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Biphenyl-DNA

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Abstract: We report on the pairing properties of oligodeoxynucleotides containing chemically functionalized biphenyl residues instead of natural nucleobases. The synthesis of the corresponding C-nucleosides with one or two nitro- or methoxy groups, resp. in the distal phenyl ring was accomplished *via* Suzuki coupling of a protected 4-bromophenyl-C-nucleoside with the correspondingly substituted phenylboronic acids or esters. The interstrand hydrophobic and/or stacking recognition properties of the aromatic units were probed by *T*m-measurements on duplexes containing single or triple incorporations. We found a strong dependence of duplex stability from the nature of the substituents (donor *vs*. acceptor). The stability of duplexes with one biphenyl-pair increases in the order of donor–donor < acceptor–donor < acceptor–acceptor pairs. On the other hand in the triply modified duplexes stability increases in the order acceptor–acceptor < donor–acceptor < donor–donor pairs. It thus appears that interactions to the nearest neighbor natural base-pairs are preferred by acceptor modified biphenyls while the interactions between biphenyls are most stable with opposing donor modified biphenyls.

Keywords: Biphenyl-DNA · DNA · Hydrophobic base-pairs · Hydrophobic interactions · Stacking interactions · Suzuki coupling ·

Introduction

Aromatic replacements of nucleobases devoid of hydrogen-bond forming capability in DNA recently became of interest in the context of studying the energetic effects of aromatic stacking on DNA duplex stability,[1] in the context of probing DNA polymerase function,[2] and in the context of the discovery of novel orthogonal base-pairs for the extension of the genetic alphabet.[3] In this context we recently found that introduction of an increasing number of biphenyl-pairs in the center of an oligonucleotide duplex leads to an increase in duplex stability much in the same way as observed for natural base-pairs.[4,5] We hypothesized that the increase in stability per biphenyl-pair arises from interstrand stacking or hydrophobic interactions of opposing biphenyl residues (zipper motif).

The replacement of natural base-pairs by non-hydrogen bonding aromatic units such as biphenyl residues offers in principle the possibility of bringing novel function into the DNA double helix by maintaining at the same time the basic structural and biophysical properties of DNA. This is of interest in applications of modified DNA in diagnostics or in the material sciences. In a first set of experiments we decided to decorate the distal phenyl ring of the biphenyl units with one or two electron acceptor or donor groups and to determine their effects on duplex stability (Fig. 1). With this we wanted to explore the tolerance of the zipper recognition motif towards chemical substitution (steric constraints) as well as identifying the effects of electron rich or poor π-systems on duplex affinity (electronic tuning).

Results and Discussion

The syntheses of the corresponding C-nucleosides with one or two nitro- or methoxy groups, resp. in the distal phenyl ring (Fig. 1) were accomplished *via* Suzuki coupling of a protected 4-bromophenyl-C-nucleoside with the correspondingly substituted phenylboronic acids or esters.[6] Oligodeoxynucleotides were prepared by standard solid-phase oligonucleotide chemistry using the appropriate phosphoramidite building blocks of the four biphenyl-C-nucleosides **1a-d**. Two sequence contexts were chosen (Fig. 2). One sequence contained a single incorporation of a biphenyl-pair and the other contained

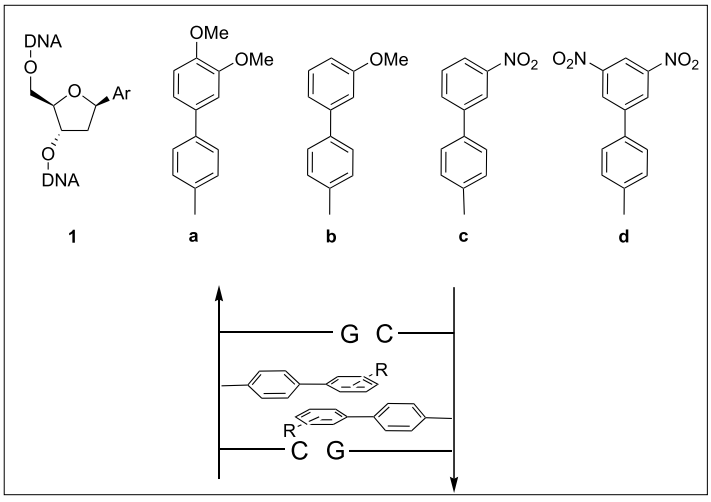

Fig. 1. Chemical structures of the biaryl-C-nucleosides, the mutual molecular recognition properties of which were investigated in the context of oligonucleotide duplexes (top); graphical representation of the zipper motif as identified by NMR-spectroscopy (bottom)

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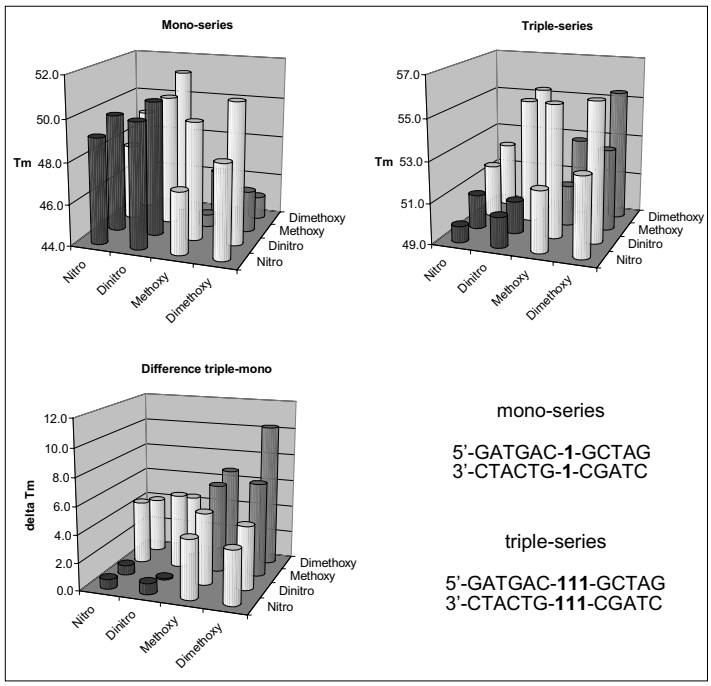

Fig. 2. Plot of the *T*m-values of the mono- and the triple-substituted duplexes as well as a plot of the Δ*T*m values of triple- and mono-substituted series. The *T*m of the deletion mutant duplex (without **1**) is 45.0 °C. Experimental conditions: c (duplex) = 1.2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0.

three contiguous biphenyl-pairs. Sequences were designed as to contain only one type of substituted biphenyl-C-nucleoside per strand (no mixed biphenyls in the same strand). Due to the fact that the sequences were not self-complementary, all permutational arrangements of donor and acceptor substituted biphenyls were possible and no interference from monomolecular hairpin formation had to be taken care of.

We recorded UV-melting curves for all possible duplexes under identical buffer conditions and at equal duplex concentrations. The corresponding *T*m-data were graphically reproduced in Fig. 2. As a standard the same duplex containing no biphenyl-pair (deletion mutant) showed a *T*m of 45 °C. As can be seen from the graphical representation in the case of the mono-series, none of the biphenyl-pairs destabilizes the parent duplex. In contrary, most of the biphenyl-pairs increase duplex stability by up to 6 °C per pair which is more than the stabilization by an additional AT base-pair for which a *T*m of 47.9 °C was determined. It becomes immediately evident that the acceptor modified biphenyls stabilize the duplex most (4–6 °C). The mixed donor–acceptor pairs also stabilize the duplex, showing though a larger *T*m diversity (2–6 °C). Interestingly donor-modified biphenyls do not significantly stabilize the duplex. Here the *T*m increases are in the range of 0–2 °C. The picture becomes different in the triple-modified series. As expected, all triple-modified duplexes are more stable that the mono-modified ones. However, also in these cases substantial differences in *T*m as a function of the nature of the biphenyl substituents are observed. Interestingly, any additional acceptor biphenyl-pair added to the first one does not lead to a significant increase in thermal duplex stability. The opposite picture is observed for the donor-substituted systems. Here the additional incorporations lead to higher stability with the dimethoxy-substituted arrangement being the most stable one. Relative to the deletion mutant, the *T*m increase for the dimethoxy-substituted duplex amounts to roughly 10 °C (3.3 °C per biphenyl pair). Again the mixed donor–acceptor biphenyl-pairs are located in between, with the dinitro-donor arrangements being more stable (even of equal stability to the dimethoxy system) than the mononitro-donor arrangements.

Clearly, two different interactions of the biphenyl residues have to be taken into account when trying to analyze these results. First, the interaction of the biphenyl residues with the neighboring natural base-pairs and second the interaction of the biphenyls with each other. The relative interactions with the neighboring natural base-pairs clearly dominate in the mono-series, while both effects are operative in the triple-series. To determine the contribution to thermal stability of biphenyl–biphenyl interaction exclusively we plotted the Δ*T*m values between the triple- and the mono-series. As can be seen from Fig. 2 it clearly emerges that acceptor-biphenyl interactions do not contribute much to duplex stability while donor-biphenyl interactions do significantly. Interestingly, donor–acceptor interactions are again in-between.

Based on the zipper model for which structural proof exists to some extent by NMR-spectroscopy,[7] the following points can be made:

i) Distal substitution of the biphenyl rings markedly influences the stabilizing properties of the corresponding pairs with Δ*T*m of up to 6 °C. The *T*m data are in agreement with the interpretation that the differences in thermal stability do not significantly arise from direct steric effects of the substituents. This clearly shows that there is plenty of freedom in the design of substituents without obstructing the overall recognition motif.

ii) Stacking of the biphenyls onto the natural base-pairs follows the order acceptor–acceptor > acceptor–donor > donor–donor. This may be due to advantageous electrostatic or dispersive interactions with the nearest neighbor guanines.

iii) Interactions of biphenyls with each other follows exactly the opposite order with donor–donor > donor–acceptor > acceptor–acceptor. At this point there is no structural model at hand that characterizes the geometry of the biphenyls in a multiple-substituted duplex in sufficient detail. It seems however that the additional stability cannot come from π-stacking or dispersive interactions alone.

Conclusions

We have shown that a substantial portion of the central part of a DNA duplex can be replaced with biphenyl residues bearing a variety of functional groups with different affinity profiles. This allows for the introduction of novel function and property into the well-ordered DNA double-helical scaffold which might be of considerable interest in the field of material sciences and for DNA diagnostics. Furthermore the differences in affinity as a function of the substitution pattern of the biphenyls theoretically open up a way to generate a primitive genetic code in which the selectivity of recognition does not depend on H-bonds but entirely on hydrophobic interactions.

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