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An Asymmetric Structure of the *Bacillus subtilis* Replication Terminator Protein in Complex with DNA

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In *Bacillus subtilis*, the termination of DNA replication *via* polar fork arrest is effected by a specific protein:DNA complex formed between the replication terminator protein (RTP) and DNA terminator sites. We report the crystal structure of a replication terminator protein homologue (RTP.C110S) of *B. subtilis* in complex with the high affinity component of one of its cognate DNA termination sites, known as the *TerI* B-site, refined at 2.5 Å resolution. The 21 bp RTP:DNA complex displays marked structural asymmetry in both the homodimeric protein and the DNA. This is in contrast to the previously reported complex formed with a symmetrical *TerI* B-site homologue. The induced asymmetry is consistent with the complex's solution properties as determined using NMR spectroscopy. Concomitant with this asymmetry is variation in the protein:DNA binding pattern for each of the subunits of the RTP homodimer. It is proposed that the asymmetric "wing" positions, as well as other asymmetrical features of the RTP:DNA complex, are critical for the cooperative binding that underlies the mechanism of polar fork arrest at the complete terminator site.

Keywords: winged-helix; DNA replication; replication terminator protein; polar fork arrest; cooperative binding

Introduction

The final phase of DNA replication in *Bacillus subtilis* begins with the arrest of clockwise and anticlockwise replication forks at a distinct region of its circular chromosome known as the terminus region. This region is bounded by DNA motifs, known as Terminator (*Ter*) sites, to which two copies of the homodimeric replication terminator protein (RTP) bind. The 2:1 complex of RTP (i.e. two dimers) and DNA (the *Ter* site) is able to effect the arrest of replication forks by impeding the progression of the replicative machinery headed by the replicative helicase. Importantly, the action of the *Ter* complex is polar in nature; that is, it allows the replication fork to pass unimpeded if it approaches from the "permissive" direction yet halts its approach from the other "blocking" direction.[1] Nine terminator sites (*Ter* sites) are arranged that allow replication forks to pass into the terminus region of the chromosome but not to leave.[1] This forms a "replication fork trap" so that the approaching replication forks are always forced to fuse in the terminus region.

The target *Ter* sites of RTP are 30 bp sequences comprising two imperfect inverted 16 bp repeats named the A site and B site that overlap at a highly conserved trinucleotide sequence.[2] The A site and B sites each bind one dimer of RTP. The RTP-*Ter* complex is capable of arresting replication fork progression only when orientated such that the B site is proximal to the approaching replication fork.[3] Whilst the two RTP molecules that comprise the complete RTP-*Ter* complex may be structurally symmetrical their binding to the A and B sites is not equivalent. Indeed, binding to the A site alone does not occur without prior filling of the B site through an as yet poorly understood cooperative binding interaction.[4] Furthermore, binding to the B site alone is not sufficient to halt the advancing helicase.[5,6] It is only when both A and B sites are filled ($K_D = 3 \times 10^{-11}$ M) that fork arrest occurs.[7]

The classic winged-helix motif is a compact α/β structure with αβααββ topology where "wings" project from the loop between β2-β3 strands and sometimes also following β3.[8] RTP conforms to this definition except that each subunit lacks a well formed β1-strand (referred to here as the β1-loop) and it possesses an additional long α4-helix

Abbreviations used: RTP, replication terminator protein; RMSD, root mean square deviation; ds, double-stranded; HSQC, heteronuclear single quantum coherence.

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following the β3-strand. This extra helix serves as a dimerisation interface, forming an antiparallel coiled coil with its α4-helix counterpart from the other subunit. The α3-helix or recognition helix of the winged-helix motif is responsible for specific binding to the major groove of DNA. The wings of most winged helix proteins are associated with providing additional protein:DNA contacts through binding the phosphate backbone of the minor groove, although they have also been implicated in protein: protein interactions.[9] Biochemical analysis of RTP has suggested that the wings are involved in both protein and DNA contacts.[9,10]

A polar replication terminator system has also been characterised in *Escherichia coli*.[11] The *E. coli* chromosome contains ten terminator sites *TerA–TerJ*, each of which is an asymmetric sequence of 23 bp, that binds one monomer of Termination utilisation substance (Tus).[12] Tus binds the *E. coli Ter* with a very high affinity, with an estimated K_D for the Tus:*TerB* complex of 3.4×10^{-13} M.[13] The crystal structure of Tus in complex with bases 4–19 of *TerA* revealed an asymmetric protein structure presenting two very different faces to the approaching replisome.[14] It was shown that the Tus:*Ter* complex blocked the action of the DnaB helicase,[15] through a mechanism coupled to helicase catalysed strand separation.[16] It was shown that the translocation of the helicase along the DNA formed a stable locked complex at the non-permissive end of the Tus:*Ter* complex *via* the shifting of the C(6) nucleotide into a cytosine specific binding site on Tus.[16] This renders the Tus:*Ter* complex a polar non-specific block to helicase translocation along the DNA.

Early expectations were that the mechanism of the *B. subtilis* terminator complex would be similar to that of its *E. coli* counterpart. However, relating the mechanisms of the two systems is complicated, as they share no similarity in sequence or structure. Further, when the crystal structure of apo-RTP was determined[17] it revealed that RTP is a symmetric homodimer. This raises the question: how does this symmetric protein produce an asymmetric function?

Identifying the basis of this functional asymmetry and furthermore the mechanism by which the helicase is impeded in *B. subtilis* has been the focus of debate in the literature.[9,18–21] It has been proposed that polarity either occurs through the differential binding affinity and cooperative binding of the RTP molecule at the A and B sites or through significant conformational changes to the RTP molecules that render the whole terminator complex asymmetric.[4] Previously, we have reported the structure of an RTP.C110S mutant in complex with a symmetric RTP B site (*sRB*) sequence.[22] This B site contains six base-pair changes compared with the native RTP B site (*nRB*) from *TerI* (the first *Ter* site that the clockwise replication fork encounters), rendering the sequence symmetric. The RTP:*sRB* interaction was shown to have a similar binding affinity, yet of note, a reduced fork arrest capability, compared to the RTP:*nRB* interaction. Both NMR and crystallographic structural analysis of RTP.C110S:*sRB* revealed a symmetric RTP bound to symmetric DNA. Importantly, this structure revealed the orientation of RTP when bound upon DNA and suggested how two molecules of RTP could be juxtaposed when bound to a complete *Ter* site.[22] Nevertheless, it did not reveal whether RTP bound to the *nRB* site would display structural asymmetry. Here we report the structure of RTP.C110S in complex with the *nRB* binding site. Interestingly, both the protein and the DNA are asymmetric.

Results

Overall structure of RTP:*nRB*

We have determined the crystal structure of RTP.C110S in complex with a 21 bp, native RTP B site oligonucleotide (*nRB*) (see Figure 1(a)). The double-stranded (ds) DNA sequence represents the 21 bp B site of the *TerI* terminator.[2] Of these, 19 base-pairs are visible in the electron density with the first two base-pairs at the upstream end (the end that overlaps with the A site) disordered. There is some evidence in the electron density of the possible position of the phosphate backbone and bases. However, the electron density could not be readily interpreted and this part of the complex is not included in the crystal structure. The dsDNA is positioned in the asymmetric unit so that the upstream end interacts with the downstream end of an adjacent dsDNA. This arrangement is not uncommon in crystals containing dsDNA, allowing base stacking to occur between the bases of abutted DNA molecules.[23]

All residues, except the first seven from the N terminus of each RTP.C110S monomer are clearly defined in the electron density. A portion of the crystal structure and electron density is shown in Figure 1(b). Crystallographic information is summarised in Table 1. The overall structure of the protein is that of a homodimeric winged-helix similar to that first reported for apo-RTP by Bussiere *et al.*,[17] and confirmed for RTP.C110S in both apo and dsDNA-bound forms in our own studies.[22,24] Notably, while RTP.C110S formed as a symmetric dimer in our previous structural studies, in the current study RTP.C110S complexed with *nRB* has distinctly asymmetric features and correspondingly asymmetric contacts with the target *nRB* (Figure 1(a)).

It is of note that the length of the *nRB* dsDNA is identical to that used for the study of RTP.C110S:*sRB* and there are only six base-pair differences between the two 21 bp sequences (Figure 1(c)). Despite this, RTP.C110S:*nRB* formed an asymmetric structure whilst RTP.C110S:*sRB* formed a virtually symmetric structure.

One β2-loop-β-3 strand or wing is positioned downwards ("wing-down" monomer) and makes