Bacillus subtilis division protein DivIVA – screen for stable oligomer state conditions

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The cell division protein DivIVA is predicted to be a coiled-coil, tropomyosin–like protein, that self-associates both *in vivo* and *in vitro* into oligomers of up to 10-12 monomers. A simple and quick screen for conditions supporting the stable oligomer structure has been developed revealing that DivIVA forms a homogeneous oligomer in the presence of PEGs (PEG 4K or PEG 8K and PEG 1K).

Keywords: **DivIVA, oligomerization, stability, crystallization conditions**

1. Introduction

A central problem in cell division is the nature of the mechanism that ensures correct placement of the division septum. In vegetatively growing cells of the Gram-positive bacterium *Bacillus subtilis* division site selection is controlled by the MinCD/DivIVA system (Cha & Stewart, 1997; Edwards & Errington, 1997). MinC and MinD are inhibitors of cell division and this inhibition seems to be mediated through DivIVA protein, which controls the topological specificity of MinCD activity and thus the positioning of the septum. During vegetative growth, DivIVA ensures that MinCD specifically inhibits division at the cell poles while allowing division at the mid-cell.

B. subtilis can also undergo an asymmetric septation during the adaptive response to hostile conditions, called sporulation. This process involves a switch in positioning of the division machinery from its normal mid-cell position to the cell poles. The role of DivIVA in sporulation septation is still unclear, although it was shown recently that DivIVA plays an important role in localizing or maintaining the *oriC* region of the chromosome near the cell pole of the sporulating cell (Thomaides *et al*., 2001).

DivIVA is a 19.5 kDa cytoplasmic protein. Secondary structure prediction algorithms indicate that, the central region of DivIVA might form an α-helical coiled-coil structure (Edwards *et al*., 2000). This structure is characterized by heptad repeats with the residues at the first and fourth positions, being hydrophobic and the remaining residues being hydrophilic (Lupas *et al*., 1991). Such structures could be used for oligomerization as in tropomyosin (Lupas, 1996).

Our previous results of gel filtration and analytical ultracentrifugation revealed oligomerization of DivIVA into a 10–12 mer (Muchová *et al*., 2002). However the analytical ultracentrifugation observations also suggested the presence of lower order oligomers ranging from dimers upwards depending on the experimental conditions. Here we describe a simple and quick screen for conditions supporting stable oligomeric structure as a basis for crystallization of DivIVA in its biologically active quartenary structure.

2. Material and methods

2.1. Expression and purification of DivIVA

DivIVA was overexpressed in *E. coli* strain BL21 (DE3) transformed with plasmid pETIVA (Muchová *et al*., 2002). Isolation and purification of DivIVA were carried out essentially as reported previously (Muchová *et al*., 2002). Briefly, cell cultures were grown in LB media containing 30μg ml^{-1} kanamycin to an optical density of 0.6 at 600nm, when protein expression was induced by the addition of IPTG to final concentration of 1mM. After 3h further growth the cells were harvested by centrifugation and frozen at -80˚C. Cells were resuspended in buffer A (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 1mM EDTA, 1mM DTT, 1 mM PMSF), lysed by sonification and cell extract was clarified by centrifugation at 100 000g for 30 minutes. The soluble cell extract was applied to Q Sepharose Fast Flow column and DivIVA was eluted with a linear gradient of 0.1M–0.5M NaCl. After dilution of fractions containing DivIVA procedure was repeated using an FPLC Mono Q HR 10/16 column. Fractions with DivIVA, which eluted at 0.3 M NaCl, were pooled and concentrated before loading onto a Pharmacia Superose 12 gel filtration column.

2.2. Screen for stable oligomer conditions

For screening and crystallization experiments, the buffer was exchanged into 10 mM Tris-HCl pH 8.0, 1 mM DTT, 100 mM NaCl and protein was concentrated using Centricon-10 (Amicon) to 5 mg ml^{-1}. The protein was mixed with set up using solutions from the Molecular Dimension Limited New Crystal Growth Screens combined with buffers in the pH range 5.5 to 8.5 and at concentration 100 mM. 2 μl of sample were mixed with 2 μl of reservoir solution and the mixture was incubated at 18˚C for 24 hours. Prior to further analysis, it was centrifuged briefly (~10s) at 14 000g. Supernatants were analyzed by 10% blue native polyacrylamide gel electrophoresis and pellets by 12.5% SDS PAGE. Initial crystallization conditions were screened by hanging-drop vapour diffusion.

3. Results and discussion

DivIVA was purified to homogeneity as judged by Coomassie blue staining of SDS polyacrylamide gels (Fig. 1A). DivIVA was eluted with buffer A from a Superose 12 gel filtration column at concentration of 0.5 mg ml^{-1} as high molecular mass species and were examined by analytical ultracentrifugation and blue native polyacrylamide gel electrophoresis (Muchová *el al*., 2002).

One of the prerequisites for protein crystallization is a homogeneous preparation of the molecule to be crystallized. To assess sample homogeneity, we have analyzed the propensity of DivIVA to form oligomers in a variety of different buffers by blue native gel electrophoresis.

Purified DivIVA forms oligomers consisting of 10-12 monomers (Fig. 1B, lane 1). Incubation of DivIVA in 2.7 M $(NH_4)_2SO_4$ at pH 5.5, 6.5 and 8.0 as well as in 1.5 M $(NH_4)_2SO_4$ at pH 8.0 causes partial disruption of the 10-12 mers and the appearance of monomers. The proportion of monomers increases as the concentration of the salt is increased (Fig. 1B, lanes 4-5). The same pattern of behavior is observed in 1.8 M Li_2SO_4 and 0.8 M Li_2SO_4 at various pHs (Fig. 1B, lanes 6-7 and unpublished observations). Addition of 15% polyethylene glycol (PEG) 4000, or both 10%PEG 8000 and 10% PEG 1000 to 0.2 M Li_2SO_4 stabilizes

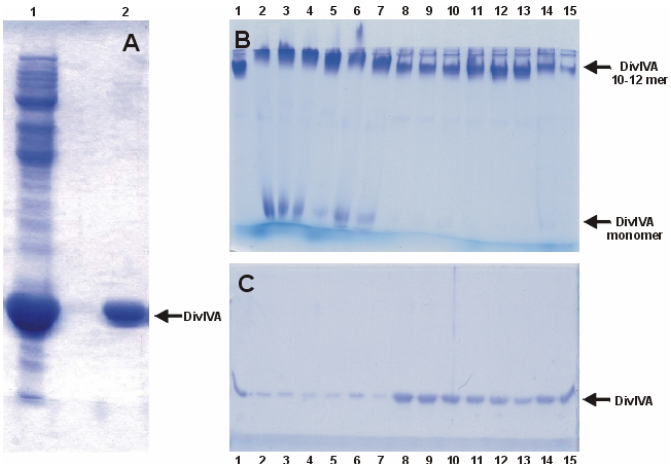

Figure 1 Electrophoretic analysis of DivIVA. ***A.*** 12.5% SDS PAGE. Lanes: 1 total cell protein from induced *E. coli* BL 21 (DE3) harbouring pETIVA; 2. DivIVA fraction after Superose 12 gel filtration column. ***B.***10% blue native polyacrylamide gel electrophoresis of 10 µg DivIVA in Lanes: 1. fraction after Superose 12 gel filtration column; 2–4. 2.7 M $(NH_4)_2SO_4$ pH 5.5, pH 6.5, pH 8.0; 5. 1.5 M $(NH_4)_2SO_4$ pH 8.0; 6. 1.8 M Li_2SO_4 pH 8.0; 7. 0.8 M Li_2SO_4 pH 8.0; 8–10. 0.2 M Li_2SO_4, 10% PEG 8K, 10 % PEG 1K pH 5.5, pH 6.5, pH 8.0; 11. 0.3 M NaAc, 10% PEG 8K, 10 % PEG 1K pH 8.0; 12. 0.2 M KBr, 10% PEG 8K, 10 % PEG 1K pH 8.0; 13. 0.2 M KSCN, 10% PEG 8K, 10 % PEG 1K pH 8.0; 14. 0.8M Na Formate 10% PEG 8K, 10 % PEG 1K pH 8.0; 15. 0.2 M $MgCl_2$ 10% PEG 8K, 10 % PEG 1K pH 8.0. ***C.*** 12.5% SDS PAGE of the pellets of the same samples as in Fig. 1B.

oligomers of DivIVA (Fig. 1B, lanes 8-10) and prevents the decomposition to monomers. However, it appears that PEG promotes aggregation of the protein as a significant fraction of the protein was found in the pellet following centrifugation of the solution at 14,000 g (Fig 1C, 8-10). The consequence of using PEGs for oligomeric state of DivIVA is evident also in conditions where 0.2 M Li_2SO_4 is substituted by 0.3 M NaAcetate, 0.2 M KBr, 0.2 M KSCN 0.8 M NaFormate or 0.2 M $MgCl_2$ pH 8.0 (Fig. 1B, lanes 11-15). As in the case of 0.2 M Li_2SO_4 much higher order molecular forms have been detected (Fig. 1C, lanes 11-15) but in different amounts depending on salt used.

The 10-12-mer of DivIVA was the only observed form in our previous *in vivo* experiments what suggests the importance of proper oligomerization state of DivIVA for its biological function (Muchová *et al*., 2002). Indeed, the mutation (Leu120Pro) that disrupts such oligomer formation is not functional in cell division. To assess the stability of DivIVA under various settings, we have developed a simple method that allows rapid characterization of sample homogeneity. Most importantly, this screen can be helpful in finding optimal conditions for crystallization of DivIVA in defined oligomeric state, which would represent the biologically active form of the protein. The data from these studies have been useful in our preliminary crystallization experiments, which allowed us already to obtain small protein crystals at conditions supporting 10-12-mer (data not shown). Further screening and crystallization experiments are presently underway.

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