Crystal Structure of Escherichia coli Primosomal Protein PriB

PriB is required for assembly of Escherichia coli φX-type primosome, a mobile multi-enzyme complex responsible for the initiation of DNA replication. Here we report the 2.1 Å-resolution crystal structure of E. coli PriB by multi-wavelength anomalous diffraction using a mercury derivative. The monomer of PriB shares structurally similarity to the DNA-binding domain of single-stranded DNA-binding protein (SSB). However, PriB functions as a dimer in cell, not a homotetramer like SSB. By combining the PriB structural information to biochemical studies, we propose that the potential tetramer formation surface and several other regions of PriB may participate in protein-protein interaction during primosome assembly. These findings may provide insight into the role of PriB in E. coli DNA replication.

A bacterial primosome is a multi-enzyme complex that participates in DNA replication. It travels along the lagging strand template, unwinds the duplex DNA, and primes the Okazaki fragments that are required for replication fork progression. Escherichia coli φX-type primosome was originally discovered as an essential component for the replication of bacteriophage φX174 and CoIE1-type plasmids. It is now clear that the primary role of the φX-type primosome is to restart the stalled replication fork at oriC after encountering DNA damage.

PriB is one of the seven essential proteins (PriA, PriB, PriC, DnaB, DnaC, DnaT, and DnaG) for the assembly of the φX-type primosome. And PriB can also responsible for the initiation of DNA (ssDNA) in the presence or absence of single-stranded DNA-binding protein (SSB) in vitro. Although, PriB is generally considered to be a structural component of the φX-type primosome, the function of this protein in vivo is not fully understood at the molecular level. Recent studies on sequence comparisons and operon organization analyses have shown that PriB evolved from SSB via gene duplication with subsequent rapid sequence diversification. SSB has long been known for its importance in DNA replication. It raises an interesting question as to how PriB participates in DNA replication in a fashion different from that of its ancestor. In this article we present the crystal structure of the E. coli PriB at 2.1 Å resolution.

The structure of PriB was determined using multi-wavelength anomalous diffraction (MAD) phasing applied to mercury. The MAD data collection was conducted using a sodium p-hydroxymercuribenzoate derivative crystal at Beamline 17B2 of NSRRC. The MAD data were collected at four wavelengths under cryogenic conditions.

The PriB crystal belongs to the orthorhombic space group P212121, with two molecules in an asymmetric unit. These two polypeptide chains form a dimer with a non-crystallographic 2-fold symmetry (Fig. 1(a)). PriB contains 104 amino acid residues. A PriB monomer is a single domain protein topologically identical to the well characterized OB (oligonucleotide-binding) fold. Briefly, the PriB monomer structure has two pleated β-sheets capped by a small α-helix located between the third and the fourth strands to form a β-barrel (Fig. 1(b)). Between the anti-parallel strands PriB has three flexible β-hairpin loops termed L12, L23, and L45 that protrude from the central core (Fig. 1(c)).

Despite the relatively low sequence identity with other single-stranded DNA-binding proteins (SSBs), the PriB monomer displays high structural similarity to the ssDNA-binding domains of E. coli SSB (EcoSSB) and H. sapiens (HsmtSSB). The tracings of the C atoms of these proteins are shown in Fig. 1(c) and demonstrate clearly that the globular core of these proteins has similar conformation. However, unlike other SSBs, the three loop regions of PriB are distinct in their conformation and orientation with respect to the core. Sequence alignment reveals two gaps that significantly shorten the L23 and L45 hairpin loops of PriB. L45 of EcoSSB has been shown to be involved in interface interactions between dimers for the one of the tetramer formation. L45 of PriB, however, was not stabilized through crystal packing as shown in EcoSSB.

Both HsmtSSB and EcoSSB function as homotetramers in solution. In contrast to these SSBs, the PriB molecules form dimers in solution and crystal (Fig. 1(a)). The two monomers in the PriB dimer are related by a non-crystallographic 2-fold axis that is similar to that of the EcoSSB or HsmtSSB dimer (Figs.1(a) and 2). However, a symmetrical pair of intermolecular disulfide bridges further stabilizes the PriB dimer. In addition, sidechains of Met-50 and Met-90 from both subunits form a methionine cluster (Fig. 1(a)) that locates between the two intermolecular disulfide bridges. These intermolecular interactions have not...
been observed in other SSBs. In addition, the arm region (L₁, and parts of the connecting β₂ and β₃) of PriB bends to one side of the paired monomer with intimate contacts (Fig. 1(a)). This close state structural feature is unique and has never been reported for crystal structures of SSBs, even for those that bind with ssDNA.

PriB does not form a tetrameric structure in crystal or solution. In EcoSSB or HsmtSSB the homotetramer is a dimer of dimers with a 222 symmetry and has molecular 2-fold dyads along three intersecting axes (Fig. 2(a)). Two β-strands, β₁, and β₄, are important for the homotetramer formation. However, residues in β₁, and β₄ of PriB share only low sequence similarity to those of EcoSSB or HsmtSSB. In addition, the charge distribution of PriB at the potential tetramer formation surface is considerably different from that in EcoSSB or HsmtSSB (Fig. 2(b)). A priB triple mutant (G56E, H57N, and E58K) has been reported to be defective in the cellular replication of CoIE1-related plasmids. Coincidentally, Glu-58 is located at the potential tetramer formation surface of PriB (Fig. 2(b)). Therefore, the negatively charged area containing Glu-58, Glu-95, and Glu-98 on the potential tetramer formation surface can possibly be a determinant for protein-protein interaction between PriB and other proteins during primosome assembly.
Because PriB has a structural resemblance to SSBs, PriB may use similar strategies to recognize ssDNAs or ssRNAs. The PriB dimer contains a pair of 2-fold related grooves that may define the path of the bound single-stranded oligonucleotide (Fig. 3(a)). The oligonucleotide-binding residues on EcoSSB have their functional homologs on the putative oligonucleotide-binding surface of PriB (Fig. 3(b)). In addition, there are four histidine residues (His-26, His-43, His-81, and His-93) located on the putative oligonucleotide-binding surface of PriB (Fig. 3(b)). Histidine residue sidechains could provide not only stacking forces to pair with rings but also positive charges to attract the oligonucleotide phosphate backbone. This histidine effect is a unique feature to PriB and not found in SSBs. It may explain the different in oligonucleotide-binding properties between PriB and SSBs.

PriB has two free cysteine residues, Cys-12 and Cys-27, which are not involved in disulfide bridge formation (Fig. 3(a)). The biological roles of Cys-12 and Cys-27 are not clear, and it is noticeable that there is no cysteine residue in EcoSSB or in HmSSB. One could speculate that the region containing Cys-12 or Cys-27 may be a potential protein-protein interface for PriB to interact with other proteins during primosome assembly. Cys-12 of PriB may be directly involved in the oligonucleotide-binding of PriB because it sits on the putative oligonucleotide-binding surface. A redox change on the sulphydryl group of Cys-12 might alter the oligonucleotide-binding affinity of PriB. Interestingly, the sulphydryl modification agent NEM can inactivate the in vitro primosomal replication activity of PriB. This finding infers that the redox state of free cysteine residues may influence the role of PriB in primosome assembly. However, these speculations need to be confirmed by further biochemical studies.

In the present study, we determined the three-dimensional structure of PriB from E. coli at 2.1 Å by x-ray crystallography. In addition, we propose several regions that may be involved in protein-protein interactions during primosome assembly. The high-resolution structure of PriB presented here offers a starting point for further studies on primosome assembly. The structure also provides the first framework for understanding the structure of other PriB from the same superfamily. The atomic coordinates and structure factors (code 1V1Q) have been deposited in the Protein Data Bank (http://www.rcsb.org/).
Fig. 3: (a) Stereo view of the putative functional residues on a PriB dimer. (b) Schematic presentation demonstrates the conservation of functional residues between PriB and EcoSSB. The oligonucleotide-binding domain is projected as a saddle-like area. The green area is contributed from one monomer, and the cyan area is from the other. The locations of functional residues are assigned according to their three-dimensional relationships.

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