁺] was determined for 2:1 mixtures of either [(L)-rU]₁₂ or [(D)-rU]₁₂ with poly(rA). The stabilities of both complexes show a linear dependence on [Na⁺], with a change of approximately 15 °C for each 10-fold change in cation concentration (Figure 5). This is essentially identical to previous observations on oligomer triplexes.²¹

Base pairing was also examined using NMR. Examination of a 2:1 mixture of [(L)-rU]₁₂ and poly(rA) in 90:10 H₂O/D₂O containing 0.1 M NaCl revealed two poorly resolved imino proton resonances at δ 13.0 and 12.6 and a third resonance of roughly

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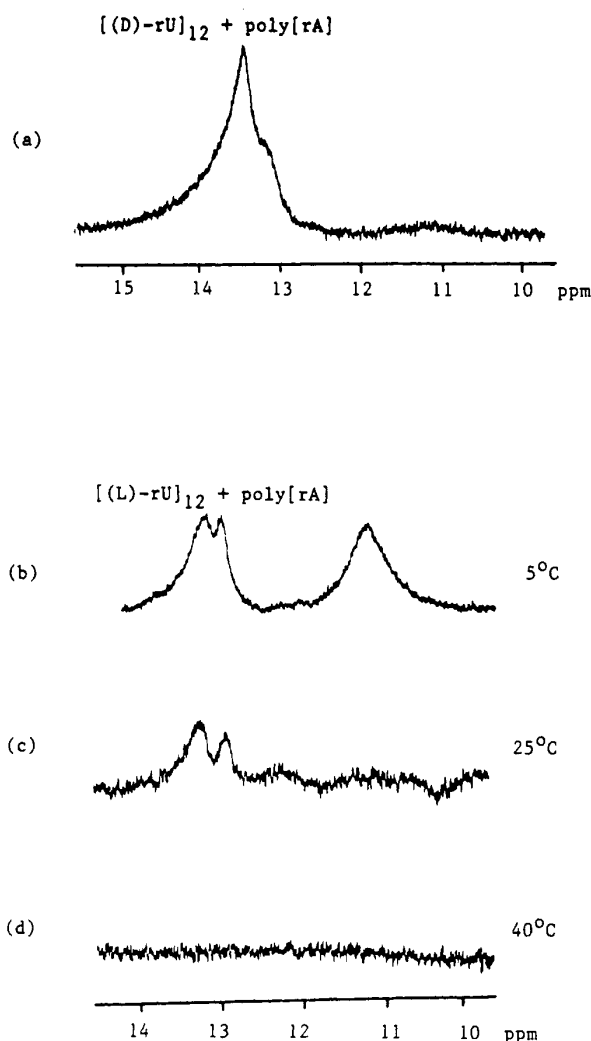


Figure 6. ^1H -NMR spectra of imino proton resonances in 90:10 $\text{H}_2\text{O}/\text{D}_2\text{O}$, 0.1 M NaCl, 5 mM MgCl_2 , 10 mM phosphate, pH 6.8: (a) 2:1 [(D)-rU] $_{12}$ + poly(rA), 5 $^\circ\text{C}$; (b) 2:1 [(L)-rU] $_{12}$ + poly(rA), 5 $^\circ\text{C}$; (c) 2:1 [(L)-rU] $_{12}$ + poly(rA), 25 $^\circ\text{C}$; 2:1 [(L)-rU] $_{12}$ + poly(rA), 40 $^\circ\text{C}$.

equal intensity to the sum of the first two at δ 11.0 (Figure 6). No change was observed upon addition of 5 mM MgCl_2 . The imino resonances disappeared upon increasing the sample temperature to 40 $^\circ\text{C}$, consistent with the expected melting behavior. The corresponding spectrum of 2[(D)-rU] $_{12}$:poly(rA) revealed a single resonance of complex line shape centered at 13.5 ppm.

Selectivity in hybridization was indicated by a lack of hyperchromic transitions with mixtures of [(L)-rU] $_{12}$ and either poly(rG) or poly(rC) in 1 M NaCl.

Nuclease Resistance. Uracine oligomers were initially tested for resistance toward purified ribonuclease A, which prefers cleavage at uridine. Treatment of [(D)-rU] $_{12}$ with RNase A led to rapid degradation within 30 s as indicated by reversed-phase HPLC analysis. Treatment of [(L)-rU] $_{12}$ under the same conditions revealed no evidence of degradation after 48 h. To test for the occurrence of unknown cellular nucleases which might degrade (L)-RNA, the oligomers were treated with whole cell extracts from L-cells (mouse fibroblast cells). Under conditions where [(D)-rU] $_{12}$ was completely degraded in 2 h, no evidence for degradation of [(L)-rU] $_{12}$ was noted over 4 h (Figure 7). The inability of T4 polynucleotide kinase to recognize and end-label (L)-DNA or (L)-RNA has made the use of the more sensitive electrophoretic method for detecting degradation impossible, so that a small amount of degradation could still be occurring.

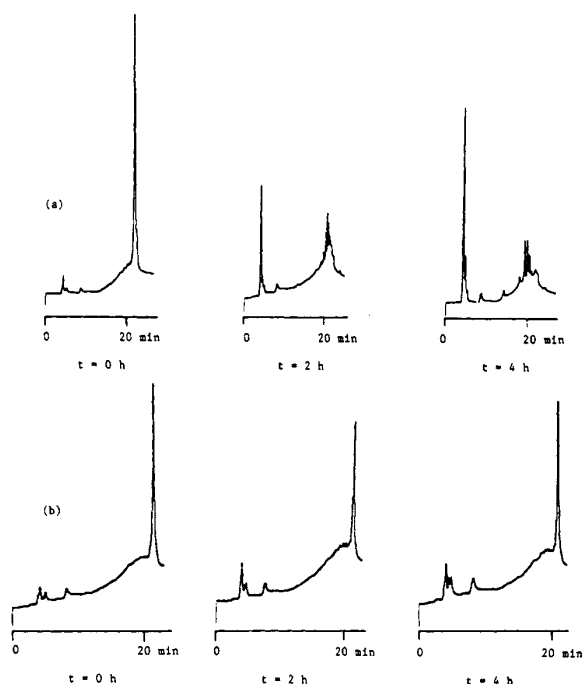


Figure 7. Degradation of rU oligomers by total cellular extracts of mouse fibroblasts (L-cells). Reversed-phase HPLC traces (monitored by absorbance at 260 nm) are shown for (a) [(D)-rU] $_{12}$ and (b) [(L)-rU] $_{12}$ at the indicated times after mixing with protein. The full-length oligomers elute at 21 min into the gradient.

Discussion

The antisense strategy for control of cellular or viral gene expression relies upon sequence-specific recognition of an RNA message, with subsequent interference with translation or destruction of the message through elicitation of RNase H activity. The antisense oligomer may also be used to direct the action of an RNA damaging agent. A major concern is the development of modified oligomers having increased metabolic stability while maintaining the solubility, transport, and recognition properties of the parent oligonucleotides. Inversion of stereochemistry has a long history as a design strategy in increasing the stability of peptide pharmaceuticals, as the "unnatural" configuration greatly decreases peptidase activity. A similar increase in metabolic stability is anticipated for enantiomeric oligonucleotides on the basis of previous results using purified enzymes.³

(L)-RNA was chosen as a design for metabolically stable antisense agents on the basis of molecular modeling calculations, which indicated that heterochiral duplexes could be reasonably stable in parallel-stranded, A-like conformations. (L)-DNA has been found to hybridize either weakly (with respect to the cognate natural systems) or not at all with natural RNA and not with DNA,³ in agreement with this model. The current results corroborate this and indicate that the destabilization of the mixed-enantiomer DNA:RNA duplex is sufficiently great that hybridization is not observable until the (L)-dU oligomers become quite long. The observation of hybridization between [(L)-dA] $_6$ and poly(rU) may be due to the high stacking energy for purines as compared with pyrimidines, resulting in oligomer-oligomer stacking along the poly(rU) strand to make an effectively longer double helical segment. Such inter-oligomer interactions would be less effective with U oligomers. In agreement with this, the oligo(U):poly(A) systems studied here form structures or substantially lower stability than those formed by the complementary oligo(A):poly(U) systems. Indeed, the oligo(U):poly(A) system shows T_m values consistent with values expected for the simple oligomeric system from free-energy parameters.²²

The expected preference for an A-like conformation in mixed-enantiomer complexes suggests that (L)-RNA should form

more stable complexes with natural RNA than does (L)-DNA. Early work of Tazawa et al. demonstrated the possibility of base pairing between [(L)-rA]₂ and poly(rU),²³ although extension of these results to oligomeric and polymeric systems is uncertain. Mixed length (2'-5')/(3'-5') oligomers of (L)-rU have been previously prepared by uncontrolled chemical polymerization,⁶ and one defined (L)-RNA pentamer has been reported.²⁴ ¹H-NMR data for a mixture of the (L)-RNA pentamer with its natural-configuration complement has been interpreted as being indicative of base pairing, but as the same spectrum was observed with both parallel and antiparallel orientations of the complement and these did not change with temperature as expected, the interpretation of this data is unclear.¹³ In the present case, resonances due to the hydrogen-bonded imino protons are observed at low temperatures, but disappear by 40 °C as expected.

(L)-RNA is readily synthesized using the silyl methodology developed by Ogilvie.²⁵ Oligomers of at least 12 nucleotides were prepared so as to investigate hybridization behavior over a full turn of the double helix. This is important for consideration of the effects of helical distortion on the formation of DL complexes of sufficient length for antisense purposes. Whereas short (2-6 base pairs) regions of DL hybrids may form readily, the distortion induced by this may hinder formation of more extensive complexes. Hybridization has also been investigated using both thermal denaturation and ¹H-NMR spectroscopy.

Triplex formation is observed for both the [(L)-rU]₁₂:poly(rA) and [(D)-rU]₁₂:poly(rA) systems, although the hyperchromicity of the DLL system is substantially less than that of the DDD system. The observation of two distinct imino resonance types in the ¹H-NMR spectrum of the DLL complex is consistent with this interpretation. Surprisingly, the corresponding spectrum of DDD revealed a single resonance, although this may be the result of accidental overlap under the experimental conditions. Triplex formation has been demonstrated previously for the natural DNA:DNA, DNA:RNA, and RNA:RNA A:U(T) systems, although in the DNA:RNA case triplexes are observed with poly-(dA):poly(rU) but not with poly(rA):poly(dT).²⁰ The difference in hyperchromicity upon melting of the complexes is most likely due to lessened base stacking within the mixed-enantiomer systems.

The poly(rA):[(L)-dU]₂₀ system also shows a much diminished hyperchromicity compared with the corresponding DD duplex system. Thus while the base pairs were constrained to be well stacked in the modeling calculations, they are probably less so in reality. In contrast, the [(L)-dA]₆:poly(rU) system appears to have identical hyperchromicity as that of the cognate natural triplex.^{3b} The reasons for this difference are not clear at present.

The increased stability of mixed-enantiomer RNA:RNA hybrids over mixed-enantiomer DNA:RNA hybrids is demonstrated by the lack of observable hybridization in mixtures of [(L)-dU]₁₂ and poly(rA) or mixtures of [(L)-rU]₁₂ and poly(dA) at the strand concentrations studied. Hybridization does occur, as indicated by the poly(rA):[(L)-dU]₂₀ system, but it is quite weak. In agreement with this, the triplex between [(L)-dA]₆ and poly(rU) formed in the presence of 10 mM Mg²⁺ has been found to be 25° less stable than the cognate homochiral triplex.^{3b} Thus, (L)-DNA oligomers have only weak affinities for their natural complements in comparison with their natural cognates. The stability of homopolymeric A/U mixed-enantiomer systems appears to increase in the order DNA:DNA < DNA:RNA < RNA:RNA.

[(L)-rU]₁₂ is resistant to both high concentrations of ribonuclease A and total cellular extracts. It appears as though there may not be common cellular ribonucleases with lowered stereospecificity. These results are in accord with early observations on the resistance of heterogeneous (2'-5')/(3'-5') oligomers of (L)-rU to degradation by snake venom phosphodiesterase.⁶ Furthermore, we have found that (L)-nucleic acids are not recognized by T4 polynucleotide kinase (data not shown).

These results show that (L)-RNA effectively recognizes natural-configuration RNA sequences but does not seem likely to interact with cellular RNA binding proteins. Because of these unique properties (L)-RNA may prove useful as a metabolically stable carrier of genetic information, targeting agent for drug delivery, and specific inhibitor of pre-mRNA splicing or gene expression.

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