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Effect of the Universal Base 3-Nitropyrrole on the Selectivity of Neighboring Natural Bases

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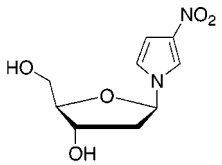
ABSTRACT



dR3-NP

The effect of the universal base 3-nitropyrrole on the pairing selectivity of neighboring nucleosides has been determined for every combination of complementary and neighboring nucleosides. In a subset of cases the discriminatory ability of the neighboring nucleoside for its Watson–Crick complement is compromised. The results have implications for the hybridization of oligonucleotides that contain 3-nitropyrrole and suggest caution in the use of oligonucleotides that contain other universal bases.

Nondiscriminatory base analogues, or universal bases, are potentially of great utility for a variety of manipulations of DNA. In particular, there is significant interest in using universal bases in degenerate primers either for pcr[1] or for Sanger-based sequencing methods.[1,2]

A number of nucleoside analogues have been investigated for their universal behavior. The focus of the majority of published studies has been to verify the nondiscriminatory hybridization properties of the universal base when incorporated into selected oligonucleotides[3] or peptide nucleic acids.[4] However, none of the purported universal bases has been subjected to a systematic investigation.

Because it is well established that nearest-neighbor sequence context affects the extinction coefficient,[5] duplex stability,[6] and mismatch stability[7] of oligonucleotides composed of only the natural bases, it is reasonable to believe that the sequence context in oligonucleotides containing a "nonnatural base" would also influence these properties. If oligonucleotides containing universal base candidates are to be generally useful, their hybridization properties must be fully understood.

We are interested in applying gapped probes,[8] containing universal bases, to sequencing by hybridization. To determine the suitability of various universal bases for this application, we have undertaken a thorough characterization of the

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hybridization behavior of oligonucleotides containing universal base candidates. Herein we report the effects of the 3-nitropyrrole base analogue on the stability of duplexes that have all four natural bases in the position opposite the universal base and that have all possible matches and mismatches of cognate base pairs in the adjacent positions.

To probe the behavior of nucleosides that are 3′ of the 3-nitropyrrole residue, we examined the hybridization properties of four 16-mer oligonucleotides (sequence **1** in Figure 1). Each oligo contained one 3-nitropyrrole (designated *)

5′-G TCC TCC * X TAC GAT G-3′ (**1**)
3′-C AGG AGG Y_1Y_2 ATG CTA C-5′ (**2**)

5′-G TCC TCC A X TAC GAT G-3′ (**3**)
3′-C AGG AGG T Y_2 ATG CTA C-5′ (**4**)

Figure 1. Structures of oligonucleotides used in thermal denaturation studies to probe effects 3′ of a 3-nitropyrrole residue. X and Y are A, G, C, T and * is a 3-nitropyrrole residue. Note that **2** and **4** are written in the 3′ to 5′ orientation reading left to right.

at position 8 (counting from the 5′ end) and one of the four natural bases (designated X) at the site 3′ to the 3-nitropyrrole (position 9). Each of these oligonucleotides **1** was hybridized with sixteen 16-mers composed of complementary oligonucleotides containing all possible combinations of natural bases at positions 8 and 9. This set of oligos is represented by sequence **2** in Figure 1.

For comparison, we also measured the effect on duplex stability of changes in the 3′-neighboring base pair with similar duplexes, formed from **3** and **4**, that contain an A/T base pair in place of the 3-nitropyrrole and its partners. Four oligos in which the 3-nitropyrrole in **1** was replaced with an adenosine (sequence **3** in Figure 1) were used to form duplexes with the four oligonucleotides **4**.

A similar set of experiments was performed to investigate the effect of a 3-nitropyrrole on 5′ neighboring nucleosides. In this series, we examined the hybridization properties of four 16-mer oligonucleotides that contained a 3-nitropyrrole at position 9 and one of the four natural bases at position 8 (sequence **5** in Figure 2)

5′-G TCC TCC X * TAC GAT G-3′ (**5**)
3′-C AGG AGG Y_1Y_2 ATG CTA C-5′ (**2**)

5′-G TCC TCC X A TAC GAT G-3′ (**6**)
3′-C AGG AGG Y_1 T ATG CTA C-5′ (**7**)

Figure 2. Sequences of oligonucleotides used in thermal denaturation studies to probe effects 5′ of a 3-nitropyrrole residue. X and Y are A, G, C, T and * is a 3-nitropyrrole residue. Note that **2** and **7** are written in the 3′ to 5′ orientation reading left to right.

Each of the four oligonucleotides **5** containing a 3-nitropyrrole was hybridized with each of the 16 complementary oligonucleotides **2**. As before, control duplexes, formed from sequences **6** and **7**, were also studied.

The melting temperatures of the 64 3-nitropyrrole-containing duplexes and the 16 control duplexes used to investigate the effect of a 3-nitropyrrole on the 3′ neighboring nucleoside are plotted in Figure 3. Each of the four graphs in Figure 3 contains five sets of data consisting of one set of controls and four sets of 3-nitropyrrole experiments.

The control data in each graph (dark blue squares) show the melting temperatures of the four duplexes formed between an oligonucleotide (**3**) with a fixed X and oligonucleotides **4** where Y_2 is varied among the four natural bases.

The four 3-nitropyrrole data sets in each graph represent the four possible bases X in **1**. Similar to the control, each data set shows the T_m values of duplexes formed between **1** with a fixed base at X and oligonucleotides **2** with Y_1 fixed and Y_2 varied among the four natural bases. The rectangular box in each graph encompasses data for the fully complementary duplexes (X and Y_2 are Watson−Crick base pairs).

For example, in Figure 3A, the control in dark blue shows the melting temperatures of the four duplexes formed from 5′-GTCCTCCA**G**TACGATG-3′ and 5′-CATCGTAY_2TGGAGGAC-3′. **G** indicates the position opposite Y_2 in the duplexes. The fully complementary duplex Y_2 = C has the highest melting temperature while the single mismatches Y_2 = A, G, T have lower melting temperatures.

Similarly the melting temperatures for duplexes formed with 5′-GTCCTCC***G**TACGATG-3′ and 5′-CATCGTA**Y_2A**GGAGGAC-3′ are shown in red. In this case the duplex has an A opposite the 3-nitropyrrole indicated by the asterisk. Again, the fully complementary duplex, Y_2 = C, has the highest melting temperature. Melting temperatures of related duplexes in which **Y_2A** in **2** is replaced with **Y_2G**, **Y_2C**, and **Y_2T** are plotted as a function of Y_2 with yellow triangles, cyan circles, and green asterisks, respectively. Any single line shows the effect on the T_m of changing the base opposite the base 3′ of the 3-nitropyrrole. The spread of T_m values at a given Y_2 for the four sets of 3-nitropyrrole data indicates how universal the 3-nitropyrrole is in that context.

The other graphs in Figure 3 show data for duplexes formed from **1** and the collection of oligos **2** and from **3** with the collection of oligos **4** in which X is A, T, or C.

The melting temperatures of the 64 3-nitropyrrole-containing duplexes and the 16 control duplexes used to investigate the effect of a 3-nitropyrrole on the 5′ neighboring nucleoside are plotted in Figure 4.

Several points are evident from the compiled data. First, as expected from, and consistent with, previous studies of 3-nitropyrrole-containing oligonucleotides, the replacement of a natural base with a 3-nitropyrrole residue results in a lowering of the T_m of the complementary duplex by 5−10 °C. Compare, for example, the control represented by the dark blue trace in Figure 3A which contains only natural bases with the four nearly superimposed traces below it.

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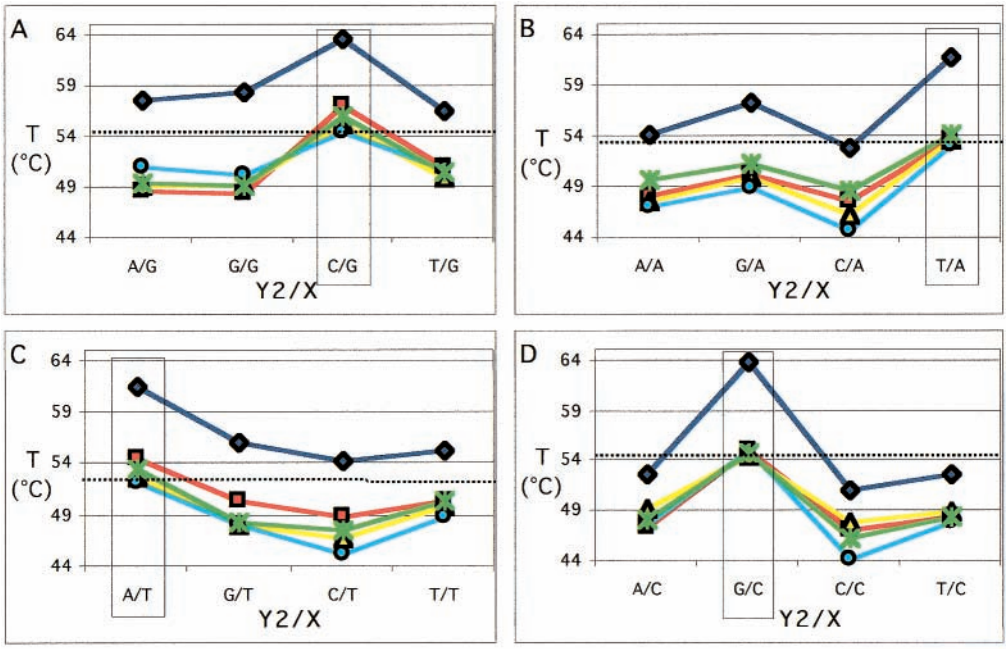

Figure 3. Thermal denaturation results for control duplexes formed with oligonucleotides **4** and **3** and 3-nitropyrrole-containing duplexes formed between oligonucleotides **1** and **2**. Each graph contains data for a different nucleoside positioned 3′- of the 3-nitropyrrole: (blue ◆) control, (red ■) Y1 = A, (yellow △) Y1 = G, (cyan ●) Y1 = C, (green ∗) Y1 = T. The rectangular box highlights the fully complementary duplex. The dashed line indicates the lowest melting temperature of the fully complementary duplex for oligos containing a 3-nitropyrrole.

Second, the comprehensive experiments described here demonstrate that the behavior of 3-nitropyrrole is indeed "universal" in most but not all contexts. For those duplexes in which Watson−Crick base pairs flank the 3-nitropyrrole,

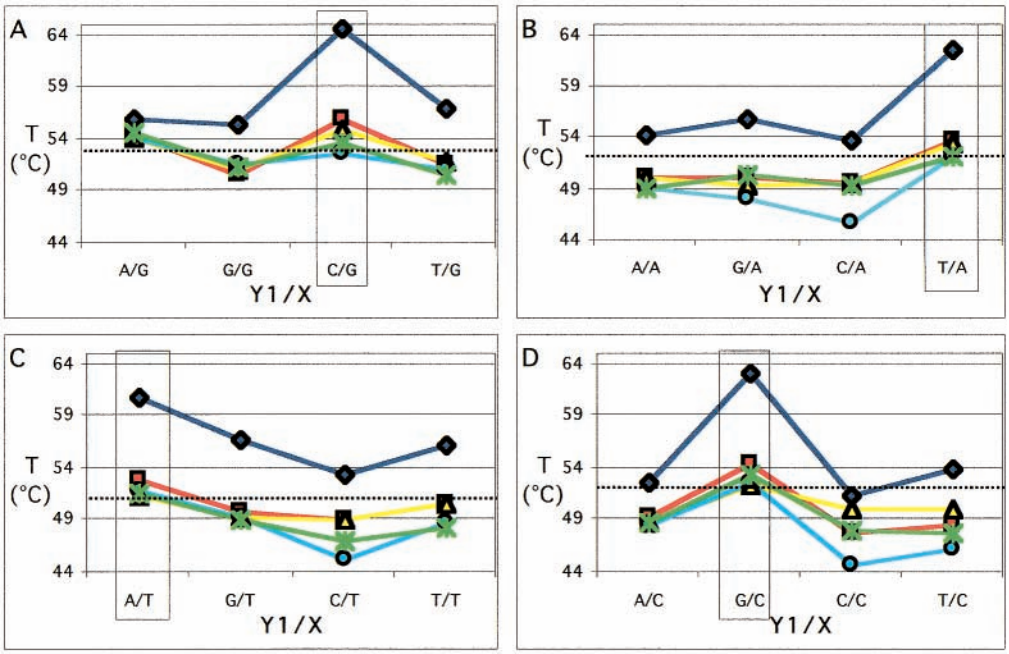

Figure 4. Thermal denaturation results for control duplexes formed with oligonucleotides **6** and **7** and 3-nitropyrrole-containing duplexes formed between oligonucleotides **5** and **2**: (blue ◆) control, (red ■) Y2 = A, (yellow △) Y2 = G, (cyan ●) Y2 = C, (green ∗) Y2 = T. The rectangular box highlights the fully complementary duplex. The dashed line indicates the lowest melting temperature of the fully complementary duplex for oligos containing a 3-nitropyrrole.

indicated in the graphs by the rectangular boxes, the largest spread in T_m is 3.3 °C (Figure 3A). When a mismatch occurs with the base neighboring the 3-nitropyrrole, the spread in T_m may be larger (up to 7.9 °C, Figure 4D, Y_1 = C).

Finally, comparison of duplex stability of 3-nitropyrrole-containing duplexes with control duplexes shows that the discriminatory behavior of specific sites in 3-nitropyrrole-containing oligos usually parallels the behavior in natural base containing oligos. However, the degree of selectivity, as measured by the difference in melting temperature between the Watson−Crick base pair and the most stable mismatch at the adjacent site, is usually reduced for 3-nitropyrrole-containing duplexes.

The diminished selectivity has ramifications for the application of oligonucleotides containing 3-nitropyrroles. If the presence of the universal base reduces the selectivity of neighboring bases too much, hybridization with incorrect sequences will occur. To preclude hybridization of 3-nitropyrrole-containing oligonucleotides to incorrect sequences, the lowest melting complementary duplex must have a T_m higher than that of the most stable mismatch. In each graph in Figures 3 and 4, the temperature of the lowest melting Watson−Crick duplex is shown with a horizontal dotted line.

In all cases but two the selectivity of the lowest melting Watson−Crick match is at least 1.7 °C higher than that of the highest melting mismatch. The two exceptions are when a G or a C is 5′ of the universal base (Figures 4A and 4C, respectively). When guanosine is at 5′ of the 3-nitropyrrole (Figure 4A), the specificity of the G residue is reduced to the point where the G/C Watson−Crick base pair and G/A mismatch are isoenergetic. When cytosine is at 5′ of the 3-nitropyrrole (Figure 4C), the difference in T_m for the lowest melting complementary duplex (a G opposite the 3-nitropyrrole) and the highest melting mismatch (Y1/X = T/T, with an A opposite the 3-nitropyrrole) is only 0.7 °C.

The experiments reported in this Letter are the first investigation of the effect on nearest neighbors of a universal base. They clearly indicate that universal bases can affect the pairing behavior at adjacent positions.

The data show the potential and also the limitations of the 3-nitropyrrole as a universal base; they also emphasize the importance of understanding the behavior of universal bases in all sequence contexts. It is likely that other universal base candidates will have their own characteristic effect on oligonucleotide hybridization; and it is possible that one or more will have close to "ideal" behavior. On the other hand, for at least some applications, nonideal behavior may be adequate so long as the contribution of the universal base to hybridization and its effects on nearest neighbors in all sequence contexts are taken into account.

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Supporting Information Available: Experimental details for thermal denaturation experiments, discussion of data fitting to determine melting temperatures of duplexes, and tabulated data from thermal denaturation studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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