DNA Recognition

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Stable Cyclohexyl–Phenyl Recognition in the Center of a DNA Duplex**

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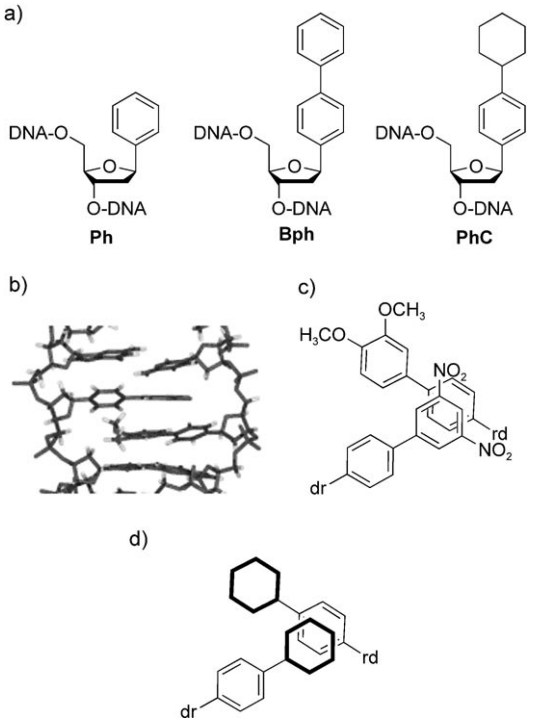
Molecular recognition of biopolymers such as complex carbohydrates, proteins, and nucleic acids is largely dominated by hydrogen bonding, aromatic stacking, and hydrophobic interactions. Whereas the structural and energetic impact of hydrogen bonding is generally well appreciated, the understanding and prediction of the structural and energetic impact of stacking or hydrophobic interactions on biomolecular folding in water is comparably poor. Well-known exceptions are, in the case of proteins, hydrophobic interactions of nonpolar amino acid side chains, which can lead to highly ordered structural motifs, such as the leucine zipper,[1,2] and in the case of DNA, the double-helical Watson–Crick structure, in which the two nucleic acid strands are held together by intra- and interstrand aromatic stacking interactions between the nucleobases as the primary source of stability and by hydrogen bonding between the bases as the source of selectivity.[3]

In attempts to probe aromatic stacking interactions[4,5] or to probe enzyme/nucleobase recognition,[6–8] and motivated by the search for novel orthogonal base pairs for the extension of the genetic alphabet,[9–11] the recognition properties of aromatic hydrophobic units as nucleobase replacements in oligonucleotide duplexes have been investigated in the recent past. Some of these aromatic units, devoid of hydrogen-bond-forming capability, stabilize duplexes to the same extent as or even more than a standard DNA base pair.[12] We recently set out to investigate this recognition behavior in more detail for biphenyl (Bph) as a nucleobase replacement, with the reasoning that zipperlike interstrand aromatic stacking interactions of the Bph residues would play an important role.[13–15] Indeed, we found that duplex stability increases with increasing numbers of biphenyl pairs. A recent ^{1}H NMR analysis of a biphenyl-modified duplex confirmed the zipperlike arrangement and provided details of the molecular interaction of the Bph units.[16] Specifically, direct aromatic stacking contacts were observed between the distal phenyl ring of one Bph unit and the proximal phenyl ring of the complementary Bph unit. An idealized structure of the pair is given in Scheme 1c.



Scheme 1. a) Chemical structures of oligonucleotides containing C-nucleosides with either a phenyl (Ph), a phenylcyclohexyl (PhC), or a biphenyl (Bph) residue as a base replacement. b) Section of an NMR-determined structure of a DNA duplex with a pair of functionalized biphenyl residues in opposing positions.[16] The two biphenyl rings recognize each other by interstrand stacking interactions in a zipper-like motif. c) Idealized representation of the structural arrangement of the biphenyl residues in this duplex. The distal phenyl ring of one unit stacks on the proximal phenyl ring of the other unit. d) Expected arrangement in the case of PhC units. The reduced cyclohexyl rings are drawn in bold to highlight the difference to the aromatic rings. dr/rd: deoxyribose.

To further evaluate hydrophobic interactions, we now wished to investigate the energetic contribution of self-pairing of a biphenyl derivative, the molecular recognition of which is based on aromatic/aliphatic interactions. On the basis of the previous NMR structure, we reasoned that this should be possible if the distal ring of the biphenyl unit is reduced to a cyclohexane unit (Scheme 1a, PhC). Consequently, the arrangement of the two units in the duplex would bring a phenyl and a cyclohexyl unit into close contact (Scheme 1d). Herein, we report on the synthesis and recognition properties of singly and triply modified PhC-containing duplexes.

Details of the synthesis of the C-nucleoside containing the PhC unit are described in the Supporting Information. Briefly,

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commercial phenylcyclohexane was brominated at the *para* position of the phenyl ring and the product was converted into the lithiated derivative, which was added to 3′,5′-*O*-*tert*-butyldimethylsilyl-protected ribonolactone. After reduction and deprotection, the corresponding phosphoramidite building block was prepared by standard methods. This building block was then incorporated into oligonucleotides by standard DNA synthesis, and the pairs of complementary oligonucleotides depicted in Table 1 and Table 2, containing either one or three consecutive PhC residues, were prepared (see the Supporting Information). For comparison, the corresponding oligonucleotides containing biphenyl (Bph) and phenyl (Ph) units were also prepared by using published procedures.[14,17,18]

We first investigated the thermal melting properties of the dodecamer duplexes containing one modified pair in the center by UV-melting-curve analysis (Table 1). We found that

Table 1: Thermal melting data (T_m) from UV-melting curves (260 nm) of dodecamer duplexes containing one hydrophobic pair in the center of the helix.

		5′-d(GATGAC-**X**-GCTAG) 3′-d(CTACTG-**Y**-CGATC)			
Entry	**X–Y**	T_m[a] [°C]	Entry	**X–Y**	T_m[a] [°C]
1	–	45.0[b]	7	Bph–PhC	43.4
2	T–A	47.9[b]	8	Ph–PhC	40.3
3	PhC–PhC	45.4	9	PhC–Ph	39.9
4	Bph–Bph	42.5[b]	10	Ph–Bph	40.3
5	Ph–Ph	32.9	11	Bph–Ph	41.0
6	PhC–Bph	44.0			

[a] $c = 1.2$ μM in 10 mM NaH_2PO_4/150 mM NaCl (pH 7.0); estimated error = ±0.5 °C. [b] Data taken from reference [15].

one PhC pair (Table 1, entry 3) had essentially no influence on the T_m value compared to that of the corresponding deletion mutant (Table 1, entry 1) and was only 1.5 °C less stable than a duplex containing an A–T base pair (Table 1, entry 2). Interestingly the PhC-pair-containing duplex was more stable by 2.9 °C than one containing a Bph pair (Table 1, entry 4). For a control experiment, we also determined the T_m value of a duplex containing two simple phenyl residues, devoid of the potential for interstrand stacking (Table 1, entry 5). As expected, this duplex was significantly less stable, by 12.5 and 9.6 °C relative to the PhC- and the Bph-containing duplexes, respectively. We also collected T_m data for duplexes with mixed arrangements of hydrophobic residues. Incorporation of a Ph residue opposite a PhC or a Bph unit (Table 1, entries 8–11) led to T_m values that were only slightly lower (2–5 °C) than those of the pure PhC or Bph duplexes and were significantly higher (7–9 °C) than that of the pure Ph duplex. This result is in support of the previous structure, in which the distal ring of one unit interacts with the proximal ring of the other unit (Scheme 1c and d). The mutual arrangement of the two units within the strands seemed to play a minor role in the stability because the differences in the T_m values between all of the permutational arrangements were only about 1 °C (Table 1, entries 8–11). The stability of duplexes with a PhC–Bph pair (Table 1, entries 6 and 7) were intermediate to those with the corresponding homopairs. From these experiments, we conclude that the molecular recognition of a cyclohexyl ring by a phenyl ring leads to slightly enhanced thermal duplex stability relative to that from the recognition of two phenyl rings.

To test whether this recognition motif is extendable, we also investigated duplexes with three consecutive modified units in each strand in a comparable manner (Table 2). For the

Table 2: Thermal melting data (T_m) from UV-melting curves (260 nm) of tetradecamer duplexes containing three hydrophobic pairs in the center of the helix.

		5′-d(GATGAC-**XXX**-GCTAG) 3′-d(CTACTG-**YYY**-CGATC)			
Entry	**X–Y**	T_m[a,b] [°C]	Entry	**X–Y**	T_m[a,b] [°C]
1	T–A	51.0 (3.1)	6	Bph–PhC	51.4 (8.0)
2	PhC–PhC	54.7 (9.3)	7	Ph–PhC	42.0 (1.7)
3	Bph–Bph	49.9 (7.4)	8	PhC–Ph	39.5 (−0.4)
4	Ph–Ph	30.0 (−2.9)	9	Ph–Bph	39.3 (−1.0)
5	PhC–Bph	53.0 (9.0)			

[a] $c = 1.2$ μM in 10 mM NaH_2PO_4/150 mM NaCl (pH 7.0); estimated error = ±0.5 °C. [b] Values in parentheses are ΔT_m values between the monomodified (Table 1) and the triple-modified duplexes.

homopairs, it became clear that the thermally most stable arrangement was that with the pure PhC pairs (Table 2, entry 2), followed by that with the Bph pairs (Table 2, entry 3; $\Delta T_m = -4.8$ °C). Remarkably, the PhC duplex was more stable by 3.7 °C than a control duplex containing 3 T–A base pairs (Table 2, entry 1). The duplexes with three mixed Bph–PhC pairs (Table 2, entries 5 and 6) again led to intermediate stabilization. All four duplexes (Table 2, entries 2, 3, 5, and 6) showed substantial stabilization relative to the monomodified duplexes ($\Delta T_m = +7.4$–9.3 °C), which indicates that the stabilizing contribution is additive. As expected, the duplex with three Ph homopairs (Table 2, entry 4) was less stable by almost 25 °C than that with three PhC pairs. A comparison of the triple-modified duplex with the monomodified duplex showed no increase in stability in this case, which indicates that there is no mutual recognition of the Ph units.

A similar situation was encountered in the case of the mixed Ph–PhC or Ph–Bph duplexes (Table 2, entry 7–9). In these cases, the differences in the T_m values between the triple-modified and the monomodified duplexes were again negligible and indicated no incremental stabilization. The bicyclic skeleton is therefore a minimal requirement to efficiently propagate molecular recognition beyond the junction site with the natural base pairs through multiple hydrophobic pairs, probably by interaction of all of the structural elements of the bicycle with each other.

Next, we determined the thermodynamic data for duplex formation from UV-melting curves by van't Hoff analysis (the concentration-variation method)[19] for the mono- and the triple-modified duplexes with the homo-PhC and homo-Bph pairs (Table 3). The free enthalpies of duplex formation (ΔG) at room temperature essentially confirmed that the thermal stabilities (T_m) also reflect the thermodynamic stabilities. The enthalpies of duplex formation (ΔH) were distinctly more

Table 3: Thermodynamic parameters of duplex formation from van't Hoff analysis of UV-melting curves (concentration-variation method).[a]

		5′-d(GATGAC-(**X**)$_n$-GCTAG) 3′-d(CTACTG-(**Y**)$_n$-CGATC)		
n	**X–Y**	$\Delta G^{298\,°C}$ [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	ΔS [cal K^{-1} mol^{-1}]
1	PhC–PhC	−15.3	−95.9	−208
3	PhC–PhC	−19.1	−110.5	−307
1	Bph–Bph	−13.5	−79.7	−221
3	Bph–Bph	−15.7	−82.6	−225

[a] $c = 0.5$–15 μM in 10 mM NaH_2PO_4/150 mM NaCl (pH 7.0); quality of the average linear fit: $R^2 > 0.99$.

favorable for the PhC-containing duplexes than the Bph-modified duplexes.

Subtraction of the enthalpies of the mono- from those of the triple-modified duplexes in each series ($\Delta\Delta H$) compensates for interactions of the modified residues with neighboring natural base pairs, which occur to an equal extent in both duplexes, and the $\Delta\Delta H$ values therefore only reflect energy contributions arising from the mutual interactions of the modified residues. These $\Delta\Delta H$ values clearly show that the PhC/PhC interactions stabilize the triple-modified duplexes by 14.6 kcal mol^{-1}, whereas the Bph/Bph interactions only contribute 2.9 kcal mol^{-1} to duplex stability. On the basis of the structural model given in Scheme 1, this indicates that phenyl/cyclohexyl interactions are enthalpically more favored than phenyl/phenyl interactions.

To test whether the general structural motif of biphenyl recognition, as experimentally determined earlier,[16] also applies here, we measured the 1D ^{1}H NMR spectra of the duplex containing a PhC pair in D_2O at different temperatures below and above the melting temperature of the duplex and followed the shift of the cyclohexyl and phenyl protons (Figure 1, gray-shaded regions).

From these spectra, it was apparent that the signals of the phenyl protons undergo a shift of approximately 0.4 ppm toward the lower field upon an increase in temperature. (For comparison, the ^{1}H NMR spectrum of the free PhC nucleoside is contained in the Supporting Information.) The shift was sigmoidal in nature, which reflects the cooperative melting of the duplex. The T_m value determined by NMR spectroscopy was about 10–15 °C higher than that measured by UV analysis, a result that is due to the higher concentration of the duplex in the NMR sample. A similar observation was made for the cyclohexyl protons, whose signals shift in a cooperative manner by up to 0.8 ppm toward the lower field upon duplex dissociation. CD spectra of the PhC-modified duplexes were also recorded (Figure S2 and S3 in the Supporting Information). As expected, they showed no major deviation from the regular B-DNA form. From this, we conclude that the PhC residues are closely packed and shielded but do not obstruct the overall B conformation of the double helix. These facts indicate a similar molecular arrangement to that found for the biphenyl residues.

In conclusion, we provide here first evidence that within the highly ordered structure of the DNA double helix, nonaromatic hydrophobic residues such as a phenylcyclohexyl unit can be sandwiched in between the base stack in a repetitive fashion, with a favorable contribution to duplex stability. On the basis of the known structure of a singly modified biphenyl duplex, molecular recognition is likely to proceed by CH/π cyclohexyl/phenyl interactions of two opposing residues. Such interactions were calculated to be relatively weak (approximately 30% of a standard hydrogen bond) and mainly composed of attractive dispersion and not electrostatic forces, despite their highly oriented nature (CH bond parallel to the π system).[20,21] Our results are in line with previous findings on steroid/DNA base-pair interactions,[22] as well as a recent report on carbohydrate/phenyl interactions in a dangling-end DNA duplex model.[23]

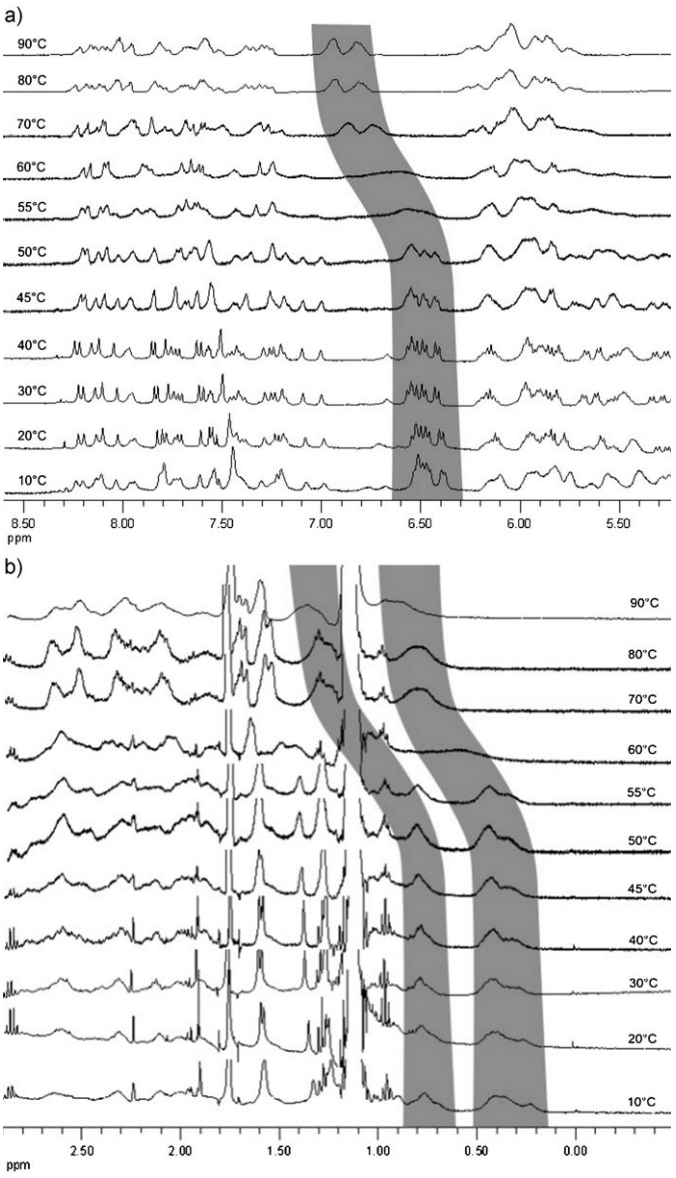

Figure 1. Temperature-dependent ^{1}H NMR spectra (500 MHz, 10–90 °C) for the duplex containing one PhC pair (Table 1, entry 3). a) Region of the aromatic and anomeric protons; b) region of the aliphatic and C2′ protons. Shaded in gray are the phenyl protons (a) and the cyclohexyl protons (b). Experimental conditions: 0.9 mM duplex in 10 mM NaH_2PO_4/150 mM NaCl in D_2O. The residual H_2O signal was suppressed by continuous-wave saturation.

General interest in our findings occurs for at least the following two reasons. The results presented herein show that the DNA double helix is an ideal scaffold to structurally investigate and quantify hydrophobic interaction energies of nonpolar organic molecules in water. Detailed structural and energetic knowledge of such interactions is important to understand their impact in the molecular recognition and folding of biomolecules. On the other hand, the structure of the Watson–Crick double helix of DNA is able to adapt for the incorporation of even saturated hydrocarbon units without a breakdown in duplex stability. This extends the structural space of novel functional units with which oligonucleotides can be equipped for applications in the field of DNA-based self-assembling nanomaterials.

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