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$FtsZ_{Dr}$, a tubulin homologue in radioresistant bacterium *Deinococcus radiodurans* is characterized as a GTPase exhibiting polymerization/depolymerization dynamics *in vitro* and FtsZ ring formation *in vivo*

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ABSTRACT

The GTPase-dependent polymerization/depolymerization dynamics of FtsZ regulate bacterial cell division *in vivo*. *Deinococcus radiodurans* is better known for its extraordinary radioresistance and therefore, the characterization of FtsZ of this bacterium ($FtsZ_{Dr}$) would be required to understand the mechanisms underlying regulation of cell division in response to DNA damage. Recombinant $FtsZ_{Dr}$ bound to GTP and showed GTPase activity. It produced bundles of protofilaments in the presence of either GTP or Mg^{2+} ions. But the formation of the higher size ordered structures required both GTP and Mg^{2+} *in vitro*. It showed polymerization/depolymerization dynamics as a function of GTP and Mg^{2+}. Interestingly, ATP interacted with $FtsZ_{Dr}$ and stimulated its GTPase activity by ~2-fold possibly by increasing both substrate affinity and rate of reaction. $FtsZ_{Dr}$-GFP expressing in *D. radiodurans* produced typical Z ring perpendicular to the plane of first cell division. These results suggested that $FtsZ_{Dr}$ is a GTPase *in vitro* and produces typical Z ring at the mid cell position in *D. radiodurans*.

1. Introduction

Cell division in bacteria is a highly ordered process involving a large number of proteins forming a higher order complex called the divisome (Margolin, 2005). FtsZ is one of the highly conserved proteins of this complex. FtsZ orthologs have been identified in bacteria, archea, chloroplasts and the mitochondria of some algae and amoebae (Gilson and Beech, 2001). FtsZ like tubulin is a GTPase and undergoes GTP dependent polymerization in head-to-tail fashion (de Boer et al., 1992; Bramhill and Thompson, 1999; Oliva et al., 2004). The GTPase activity of FtsZ and its effect on polymerization and depolymerization dynamics is regulated differently in different bacteria (Adams and Errington, 2009). *In vitro* FtsZ-GTP produces different lengths of polymer, which after attaining a critical length undergo GTP hydrolysis and results in depolymerization, and thereby the release of monomer in the form of FtsZ-GDP (Scheffers et al., 2002). The protofilaments may exist as single filaments or laterally associate to form bundles and sheets in the presence of added cofactors and/or proteins (Yu and Margolin, 1997; Mukherjee and Lutkenhaus, 1999; Hale et al., 2000; Erickson et al., 2010). In certain cases, lateral interactions can affect the GTPase activity and stability of the protofilaments. *In vivo*, FtsZ polymerizes into ring like structure termed the Z-ring at the cell division site, which undergoes depolymerization in a tightly regulated manner and brings about cytokinesis. Negative regulatory systems like 'Min' and 'NOC' (nucleoid occlusion) bring about both spatial and temporal regulation of Z ring formation *in vivo* (Barak and Wilkinson, 2007). The FtsZ ring dynamics is intrinsic to the structure of this protein and its stability is influenced by surrounding microenvironment. Therefore, the regulation of polymerization and depolymerization dynamics of FtsZ should presumably be different in different bacteria and influence the rates of cell division under different growth conditions.

Deinococcus radiodurans, an extraordinarily radioresistant bacterium grows as diplococci and tetracocci with a doubling time of ~90 min under normal growth conditions (Slade and Radman, 2011). It survives the lethal and mutagenic effects of several DNA-damaging agents including radiation and desiccation without a measurable loss of cell viability (Battista, 2000). *D. radiodurans*

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genome encodes almost all the core components of divisome, including FtsZ and FtsA, and associated regulatory proteins like MinCDE and DivIVA (White et al., 1999). Cells exposed to γ radiation stop growth until DNA damage is repaired (Cox and Battista, 2005). Transcriptome analysis has shown that the transcription of annotated divisome genes is unaffected by γ radiation (Liu et al., 2003). Also, the genome of this bacterium lacks homologs of SulA (Trusca et al., 1998), YneA (Mo and Burkholder, 2010), DivS (Ogino et al., 2008) and Rv2719c (Chauhan et al., 2006) proteins that are known SOS-response and cell division inhibitors in *E. coli*, *B. subtilis*, *Corynebacterium glutamicum* and *Mycobacterium tuberculosis*, respectively. Interestingly, *D. radiodurans* does not exhibit a typical SOS response. However, a eukaryotic type Ser/Thr protein kinase (DR2518) having roles in radioresistance and DNA double strand break (DSB) repair in *D. radiodurans* has been characterized (Rajpurohit and Misra, 2010). Recently, it has been shown that the kinase activity of DR2518 (RqkA) and phosphorylation of PprA a DNA repair protein are required for radioresistance in *D. radiodurans* (Rajpurohit and Misra, 2013). Therefore, the mechanisms of cell division regulation in *D. radiodurans* would be worth investigating.

Here we report the detailed characterization of FtsZ from *D.radiodurans* and demonstrate that $FtsZ_{Dr}$ is a GTP-binding protein with GTPase activity that requires both GTP and Mg^{2+} for its polymerization. Sedimentation analysis and transmission electron microscopy data provided evidence about the polymerization of $FtsZ_{Dr}$ *in vitro*. It showed slow polymerization/depolymerization dynamics and poor GTPase activity. ATP binding with $FtsZ_{Dr}$ increased its affinity to GTP and stimulated GTPase activity *in vitro*. $FtsZ_{Dr}$-GFP expressing in *D. radiodurans* produced typical FtsZ ring. *E. coli* expressing $FtsZ_{Dr}$-GFP on a multicopy plasmid produced a significantly large population of longer cells as compared to non recombinant cells. These results suggested that FtsZ of *D. radiodurans* is active both *in vitro* and *in vivo* and it seems to be different from other characterized FtsZ proteins with regard to its polymerization/depolymerization dynamics and GTPase activity.

2. Experimental

2.1. Bacterial strains, plasmids and materials

D. radiodurans R1 (ATCC13939) was a gift from Professor J. Ortner, Germany (Schaefer et al., 2000). The *E. coli* strains DH5α and NOVABLUE were used for cloning and *E. coli* strain BL21 (DE3) pLysS was used for the expression of recombinant protein. *E. coli* was grown in LB and *D. radiodurans* was grown in TGY medium with shaking at 180 rpm at 37 °C and 32 °C, respectively. Recombinant *E. coli* harboring expression vectors and their derivatives were grown in the presence of antibiotics as required. Shuttle expression vector pVHS559 (Charaka and Misra, 2012) and its derivatives were maintained in *E. coli* strain NOVABLUE in the presence of spectinomycin (40 μg/ml) or spectinomycin (75 μg/ml), in the case of *D. radiodurans*. All recombinant techniques were performed as previously described (Sambrook and Russell, 2001). For microscopic examination, *D. radiodurans* and *E. coli* cells harboring recombinant plasmid were grown at 32 °C or 37 °C in TGY or LB mediums respectively, and induced with 2 mM or 200 μM IPTG, respectively. Antibodies against FtsZ of *D. radiodurans* were commercially produced in rabbit (MERCK Millipore, India) and GFP antibodies were from Roche Biochemical Germany. Molecular biology grade chemicals and enzymes were procured from Sigma Chemicals Company, USA, Roche Biochemicals, Mannheim, Germany, New England Biolabs, USA and Bangalore Genie, India.

2.2. Construction of expression plasmids

Genomic DNA from *D. radiodurans* was prepared as described earlier (Battista et al., 2001) and 1116 bp of DR_0631 ($ftsZ_{Dr}$) were PCR amplified using forward primer (5′ GGAATTCCATATGCAAGCAGCCAGAATTCGCGT3′) and reverse primer (5′ CGGGATCCTTACTGTCCGCCGA CGT3′) with *Nde*I and *Bam*HI sites incorporated at their 5′ends, respectively. PCR product was subsequently cloned at compatible ends in pET28a (+) (Novagen) to get pFTSZdr. *E. coli* BL21 (DE3) was transformed with pFTSZdr and inducible expression of recombinant $FtsZ_{Dr}$ was confirmed by SDS-PAGE. The 1116 bp $ftsZ_{Dr}$ was PCR amplified using forward primer (5′GCGGGATCCATGCAAGCAGCCAGAATTC3′) and reverse primer (5′CGCTCTAGACTGTCCGCCGACGT3′). The PCR product was cloned at *Bam*HI and *Xba*I sites in pDSW208 (Weiss et al., 1999) to yield pDSFTZ. The 1.8 kb $FtsZ_{Dr}$ -GFP was further PCR amplified using forward primer (5′GCGAGCTCATGCAAGCAGCCAGAATTCGCGT3′) and reverse primer (5′CGCTTAAGTTATTTGTATAGTTCATCCA3′) and cloned at *Sac*I and *Afl*II sites in pVHS559 (Charaka and Misra, 2012) and pFTSZGFP was obtained. The pFTSZGFP was transformed into both *E. coli* and *D. radiodurans* plasmids and the expression of $FtsZ_{Dr}$-GFP fusion was confirmed by immunoblotting using antibodies against $FtsZ_{Dr}$ and GFP. Correctness of coding sequence of FtsZ in both pFTSZdr and pFTSZGFP was confirmed by sequencing.

2.3. Purification of recombinant $FtsZ_{Dr}$

E. coli BL21 (DE3) pLysS cells containing pFTSZdr were grown to OD_{600nm} ~0.3–0.4 in LB broth supplemented with kanamycin (25 μg/ml). Protein expression was induced with 0.5 mM IPTG for 3 h. Cells were centrifuged at 5000 rpm for 10 min and pellets were stored at −20 °C. Recombinant $FtsZ_{Dr}$ was purified by immobilized metal affinity chromatography (IMAC) using previously described protocols (Kota et al., 2010). In brief, the cells were incubated in Buffer A (15 mM Tris–HCl, pH 7.6, 100 mM NaCl and 10% glycerol) supplemented with 10 mM imidazole, 2 mM EDTA, 0.5 mg/ml lysozyme, 1 mM PMSF and protease inhibitor cocktail at 37 °C for 30 min and then on ice for 1 h. Cells were then sonicated for 10 min at 30 s pulses with intermittent cooling for 30 s at 25% amplitude, and centrifuged at 11,000 rpm for 45 min at 4 °C. The cell free extract containing recombinant $FtsZ_{Dr}$ was dialyzed overnight in buffer A containing 10 mM imidazole and loaded onto column packed with fast flow chelating sepharose (GE Healthcare) charged with $NiCl_2$ and equilibrated with buffer A containing 10 mM imidazole. The column was washed with 20 column volumes of buffer A containing 50 mM imidazole. Protein was eluted with buffer A containing 250 mM imidazole. Fractions were analyzed by SDS-PAGE and fractions containing $FtsZ_{Dr}$ were pooled and repurified by IMAC as described above. Proteins were then eluted using a stepwise gradient of buffer A containing 100 mM, 200 mM, 300 mM and 400 mM imidazole. All the fractions were analyzed on 10% SDS-PAGE. Fractions containing $FtsZ_{Dr}$ with more than 95% purity were pooled. The histidine tag was removed from $FtsZ_{Dr}$ by incubating the purified protein with 0.03 U of biotinylated thrombin (Product number 69672, Novagen) on ice for 60 min and analyzed by SDS-PAGE to confirm the removal of (His)6 tag from protein. Thrombin was removed by Thrombin Cleavage Capture Kit (Product number 69203, Novagen) using kit's protocol. The cleaved protein was concentrated with 10 kDa cut off spin column and then centrifuged protein sample was centrifuged at 16,000 rpm for 60 min to remove aggregates. $FtsZ_{Dr}$ was dialyzed in buffer A containing 10 mM Tris–Cl pH 7.6, 50 mM KCl, 50% glycerol and 1 mM PMSF and stored at −20 °C. Protein concentration was determined by Bradford assay.