RNA duplexes with biphenyl substituents as base replacements are less stable than DNA duplexes†

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Introduction of a hydrophobic biphenyl-*C*-nucleotide pair into a 11-mer RNA duplex is associated with a net penalty in the free energy of duplex formation of 2.0 kcal mol^{-1} or 10 °C in T_m, relative to DNA. These differential stabilities are of relevance with respect to the transcriptional and translational aspects of hydrophobic base-pairs.

Unnatural pairs of heteroaromatic base surrogates in DNA, devoid of the capability to form hydrogen bonds, have recently been investigated as universal bases,[1–3] as tools to probe base-recognition and insertion fidelity of DNA polymerases,[4–8] as reporter units,[9,10] and as novel, orthogonal base-pairs for expanding the genetic alphabet.[11–15] Within the latter context it was shown that shape mimics of natural base-pairs, as *e.g.* the difluorotoluene–methylindole pair, are structurally accommodated in a DNA double helix in a side-by-side fashion without distortion of the helix.[16] Such pairs were shown to significantly destabilise a DNA duplex. On the other hand, non shape mimics with extended surface areas, as *e.g.* the propynyl isocarbostyril (PICS) pair,[11] can lead to duplex stabilities that match or even excel those of natural base-pairs, due to partial interstrand stacking interactions between the units.[17–19]

In the context of expanding the genetic code, such unnatural base-pairs should not only recognise each other and be efficiently replicated on the DNA level. They also have to be efficiently transcribed and translated. In the latter two processes, DNA–RNA and RNA–RNA duplexes are central structures along the information transfer. Thus the critical question arises, how stability (and enzymatic processing) varies with respect to the nature of the backbone (RNA *vs.* DNA). So far there is only little data available on RNA containing non-hydrogen bonding base analogues. It was shown that oligoribonucleotides containing fluorophenyl–fluorobenzimidazol pairs (isosteric to natural base-pairs) destabilise RNA duplexes by *ca.* 5–7 °C in T_m.[20,21] However, no data exist so far on RNA duplexes with partially intercalating aromatic pairs.

We recently found that biphenyl *C*-deoxynucleosides (dBph, Fig. 1) form stable self-pairs in DNA duplexes due to interstrand stacking interactions of the phenyl rings.[17,18] In the sequence context given in Fig. 1 we found that this motif is extendable to at least 7 pairs with increasing thermal stability of the duplex.[22] Thus, this motif can serve as a model to study the energetics of intercalative base arrangements as a function of the nature of the backbone (RNA or DNA). Here we set out to study the thermal and thermodynamic properties of this motif in RNA and RNA–DNA mixed duplexes and to compare the data with that of DNA duplexes.

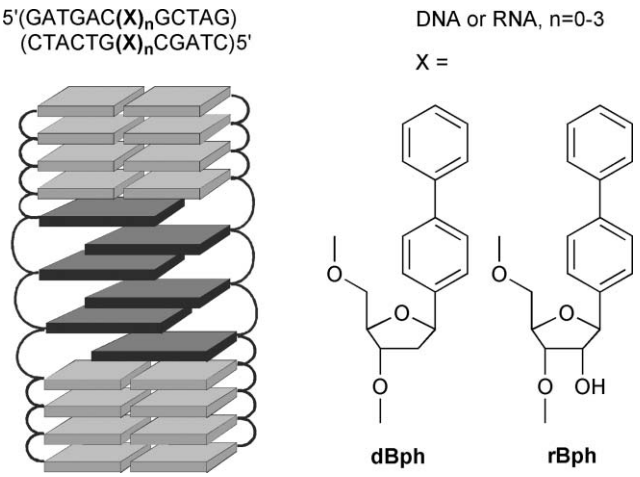

Fig. 1 Sequence information (top), chemical structures of the deoxyribo- and ribonucleoside residues containing the biphenyl base substitutes (right), and schematic representation of the interstrand stacking motif (left).

Synthetic details and analytical data for the C-nucleoside rBph, its building block for RNA synthesis as well as the corresponding oligoribonucleotides (Fig. 1, n = 0–3), are contained in the electronic supplementary information.† Results for the DNA series were, in part, already reported previously.[18,22] The thermodynamic data for duplex formation in both the DNA and RNA series were obtained from $1/T_m$ *vs.* ln(c) plots (van't Hoff plots, see supplementary information†). Thermal melting (T_m) and thermodynamic data are summarized in Table 1.

Introduction of one rBph-pair into the RNA duplex is associated with a drop in T_m by 10 °C and a loss in the free energy of duplex formation ($\Delta\Delta G^{25\ °C}$) of 2.5 kcal mol^{-1}, relative to the unmodified duplex. On the other hand, introduction of one dBph-pair into the DNA duplex leads to a decrease in T_m of only 2.5 °C and a $\Delta\Delta G$ of 0.5 kcal mol^{-1}. Additional Bph-pairs were then introduced into the duplex to investigate whether a stability enhancing interaction between the aromatic residues, also occurs in the RNA context. Indeed, introducing a second and a third consecutive Bph-pair leads to partial recovery of the thermal and thermodynamic stabilities in RNA. While in the case of DNA, three Bph-pairs lead to a T_m that is higher by almost 5 °C relative

† Electronic supplementary information (ESI) available: synthesis and analytical data of monomer rBph and oligoribonucleotides, and van't Hoff plots for the determination of thermodynamic data. See http://www.rsc.org/suppdata/cc/b4/b419278h/

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Table 1 Thermal melting data (T_m) from UV–melting curves (260 nm) and free enthalpy of duplex formation from van't Hoff plots of DNA or RNA duplexes with the sequence indicated in Fig. 1

n^a	DNA $T_m{}^b$	RNA $T_m{}^b$	$\Delta G^{25°c}$ (DNA)	$\Delta G^{25°c}$ (RNA)	$\Delta\Delta G^d$ (DNA)	$\Delta\Delta G^d$ (RNA)
0	45.0	57.1	−13.2	−18.1	—	—
1	42.5	47.1	−12.7	−15.6	+0.5	+2.5
2	46.9	51.9	−13.9	−16.9	−1.2	−1.3
3	49.9	53.2	−14.9	−17.7	−1.0	−0.8

[a] conditions: 10 mM NaH_2PO_4, 0.15 M NaCl, pH 7.0; T_m [°C], estimated error: ± 0.5 °C. [b] T_m values at 1.2 μM. [c] [kcal mol^{-1}]. [d] $\Delta\Delta G = \Delta G_{n+1} - \Delta G_n$.

to the unmodified duplex, the same situation in the RNA series is still associated with a reduction in T_m by *ca.* 4 °C. It emerges, however, that introduction of any Bph-pair after the first one leads to a similar increase in thermal stability in both backbone series, indicating a similar mode of interaction of the additional hydrophobic pairs.

We measured CD spectra of the RNA duplexes in order to screen for major changes in duplex structure upon introduction of rBph-pairs (Fig. 2). As expected, the unmodified RNA duplex shows the typical fingerprint of an A-conformation. Introduction of one or three rBph-pairs does not significantly change this pattern. The duplex with $n = 1$ leads to a slight red shift of the ellipticity maximum, which is not the case for the duplex with three rBph-pairs and for the corresponding DNA duplexes.[22] Thus introduction of only one Bph-pair seems to be associated with a more pronounced structural effect in the RNA series compared to the DNA series.

In order to determine the effect on duplex stability of a hydrophobic residue in an internal bulge position, in DNA relative to RNA and to determine the effect of one Bph-pair insertion into a DNA–RNA hybrid, we measured the thermal stabilities of the duplexes listed in Table 2. Introduction of a Bph-unit in a DNA bulge stabilises the duplex relative to the unmodified duplex by 0.9 °C, while the same sequence arrangement in the RNA leads to a destabilisation by 8.0 °C (Table 2, entries 1 and 2). The increase in stability in the former case is an indication for intercalation of the Bph-residue into the base-stack. In the case of RNA, intercalation is either associated with a significant energetic penalty or does not occur. The introduction of a complete Bph-pair into a DNA–RNA hybrid (Table 2, entry 3) again leads to a decrease in T_m, relative to the unmodified duplex, by 7.4 °C, which

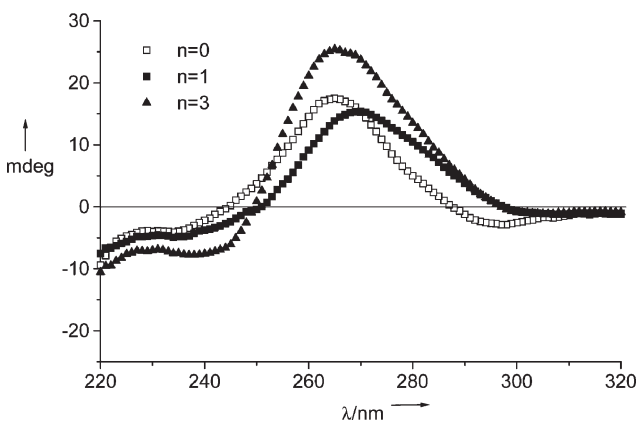

Fig. 2 CD spectra of RNA-duplexes from Table 1 (n = 0, 1, 3), c = 3.6 μM, in 10 mM NaH_2PO_4, 0.15 M NaCl, pH 7.0, T = 20 °C.

Table 2 Thermal melting data (T_m) from UV–melting curves (260 nm) of DNA or RNA duplexes with one Bph-residue in a bulge position, or of a DNA–RNA hybrid containing one Bph-pair

Entry	Structure	Duplex	$T_m{}^a$
1	DNA bulge	5′-d(GATGAC **Bph** GCTAG)-3′ 3′-d(CTACTG' − 'CGATC)-5′	45.9 (45.0)
2	RNA bulge	5′-r(GAUGAC **Bph** GCUAG)-3′ 3′-r(CUACUG' − 'CGAUC)-5′	49.1 (57.1)
3	DNA–RNA hybrid	5′-d(GATGAC**Bph**GCTAG)-3′ 3′-r(CUACUG**Bph**CGAUC)-5′	36.1 (43.5)

[a] conditions: c = 1.2 μM in 10 mM NaH_2PO_4, 0.15 M NaCl, pH 7.0. estimated error in T_m: ± 0.5 °C; in parenthesis: T_m data of parent, non-modified duplex.

corresponds to ¾ of the loss in T_m relative to a pure RNA duplex. Thus we conclude that any accommodation of a Bph-residue in a duplex that contains at least one RNA strand leads to reduced thermal stability relative to a pure DNA duplex.

It appears that interstrand stacking recognition of a single Bph-pair is associated with a net penalty in free energy of duplex formation of 2.0 kcal mol^{-1} in RNA relative to DNA. A possible explanation for this could lie in the different conformational families (A *vs.* B) in which the geometry and the extent of overlap of the aromatic units of both strands lead to less efficient stacking in the case of RNA. Another reason could be the reduced flexibility and dynamics of the RNA backbone relative to DNA. Indeed, it has been shown before that classical aromatic intercalators tend to stabilise double stranded DNA more than RNA duplexes.[23] Along the same lines it was shown recently that an oligodeoxynucleotide containing a covalently attached pyrenyl intercalator, pairs preferentially to a DNA relative to an RNA complement.[24] In the context of more complex nucleic acid structures it was also shown that the i-motif, consisting of pairwise intercalated C–C^+ nucleotide-pairs, is significantly more stable in DNA as compared to RNA.[25]

In conclusion, the data presented here show that interstrand intercalation of hydrophobic base-pairs that can adopt stabilities of natural base-pairs in DNA, can be significantly less stable in RNA duplexes or DNA–RNA hybrids.

These results are of interest in the following context. They indicate that a hydrophobic base-pair that is not a shape mimic of a natural base-pair, and that takes its interaction energy mainly from interstrand stacking, may compromise the process of transcription and translation due to significant changes in base–base affinity, by changing from the DNA to the RNA backbone and consequently from a B to an A-helical structure. Thus, this finding is of importance for the future design of hydrophobic base-pairs aiming at expanding the genetic alphabet. Furthermore, the fact that an additional hydrophobic base in a bulge position within

a DNA probe does not alter stability with a fully complementary DNA but discriminates fully complementary RNA, opens a way into backbone specific targeting of nucleic acids and may be useful for specific DNA recognition in mixed, DNA and RNA containing environments.

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