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Review

Non-natural nucleotides as probes for the mechanism and fidelity of DNA polymerases

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ABSTRACT

DNA is a remarkable macromolecule that functions primarily as the carrier of the genetic information of organisms ranging from viruses to bacteria to eukaryotes. The ability of DNA polymerases to efficiently and accurately replicate genetic material represents one of the most fundamental yet complex biological processes found in nature. The central dogma of DNA polymerization is that the efficiency and fidelity of this biological process is dependent upon proper hydrogen-bonding interactions between an incoming nucleotide and its templating partner. However, the foundation of this dogma has been recently challenged by the demonstration that DNA polymerases can effectively and, in some cases, selectively incorporate non-natural nucleotides lacking classic hydrogen-bonding capabilities into DNA. In this review, we describe the results of several laboratories that have employed a variety of non-natural nucleotide analogs to decipher the molecular mechanism of DNA polymerization. The use of various non-natural nucleotides has lead to the development of several different models that can explain how efficient DNA synthesis can occur in the absence of hydrogen-bonding interactions. These models include the influence of steric fit and shape complementarity, hydrophobicity and solvation energies, base-stacking capabilities, and negative selection as alternatives to rules invoking simple recognition of hydrogen-bonding patterns. Discussions are also provided regarding how the kinetics of primer extension and exonuclease proofreading activities associated with high-fidelity DNA polymerases are influenced by the absence of hydrogen-bonding functional groups exhibited by non-natural nucleotides.

1. Introduction

DNA polymerases are responsible for chromosome replication, and many play essential roles in DNA repair and recombination. These enzymes add mononucleotides onto the 3′-end of a primer strand using the complementary strand as a template (Fig. 1A). Viewing DNA in this simple two-dimensional projection gives the impression that hydrogen-bonding interactions between the template base and the base of the incoming nucleotides are the most powerful physical forces that stabilize the conformation and structure of nucleic acid. By inference, these hydrogen-bonding interactions are thought to be the primary determinants in base pair recognition during the polymerization reaction. In this case, the mutual recognition of adenine (A) by thymine (T) and of guanine (G) by cytosine (C) involves hydrogen-bonding interactions between each partner (Fig. 1B). At the atomic level, the non-sp2 hybridized amino groups are good hydrogen bond donors (denoted as ***d***) while the oxo and the sp2 hybridized amino groups within the heterocyclic rings are hydrogen bond acceptors (denoted as ***a***). For these preferred tautomers, the pattern for an A:T base pair uses complementarity ***d*a*(−)*** to ***a*d*a*** hydrogen-bonding interactions while the G:C base pair uses complementarity ***a*d*d*** to ***d*a*a*** interactions. These base pairing patterns are commonly referred to as Watson-Crick base pairs and are the predominant pattern used to stabilize DNA.[1]

Although hydrogen-bonding interactions are a prominent feature that influences the conformation and tertiary stucture of DNA, other physical features such as π–π stacking interactions, desolvation/

Abbreviations: dNTP, deoxynucleoside triphosphate; A, adenine; C, cytidine; G, guanine; T, thymine; dAMP, adenosine-2′-deoxyriboside monophosphate; dCMP, cytosine-2′-deoxyriboside; dGMP, guanosine-2′-deoxyriboside dTMP, thymine-2′-deoxyriboside; dF, 2,4-difluorotoluene; dFMP, 2,4-difluorotoluene monophosphate; dPMP, pyrene 2′-deoxyriboside monophosphate; 5-NI nucleotide, 5-nitro-indolyl-2′-deoxyriboside triphosphate; 5-Nap nucleotide, 5-napthyl-indolyl-2′deoxyriboside triphosphate; 5-Ph nucleotide, 5-phenyl-indolyl-2′-deoxyriboside triphosphate; 5-CE-nucleotide, 5-cyclohexene-indolyl-2′deoxyriboside triphosphate; 5-CH-nucleotide, 5-cyclohexyl-indolyl-2′deoxyriboside triphosphate; dQ, 9-methyl-1*H*-imidazo-[4,5-b]pyridine; dZ, 4-methylbenzimidazole nucleoside; d3FB, 3-fluorobenzene 2′ deoxyriboside

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[1] Other base-pairing interactions such as Hoogsteen and wobble base pairs have been identified and extensively characterized (reviewed in [1]).

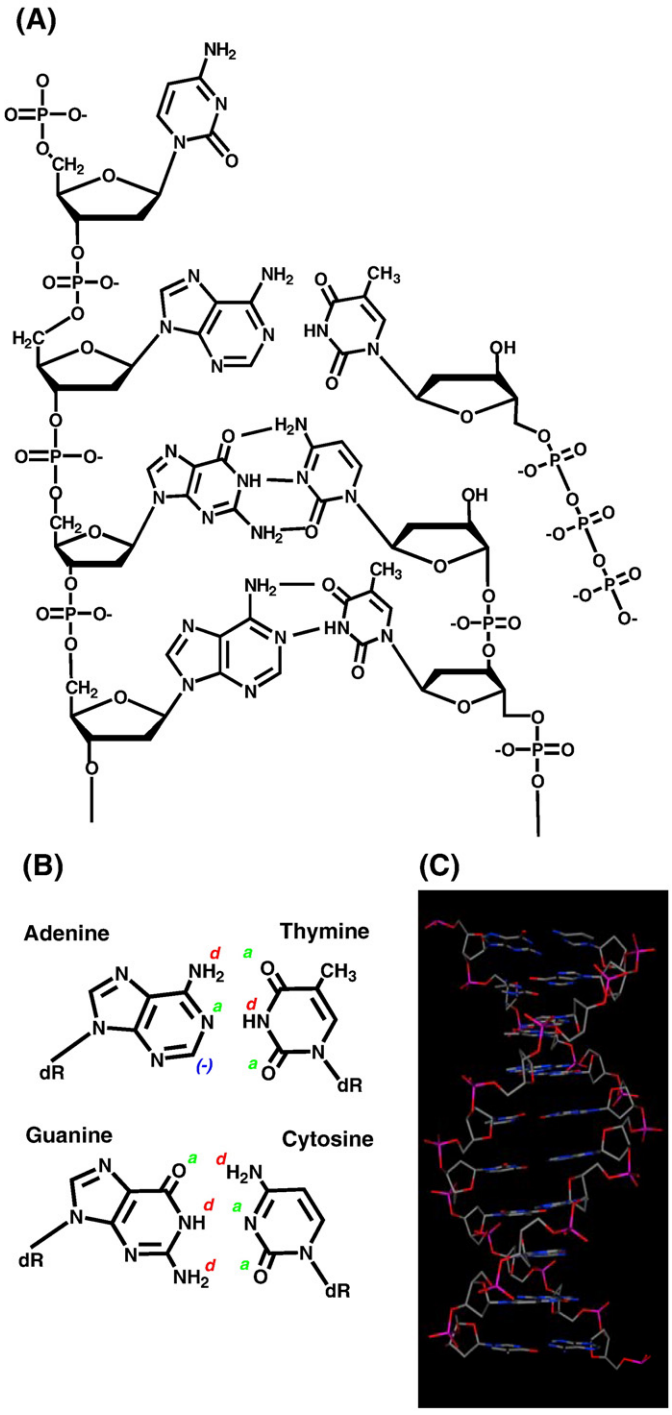

Fig. 1. (A) DNA as presented in linear, two-dimensional projections. (B) Hydrogen-bonding interactions between natural nucleobases. (C) Three-dimensional representation of typical B-form DNA highlighting the influence of hydrogen-bonding interactions, steric constraints, π-π stacking interactions, and hydrophobicity on its structure.

hydrophobic effects, and geometrical constraints contribute extensively to the stability of nucleic acid (reviewed in [1]). In solution, DNA exists as a double helix that resembles an intertwining spiral staircase where the nucleobases are stacked above and below one another (Fig. 1C). In this native form, each base is rotated ~36° around the helical axis relative to the next base pair such that roughly 10 base pairs make a complete turn of 360°. While hydrogen-bonding, π–π stacking interactions, solvation and hydrophobic effects, and geometrical constraints play important roles in defining the structure of DNA (reviewed in [2]), their roles during DNA polymerization remain remarkably elusive. One example is with respect to the roles of hydrophobicity and desolvation energies. Hydrophobicity defines the tendency of a molecule to repel water whereas desolvation energy defines the quantity of energy required to remove water from a molecule. Although these terms are sometimes used interchangeably, each provides a unique biophysical consequence toward stabilizing nucleobase interactions during DNA polymerization. For example, it is evident from the structures of duplex DNA that the interior of the helix is hydrophobic since it is devoid of water. This hydrophobic environment is essential for the formation of the correct hydrogen-bonding network between each base pair. However, creating a hydrophobic environment during DNA polymerization is challenging since desolvation must occur on the templating and incoming nucleobase.

DNA polymerases are fascinating enzymes as they maintain remarkable specificity despite the fact that the heteropolymeric nature of the genomic message dictates that the substrate requirement changes during each cycle of nucleotide incorporation. As such, it is remarkable that most polymerases are strict in their ability to selectively incorporate only one of four potential deoxynucleoside 5′-monophosphates (dNMPs) opposite a template base while being flexible enough to recognize four distinct pairing partners (A:T, G:C, T:A, and C:G). In fact, replicative DNA polymerases display incredible fidelity as they have error frequencies of only 1 mistake every 10^6 opportunities [3-5]. Even more impressive is the fact that these enzymes perform the repetitive cycle of nucleotide binding, base pairing, phosphodiester bond formation, product release, and movement to the next templating position at rates greater than 100 bp/s [6]. The underlying molecular events describing the remarkable speed and accuracy of DNA polymerases are generally defined by the rate and equilibrium constants for all the individual reactions involved in polymerization cycle. These include the binding of the substrates DNA and dNTP, conformational changes, phosphoryl transfer, and kinetic steps associated with product release. However, the details for how polymerases cope with the intricate biophysical features of both DNA and dNTP substrates are often ignored. In this review, we describe the work of several laboratories that have used various non-natural nucleotides to define the contribution of the aforementioned biophysical forces on the kinetics and selectivity of nucleotide incorporation. Each distinct section will describe the application of a unique set of non-natural nucleotides toward understanding DNA polymerization. This is provided as a historical perspective describing the rationale for designing the nucleotide analog of interest, a description of pertinent results, and a discussion of mechanistic implications. In addition, we describe the mechanistic information gained from applying these non-natural nucleotides on the behavior of DNA polymerases during primer elongation and exonuclease proofreading.

2. Lessons learned from replicating alternative hydrogen-bonding patterns

One of the first published efforts of rationally designing an alternative base pair that could be efficiently replicated was reported by Steve Benner's group [7]. The strategy was remarkably simple and straightforward: construct a base pair that is geometrically identical to an existing Watson–Crick base pair by manipulating the spatial arrangements of hydrogen-bonding acceptor–donor pairs. An intriguing base-pairing combination consisting of iso-cytosine paired with iso-guanine was initially tested (Fig. 2A). While this novel base pair has differing arrangements of hydrogen-bond acceptor–donor pairs compared to natural A:T and C:G base pairs, this combination is predicted to maintain the proper interglycosyl bond distance and angles relative to a Watson–Crick base pair. The *Escherichia coli* Klenow fragment was used to test whether each non-natural base pair could be formed enzymatically. Although the iso-cytosine:iso-guanine base pair can be formed [8], the overall fidelity of this new base pair is low as iso-guanine can be easily incorporated opposite a templating thymine and vice versa [7]. The ease for forming an iso-