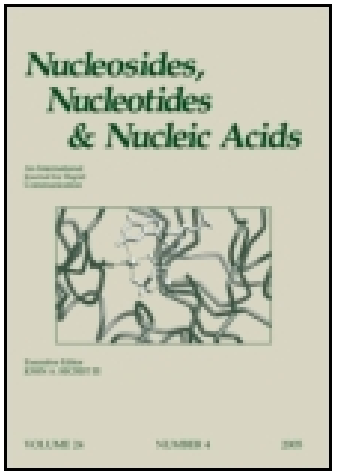

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Chirally-Modifiedoligonucleotides and the Control of Gene Expression. The Case of L-DNAS And-RNAS

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CHIRALLY-MODIFIED OLIGONUCLEOTIDES AND THE CONTROL OF GENE EXPRESSION. THE CASE OF L-DNAS AND -RNAS.

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Abstract: The affinity of L-DNAs, L-RNAs and L/D-DNAs for homopurine•homopyrimidine d.s. D-DNA and s.s. D-RNA was probed by gel electrophoresis and CD spectroscopy. It was found that the L-modified oligomers do not bind to d.s. DNA and to natural RNA that contains all four natural bases. Thus they cannot be used, in general, for the control of gene expression according to the antigene and antisense methodologies. Heterochiral complexes with 1:1 stoichiometry and low thermal stability are formed, instead, by homopurinic L-RNA or L/D-DNA and homopyrimidinic L-RNA with the W/C complementary natural RNA sequences.

INTRODUCTION

Synthetic oligonucleotides are increasingly explored as antiviral and antitumor agents for their potential to inhibit, specifically, the expression of a given gene following the antimessenger (antisense) and antigene strategies. In the former [1], the designed oligomer binds to a Watson/Crick complementary, single-stranded, segment of the target mRNA. In the latter [2], the binding occurs to a Hoogsteen complementary purine sequence, in homopurinic•homopyrimidinic regions of double-stranded DNA, leading to the formation of a triple-helical segment. Since natural oligonucleotides are rapidly degraded by nucleolytic enzymes, a large number of more stable analogues have been devised and tested [3] to verify their ability to bind to natural nucleic acids. L-oligonucleotides, i. e. sequences where the natural D-ribose is replaced by L-ribose, have soon attracted attention [4-6], because they are highly resistant to nuclease degradation [7,8]. However, their potential in gene inhibition is still unsettled, as the only reported examples [9-11] of significant

binding affinities of L-DNA and -RNA for natural nucleic acids pertain to the formation of the triple-stranded complexes L-(d)-$A_{6/12}$: 2 poly(r)U [9,11] and poly-(r)-A : 2 L-(r)- U_{12} [10]. Though these findings led the authors to suggest that "enantio-DNAs may have a characteristic ability to be RNA-specific antisense oligonucleotides" [9] and "L-RNA may prove useful as.... specific inhibitor of pre-mRNA splicing or gene expression" [10], it is obvious that the behaviour of homo sequences may not be safely generalized. As a matter of fact, we already demonstrated that mixed-sequence L-oligo*deoxyribo*nucleotides (i.e. L-DNAs containing all four canonical bases) do not bind to W/C complementary natural RNA (and DNA) [12].

Hence, to fully investigate the possible use of L-oligonucleotides in the control of gene expression, according to the antigene and antisense methodologies, we have now studied i) the affinity of homopurinic and homopyrimidinic L- and alternating L/D- oligonucleotides for a double-stranded D-DNA oligomer, and ii) the affinity of the same L-modified sequences *and* of a mixed-sequence L-oligo*ribo*nucleotide for single-stranded D-RNAs.

MATERIALS AND METHODS

D-amidites were purchased from Chem Gene and Pharmacia. Solid phase syntheses were performed on a Pharmacia Gene Assembler II plus (1.3 μmol scale) using Chem Gene supports. Triethylammonium bicarbonate (TEAB) solution was prepared by passing CO_2 gas through a solution of 2 M triethylamine (Fluka) in HPLC grade water at 0 °C, until pH 7.4 was reached. DEAE Sephacel was purchased from Pharmacia, Dowex 50WX8 from Fluka.
HPLC were performed with a Waters 600E instrument equipped with DEAE 5-PW (TosoHaas) and OD 4-PW (TosoHaas) columns. NMR spectra were recorded with a Varian VXR 200 MHz spectrometer. The following abbreviations were used in the text: DMTr, 4,4'-dimethoxytriphenylmethyl; Bz, benzoyl; ibu, isobutyryl; palm, palmitoyl; TBDMS, tert-butyldimethylsilyl; DIPEA, diisopropylethylamine; THF, tetrahydrofurane; TEAB, triethylammonium bicarbonate.

Synthesis of L-2'-deoxynucleosides

The L-2'-deoxynucleosides L-T, L-dC, L-dA and L-dG were prepared, as already reported [13], from the common intermediate 3',5'-di-O-benzoyl-2'-deoxy-L-uridine, which was obtained, on a large scale, from commercial L-arabinose, following the five-step synthesis described by Holy [14]. The protected L phosphoramidites were prepared by standard procedures [12,15-17].

Synthesis of 2',3',5'-tri-O-benzoyl-L-ribonucleosides (2a-d)

2',3',5'-tri-O-benzoyl-L-ribonucleosides **2a-d** (Scheme 1) were prepared from 1-O-acetyl-2,3,5-tri-O-benzoyl-L-ribose **1** [prepared according to a reported synthetic procedure[18]]. 12.6 g

(25 mmol) of **1**, 11.96 g (50 mmol) of N6-benzoyl-adenine and 18.9 ml (77.3 mmol) of N,O-bis-trimethyl-silylacetamide (BSA) were added to 500 ml of anhydrous 1,2-dichloroethane and the resulting mixture was refluxed for 30 min under nitrogen. After cooling to room temperature, 21.2 ml (116.8 mmol) of trimethylsilyl-trifluoromethanesulfonate (TMS-triflate) were added and the reaction was continued, under reflux, for 4 hours. The solution was then cooled to room temperature, diluted with 300 ml of dichloromethane, washed with 200 ml of 5% aqueous $NaHCO_3$, then twice with 200 ml of water. The organic layer was dried with Na_2SO_4, then concentrated under reduced pressure. The resulting foam was treated with 20 ml of dichlorometane and filtered to remove the unreacted N6-benzoyl-adenine. The resulting solution was concentrated and purified on a silica gel column using CH_2Cl_2/acetone 90/10 as eluent. 13.59 g of **2a** (19.88 mmol) were obtained. The other tribenzoyl-nucleosides were prepared by the same procedure, as summarised in Table 1.

Synthesis of protected ribo-phosphoramidites (3a-d)

Compounds **2a-d** were completely deprotected with 30% aqueous ammonia in pyridine (1/1 v/v, 12 ml/mmol) at 50 °C for 25 h. The reaction mixture was evaporated leaving a solid foam, from which the L-nucleosides were recovered after repeated washings with diethyl ether. The L-ribonucleosides were reprotected with groups suitable for the solid phase synthesis (DMTr for the 5' -OH, TBDMS for the 2'-OH, benzoyl for the amino group of cytidine and adenosine, isobutyryl for that of guanosine) [19]. As an example, the synthesis of the amidite of L guanosine is described.

Synthesis of N2 - ibu - 5'- DMTr - 2'- TBDMS - L - riboguanosine - 3'- (2-cyanoethyl-N,N - diisopropyl) phosphoramidite (3c).

N2-ibu-5'-DMTr-L-riboguanosine (2.16 g, 3.3 mmol) (obtained as described by Damha and Ogilvie[19]) and $AgNO_3$ (1.01 g, 5.94 mmol) were coevaporated three times with pyridine and dissolved in 25 ml of freshly distilled THF and 2.66 ml (33 mmol) of pyridine. After 5 min, 8 ml of a solution of TBDMSCl (0.897 g, 5.94 mmol) in THF was added under stirring in nitrogen atmosphere. After 4.5 h the reaction mixture was diluted with 50 ml of CH_2Cl_2 and filtered through celite; the celite was washed with 100 ml of CH_2Cl_2 and the combined liquid phases were concentrated to a small volume, then redissolved in 80 ml of CH_2Cl_2. This solution was washed with 5% aqueous $NaHCO_3$ (25 ml) then with brine (25 ml). The organic phase was dried with Na_2SO_4, and evaporated to dryness. The resulting foam was purified on a silica gel column (4×23 cm), by eluting with CH_2Cl_2/CH_3CN 80/20. 1.07g (1.39 mmol) of N2-ibu-5'-DMTr-2'-TBDMS-L-riboguanosine (42%) (faster isomer) and 0.85 g (1.10 mmol) of N2-ibu-5'-DMTr-3'-TBDMS-L-riboguanosine (33%) (slower isomer) were obtained. A further 11% yield of the desidered 2'-

TABLE 1. Reagents and conditions required for the synthesis of **2a-d** from 25 mmol of **1**.

	2a	**2b**	**2c** [a]	**2d** [b]
base	N6-benzoyladenine 11.96 g (50 mmol)	cytosine 5.56 g (50 mmol)	N2-palmitoylguanine 19.48 g (50 mmol) [0.97 g (2.5 mmol)]	uracil 5.6 g (50 mmol)
BSA	18.9 ml (77.3 mmol)	18.8 ml (76.9 mmol)	14.1 ml (57.7 mmol) [13 ml (53.2 mmol)]	14.0 ml (57.3 mmol)
TMS-triflate	21.2 ml (116.8 mmol)	13.4 ml (73.9 mmol)	5.7 ml (31.4 mmol) [5.4 ml (29.8 mmol)]	6.5 ml (35.8 mmol)
reaction time	4 h	3 h	4 h	3.5 h
column eluent	CH_2Cl_2/acetone 90/10	CH_2Cl_2/MeOH	CH_2Cl_2/CH_3CN/	CH_2Cl_2/acetone 90/10
recovered product	13.59 g (19.9 mmol)	12.89 g (23.2 mmol)	13.84 g (16.6 mmol)	8.45g (15.2 mmol)
yield	79.5%	92.8%	66.4%	60.8%

[a] The glycosylation reaction gives a mixture of N-7/N-9 guanosine isomers, in a 2/3 ratio; after the normal work-up, the mixture was treated again with TMS-triflate and BSA to give a mixture of N7/N9 isomers in a 1/9 ratio. The amounts of reagents used for this isomerisation reaction are indicated in square brackets.
[b] During the concentration of the methylene chloride solution, part of the uridine derivative crystallises and is isolated by filtration. The remaining crude mixture is purified by silica gel column chromatography. The recovery figures are referred to the overall yield of pure compound.

TBDMS isomer was obtained by isomerization of the 3'-TBDMS-L-riboguanosine [19]. The amidite was prepared by reacting the 2'-TBDMS isomer with N,N-diisopropyl-(2-cyanoethyl)-chlorophosphite in THF/DIPEA[20]; after work up, the amidite was purified by silica gel chromatography, by eluting with $CH_2Cl_2/Et_2O/Et_3N$ 73/25/2. Pure **3c** was obtained with a yield of 65%. All the final compounds gave ^1H and ^{31}P-NMR spectra comparable with those of the corresponding commercial D-ribophosphoramidites.

Purification of the oligonucleotide sequences

The *DNA-oligomers* were purified by anion exchange chromatography (DEAE Sephacel) using a gradient of TEAB from 0.1 M to 1.6 M. The fractions with HPLC purity higher than 90% (DEAE anion exchange performed at pH 10.0) were pooled and coevaporated several times with water to decompose the TEAB excess.

D- and L-RNA-oligomers were prepared and purified by HPLC, according to Sproat et al.[21]. Sterile glassware was used in the case of D-RNAs.

The purified oligomers were converted to the sodium salts by passing them through a column of Dowex (Na^+), analyzed by HPLC on the anion exchange column and eventually lyophilized.

PAGE experiments

PAGE was run on a 20% polyacrylamide gel, at 15 °C, in a thermostatted slab minigel unit, at 10 V/cm. The loaded samples were approximately 20 μM per strand. The following buffers were used:

A: 0.1M NaOAc, 50 mM NaCl, 10 mM $MgCl_2$ at pH 5.0 (for Py:Pu·Py triple helix experiments); **B**: 0.1 M TrisOAc, 50 mM NaCl, 10 mM $MgCl_2$ pH 7.0 (for Py:Pu·Pu triple helix experiments); **C**: 0.1M TrisOAc, 50 mM NaCl pH 7.0 (for duplex experiments).

Staining was performed by soaking the gel with 0.01% Stains-all dye in 1/1 water/formamide solution.

UV experiments

UV measurements were done with a Perkin Elmer 554 spectrophotometer equipped with a MGW Lauda RC5 thermostat and a MGW Lauda R40/2 digital thermometer; an electronic device was used to generate a linear gradient of temperature. Equimolar amounts of each strand were mixed in 0.1M Tris·HCl, 0.1M NaCl, pH 7.0; the final concentration was approximately 3.0 μM per strand. The cells were heated to 80 °C for 15 minutes, then let to cool down slowly. The thermal profile was registered using a temperature gradient of 0.5 °C/min, starting at 4 °C (moisture condensation on the cells' walls was prevented by flushing nitrogen inside the cell holder).

CD experiments

CD spectra were recorded with a Jasco J715 spectropolarimeter equipped with a thermostatted water jacket cell holder. The solutions were prepared as previously described for the UV experiments.

RESULTS AND DISCUSSION

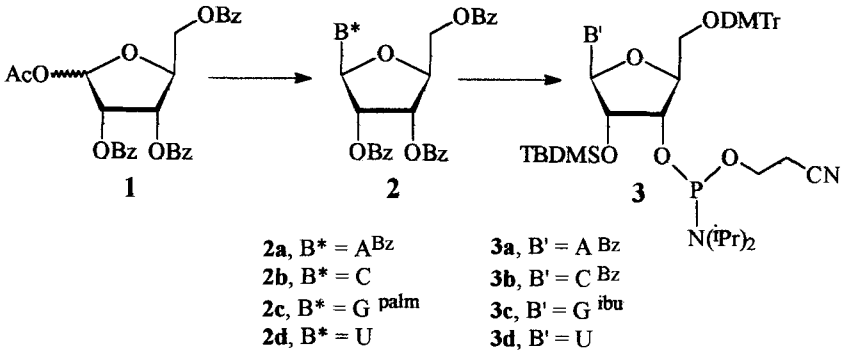
Synthesis.

The oligonucleotides listed in Table 2 were prepared by automated solid phase synthesis, using the phosphoroamidite protocols[21,22]. Palindromic homopurinic and homopyrimidinic sequences were chosen, to make their binding orientation-independent thus halving the number of binding experiments. Since commercial derivatized supports were always used, the L-sequences carried an irrelevant extra D-nucleoside (not shown) at the 3' end. DNA oligomers were purified by anion-exchange column chromatography, and RNA oligomers by HPLC. Syntheses of the L-2'-deoxynucleosides and of the corresponding phosphoroamidites were performed as previously described [12,13].

L-ribophosphoroamidites were prepared as summarised in Scheme 1. L-ribofuranoside derivative **1**, obtained from commercial L-arabinose by a four steps procedure, was condensed with the base in the presence of trimethylsilyl triflate and bis-silyl-acetamide, giving ribonucleosides derivatives **2** [18], which were converted into the suitably protected amidites **3** by standard procedures [19].

TABLE 2. Oligonucleotides used in the present study (L-nucleotides are underlined).

4	D dna PU	AAG GAA GGA AGG AAG GAA
5	D dna PY	TTC CTT CCT TCC TTC CTT
6	D rna PU	AAG GAA GGA AGG AAG GAA
7	D rna PY	UUC CUU CCU UCC UUC CUU
8	D rna	GGU CCG AGC UUG ACU ACU
9	D rna	UCA UCA GUU CGA GCC UGG
10	D rna	AGU AGU CAA GCU CGG ACC
11	L dna PY	TTC CTT CCT TCC TTC CTT
12	L/D dna PY	TTC CTT CCT TCC TTC CTT
13	L rna PY	UUC CUU CCU UCC UUC CUU
14	L dna PU	AAG GAA GGA AGG AAG GAA
15	L/D dna PU	AAG GAA GGA AGG AAG GAA
16	L rna PU	AAG GAA GGA AGG AAG GAA
17	L rna	AGU AGU CAA GCU CGG ACC



2a, B* = A^{Bz} **3a**, B' = A Bz
2b, B* = C **3b**, B' = C^{Bz}
2c, B* = G^{palm} **3c**, B' = G^{ibu}
2d, B* = U **3d**, B' = U

SCHEME 1. Synthesis of L-riboamidites.

PAGE experiments.

The occurrence of binding between the sequences, made to interact at a concentration of 20 μM per strand, was probed by PAGE retardation assay, in the following buffers:

A: NaOAc 0.1 M, NaCl 50 mM, $MgCl_2$ 10 mM (pH 5)

B: TrisOAc 0.1 M, NaCl 50 mM, $MgCl_2$ 10 mM (pH 7)

C: TrisOAc 0.1 M, NaCl 50 mM (pH 7)

Double-stranded DNA target 18: **AAG GAA GGA AGG AAG GAA**

TTC CTT CCT TCC TTC CTT

Duplex **18** (Tm= 63 °C) was combined with one equivalent of homopyrimidine sequences L-DNA **11**, L/D-DNA **12**, L-RNA **13** and D-DNA **5**, in buffer **A** at pH 5. The resulting solutions

were heated at 45 °C, slowly cooled at room temperature, then kept at 4 °C overnight. Gel electrophoresis was run in the same buffer, at 15 °C.

As shown in Fig. 1, while a complex with the expected lower mobility - the triple helix - was formed between duplex **18** and D-DNA **5** (lane 5), the natural duplex combined with the L-modified oligomers did not give rise to slower migrating species. Accordingly, two bands are present in lanes 4 and 8. In lane 3, instead, only duplex **18**, with unchanged mobility is detectable, because homopyrimidinic L/D-DNA **12** cannot be visualised by staining at the applied concentration (20 μM). As a matter of fact, the band in lane 1 was obtained only by loading it at 50 μM concentration. The same negative results (not shown) were found when duplex **18** was made to interact with the homopurinic sequences : L-DNA **14**, L/D-DNA **15**, L-RNA **16** in buffer **B** at pH 7.

Overall, this finding shows that *L-modified oligonucleotides do not form a triple helix with natural d.s. DNA*, either according to the pyrimidine•purine•pyrimidine motif or to the pyrimidine•purine•purine one. Thus it may be surmised that the reported [9-11] formation of triplexes between homopyrimidine and homopurine strands with opposite chirality can only occur when, at odd with the present examples, the only ones relevant for the antigene methodology, *the two pyrimidine strands in the triplex have the same chirality.*

Single-stranded RNA target. L-RNA **17**, containing all four canonical bases, was combined with one equivalent of D-RNAs **8** and **9** (its antiparallel and parallel W/C complements, respectively), in buffer **C** (pH 7). For comparison, D-RNA **10**, the enantiomer of sequence **17**, was combined with D-RNA **8**, its antiparallel W/C complement. After annealing, PAGE was run in the same buffer at 15 °C.

As shown in Figure 2, while the expected homochiral duplex between **10** and **8** was formed (lane 2), no retarded species was observed when the L and D complementary strands were combined (lanes 3 and 4). Thus, we can conclude that *mixed sequence L-RNAs, just like the corresponding L-DNAs [12], do not bind to antiparallel or parallel W/C complementary RNA sequences with natural chirality.*

Instead, as shown in Figure 3, when, in the same buffer as above, homopyrimidinic D-RNA **7** (lane 4) was combined with one equivalent of homopurinic L-RNA **16** (lane 2) and L-DNA **14** (lane 6) a retarded species was formed, as the only detectable product for **7** + **16** (lane 3) and together with large amounts of the free strands for **7** + **14** (lane 5). When equimolecular quantities of D-RNA **7** (lane 7) and L/D-DNA **15** (lane 9) were made to interact, two bands were found: one having the same mobility as **7** but a much higher intensity, and a very faint second one with the same mobility as **15**. This finding was considered as an indication that a complex was present as the main product and this interpretation was confirmed by Circular Dichroism spectroscopy (see below). For comparison, the natural duplex D-RNA **7**• D-RNA **6** (lane 1) and D-RNA **7**•

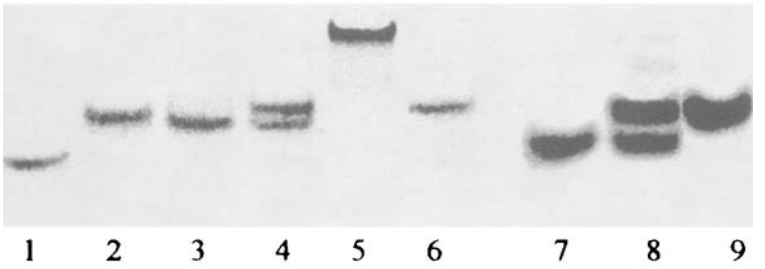


FIGURE 1. Non-denaturing PAGE, at pH 5 and 15 °C, of 1:1 mixtures of duplex **18** and homopyrimidine oligonucleotides (c = 20 μM per strand) in a buffer containing NaOAc 0.1 M, NaCl 0.05 M. $MgCl_2$ 0.01 M. Lane 1: **12** (c = 50 μM). Lane 2: **18**. Lane 3: **18** + **12**. Lane 4: **18** + **11**. Lane 5: **18** + **5**. Lane 6: **11**. Lane 7: **18**. Lane 8: **18** + **13**. Lane 9: **13**.

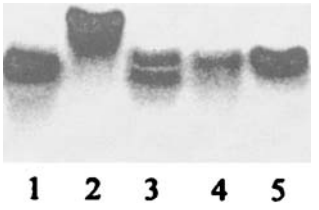


FIGURE 2. Non-denaturing PAGE of 1:1 mixtures of mixed-sequence, W/C complementary, RNAs (C = 20 μM per strand) at pH 7 and 15 °C, in the buffer containing TrisOAc 0.1M, NaCl 0.05 M. Lane 1: **8**. Lane 2: **8** + **10**. Lane 3: **8** + **17**. Lane 4: **9** + **17**. Lane 5: **17**.

D-DNA **4** (lane 10) are shown, too. The addition of two and three equivalents of L- and L/D-oligomers to D-RNA **7** did not change the overall picture, a demonstration that, under the present experimental conditions, *only heterochiral complexes with 1:1 stoichiometry are formed.*

On the contrary, no evidence of complex formation was found when homopurinic D-RNA **6** was made to interact with homopyrimidinic sequences L-DNA **11** and L/D-DNA **12** (data not shown). Obviously, D-RNA **6** and L-RNA **13** gave the expected heterochiral complex (not shown) that, being the enantiomer of that formed by D-RNA **7** and L-RNA **16** (Figure 3, lane 3), has the same mobility.

Circular Dichroism experiments.

CD spectra of an equimolecular solution (3 μM/strand) of D-RNA **7** and L/D-DNA **15** at 15 °C and 37 °C are shown in Figure 4 A, together with i) the calculated addition spectrum at 15°C (i.e. the sum of the spectra of the two separated component strands at this temperature), and ii) the spectrum of the corresponding natural duplex (D-RNA **7** • D-DNA **4**) at 15°C.

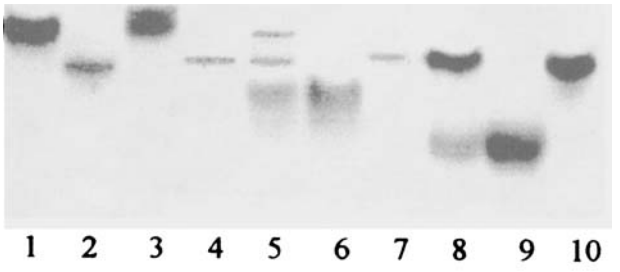


FIGURE 3. Non-denaturing PAGE, at pH 7 and 15 °C, of 1:1 mixtures of homopyrimidinic D-RNA **7** with complementary oligonucleotides (c = 20 μM per strand), in buffer containing TrisOAc 0.1 M, NaCl 0.05 M. Lane 1: **6** + **7**. Lane 2: **16**. Lane 3: **7** + **16**. Lane 4: **7**. Lane 5: **7** + **14**. Lane 6: **14**. Lane 7: **7**. Lane 8: **7** + **15**. Lane 9: **15**. Lane 10: **4** + **7**.

At low temperature, the measured spectrum of the annealed heterochiral strands is clearly different from the addition spectrum, as shown by the broad absorption centered at around 250 nm, that has disappeared at 37 °C. At this last temperature, the overall shape of the experimental spectrum is very similar to the addition spectrum. These observations indicate that the heterochiral complex D-RNA **7** • L/D-DNA **15** is the main species present at 15 °C and that its thermal stability is low.

The same type of experiment was performed for the complex D-RNA **7** • L-RNA **16**, whose formation at low temperature had been established, unequivocally, by gel electrophoresis analysis. The pertinent spectra are shown in Figure 4 B. The heterochiral complex is characterized, at 15 °C, by a wide intense absorption in the 245-280 nm region, while the spectrum measured at 37 °C, instead, is very similar to the addition spectrum, and its shape remains the same at 65°C (not shown). Figures 4 A and 4 B also show a clear difference between the spectra of the heterochiral complexes and those of the corresponding natural duplexes at 15 °C, which suggests that their conformations differ significantly.

UV melting experiments.

The thermal stability of the duplexes formed by D-RNA **7** with L-RNA **16** and L/D-DNA **15** was further studied with UV melting experiments. The absorption spectum (210-310 nm) was first recorded, after annealing, in the 10-90 °C temperature range, at 5 °C intervals, and it was found that the maximum hyperchromicity change with temperature occurred, in both cases, at around 260 nm. The denaturation curves, at this wavelenght, are reported in Figure 5.

In comparison with that of the natural duplexes, the melting of the heterochiral complexes is characterized by low cooperativity and small hyperchromicity. However, a sigmoidal profile is rather clear in both cases. Denaturation occurs, to a large extent, below 37 °C, in agreement with the result of the CD experiments.

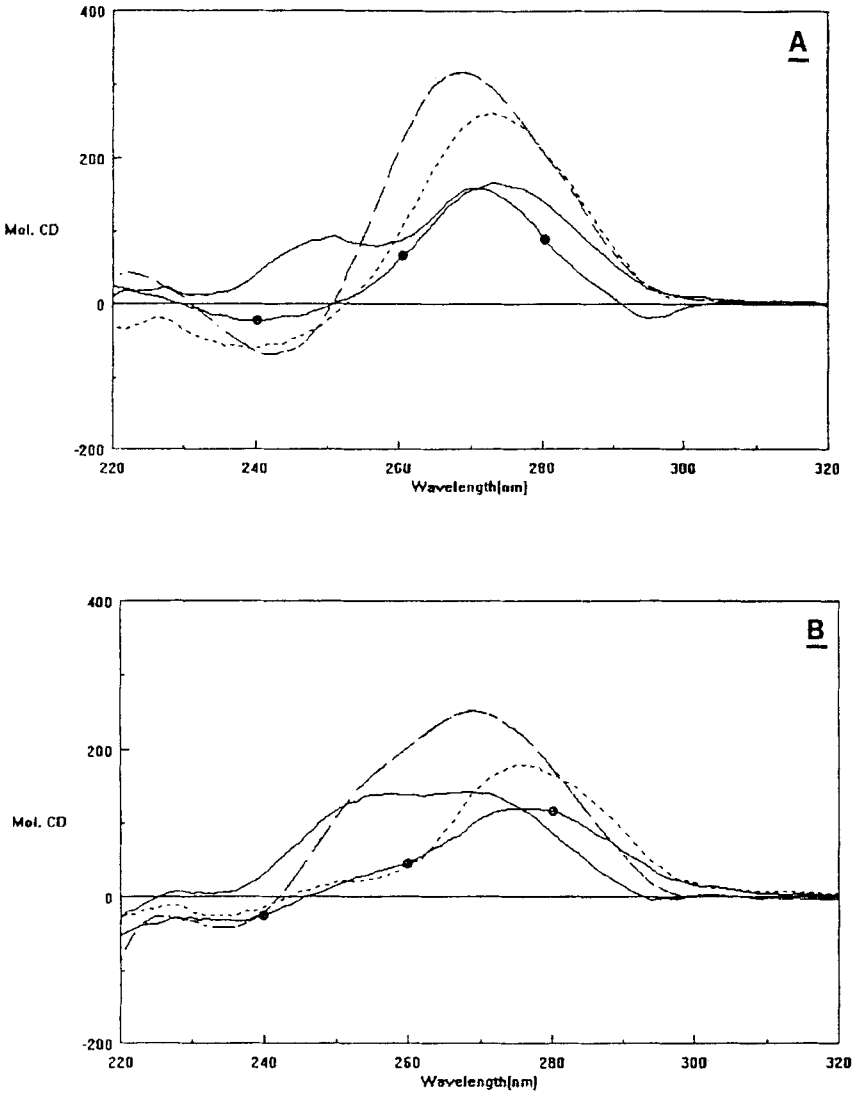


FIGURE 4. CD spectra of heterochiral and homochiral 1:1 complexes (c = 3 μM per strand), in Tris.HCl 0.1 M, NaCl 0.1M (pH 7). A) **15** + **7**; **4** + **7**. B) **16** + **7**; **6** + **7**

——————— heterochiral complex at 15 °C.

——●—— heterochiral complex at 37 °C.

— — — homochiral duplex at 15 °C.

- - - - - - - - calculated addition spectrum, at 15 °C, of the single strands used for heterochiral complex formation.

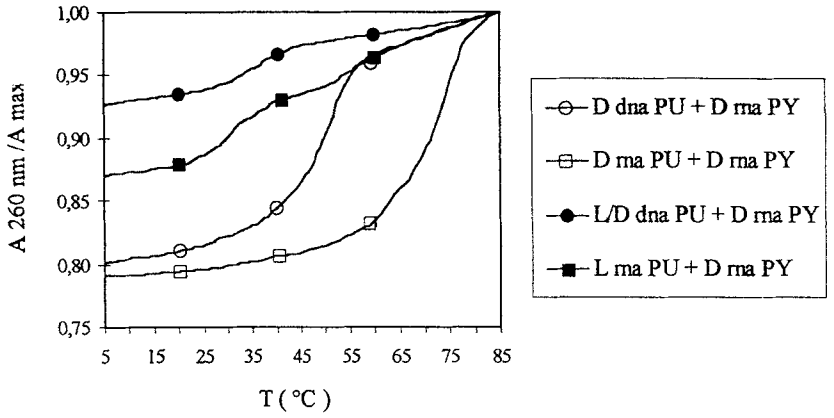


FIGURE 5. UV melting profiles of 1:1 complexes (c = 3 μM per strand) in Tris buffer (Tris.HCl 0,1 M, NaCl 0.1 M, pH 7).

This low thermal stability demonstrates that also homopyrimidinic and homopurinic L-modified oligonucleotides have a very reduced potential as antisense sequences.

Nevertheless, the present results are interesting from a structural point of view since, to the best of our knowledge, this is the first report of the existence, albeit at low temperature, of *heterochiral complexes with 1:1 stoichiometry,* as unequivocally shown by both gel electrophoresis and CD spectroscopy.

CONCLUSIONS

The present study shows that:

i) L-DNA, L/D-DNA and L-RNA are not, in general, potential antigene oligonucleotides [2], since they do not bind to a homopurine/homopyrimidine double-stranded DNA, which contains *both* purine bases.

ii) L-RNAs, like previously studied L-DNAs [12], have no general prospect as antimessenger agents, according to the antisense methodology [1], since they do not bind to natural RNA segments that contain all four canonical bases.

iii) homopurinic and homopyrimidinic L-RNA, L-DNA and L/D-DNA have only limited potential value as antisense agents. As a matter of fact, either they fail to bind a Watson/Crick complementary natural RNA that contains both canonical bases, or, when they do, the resulting complexes, of unknown structure, have low thermal stability.

To fully explore the potential of L-oligonucleotides in the specific regulation of gene expression, a project has been undertaken to probe the interaction between L-RNAs and -DNAs

and natural proteins. This study, that is also of interest in the general field of molecular recognition [23], has led to the discovery of a L-RNA sequence , which gives, with the HIV-1 *tat* protein, a complex with a dissociation constant in the nanomolar range [24].

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