Selective Recognition of Unnatural Imidazopyridopyrimidine:Naphthyridine Base Pairs Consisting of Four Hydrogen Bonds by the Klenow Fragment

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Selective recognition of complementary base pairs during DNA replication by DNA polymerase is a fundamental biological process in transmitting genetic information. Since this ubiquitous event occurs in all living matter using only two sets of base pairs consisting of adenine (A):thymine (T) and guanine (G):cytosine (C), the creation of alternative new base pair(s) other than the Watson–Crick base pairs, which could replicate selectively, would be a potentially useful contribution to the field. Starting with the pioneering work of Benner’s group,¹ the development of such base pairs has been intensely investigated to expand the genetic code and explore synthetic biology.² Throughout these aforementioned investigations, it has been suggested that shape complementarity of the purine:pyrimidine pair and hydrophobic (stacking) interactions between the nucleobases as well as the complementarity of the hydrogen bonds (H-bonds) are critical for the selective recognition of DNA polymerases.

We have been working on a project to develop new base pairs consisting of four H-bonds.³ Accordingly, we designed imidazopyridopyrimidine (Im):naphthyridine (Na) base pairs and found that the DNA duplexes with ImO:\NaO and ImO:\NaO pairs were highly thermally stabilized (±8–9 °C per pair) resulting from (1) four noncanonical H-bonds, (2) stacking ability, and (3) shape complementarity of the Im:Na pair (Figure 1).⁴ These successful results prompted us to investigate how these thermally stable base pairs are recognized by DNA polymerases. In this communication, we report the results of kinetic studies of Im:Na base pair recognition by the Klenow fragment (KF).

We began the proposed study by preparing the corresponding nucleoside 5′-triphosphates, ImO\TP, ImO\NT, NaO\TP, and NaO\TP (Supporting Information, Schemes S1 and S2), and then we examined single nucleotide insertion into a template—primer duplex (Figure S1) by KF. As can be seen in Figure 2, NaO\TP was incorporated against ImO\ in the template to afford a 21-mer sequence (Figure 2A, lane 7)⁵ while other dNTPs were not incorporated at all (lanes 2–6). When NaO\ was introduced in the template, dATP as well as ImO\TP was incorporated as the complementary 5′-triphosphate (Figure 2B, lanes 8 and 12). When the same reactions were carried out in the ImO\:NaO\ pair (Figure S2), a higher selectivity was observed.

To understand these observations quantitatively, we determined the kinetic parameters (Km = the Michealis constant, Vmax = the maximum rate of the enzyme reaction, and Vmax/Km = the insertion efficiency) of every 5′-triphosphate at various concentrations (Table 1). The quantitative analyses revealed that KF incorporated NaO\TP preferentially against ImO\ in the template, and the efficiency was 100–1000-fold higher than other dNTPs (Vmax/Km; 8.5 × 10⁸ vs 2.3 × 10⁷–5.1 × 10⁸). Although the efficiency of ImO\TP incorporation against NaO\ was slightly higher than that of NaO\TP against ImO\ (Vmax/Km; 2.5 × 10⁸ vs 8.5 × 10⁸), incorporation of dATP also showed the same efficiency (Vmax/Km; 2.9 × 10⁷ vs 2.5 × 10⁸). For the ImO\:NaO\ pair, either ImO\TP or NaO\TP was incorporated selectively against NaO\ and ImO\, respectively, in the templates although the efficiencies were ~1 order of magnitude lower than those of ImO\TP and NaO\TP (Vmax/Km; 8.5 × 10⁶ vs 2.3 × 10⁵ and 2.5 × 10⁵ vs 3.6 × 10⁴, respectively).

A careful consideration of these results indicates first that noncanonical base pairs consisting of four H-bonds were, interestingly, recognized preferentially by KF as complementary bases. Although one can imagine, for example, a T:ImO pair with three H-bonds (Figure S3), TTP incorporation was approximately a few thousand-fold less than NaO\TP, and enzymatic recognition of the pair with four H-bonds by KF was thought to act advantageously. On the other hand, the efficiency of dATP incorporation against NaO\ was almost equal to that of ImO\TP despite the fact that only two H-bonds can be expected in the A:NaO\ pair (Figure S3). This result suggests that NaO\ in the template would be recognized as a ring-expanded T analogue. For the ImO\:NaO\ pair, the selectivities against natural dNTPs were higher than those of the ImO\:NaO\ pair, although the efficiencies of the NaO\TP and ImO\TP incorporation were somewhat lower. These results can be attributed to the H-bonding pattern of ImO\:NaO\ pair. Thus, it has been suggested that interaction of the N3 of the purine base and the O2 of the pyrimidine base as proton acceptors located in the minor groove with the DNA polymerase is critical for dNTP incorporation (see A:T pair in Figure 1).⁶ In the case of the ImO\:NaO\ pair, a similar interaction is expected as depicted in Figure 1, while the proton acceptor corresponding to the O2 of the pyrimidine base is missing in the ImO\:NaO\ pair. Therefore, this unusual H-bonding pattern is thought to exhibit higher selectivity, albeit a lower efficiency for the ImO\:NaO\ pair relative to the

Figure 1. Structures of base pairs consisting of four H-bonds.

Figure 2. Single nucleotide insertion by Klenow fragment (selectivity toward natural dNTPs). (A) Incorporation of dYTP against ImO\ in template. (B) Incorporation of dYTP against NaO\ in template. In lanes 14 and 15, the results of matched and mismatched pairs of natural substrates were shown. Experimental details are described in the Supporting Information.

References:

ImON:NaNO pair. Additionally, there is a noticeable difference in efficiency between incorporation of NaNOTP (and NaONTP) against ImON (and ImNO) in the template and that of ImONTP (and ImNOTP) against NaON (and NaOTP). Thus, the former is about 1 order of magnitude less effective relative to the latter. The Im bases can be considered as ring-expanded analogues of purine toward the minor groove direction, while the Na bases are ring-expanded analogues of pyrimidine toward the major groove direction. In general, reaction by DNA polymerase tolerated steric repulsion in the major groove site in the template duplex, whereas steric repulsion in the minor groove site in the template duplex appeared to have an adverse effect. Thus, our results would seem to agree with these previous observations.

As described above, KF incorporated the noncanonical 5′-triphosphates against the complementary base in the templates. These recognitions would arise from the four H-bonds and also the shape complementarity of the Im:Na pair, which is similar to the purine:pyrimidine base pair. To the best of our knowledge, this is the first example of enzymatic recognition of base pairs possessing four H-bonds. Our results would be a contribution toward developing alternative stable base pairs to expand the genetic code and explore the synthetic biology.

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Supporting Information Available: Synthesis of noncanonical nucleoside 5′-triphosphates and conditions applied in enzymatic incorporation. This material is available free of charge via the Internet at http://pubs.acs.org.

References


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Table 1. Steady-State Kinetics Data of the Single Nucleotide Insertion by Klenow Fragment

<table>
<thead>
<tr>
<th>X</th>
<th>dYTP</th>
<th>K_m (μM)</th>
<th>V_max (% min⁻¹)</th>
<th>V_max/K_m (% min⁻¹·M⁻¹)</th>
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<tr>
<td>ImON</td>
<td>dATP</td>
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<td>dGTP</td>
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<td></td>
<td>dCTP</td>
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<td>0.93 ± 0.01</td>
<td>2.2 ± 10⁶</td>
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<td>TTP</td>
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<td>0.25 ± 0.01</td>
<td>5.1 ± 10⁵</td>
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<td>ImONTP</td>
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<td>22 ± 0.96</td>
<td>8.5 ± 10⁵</td>
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<td>NaNO</td>
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<td>20 ± 3.4</td>
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<td>dGTP</td>
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<tr>
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<td>0.12 ± 0.01</td>
<td>4.8 ± 10⁸</td>
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<td></td>
<td>TTP</td>
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<td>0.11 ± 0.015</td>
<td>5.8 ± 10⁷</td>
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<td>ImONTP</td>
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<td>21 ± 5.3</td>
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<td>2.3 ± 10⁴</td>
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</table>

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Figure 3. Single nucleotide insertion by Klenow fragment (selectivity toward noncanonical dNTPs). (A) Incorporation of dYTP against ImON in template. (B) Incorporation of dYTP against ImON in template. Experimental details are described in the Supporting Information.

In summary, we have investigated how thermally stable ImON; NaNO and ImON:NaNO pairs are recognized by KF. Although dATP and ImONTP were incorporated against NaNO in the template, these complementary base pairs, especially the ImON:NaNO pair, were recognized selectively by KF. This selectivity of these noncanonical pairs is considered to be due to the four H-bonds between the nucleobases and the shape complementarity of the Im:Na pair similar to the purine:pyrimidine base pair. To the best of our knowledge, this is the first example of enzymatic recognition of base pairs possessing four H-bonds. Our results would be a contribution toward developing alternative stable base pairs to expand the genetic code and explore the synthetic biology.

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