

Purification of a Tat leader peptide by co-expression with its chaperone

Charles M. Stevens, Mark Paetzel*

Department of Molecular Biology and Biochemistry, Simon Fraser University, South Science Building, 8888 University Drive, Burnaby, British Columbia, Canada V5A 1S6

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ABSTRACT

We present a method for the purification of the 45 residue long leader peptide of *Escherichia coli* dimethyl sulfoxide reductase subunit A (DmsA_L), a substrate of the twin arginine translocase, by co-expressing the leader peptide with its specific chaperone protein, DmsD. The peptide can be isolated from the soluble DmsA_L/DmsD complex or conveniently from the lysate pellet fraction. The recombinant leader peptide is functionally intact as the peptide/chaperone complex can be reconstituted from purified DmsA_L and DmsD. A construct with DmsA_L fused to the N-terminus of DmsD (DmsA_L-DmsD fusion) was created to further explore the properties of the leader peptide-chaperone interactions. Analytical size-exclusion chromatography in-line with multi-angle light scattering reveals that the DmsA_L-DmsD fusion construct forms a dimer wherein each protomer binds the neighboring leader peptide. A model of this homodimeric interaction is presented.

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Introduction

The twin arginine translocase (Tat)¹ facilitates the transport of large fully folded protein complexes, that often contain redox sensitive cofactors, across the plasma membrane in bacteria and the thylakoid membrane of plants [1–3]. Substrates of the Tat contain amino-terminal leader peptides that are distinct from the leader (or signal) peptides of proteins destined to be secreted by way of the general secretory (Sec) system [4]. Tat leader peptides tend to be longer, have a less hydrophobic H-region, and contain a twin arginine (RR) motif at the border between the N-region and H-region of the peptide (Fig. 1A) [5,6]. The consensus sequence for the RR motif is S/T-R-R-X-F-L-K; wherein X can be any amino acid. Another salient feature of these peptides is the net +2 charge in the C-region of the leader peptide [7].

Many substrates of the Tat system have specific molecular chaperones that bind primarily via the leader peptide [8,9], and prevent translocation until the substrate is properly folded, cofactors have been inserted and multi-protein complexes have been assembled [10,11]. The chaperones are referred to collectively as Redox Enzyme Maturation Proteins (REMPs) [12]. It has been shown

that REMPs can protect the leader peptide of Tat substrates from proteolytic degradation. This protection has been demonstrated using the model REMP TorD and the preTorA substrate [13,14]. Co-expression of the TorD protein has also been used to enhance secretion of a chimeric TorA leader peptide fused to the GFP protein [15].

Escherichia coli dimethylsulfoxide reductase subunit A (DmsA) is one of the most studied Tat substrates. We are attempting to probe the interactions between the 45 residue leader peptide from the DmsA pre-protein (DmsA_L) and its specific chaperone DmsD via binding assays and crystallographic methods. To do this, it is essential to have a method that can produce milligram quantities of the peptide. It is difficult and costly to chemically synthesize DmsA_L due to its length and hydrophobicity. Recombinant methods provide an alternative option to produce the peptide. Attempts to express DmsA_L alone using recombinant technology have failed. This study demonstrates that the REMP DmsD [16], when co-expressed with DmsA_L, aids in the production of recombinant DmsA_L.

Materials and methods

Cloning DmsA_L

The DmsA leader peptide (DmsA_L), residues 1–45 of the *dmsA* gene (UniProt ID: P18775), was PCR amplified from a plasmid that codes for the DmsA leader peptide sequence, pTDms35, a gift from Dr. R.J. Turner. The sense primer: 5'-AGCTCATATGATGAAAACGAAAATCCCTGATCGGTTATTGG-3', contains an NdeI restriction enzyme recognition site. The antisense primer: 5'-AGCTCTCGAGGCTGCCG

* Corresponding author. Fax: +1 778 782 5583.

E-mail address: mpaetzel@sfu.ca (M. Paetzel).

¹ Abbreviations used: DmsA, dimethyl sulfoxide reductase subunit A; DmsA_L, DmsA leader peptide; Tat, twin arginine translocase; RR, twin arginine motif; REMP, redox enzyme maturation protein; *E. coli*, *Escherichia coli*; TBS, tris buffered saline; LB, luria broth; Ni-NTA, nickel-nitrilotriacetic acid; PCR, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; SEC-MALS, size-exclusion chromatography in-line with multi-angle light scattering.

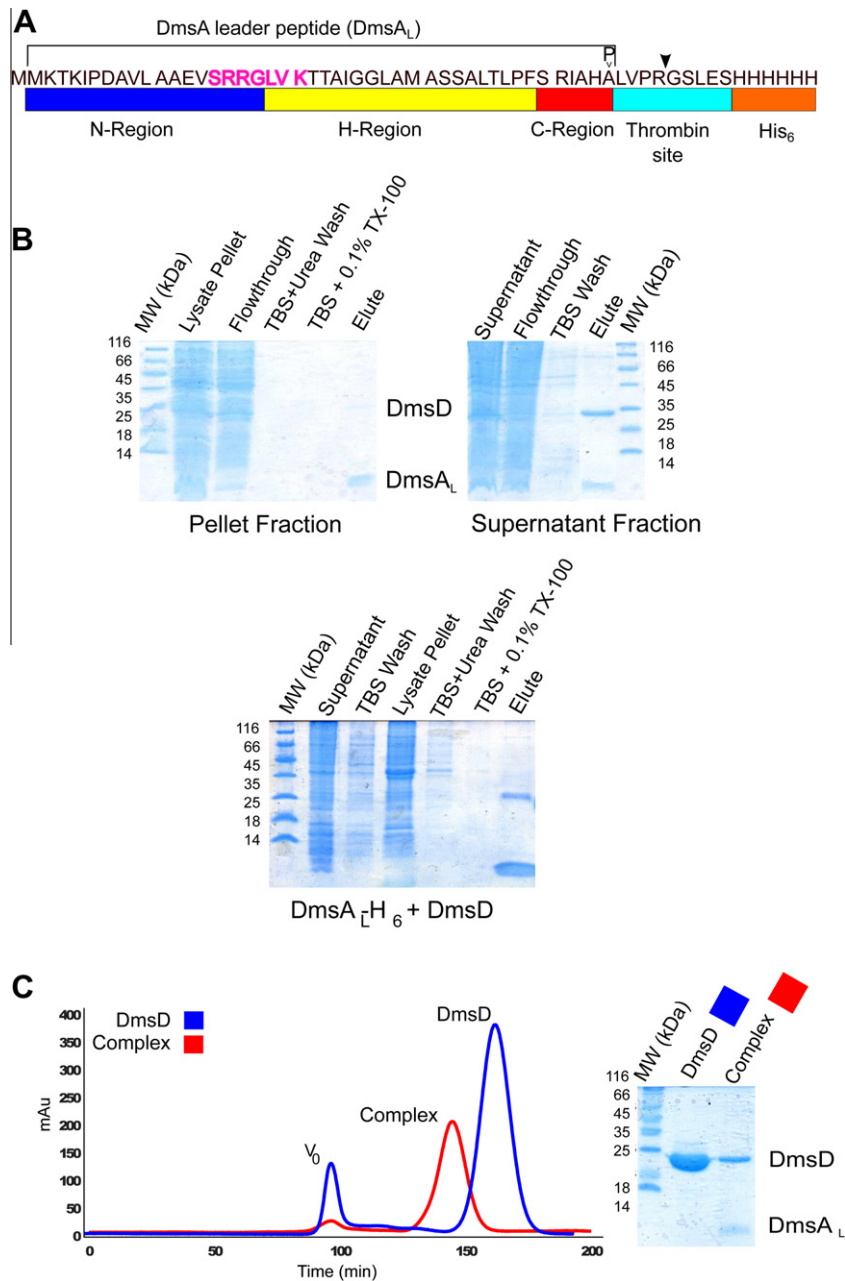


Fig. 1. (A) The expressed DmsA leader peptide construct with the twin arginine sequence in red letters. The different regions of the peptide are color coded below the sequence. In this construct, Ala45 was mutated to a proline. (B) Co-expressed DmsA_L and DmsD reveals two populations. SDS-PAGE gel (15%) stained with PageBlue shows the purification of the insoluble DmsA_L from the pellet fraction (left) and soluble DmsA_L/DmsD complex from the supernatant fraction of the cell lysate (right). The gel below shows the purification of the peptide after co-expression with untagged DmsD (combined pellet fraction and supernatant fraction) (C). Reconstitution of the DmsA_L/DmsD complex from purified components. In blue is the analytical size-exclusion chromatogram of DmsD alone. In red is the analytical size-exclusion chromatogram of the reconstituted DmsA_L/DmsD complex. Each peak was visualized by 15% SDS-PAGE gel stained with PageBlue. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

GCGGCACCAGGGGCGCAATCCGACTAAAAGGTAATG-3', contains an XhoI restriction enzyme recognition site and also encodes a C-terminal thrombin protease recognition site before the C-terminal hexahistidine tag that is present in the pET-24a vector. This primer also contains a codon change that mutates the last residue of the leader peptide (P1 site) from an alanine to a proline (Ala45Pro). We found that this mutation limited the level of proteolysis. The PCR product was purified with a GeneJet PCR purification kit (Fermentas) and inserted into the kanamycin resistant expression plasmid pET-24a (Novagen). The sequence of this C-terminal extension was: LVPRGSLEH₆ (Fig. 1A). This plasmid

pDmsA_L-H₆-24a was verified by DNA sequencing and transformed into competent BL21(DE3) host cells for expression.

Cloning DmsD with amino-terminal hexahistidine tag

The *E. coli dmsD* gene was PCR amplified from the plasmid pTDms67, a gift from Dr. R.J. Turner, using the primers 5'-ACTGCAT ATGACCCATTTTTCACAGCAAGATAATTTTCTG-3' and 5'-ACTGCATA TG ACCCATTTTTCACAGCAAGATAATTTTCTG-3'. The resulting PCR product, containing 5' NdeI and 3' XhoI restriction sites, was inserted into the ampicillin resistant pET-15b expression vector

(Novagen). The sequence for this plasmid, pH₆-DmsD-15b, was verified by DNA sequencing. The construct encoded by pH₆DmsD-15b consists of an N-terminal extension (MGSH₆SSGLVPRGSHM) containing a hexahistidine tag and a thrombin protease recognition site, followed by the 204 residues of the *dmsD* gene product (UniProt ID: P69853).

DmsD without tag construct

Site directed mutagenesis was performed to generate a DmsD construct without an N-terminal affinity-tag. The QuickChange protocol (Stratagene), primer: 5'-GAAGGAGATATACCAAGGGCAG-CAGCCATC-3' and its complement were used with the template plasmid pH₆-DmsD-15b to converted the initiation codon ATG (-19 Met) to AAG (Lys), thereby delaying translation until the first codon of DmsD. The resulting plasmid is named pDmsD-15b. Successful mutagenesis was verified by DNA sequencing.

Cloning the DmsA_L-DmsD fusion

The DmsA_L-DmsD fusion construct was generated by cloning the DmsA_L sequence (residue 45 mutated to proline as described above) using the primers: 5'-AGTCATATGATGAAAACGAAAATCCTGATGCGGTATGG-3', containing an NdeI restriction enzyme recognition site and 5'-AGCTGAATTCGGGCGCAATCCGACTAAAAGGTAA TG-3', containing an EcoRI restriction enzyme recognition site. The *dmsD* gene was amplified from the plasmid pTDms67, using the primers: 5'-ACTGGAATTCATGACCCATTTTTCACAGCAAGATAATTTT CTG-3' containing an EcoRI restriction enzyme recognition site and 5'-AGCTCTCGAGTATCGAAACAGCGGTTTAACCGCG-3' containing an XhoI restriction enzyme recognition site. The amplified and digested products were ligated into the kanamycin resistant expression vector pET-28a (Novagen) to create a construct that produces a protein consisting of an N-terminal hexahistidine tag and a thrombin protease recognition site (MGSH₆SSGLVPRGSHM), residues

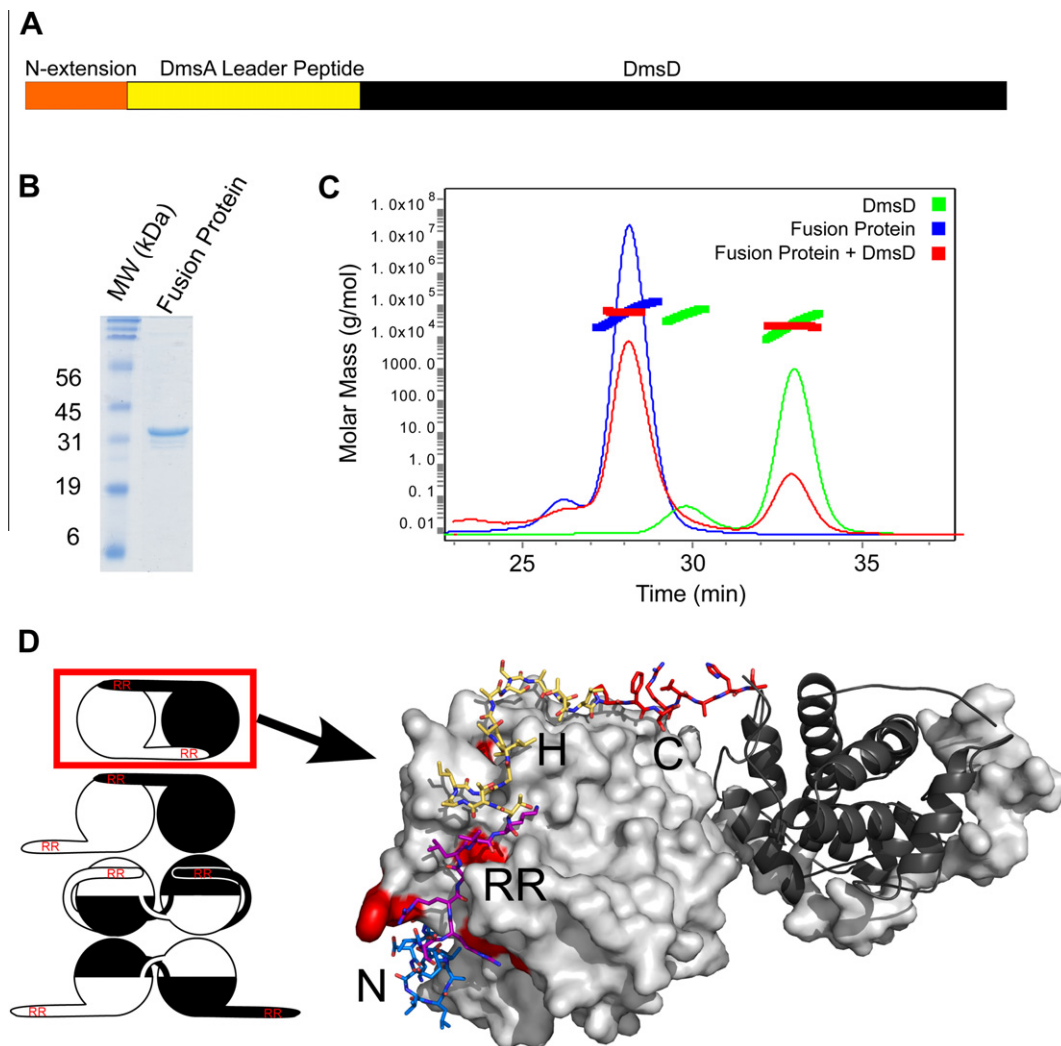


Fig. 2. (A) Schematic diagram of the DmsA_L-DmsD fusion protein construct. (B) Purified DmsA_L-DmsD fusion protein analyzed by SDS-PAGE stained with PageBlue. (C) A multi-angle light-scattering (MALS) analysis of purified DmsD (green), fusion protein (blue) and fusion protein + DmsD (red) is represented by a molar mass (g/mol) versus time (min) plot overlaid with a size-exclusion elution profile. Leader peptide moieties on the DmsA_L-DmsD fusion protein dimer are not accessible to free DmsD when analyzed by analytical size-exclusion chromatography in-line with multi-angle light scattering (SEC-MALS). This is demonstrated by the absence of a peak corresponding to 80.3 kDa or higher, which would be present if a DmsD-DmsA_L fusion protein dimer contained a free DmsA leader capable of binding a DmsD monomer. (D) Schematics of the possible interactions between the protomers in the dimeric arrangement. The boxed model is consistent with the experimental results, and a model of the DmsA_L-DmsD fusion protein is presented. In this model, each of the fusion protein monomers binds the leader peptide of the partner molecule. One molecule is shown as a white surface, with red indicating the residues shown to be important for DmsA_L binding [23] and the neighboring molecule is shown as a black ribbon, with the DmsA_L moiety shown as sticks and colored according to the region of the peptide, blue represents the N-terminal region, the hydrophobic region is yellow, and the C-terminal region is red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

1–44 of the DmsA preprotein, a proline residue, and finally, the DmsD protein. This plasmid was designated pH₆-DmsA_L-DmsD-28a. (Fig. 2A).

Expression

To prepare cells for expression of the DmsA_L-DmsD fusion, the plasmid pH₆-DmsA_L-DmsD-28a was transformed into the host strain BL21(DE3).

To prepare cells capable of co-expressing DmsA_L and DmsD, the plasmids pDmsA_L-H₆-24a (leader peptide, kanamycin resistant) and either pH₆DmsD-15b (DmsD with 6xHis-tag, ampicillin resistant) or pDmsD-15b (DmsD without the tag, ampicillin resistant) were co-transformed into the host strain BL21(DE3) and grown in LB media containing final concentrations of 0.05 mg/mL kanamycin and 0.1 mg/mL ampicillin.

To express each construct separately or to co-express the DmsA_L/DmsD complex, 10 mL of an overnight culture was diluted into 1 L of LB media containing the appropriate antibiotic, and incubated at 37 °C with shaking at 250 rpm until OD₆₀₀ = 0.6. The culture was induced with IPTG to a final concentration of 0.1 mM, incubated for a further 3 h, and harvested by centrifugation at 5000g. The cell pellet was re-suspended in 30 mL of 20 mM Tris pH 8.0, 100 mM NaCl (TBS) and stored at –80 °C. The cells were lysed by sonication for one minute at 30% amplitude using 5 s pulses at 5 s intervals. This was followed by treating the lysate with three cycles through an Avestin Emulsiflex-3C cell homogenizer. The lysate was clarified by centrifugation at 45,000g for 60 min at 4 °C, and the pellet and supernatant fractions were recovered.

Purification of DmsD, the DmsA_L-DmsD fusion or the DmsA_L/DmsD complex

To isolate DmsD, the DmsA_L-DmsD fusion protein or the soluble DmsA_L/DmsD complex, the lysate supernatant was applied to a 5 mL Ni-NTA (Qiagen) column equilibrated with TBS, washed with 10 column volumes (CV) of TBS, and eluted with 2 CV of 500 mM imidazole in TBS. The eluted protein was concentrated to 5 mL with a centrifugal filter concentration apparatus (10 kDa molecular mass cutoff, cellulose membrane, Millipore) and further purified by size-exclusion chromatography (Sephacryl S-100 HiPrep 26/60, TBS equilibrated) using an AKTA Prime system (GE Healthcare) at a flow-rate of 1.0 mL/min. Fractions containing the purified protein of interest were concentrated as before to 15 mg/mL. Hexahistidine tags were removed by incubation with a 1:1000 ratio of thrombin protease (Sigma) overnight at room temperature, followed by application of the digested protein sample to a 1 mL bed volume Ni-NTA (Qiagen) column, previously equilibrated with TBS, and washed with 4 mL of TBS. The flow-through and wash fractions were collected and further purified by size-exclusion chromatography (Sephacryl S 100 HiPrep 26/60, equilibrated with TBS, flow-rate 1.0 mL/min). Fractions containing the protein of interest were pooled and concentrated as above. The final protein concentration was measured using a NanoDrop spectrophotometer. The extension coefficient was calculated based on the protein sequence using the program ProtParam [17]. The protein purity was confirmed by the presence of a single band on a 15% SDS-PAGE gel stained with PageBlue (Fermentas).

Purification of DmsA_L

To isolate the DmsA_L peptide from the cell lysate pellet fraction, the pellet was solubilized in 8 M urea/TBS and centrifuged at 30,000g for 60 min at 4 °C. The resulting supernatant was applied to a Ni-NTA (Qiagen) column (5 mL bed volume) equilibrated with TBS containing 8 M urea, and washed with 5 CV of TBS containing

8 M urea. The urea was removed by washing with 5 CV of TBS containing 0.1% (v/v) Triton X-100 to stabilize the hydrophobic leader peptide. The peptide was eluted with 2 CV of TBS containing 0.1% (v/v) Triton X-100 and 500 mM imidazole and the resulting purified peptide was visualized using SDS-PAGE stained with PageBlue (Fermentas).

To purify the DmsA_L peptide from the culture that co-expressed the DmsA_L-H₆ with DmsD without a His₆ Tag, the same protocols were followed for lysis and clarification, followed by application of the lysate supernatant to a Ni-NTA (Qiagen) column (5 mL bed volume) equilibrated with TBS. The column was then washed with 5 CV of TBS to remove nonspecific binding of unwanted protein, and the re-solubilized lysate pellet, prepared as above, was added to the column. The column was then washed with 20 CV of TBS containing 8 M urea, and the urea removed with by washing with 5 CV of TBS containing 0.1% (v/v) Triton X-100 to stabilize the hydrophobic leader peptide. The peptide was eluted with 2 CV of TBS containing 0.1% (v/v) Triton X-100 and 500 mM imidazole and the resulting purified peptide was visualized using SDS-PAGE stained with PageBlue (Fermentas).

Amino-terminal sequencing of the DmsA_L peptide

After purification, SDS-PAGE, and blotting of the peptide sample onto a PVDF membrane, samples of the DmsA_L peptide were analyzed by N-terminal sequencing at the Ohio State University Protein Facility.

Formation of DmsA_L/DmsD complex from purified peptide and protein

The DmsA_L/DmsD complex was reconstituted by first adding the DmsA_L peptide to the Ni-NTA (Qiagen) column with a 1 mL bed volume as described above, followed by washing the column with 2 CV of TBS, and applying an excess of purified DmsD without a hexahistidine tag. This was incubated at room temperature for 30 min before being washed with 5 CV of TBS to remove the unbound DmsD protein. The complex was then eluted with 2 CV of TBS containing 500 mM imidazole, and further purified by size exclusion chromatography (Sephacryl S-100 HiPrep 26/60) using an AKTA Prime system (GE Healthcare) equilibrated with TBS and run at 1.0 mL/min. Fractions containing the proteins of interest were pooled and concentrated, and the purity was verified by visualization using SDS-PAGE stained with PageBlue (Fermentas).

Analytical size-exclusion chromatography in-line with multi-angle light scattering analysis (SEC-MALS)

SEC-MALS was carried out using a Superdex 200 size-exclusion column (GE Healthcare) in-line with a multi-angle light scattering system (Wyatt Technologies Inc.). Purified DmsD, the DmsA_L-DmsD fusion protein, and a mixture of equal volumes of DmsD and the DmsA_L-DmsD fusion protein, each at a concentration of 5 mg/mL incubated at room temperature for 30 min, were assayed to probe the association of the dimeric DmsA_L-DmsD fusion protein. A 100 µL sample of purified protein (5 mg/mL) was injected and resolved at a flow rate of 0.5 mL/min in a TBS buffer at pH 8.0. The molecular masses for each protein population in each sample were determined by a multi-angle light scattering DAWN EOS instrument with a 684 nm laser (Wyatt Technologies Inc.) coupled to a refractive index instrument (Optilab rEX, Wyatt Technologies Inc.). The molar mass of the protein was calculated from the observed light scattering intensity and differential refractive index using ASTRA v5.1 software (Wyatt Technologies Inc.), based on the Zimm fit method with a refractive index increment of dn/dc = 0.185 mL/g.

Molecular model for the DmsA_L-DmsD fusion protein

A model for the DmsA_L-DmsD fusion protein dimer was constructed based on the crystal structure of the *E. coli* DmsD monomer (PDB ID: 3EFP) [18]. The molecules were positioned such that, in the dimer, the leader peptide moiety from each molecule was bound by the partner molecule in a manner consistent with a molecular dynamics simulation of the binding event [18]. Coordinates were manipulated using Coot [19] and the final model was energy minimized, using the steepest descents algorithm in the GROMACS 3.3 suite of molecular dynamics programs, so that the maximum force on any atom did not exceed 250 kJ mol⁻¹ nm⁻¹ [20]. A periodic simulation box, 9 Å larger than the protein complex along each dimension, was formed and the system was solvated using the spc216 water model. The net charge of the system was made zero by replacing randomly selected water molecules with Na⁺ or Cl⁻ ions. The system was energy minimized, as before, to a maximum force of 1000 kJ mol⁻¹ nm⁻¹ and the system was simulated for 1 ns using a 0.002 ps time-step with all protein atoms constrained. In subsequent simulation steps, the constraints were replaced by LINCS bond length and angle restraints. The simulation was run on the Westgrid computing cluster “matrix” for a total simulation time of 6 ns, followed by steepest descent energy minimization to a maximum force of 250.0 kJ mol⁻¹ nm⁻¹. This simulation used the GROMOS96 43a1 force-field and was run in an environment that holds the number of atoms, temperature and pressure of the system constant. Interactions were calculated using a twin range pair list with a long range cut-off of 10 Å and a short range cutoff at 0.8 Å. Temperature and pressure coupling used the Berendsen method at 300 K, with a τ_T value of 0.1 and a τ_P value of 1.0. The figure was generated by converting the output of the simulation from the GROMACS file format to the PDB format with editconf [20] and the image was rendered using Pymol [21].

Results and discussion

Expression and purification

DmsD, the Tat chaperone (REMP) for DmsA, was successfully over-expressed from the plasmid pH₆-DmsD-15b with a yield of approximately 2.5 mg of pure protein per liter of culture. The purified DmsD was visualized as a single band by SDS-PAGE stained with PageBlue. Attempts to express DmsA leader peptide (DmsA_L) from the plasmid pDmsA_L-H₆-24b failed to produce detectable quantities of product when expression was carried out at 37 °C with induction at OD₆₀₀ = 0.6 for 3 h, or at 25 °C, with induction at OD₆₀₀ = 1.0 overnight. However, co-expression of the DmsD and DmsA_L, followed by purification of the lysate supernatant fraction, produced the DmsA_L/DmsD complex visible as two distinct bands on an SDS-PAGE gel (Fig. 1B) with a yield of approximately 1 mg of pure protein per liter of culture. Isolation of the lysate pellet fraction produced almost pure DmsA_L based on SDS-PAGE gel results, a faint band corresponding to the DmsD molecular mass was visible (Fig. 1B). The absence of tryptophan residues in the

peptide makes direct spectrophotometric measurement of the concentration difficult, yet based on the concentration of DmsD recovered after assembly of the DmsA_L/DmsD complex (discussed below), and assuming a 1:1 M ratio of DmsA_L (6.4 kDa) to DmsD (23.3 kDa) in the complex, approximately 0.02 μMol of peptide (129 μg) was recovered from the pellet fraction from each liter of culture, and a further 0.04 μMol (230 μg) of peptide was recovered from the supernatant fraction from each liter of culture.

Amino-terminal sequencing of the first six residues of the purified DmsA_L recovered from the co-expressed protein complex yielded the sequence: MMKTKI, which corresponds exactly to the DmsA_L amino-terminal sequence (with an additional N-terminal methionine residue that was introduced by the pET-24a expression vector).

Reassembly of the DmsA_L/DmsD complex

The purified DmsA_L appears to be functional, in that it is able to bind to its chaperone DmsD. Adding tag-free DmsD to a Ni-NTA column containing bound DmsA_L yielded a population of the DmsA_L/DmsD complex that was distinct from free DmsD when analyzed by size-exclusion chromatography and could be visualized by SDS-PAGE (Fig. 1C).

The DmsA_L-DmsD fusion protein

The DmsA leader peptide fused to the N-terminus of DmsD (DmsA_L-DmsD fusion protein) (Fig. 2A) was purified such that it appeared as a single band when analyzed by SDS-PAGE (Fig. 2B) with an approximate yield of 0.75 mg per liter of culture. Analysis of this fusion protein by SEC-MALS reveals that it purifies as a dimer (Fig. 2C). Incubation of the DmsA_L-DmsD fusion protein with DmsD followed by SEC-MALS analysis reveals no higher order species, suggesting that there are no exposed leader peptide moieties for DmsD to bind (Fig. 2C). It is likely that each N-terminal leader peptide is bound to the neighboring DmsD in the fusion homodimer, and that the dimer is likely held together via this *trans*-interaction. If there was a single DmsD molecule attached to the DmsA_L-DmsD dimer molecule, the calculated molecular mass would be approximately 80.3 kDa, and it would be visible within the 10 kDa to 600 kDa separation range of the Superdex 200 column. The molecular masses determined from SEC-MALS and SEC alone for each of the proteins is reported in Table 1. The observed molecular masses differ somewhat from those calculated but no major peaks are observed that correspond to higher order complexes. When DmsD alone was analyzed, a higher molecular mass peak was observed at the expected elution point of a homodimeric DmsD species that has been previously characterized [22].

The model for the DmsD-DmsA_L dimer generated by docking and molecular dynamic simulation provides a proposed relative orientation for the DmsD molecules within the dimer based on the mutual docking of the amino-terminal DmsA_L leader peptide of the neighboring protomer within the dimer. The protein-protein interactions in the DmsA_L-DmsD fusion protein dimer were

Table 1
Calculated and observed molecular mass.

Protein	Calculated (kDa) ^a	MALS (kDa) ^b	SEC (kDa) ^c
DmsD monomer	23.3	22.3	21.5 (Superdex 200) 22.8 (Sephacryl S-100)
DmsA _L /DmsD complex	28.5	N/A	32.7 (Sephacryl S-100)
DmsD dimer	46.6	52.8	39.1 (Superdex 200)
DmsA _L -DmsD fusion dimer	57.0	43.6	52.0 (Superdex 200)

^a See methods section for a description of the amino acid sequence for each construct.

^b MALS: multi-angle light scattering.

^c SEC: size-exclusion chromatography (The column type used for each analysis is given). A standard curve was generated using the low molecular weight calibration kit (LMW), Amersham Pharmacia Biotech.

modeled and subjected to molecular dynamics simulation. The resulting model is consistent with the leader peptide of each of the protomers in the dimer being bound by the partner protomer and predicts their relative orientation (Fig. 2D).

The interaction between the residues of DmsA_L and the DmsD surface is consistent with those observed previously when the same analysis was performed for the DmsD alone and DmsA_L [18], and the modeled interactions are consistent with the DmsD contact sites proposed from previous site-directed mutagenesis studies (Fig. 2D) [23]. The twin arginine motif is located within a crevice formed by three conserved surface loops that are observed to bind ligands in the X-ray crystal structure of *E. coli* DmD (77–87, 93–100, 113–127) (PDB ID: 3EFP) [18].

The N-terminal region of the DmsA_L peptide interacts with the C-terminal region of DmsD. This includes the previously described hot pocket residues, and the area between helix 5 and the loop between helices 1 & 2 [23], which contacts the arginine of the SRRGLVK motif. Helix 5 has been shown by mutagenesis to be important for the homologous TorD/TorA_L interaction [9]. The hydrophobic region of the DmsA_L peptide also interacts with helix 5, the loop between helices 4 and 5, and residues on helices 2 and 4. Each of the leucine residues in the hydrophobic region of the DmsA_L peptide interact with hydrophobic pockets on the surface of the DmsD molecule. This is consistent with mutagenesis experiments that show conserved leucine residues in Tat leader peptides are critical for chaperone binding [2,24]. The C-terminal region of the DmsA_L peptide interacts in part with the C-terminal region of helix 2, though a large portion of the C-terminal region acts as a linker between partner protomers.

Concluding remarks

These methods provide a means to recombinantly produce a Tat leader peptide through co-expression and co-purification with its chaperone protein. In principle, this technique should be applicable to any of the REMP family proteins and possibly other peptide binding molecular chaperones.

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