Polymerase Recognition of 2-Thio-iso-Guanine•5-Methyl-4-Pyrimidinone (iGs•P) – A New DD/AA Base Pair

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ABSTRACT

Polymerase specificity is reported for a previously unknown base pair with a non-standard DD/AA hydrogen bonding pattern: 2-thio-iso-guanine•5-methyl-4-pyrimidinone. Our findings suggest that atomic substitution may provide a solution for low fidelity previously associated with enzymatic copying of iso-guanine.

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The distinct hydrogen-bonding patterns found in natural A•T and G•C Watson-Crick base pairs enable the storage and transfer of genetic information in all terran organisms. Over the past two-and-a-half decades, considerable effort has been expended to increase the number of functional Watson-Crick-like base pairs using non-standard hydrogen bonding patterns, or other types of non-covalent interactions. These efforts have led to non-standard DNA base pairs capable of enzymatic replication in the context of PCR and a semi-synthetic organism, as well as an enhanced understanding of the physical principles underlying both base-pairing and replication.^{1,2}

The iG•iC base-pair was postulated over fifty years ago as an extension of the natural set of Watson-Crick hydrogen bonding patterns.³ Subsequent assessment of this proposal showed that the iG•iC pair was incompletely functional for replication and translation owing to the formation of mispairs between iG and T.⁴ The tendency of iG to pair with both iC and T has been attributed to the coexistence of N1-H and O2-H iG tautomers.⁵ Several strategies have been investigated to date to improve the fidelity of iG•iC, including the replacement of iG with isoinosine,⁶ 7-deaza-iG,⁷ 7-halo-7-deaza-iG,⁸ 8-aza-iG,⁹ and HNA-iG.¹⁰ While several of the nucleobase analogs increased the N1-H tautomer content of iG, none of the iG alternatives examined enable replication in the absence of high levels of iG>A transitions.

Sulfur modified nucleobases occur naturally in tRNA, and include 2-thio-U, 4-thio-U and 2-thio-C, among others.¹¹

Moreover, the anticipated prebiotic accessibility of sulfur-modified nucleobases suggests that they are fundamental constructs.¹² Beyond tRNA, the effect of sulfur substitution on DNA has been investigated, including: 2-S-T,¹³ 4-S-T,^{13,14} 2-S-C,¹⁵ and 6-S-G.¹⁶ As part of the continuing search for an iG replacement structure that exhibits fidelity for its nonstandard pyrimidine complement, we report on 2-thio-iG (iGs) interactions with 5-methyl-4-pyrimidinone (P) and iC, both in a DNA double helix and under the direction of a DNA polymerase, including

Figure 1. The 2-thio-iso-guanine•5-methyl-4-pyrimidinone (iGs•P) and iso-guanine•5-methyl-iso-cytosine (iG•iC) base-pairs.

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faithful enzymatic copying of the iGs•P base pair (Figure 1).

Table 1. Melting temperatures (T_m values) from UV monitored thermal denaturation of DNA double helices. Samples contained 2.5 μ M of each oligonucleotide strand, NaCl (1M), Na_2HPO_4 (10mM, pH=7.0), EDTA (0.1mM, pH=7.0). Errors in T_m values are estimated as $\pm 0.5^\circ\text{C}$.

5'-d-GTTTXXXXTTC 3'-d-CAAAYAAAG				
X/Y	P	iC	C	T
iGs	21.8	23.4	<10	<10
iG	20.1	29.7	17.9	22.9
G	16.5	20.3	32.6	<10
A	<10	17.1	<10	26.0

5'-d-AGGGAGXGAAAG 3'-d-TCCCTCYCTTTC				
X/Y	P	iC	C	T
iGs	50.5	53.6	45.2	45.3
iG	50.0	60.1	48.3	51.4
G	50.3	50.6	56.3	44.9

Before conducting polymerase studies, the iGs•P and iGs•iC base-pair and mismatch stabilities were assessed by UV-monitored thermal denaturation of DNA double helices. Towards this end, we synthesized complementary oligonucleotides containing the iGs, iG, P and iC nonstandard nucleobases. iGs was synthesized and incorporated into DNA using the procedure we reported recently.¹⁷ DNA oligomers bearing P were synthesized from the corresponding phosphoramidite, which was prepared as outlined by Rajur and McLaughlin.¹⁸ Table 1 summarizes the results from the thermal denaturation studies.

Figure 2. A) The polymerase catalyzed reaction studied, including primer and template sequences. B) Autoradiograph of the results of primer extension using templates containing iGs or iG and only those dNTPs corresponding to the nucleosides indicated above each lane, 9U AMV reverse transcriptase, 37°C, 1 h.

Based on the two duplexes studied, the relative base-pair stabilities were observed on average as: $\text{G}\cdot\text{C} \sim \text{iG}\cdot\text{iC} > \text{A}\cdot\text{T} > \text{iGs}\cdot\text{iC} > \text{iG}\cdot\text{P}$. While it was not always possible to detect mismatches in the case of the smaller nonamer duplex, the mismatches that were detected along with those observed in the dodecamer case revealed several notable features. First, iGs exhibits a leveling of the C and T mismatch stabilities in comparison to iG, which shows a greater affinity for T over C. Second, both P and iC form stronger mispairs with G than A.

Polymerase studies were performed with the template/primer combinations illustrated in Figure 2A in conjunction with AMV reverse transcriptase previously employed for iG.^{4b} Initially, the ability was probed of template iGs to direct the incorporation and extension of its intended cognate base, iC, while avoiding mispairing with T or the other natural nucleobases. The autoradiograph depicted in Figure 2B, obtained after PAGE separation of the polymerase reaction products, clearly indicates no template iGs directed incorporation of C, A, or G (lanes 2, 3, 5). Satisfyingly, iGs directed incorporation of T is nearly absent (less than 1% based on densitometry, lane 4, Figure 2B). In contrast to the result in lane 4, when iGs is replaced by a template bearing iG instead, complete incorporation of T is observed (lane 11, Figure 2B) as expected based on prior studies due to the iG enol tautomer.^{4,5} Problematically, however, iGs was found to no longer direct the incorporation of iC (iGs lane 6 vs. iG lane 12, Figure 2B).

Figure 3. Autoradiograph of the results of primer extension using templates containing iGs, A or G and only those dNTPs corresponding to the nucleosides indicated above each lane, 9U AMV reverse transcriptase, 37°C, 1 h.

To circumvent the inability of iGs to direct the incorporation of iC, we elected to remove the 2-amino group of the pyrimidine component by replacing iC with P (Figure 1). The outcome of this pyrimidine 2-substituent size reduction ($\text{NH}_2 \rightarrow \text{H}$) was expected to be a base pair possessing N9-N1 and C1'-C1' distances identical to natural Watson-Crick pairs. Reactions catalyzed with AMV reverse transcriptase exploring the replacement of diCTP with dPTP are shown in Figure 3. As a point of reference, lane 5 in Figure 3 corresponds to lane 4 of Figure 2B, demonstrating the nearly absent pairing between iGs and T (less than 1% based on densitometry). In contrast to the absence of incorporation of iC by iGs (lane 6, Figure 2B), ready incorporation of P by iGs is observed (lane 4, Figure 3). Following on the successful recognition of P by iGs, the absence of P mispair formation with either A or G was probed and confirmed (lanes 10 and 13, respectively, Figure 3).

In summary, we have reported faithful polymerase copying of the iGs•P base pair. In particular, we have shown polymerase directed incorporation by iGs of cognate pyrimidine P, and the absence of incorporation of P by non-cognate purines A or G. Critically, there is a near absence of polymerase directed misincorporation by iGs of T -- a fidelity issue that has plagued iG as a Watson-Crick base pair component from its inception. On the basis of our results, we suggest that iGs•P is a possible alternative to the iG•iC non-standard base pair. iGs appears to be unique among iG congeners in its ability to avoid enzyme directed interactions with T. Efforts to further explore the iGs•P base pair are in progress.

Acknowledgments

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Graphical Abstract

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