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Full Length Research Paper

A computational analysis to understand the role of DmsD in the biosynthesis of dimethyl sulfoxide (DMSO) reductase

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The role of DmsD in the biosynthesis of dimethyl sulfoxide (DMSO) reductase is quite controversial. Several studies have indicated its role as 'proof-reading chaperone', which might function to prevent the translocation of misfolded and non-cofactor containing protein. DmsD is also shown to bind to the signal peptide; however, its possible function as a 'guidance factor' for membrane targeting is ruled out by some experimental evidences. In this computational study, the interactions of DmsD with some other proteins were analyzed. The results of the analysis indicate that rather than playing the role of a chaperone directly, DmsD may influence the recruitment of GroEL, MoeB, and DnaK which can function as a chaperone for protein folding. The results of this analysis were also used together with the findings of available literature to generate a hypothetical model, proposing a possible function of DmsD in the biosynthesis of DMSO reductase.

Key words: Dimethyl sulfoxide (DMSO) reductase, DmsD, molecular docking.

INTRODUCTION

Dimethyl sulfoxide (DMSO) reductase is a membrane anchored enzyme in bacteria, which is needed for the anaerobic respiration using DMSO and some other related S-and N-oxides (Chan et al., 2008; Qiu et al., 2008). DMSO is a molybdenum cofactor containing heterotrimeric enzyme consisting of the DmsABC subunits, of which DmsA is the catalytic subunit and DmsB is the electron-transfer subunit (Rajagopalan, 1991). The DmsAB dimer is transported to the cytoplasmic membrane via the Tat system, where it attaches to the membrane-bound subunit DmsC (Chan et al., 2008). The DmsA is found to contain a specific sequence motif (S-R-R-x-F-L-K), known as 'twin-arginine' in its N-terminal leader peptide, which has been reported to be identified by the Tat translocase, leading to the translocation of DmsA across the cytoplasmic membrane in a fully-folded, cofactor-loaded state (Chan et al., 2008; Qiu et al., 2008). The molybdenum cofactor is also shown to be required for this translocation (Yoshida et al., 1991).

Two pathways, known as the Sec and the Tat pathways have been reported in Bacteria for the biosynthesis of cofactor containing proteins. Using the Sec apparatus, the apoprotein and cofactor are transported to the periplasm separately and the cofactor insertion and protein folding occur in the periplasm (Palmer et al., 2003). Tat pathway, on the other hand, is an independent translocation system with an ability to transport fully folded and oligomerized proteins across the cytoplasmic membrane (Qiu et al., 2008; Plamer et al., 2002). The core structure of the system comprises three membrane-associated proteins, TatA, TatB and TatC (Berks et al., 2000; Palmer et al., 2003). The TatBC unit acts as the twin-arginine signal peptide recognition module (Alami et al., 2000), whereas TatA is shown to form a large oligomeric ring-structure, similar to a channel (Palmer et al., 2003).

In the process of the DMSO reductase biosynthesis in bacteria, DmsD has been identified as the most potential candidate for the role of 'proofreading chaperone', which functions is to prevent the translocation of immature, misfolded and non-cofactor containing proteins (Hatzixanthis et al., 2005; Qiu et al., 2008). DmsD has

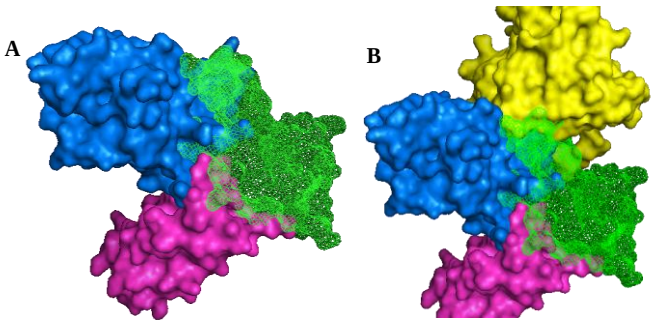

Figure 1. Binding of GroEL subunit (blue), DnaK (magenta) and MoeB (yellow) with DmsD (green). (A) Shows the binding of GroEL and DnaK and (B) shows the complete complex of four proteins. DmsD is shown in mesh format to clarify the binding sites between the proteins. Docking and modeling was done using Auto Dock and Pymol.

been reported to bind with the 'twin arginine' signal peptide of DmsA and also to TatB and TatC (Oresnik et al., 2001), indicating that this protein might have direct role in protein folding, co-factor insertion and also in membrane targeting. However, studies with chimeric proteins have shown that DmsD is not required for the interaction of the DmsA signal peptide with the Tat apparatus (Ray et al., 2003). In their experiment, Ray et al. (2003) showed that in the absence of DmsD, DmsA-GFP fusions were exported with high efficiency, ruling out the possible role of DmsD as guidance factor. Thus, the binding of DmsD to the signal peptide might influence the proper assembly of DmsA and prevent the export of misfolded or pre-folded protein (Ray et al., 2003). However, studies indicate that Tat transport mechanism does not require a correctly folded protein as substrate (Hynds et al., 1998).

The findings of Ray et al. (2003) have indicated towards another important possibility that DmsD might be required for the cofactor insertion in DmsA, similar to its homologus TorD. TorD is shown to stabilize the folding of apoTorA at high temperature and help in acquiring the Mo-cofactor (Genest et al., 2005). However, besides binding to the signal peptide and unlike the DmsD, TorD is found to interact with the mature TorA protein (Pommier et al., 1998). Some studies have also suggested the presence of a secondary binding site in the mature TorA for the binding of TorD (Jack et al., 2004). The interaction between the mature DmsA and the DmsD has not yet been reported.

Besides DmsA and TorBC, some of the key proteins have been identified to interact directly or indirectly with DsmD include elongation factor Ef-Tu, MoeB, GroEL and DnaK (Li et al., 2010). Interaction of the translation elongation factor Ef-Tu with DmsD suggests the possibility that DmsD might approach and bind to the leader peptide immediately after its synthesis, prior to its release from the Ef-Tu (Li et al., 2010). MoeB is considered to be involved with NarJ in some earlier pathway of nitrate reductase biosynthesis (Schwarz, 2005). Also mutant MoeB is found to affect DmsA maturation (Sambasivarao et al., 2002).

DnaK is well established as an *in vitro* RR-leader binding protein (Oresnik et al., 2001; Graubner et al., 2007). GroEL, in cooperation with DnaK has been shown to play role in the folding of a plant ferredoxin-$NADP^+$ reductase (Dionisi et al., 1998). Furthermore, a recent study has implicated GroEL in an interaction with NapD for the periplasmic nitrate reductase biosynthesis in *Escherichia coli* (Butland et al., 2005). Taken together, these findings suggest that DmsD might work as a 'hub-protein' in the network of interaction and facilitate the folding of DmsA by binding to it and also recruiting GroEL and / or DnaK (Li et al., 2010). In this study, a computational analysis was carried out with a view to predict possible interaction between DmsD and mature DmsA and also to find the involvement of other proteins in DMSO reductase biosynthesis.

MATERIALS AND METHODS

The crystal structure of DmsD from *Salmonella typimurium* (PDB ID: 1S9U) was analyzed using a docking (Auto Dock) and two modeling software (Pymol and Swiss PdbViewer). The homology model was generated for both mature (GI: 119351635) and precursor (GI: 16502128) DmsA from *S. typimurium* from Swiss Model, using 1dmrA as template. In order to visualize the pattern of interaction between DmsD and other proteins, some protein candidates were chosen from various protein-protein interaction studies done *in vitro* and *in silico* using bioinformatics approaches (Kostecki et al., 2010; Li et al., 2010). Although, these studies indicated towards a list of more than 300 candidates from bioinformatic analysis, only four most potential possible proteins were taken here, which include GroEL, DnaK and MoeB. The choice was done on the basis of the previous known function of these proteins.

For molecular docking, the homology model was generated for DnaK (GI: 1389758) of *S. typimurium* using 1q51A as template. 1jw9B was used as a template for modeling MoeB of the same organism (GI: 1256835). In both the cases, Swiss modeler was used. The crystal structure of GroEL was downloaded from Protein databank (PDB ID: 1J4Z).

RESULTS

When protein-protein interaction sites were analyzed using Auto Dock, no possible binding site was found between DmsD and mature DmsA. All the proteins GroEL, DnaK and MoeB were found to bind with DmsD. Moreover, they are found to form a complex (Figure 1). This indicated a possibility that rather than playing the role of a chaperon directly, DmsD may influence the recruitment of GroEL, MoeB and DnaK which function as a chaperone and lead to the folding of DmsA.

To verify this prediction, both the mature and precursor DmsA was docked with the complex and also with the individual molecules. Neither the individual candidate proteins nor the predicted complex showed any

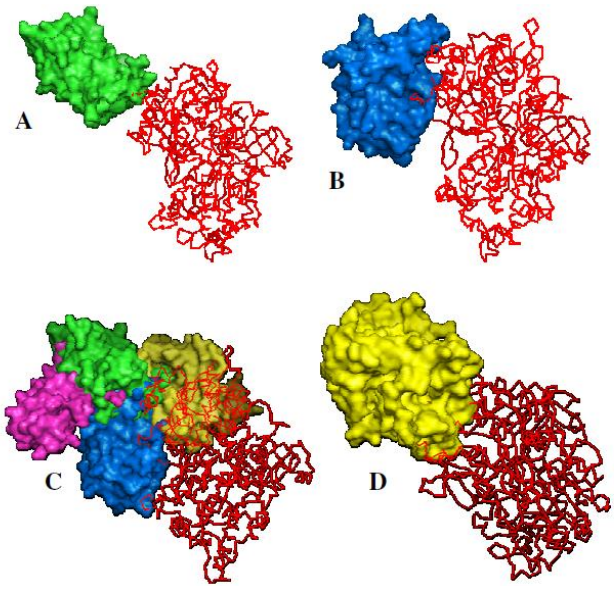

Figure 2. The precursor DmsA (apoprotein) showed to have possible binding sites with DmsD (A), GroEL subunit (B), MoeB (C) and also with the complex of four protein (D). In the protein complex, green is representing DmsD, blue is showing GroEL subunit and yellow and magenta is showing MoeB and DnaK respectively. The apo-protein is shown in red. Docking and modeling was done using Auto Dock and Pymol.

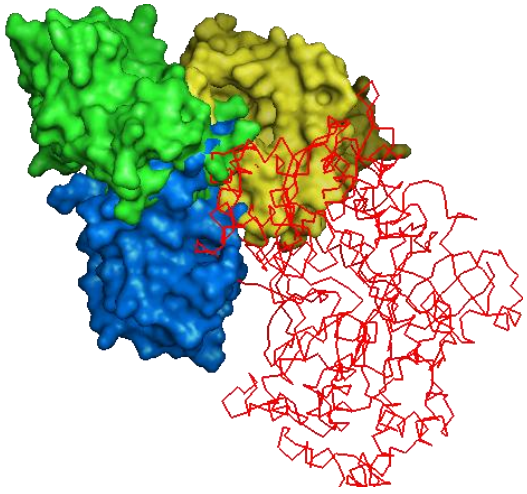

Figure 3. The final predicted protein complex that is assumed to interact with the precursor DmsA (shown in red). In the protein complex, green is representing DmsD, blue is showing GroEL subunit and yellow is showing MoeB. Docking and modeling was done using Auto Dock and Pymol.

interaction with mature DmsA. However, DmsD, GroEL, MoeB and the complex showed interaction with the precursor DmsA (Figure 2). No interaction was found between DnaK and precursor DmsA. This leads to the possibility that DmsD recruits GroEL, MoeB or other molecules before the folding of precursor DmsA and after proper folding; the mature DmsA is released from the complex. The final predicted complex is shown in Figure 3. GroEL is known to play a role in Molybdenum-iron cofactor insertion into the nitrogenase enzyme of

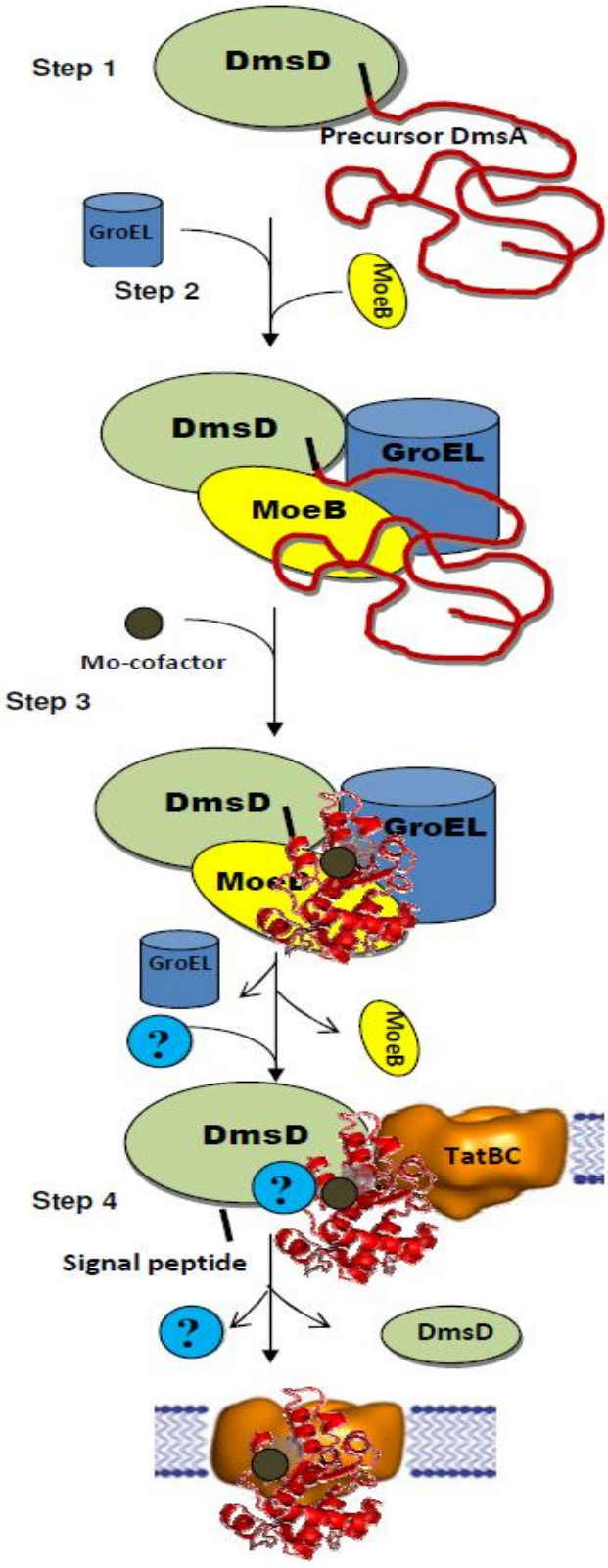

Figure 4. A hypothetical model showing the possible function of DmsD in the biosynthesis of DMSO reductase. Step 1: (Binding of DmsD); Step 2: (Binding of GroEL and MoeB); Step 3: Protein folding and co-factor insertion); Step 4: (Interaction with TatBC and release of signal peptide).

Azobacter vinelandii (Ribbe et al., 2001), which can play the similar role in DMSO biosynthesis as well.

DmsD is found to interact with TatBC complex in *E. coli* (Papish et al., 2003) and it is assumed that TatBC complex initiates a structural change within DmsD that causes the release of the DmsA signal peptide, which then can interact with Tat machinery (Winstone et al., 2006). However the factor that guides the trafficking of DmsA to TatBC is not known and the role of DmsD as a guidance factor is controversial. Thus, it is assumed that some proteins which might be present in this interaction can bind to the mature DmsA or DmsD or both as well as with TatBC complex. Taking all these information together, a hypothetical model is generated explaining the possible role of DmsD in DMSO reductase biosynthesis (Figure 4). The goal of this model is to use the pieces of information from available literature as well as the computational analysis to generate a complete over view of the scenario. The following steps from Figure 4 explain more succinctly the role of DmsD in DMSO reductase biosynthesis.

Step 1 (Binding of DmsD)

DmsD binds with the signal peptide (shown in black) of the precursor DmsA immediately after its synthesis, prior to its release from Ef-Tu factor.

Step 2 (Binding of GroEL and MoeB)

DmsD influence the recruitment and binding of GroEL and MoeB, forming a complex with the precursor DmsA.

Step 3 (Protein folding and co-factor insertion)

GroEL and MoeB mediate the insertion of Mo-cofactor and guide the proper folding of DmsA. GroEL plays the role of molecular chaperone and the protein folding is done inside the GroEL. GroEL and MoeB are released after this step. DmsD remains bound to the signal peptide.

Step 4 (Interaction with TatBC and release of signal peptide)

DmsD presents the bound DmsA to TatBC complex. This step might have the involvement of an unknown protein, which can bind to the mature DmsA and can act as a guidance factor to guide the binding of the complex with TatBC. The interaction between DmsD, TatBC and mature DmsA cause conformational change in DmsD causing the release signal peptide. DmsD and the unknown protein are also released later in this step.

DISCUSSION

Tat pathway requires a protein to be completely folded and assembled with all subunits and cofactors for its translocation. DmsD is thought to be a regulator of the process, possibly involved in proper folding of DmsA and also to its targeting to membrane bound Tat-complex. However, the exact function of DmsD is controversial and needs further analysis. In this study, a small docking analysis was also carried out which corroborated the involvement of GroEL and MoeB with DmsD for the folding of DmsA. GroEL may play a role in co-factor insertion. Considering all the piece of information from published literature, a hypothetical model was also generated explaining the possible role of DmsD. However, the model indicates the necessity of a guidance factor, which could be DmsD itself or some new protein, which is yet to be discovered. Further studies are needed in this area to end the controversies and to reveal the complete picture of the scenario.

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