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NMR investigation of DNA primer–template models: Structural insights into dislocation mutagenesis in DNA replication

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Abstract Slipped frameshift intermediates can occur when DNA polymerase slows or stalls at sites of DNA lesions. However, this phenomenon is much less common when unmodified DNA is replicated. In order to study the effect of templating bases on the alignment of primer–templates, NMR structural investigation has been performed on primer–template oligonucleotide models which mimic the situation that dNTP has just been incorporated opposite template. NMR evidence reveals the occurrence of misalignment when dGTP is incorporated opposite template T with a downstream nucleotide C. Depending on the template sequence, further extension of the primer can lead to realignment.

Keywords: NMR; DNA; Low fidelity polymerase; Primer–template; Misalignment; Bulge

1. Introduction

A DNA sequence has long been considered as a piece of passive substrate that awaits processing by enzymes. During transcription, the genetic sequence information is read by DNA transcriptase for protein synthesis. During DNA replication, the sequence is copied by DNA polymerases. The fidelity of replication is related to selectivity of DNA polymerase, exonucleolytic proofreading function and post-replication mismatch repair activity [1]. Highly processive and accurate DNA synthesis is required for maintaining genetic information and avoiding mutations that can initiate and promote human diseases over generations. On the contrary, negative consequences of mutations are essential to facilitate translesion synthesis of otherwise replication-blocking lesions. They are counterbalanced by the requirement for mutations in evolution, fitness and immunological diversity [2].

Continuous efforts in DNA polymerase research improve our understanding of the replication fidelity. The discovery of Y-family polymerases that are able to efficiently bypass damaged template of bulky DNA adducts and replicate undamaged DNA with a 10- to 100-fold increase in error rate compared to other family polymerases provides new mechanistic pathways for evolution [3–7]. The Y-family polymerases have no detectable sequence similarity with other family polymerases and lack any intrinsic exonucleolytic activity for proofreading [8]. Their shapes resemble a right-hand with fingers, palm, thumb and a unique C-terminal little finger domain [9,10]. Unlike other family polymerases, their active sites are open and spacious that only a few van der Waals contacts are present with the replicating base pairs. A bulky adduct, a wobble or Hoogsteen base pair is thus allowed at or beyond the loose active site [11–13]. Recently, crystal structures of the low-fidelity polymerases containing a misaligned DNA template in active sites have been determined [12,14]. The results support the accommodation of misaligned primer–template in the loose active site [15], which ultimately can lead to dislocation mutagenesis [16–18]. Yet whether misalignment of primer–template is due to the presence of the polymerase or due to the template sequence remains elusive.

It is well known that slipped frameshift intermediates can occur when the polymerase slows or stalls at sites of DNA lesions [19–25], but such effects are much less common when unmodified DNA is replicated. In order to study how templating bases affect the alignment of primer–templates, we have investigated the base pair structures at the replicating sites of a set of primer–template oligonucleotide models using NMR spectroscopy. Since the most striking violation of Watson–Crick base pairing rules is exhibited by the human Y-family polymerase iota (Polι), which inserts dGTP opposite template T even more efficiently than inserts A opposite T [26–28], we have initially synthesized and purified two 15-nucleotide DNA sequences, mimicking the intermediate replicating products that either dATP or dGTP has just been incorporated opposite template T (Fig. 1A).

2. Materials and methods

2.1. Sample design

All DNA samples were designed to contain a 5′-GAA loop that connects the primer and template in order to simplify the sample preparative work. These samples mimic the primer–templates that deoxyribonucleotide triphosphate (dNTP) has just been incorporated to templates. The 5′-overhang region of the samples represents template sequences whereas the 3′-terminal nucleotide represents the newly incorporated dNTP at the end of primers.

2.2. Sample preparation

All DNA samples were synthesized using an Applied Biosystems Model 392 DNA synthesizer and purified using denaturing polyacryl-

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Abbreviations: NMR, nuclear magnetic resonance; dNTP, deoxyribonucleotide triphosphate; T_m, melting temperature; ΔH°, change of enthalpy; ΔS°, change of entropy; ΔG°, change of Gibbs free energy; NOE, nuclear Overhauser effect; 2D NOESY, two-dimensional nuclear Overhauser effect spectroscopy; polι, polymerase iota; WATERGATE, water suppression by gradient-tailored excitation

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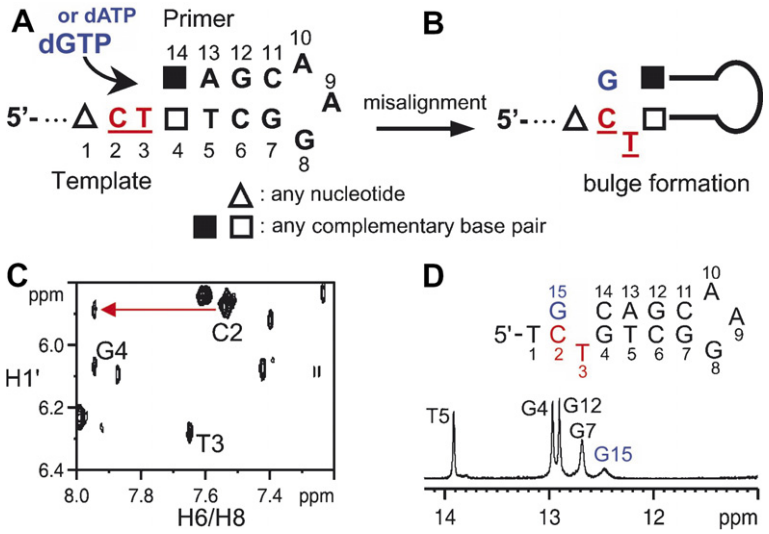

Fig. 1. (A) DNA sequence mimics the intermediate replicating product. (B) Misalignment leads to the formation of GC base pair and a T bulge. (C) The NOESY fingerprint region shows an unusual cross-peak between C2 and G4. (D) The presence of G15 imino signal at 5 °C shows there is C2–G15 Watson–Crick base pair.

amide gel electrophoresis and diethylaminoethyl Sephacel anion exchange column chromatography. NMR samples were prepared by dissolving 1 mM purified DNA samples into 500 μl buffer solution containing 150 mM sodium chloride, 10 mM sodium phosphate at pH 7 and 0.1 mM 2,2-dimethyl-2-silapentane-5-sulfonic acid.

2.3. NMR analysis

All NMR experiments were performed using either a Bruker ARX-500 or AV-500 spectrometer operating at 500.13 MHz and acquired at 25 °C unless stated otherwise. 1D imino spectra were acquired using water suppression by gradient-tailored excitation (WATERGATE) pulse sequence [29,30]. 2D NOESY experiments on samples in 100% D_2O buffer solution and 2D WATERGATE-NOESY experiments on samples in 90% H_2O/10% D_2O buffer solution were performed at 300 ms mixing time and 4k × 512 data sets were collected. The acquired data were zero-filled to give 4k × 4k spectra with cosine window function applied to both dimensions.

2.4. UV optical melting study

UV absorbance data at 260 nm were measured versus temperatures from 25 to 95 °C at a heating rate of 0.8 °C/min using a HP 8453 Diode-Array UV–Vis spectrophotometer. The DNA sample concentration was kept at 5 μM and a 10 mm path length cuvette was used. Thermodynamic parameters were determined from the melting curves using the software MELTWIN version 3.5 (available from Jeffrey A. McDowell at www.meltwin.com).

3. Results and discussion

In this work, we mainly used 1D imino proton and 2D NOESY analysis to probe the base pair structures at the replicating sties of the primer–template models. Since imino proton observation is only feasible when its exchange with the solvent is slow on the NMR time scale, the appearance of imino signals indicates the presence of base pairings. On the other hand, the presence of a NOESY cross-peak between two protons in different nucleotides indicates the two nucleotides are close in space. In order to obtain structural results, sequential assignments of the aromatic, sugar and imino protons were made by investigating the fingerprint regions in 2D NOESY and WATERGATE-NOESY spectra (Appendix A S1–S3).

When dGTP has just been incorporated opposite 5′-CT template sequence, we have found the formation of a GC Watson–Crick base pair and a T bulge due to misalignment (Fig. 1B). This is supported by the presence of an unusual C2H1′-G4H8 NOE (Fig. 1C), which indicates C2 and G4 are close in space. In regular B-type DNA structures, it is expected that sequential connectivities between consecutive nucleotides will be present in 2D NOESY spectrum [31]. No sequential C2-T3 and T3-G4 NOEs have been observed in the H6/H8–H1′ fingerprint region of the NOESY spectrum. The appearance of G15 imino proton signal at lower temperatures indicates the presence of G15–C2 base pair and further consolidates the formation of the misaligned structure (Fig. 1D).

To compare the thermodynamic stabilities of the misaligned sample with the one containing a normal Watson–Crick base pair, UV optical melting analysis of the normal AT and the mismatched GT products has been performed. Interestingly, both products possess similar ΔG°_{37} values, indicating their stabilities are similar (Table 1). This is because in the misaligned structure, the formation of GC Watson–Crick base pair outweighs the destabilization of the T bugle. Similar misalignment has also been observed in a crystal structure in which an incoming nucleotide ddGTP base paired with the nucleotide C in template 5′-CG rather than forming a GG mismatch [12].

In order to investigate if misalignment depends on the neighbor nucleotides of the 5′-CT motif and the length of the template sequence, we have (i) substituted the base pair G4–C14 with C4–G14, A4–T14 and T4–A14, (ii) changed the type of nucleotide downstream to 5′-CT, i.e. from 5′-TCT to 5′-CCT, 5′-GCT and 5′-ACT, and (iii) lengthened the template from 5′-ACT to 5′-ACACT, 5′-ACCACACT and 5′-ACCACCACACT. In all cases, the 2D NOESY spectra reveal an unusual NOE corresponding to the misaligned structures (Appendix A S4–S6). This suggests that the neighbor nucleotides of the 5′-CT motif and the length of template do not affect the formation of misaligned structures. However, when the nearest nucleotide downstream to template T was changed from C to other types of nucleotides, no misalignment was