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Selective Pairing of Polyfluorinated DNA Bases

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Recent studies of polyfluorinated organic compounds have revealed useful selective interactions between such "fluorous" species relative to their interactions with water or the parent hydrocarbons.[1] Although the origins of this selective interaction are not fully understood, it has been established that perfluorinated hydrocarbons are significantly more hydrophobic than the analogous hydrocarbons.[2] The shielding of strongly hydrophobic surfaces from solvent may explain selective polyfluorinated side-chain pairing, recently employed successfully in peptide−peptide interactions,[3] and in enhanced stability of proteins with fluorinated amino acids.[4] Fluorocarbon interactions have been gaining widespread utility and interest, especially in aiding separations of catalysts, reagents, substrates, and products.[1]

We sought to establish whether such fluorocarbon selectivity could be harnessed in pairing of DNA bases, as an alternative to other known modes of pairing. A number of laboratories have investigated molecular strategies for selective base pairing, using approaches other than that of nature itself, namely Watson−Crick hydrogen bonding. Such work has yielded fundamental information on DNA biophysics and biology, and applied utility in expansion of the genetic alphabet.[5] Benner proposed over a decade ago that hydrogen-bonding schemes beyond the standard Watson−Crick arrangement could be used in selective pairing.[6] In more recent years, strategies that avoid hydrogen bonding altogether have been introduced, employing selective pairing of hydrocarbon "bases" with one another.[7] Such selectivity can arise from the avoidance of the energetic cost of desolvation of polar natural bases and from the advantageous burying of hydrophobic surface area.

Although nucleic acid base analogues with fluorine substituents have been reported recently,[8−10] there is as yet no report on whether highly fluorinated DNA bases might display a selective interaction with one another, analogous to the pairing of polyfluorinated peptides. A recent report described pentafluorobenzene as a DNA base replacement,[9] but no selective pairing was observed in oligonucleotides. Subsequent studies have revealed, however, that pentafluorobenzene is strongly destabilizing to helical DNA because of an unfavorable effect of two ortho fluorine substituents.[10] This suggested that selective pairing might still be possible if other highly fluorinated DNA base analogues were to be examined, as long as this bis-ortho effect were avoided.

Examination of DNA models suggested a number of polyfluorinated DNA base replacements as possible candidates for selective fluorous pairing. The modeling suggested that 2,3,4,5-tetrafluorobenzene (abbreviated **^{F}B**) might pair opposite itself without distorting the helical geometry. Moreover, this compound has recently been shown to stack quite strongly at the ends of DNA helices.[10] A second candidate was the previously unknown 4,5,6,7-tetrafluoroindole (**^{F}I**), which was readily synthesized as an *N*-nucleoside species (see Supporting Information (SI)). These two deoxyribosides were prepared as their 5′-trityl, 3′-phosphoramidite derivatives for incorporation into DNA by automated synthesizer.

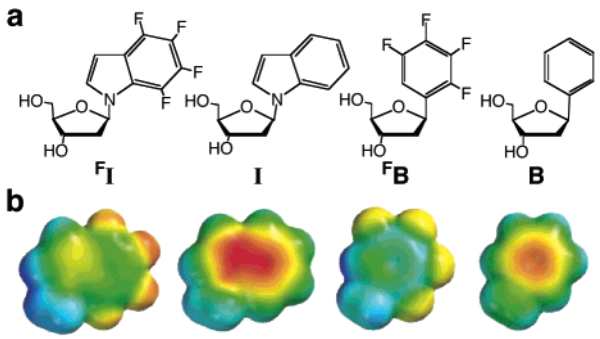

Figure 1. Structures of fluorinated and hydrocarbon DNA base replacements. (a) Chemical structures of the four nucleosides in the study. (b) Electrostatic surface potentials of bases with methyl groups at the point of attachment to deoxyribose. Calculated with Spartan '02 (Wavefunction, Inc.) using the AM1 Hamiltonian.

For comparison we also prepared the non-fluorinated hydrocarbon analogues, phenyl (**B**)[11] and indole (**I**)[12] glycoside derivatives (Figure 1).

To test pairing preferences of the four unnatural base replacements, we placed them in short oligonucleotides and paired these strands with complementary partners containing either natural bases or unnatural analogues at single or double positions. Stabilities of the duplexes were evaluated by thermal denaturation monitored by UV absorbance, in a pH 7.0 buffer containing 1.0 M NaCl, 10 mM Na·phosphate, and 0.1 mM EDTA. Melting temperatures were determined from the inflection points in the curves, and free energies were obtained by van't Hoff plots of the data at multiple DNA concentrations.

Initial experiments pairing the two fluorinated nucleosides opposite natural DNA bases in a 12-bp duplex confirmed they pair with low stability opposite the hydrophilic nucleobases (see SI). However, when paired opposite themselves, a significant degree of stability was regained for both compounds. This confirms that the pairing of the polyfluorinated bases operates selectively in the context of natural DNA, thus displaying significant levels of orthogonality. The **^{F}I**−**^{F}I** pair in this context is nearly as stable as the natural T−A pair. The mild-to-moderate destabilization of the duplex by these pairs (as compared to natural base pairs) is consistent with several previous nonpolar DNA base-pair analogues and is most likely due to the energetic cost of desolvation.[13]

We then began more detailed studies with a new sequence, increasing the nonpolar pair content to 2/12 (17%) to emphasize differences among various combinations of the nonnatural bases. The data confirm that all the self-complementary sequences give the expected concentration dependence, confirming two-stranded duplexes (as opposed to self-folded hairpins (see SI)). Overall, the results show (Table 1, Figure 2) that the fluorinated bases pair selectively with each other, as compared to the hydrocarbon−hydrocarbon pairing or the mixed fluorocarbon−hydrocarbon pairing. For example, the tetrafluorobenzene−tetrafluoroindole pair (**^{F}B**−**^{F}I**) is more stable than the similar benzene−indole pair lacking

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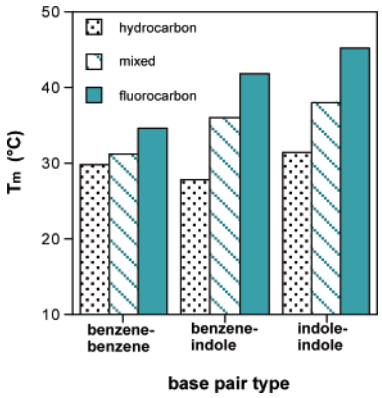

Figure 2. Histogram of base-pair stabilities as measured for double substitution of the pair into a 12-bp duplex (see Table 1).

Table 1. Thermodynamic Data for Duplexes Containing Fluorous and Hydrocarbon Bases[a]

base pair[b] (X•Y)	T_m [c] (°C)	$\Delta G°_{37}$ [d] (kcal/mol)	$\Delta\Delta G°_{37}$ [e] (kcal/mol)
B•B	29.8	−6.7 ± 0.2	−1.0 ± 0.4
^{F}B•^{F}B	34.6	−7.3 ± 0.1	−1.6 ± 0.3
I•I	31.5	−7.2 ± 0.2	−1.5 ± 0.4
^{F}I•^{F}I	45.2	−8.8 ± 0.1	−3.1 ± 0.3
B•I	27.8	−6.7 ± 0.1	−1.0 ± 0.3
^{F}B•^{F}I	41.8	−8.2 ± 0.1	−2.5 ± 0.3
T•A	58.1	−11.9 ± 0.3	−6.2 ± 0.5
T•C	20.3	−5.7 ± 0.2	−

[a] Conditions: 1 M NaCl, 10 mM phosphage (pH 7.0) with 0.1 mM EDTA. [b] Sequence is 5′- CGGXAGCTYCCG (self-complementary). [c] T_m values are at 5.0 μM. [d] Averages of values from van't Hoff and curve fitting methods. [e] Values resolve to the least stable duplex (the T−C mismatch).

fluorine; the difference is a significant 14 °C in T_m and 1.5 kcal/mol in free energy. The mixed versions of these pairs, placing hydrocarbon opposite fluorocarbon, resulted in pairing stabilities falling between those of the fully fluorinated and nonfluorinated pairs. The two other cases also confirm the selective pairing effect: the ^{F}**B**−^{F}**B** pair is more stable than the all-hydrocarbon **B**−**B** pair, and ^{F}**I**−^{F}**I** is more stable than **I**−**I**. The fluorinated ^{F}**I**−^{F}**I** pair is the most stable of the series, while the hydrocarbon **B**−**B** pair is the least stable. The difference between these extremes is 15.4 °C (2.1 kcal/mol), illustrating the significant degree by which structure and polyfluorination can affect base-pair stability.

To seek evidence for the origins of this selectivity, we examined individual properties of the four nonpolar nucleoside analogues. Stacking was evaluated by the standard dangling end approach using a 6-bp self-complementary DNA. Results showed (Table S3, SI) that the two polyfluorinated analogues do, in fact, stack more strongly than the two parent hydrocarbons. ^{F}**B** stacks 1.0 kcal/mol more favorably than **B**, and ^{F}**I**, 1.1 kcal/mol more favorably than **I**.

We explicitly examined hydrophobicity of the four nucleosides by partitioning between 1-octanol and water. The data are as follows: **B** (log P = 0.77 ± 0.10); ^{F}**B** (1.39 ± 0.10); **I** (0.99 ± 0.10); ^{F}**I** (1.66 ± 0.10). The results confirm what has been previously reported for polyfluorinated saturated hydrocarbons: namely, that they are more hydrophobic than their hydrocarbon variants.[14] For the present compounds, the order of hydrophobicity is ^{F}**I** > ^{F}**B** > **I** > **B**. This correlates well with the stabilities of their self-pairs as well as with their relative stacking abilities.[10]

Taken together, the data suggest that this selective pairing may be due to solvent avoidance of these specially hydrophobic structures on formation of a duplex relative to the more exposed single strands. Placing them in pairs opposite one another buries large fractions of the flat π surfaces and significant parts of the edges facing one another as well (see Figure S2, SI). Thus, the basic physical origins of the selective interaction appear to be similar to those seen recently in selective fluorinated peptide interactions.[3]

Our findings suggest that polyfluoroaromatic base pairing might be employed as a new, selective approach to pairing in DNA that is orthogonal to that of the natural genetic system. Future structural studies could shed light on the orientations of the base analogues in DNA. Also of interest is whether such fluorocarbon pairing selectivity could exert significant effects in the enzymatic replication of DNA.

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Supporting Information Available: Details of synthesis and thermodynamics methods (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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