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Identification of the SSB Binding Site on *E. coli* RecQ Reveals a Conserved Surface for Binding SSB's C Terminus

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RecQ DNA helicases act in conjunction with heterologous partner proteins to catalyze DNA metabolic activities, including recombination initiation and stalled replication fork processing. For the prototypical *Escherichia coli* RecQ protein, direct interaction with single-stranded DNA-binding protein (SSB) stimulates its DNA unwinding activity. Complex formation between RecQ and SSB is mediated by the RecQ winged-helix domain, which binds the nine C-terminal-most residues of SSB, a highly conserved sequence known as the SSB-Ct element. Using nuclear magnetic resonance and mutational analyses, we identify the SSB-Ct binding pocket on *E. coli* RecQ. The binding site shares a striking electrostatic similarity with the previously identified SSB-Ct binding site on *E. coli* exonuclease I, although the SSB binding domains in the two proteins are not otherwise related structurally. Substitutions that alter RecQ residues implicated in SSB-Ct binding impair RecQ binding to SSB and SSB/DNA nucleoprotein complexes. These substitutions also diminish SSB-stimulated DNA helicase activity in the variants, although additional biochemical changes in the RecQ variants indicate a role for the winged-helix domain in helicase activity beyond SSB protein binding. Sequence changes in the SSB-Ct element are sufficient to abolish interaction with RecQ in the absence of DNA and to diminish RecQ binding and helicase activity on SSB/DNA substrates. These results support a model in which RecQ has evolved an SSB-Ct binding site on its winged-helix domain as an adaptation that aids its cellular functions on SSB/DNA nucleoprotein substrates.

Keywords: RecQ; helicase; single-stranded DNA-binding protein; genome maintenance; protein complex

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Introduction

RecQ DNA helicases are evolutionarily conserved enzymes with diverse roles in genome maintenance.[1–5] In bacteria, RecQ proteins aid in recombination, stalled replication fork processing, SOS signaling, and antigen variation pathways.[6–12] In humans, individual mutation of three of five RecQ helicase genes (*BLM*, *WRN*, and *RECQ4*) gives rise to Bloom, Werner, and Rothmund–Thompson syndromes, respectively.[13–15] These conditions are marked by genome instability manifested as chromosome breakage and genomic rearrangements that are linked to cancer predisposition, underscoring the importance of the RecQ family of enzymes in maintaining the integrity of genomic DNA.

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Abbreviations used: SSB, single-stranded DNA binding protein; RPA, replication protein A; OB, oligosaccharide binding; ssDNA, single-stranded DNA; RecQ-WH, RecQ winged-helix; HSQC, heteronuclear single quantum coherence; 3D, three-dimensional; NOESY, nuclear Overhauser enhancement spectroscopy; dsDNA, double-stranded DNA; EMSA, electrophoretic mobility shift assay; EDTA, ethylenediaminetetraacetic acid; NIH, National Institutes of Health.

RecQ proteins associate with a large number of heterologous proteins, often functioning as components in multiprotein complexes.[1,5,16–18] Direct interaction with single-stranded DNA-binding protein (SSB) [called replication protein A (RPA) in eukaryotes] is a shared feature among bacterial and eukaryotic RecQ proteins. RecQ proteins known to associate with their cognate SSB include *E. coli* RecQ,[19] and human BLM,[20] WRN,[21] and RecQ1.[22,23] In addition, human RecQ5β is strongly stimulated by, and most likely associates with, RPA.[24] Due to the conservation of RecQ interactions with SSB, a physical and functional understanding of how these complexes assemble to act on DNA substrates is critical for appreciating the broad cellular roles of RecQ proteins.

In *E. coli* and nearly all other bacteria, SSB function as homotetramers, each subunit of which comprises an N-terminal oligonucleotide/oligosaccharide binding (OB) fold linked to a structurally dynamic C-terminal tail[25–29] (Fig. 1a). Whereas the OB fold is responsible for tetramerization and single-stranded DNA (ssDNA) binding, the C-terminal tail region ends with a highly conserved amphipathic peptide sequence (SSB-Ct) that associates with a diverse array of genome maintenance enzymes[31,32] (Fig. 1a). Interactions made with the SSB-Ct element are critical to proper genome maintenance, and alterations in the SSB-Ct sequence can severely affect *E. coli* viability.[33–38] To date, every bacterial SSB-interacting protein examined binds to SSB-Ct, including *E. coli* RecQ,[19,31] although structural data indicating how such complexes are formed are only available for *E. coli* exonuclease I.[32] Additional studies are necessary to map the conserved structural features that define SSB-Ct binding sites.

E. coli SSB is important for stimulating *E. coli* RecQ helicase activity and for aiding in joint activities between RecQ and other genome maintenance enzymes. SSB stimulates RecQ in at least two ways. First, SSB passively stimulates RecQ activity by binding and sequestering ssDNA generated by RecQ helicase activity and by preventing the formation of nonproductive ssDNA/RecQ complexes that inhibit the enzyme.[39,40] Second, SSB actively stimulates RecQ by recruiting RecQ to its DNA substrate and/or by helping retain the enzyme on DNA through direct physical interaction.[19] For active stimulation, interaction between SSB and RecQ is mediated by direct contacts made between the SSB-Ct sequence and the RecQ winged-helix (RecQ-WH) domain (Fig. 1a).[19] Interestingly, the winged-helix domain of WRN is also important for contacting RPA;[41] however, this interaction presumably occurs via a different mechanism, as RPA lacks the amphipathic SSB-Ct sequence that defines the bacterial SSB protein interaction site. Nonetheless, it is striking that, for both *E. coli* RecQ and WRN, their respective winged-helix domains play important roles in complex formation with cognate SSB. SSB is also a component in more complicated reactions, including recombination initiation with RecQ and RecA,[42] DNA catenation and supercoiling, and converging replication fork resolution with RecQ and topoisomerase III.[6,43,44]

In this study, we have used NMR to identify the SSB-Ct binding site on the RecQ-WH domain. This site, which is on the opposite face of the proposed DNA binding site of the domain,[30] forms a pocket that includes nonpolar and electropositive elements that resemble those found in the exonuclease I SSB-Ct binding site.[32] Using NMR data and additional structural information from the *E. coli* RecQ catalytic core X-ray crystal structure[30] as guides, we constructed a panel of RecQ variant proteins with amino acid substitutions that were predicted to impair association with SSB. This panel was used to determine the extent to which alteration of particular residues influences RecQ binding to SSB, and RecQ DNA binding and unwinding in the presence and in the absence of SSB. Additional experiments using SSB variant proteins with altered SSB-Ct elements assessed the effect of altering SSB in the same reactions. Each of the RecQ variant proteins had deficiencies in binding SSB protein, with several arginine and glutamine substitutions having the greatest impact. RecQ proteins bearing one or two amino acid changes that diminish SSB binding exhibited modest nucleic acid binding defects, but more pronounced defects in binding to SSB-coated DNA. These RecQ variants retained the ability to be stimulated by SSB, albeit to a lesser extent than wild-type proteins. Nonstandard SSB-Ct elements also mildly inhibited the helicase activity of wild-type RecQ protein on a partial-duplex DNA substrate. We postulate that SSB-Ct binding to the site identified in this study influences RecQ-mediated DNA unwinding of cellular nucleoprotein substrates.

Results

NMR studies identify RecQ-WH domain residues involved in SSB-Ct binding

Our earlier results indicated that *E. coli* RecQ and SSB form a complex that is mediated by stoichiometric binding of the RecQ-WH domain to the SSB-Ct element,[19] although the precise SSB-Ct binding site on the RecQ-WH domain was not defined. A structural approach was therefore taken to map the SSB-Ct binding site on RecQ. We initially attempted to map the site by soaking the SSB-Ct peptide into crystals of the *E. coli* RecQ catalytic core domain (which includes the RecQ-WH domain), but electron density for the peptide was never observed in these experiments (data not shown). Since the isolated RecQ-WH domain binds the SSB-Ct peptide with the same specificity and with nearly the same affinity as the full-length RecQ protein,[19] we used NMR to define the SSB-Ct binding site on this small (116-residue) domain.

^{15}N- and ^{15}N-,^{13}C-labeled RecQ-WH domain samples provided well-dispersed NMR spectra that allowed determination of over 90% of the