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Multidrug transporter MexB of *Pseudomonas aeruginosa*: overexpression, purification, and initial structural characterization

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Abstract

Structural and functional characterization of the multidrug transporter, MexB, of *Pseudomonas aeruginosa* is significantly restricted due to a low yield of approximately 0.1 mg/L of culture from natural sources. To facilitate structural studies of this medically important transporter protein, we developed a large-scale system for expression of the genetically engineered recombinant, MexB, in the *Escherichia coli* cell. Using the system, the eventual yield of MexB attained was about 10 mg/L of culture. The optimized purification protocol in the presence of dodecyl β-D-maltoside allowed isolation of highly homogeneous MexB. The oligomeric state of the protein in detergent solution has been characterized to verify that the native state of the purified protein has been preserved. The molecular mass of the protein–detergent complex was found to be 380–450 kDa. The MexB-dodecyl β-D-maltoside mass ratio was determined to be 1.8 ± 0.05. Taking into account the monomeric MexB molecular mass deduced from its amino acid sequence (112.8 kDa), we concluded that the purified MexB exists as the homotrimer in the surfactant solution. Circular dichroism analysis of MexB showed dominance of the α-helix structures. High yield, homogeneity, and stability of MexB position it as a good candidate for structural and functional characterization.

Keywords: Xenobiotic transporter; Membrane protein; Expression; Purification; Native state; Antibiotic; Expression vector

Pseudomonas aeruginosa shows significant degrees of intrinsic resistance to a wide variety of antimicrobial agents, including most β-lactams, tetracyclines, chloramphenicol, and fluoroquinolones. This resistance is mainly attributable to expression of the antibiotic efflux pump [1–6]. This is a problem in hospitals because *P. aeruginosa* is a major opportunistic pathogen and a leading cause of hospital-acquired infections and mortality [7]. This pathogenic bacterium encodes several family efflux pumps, including MexAB-OprM, of the resistance-nodulation-cell division (RND)[1] [8]. This pump consists of the inner membrane-spanning proton-antibiotic antiporter, MexB, the membrane fusion protein MexA that is assumed to connect the inner and outer membranes, and outer membrane-associated lipoprotein, OprM [9]. The MexB subunit consists of 1046 amino acid residues, has a molecular mass of about 113 kDa, and is encoded by the second gene in the *mexAB-oprM* operon. It was shown previously that the MexB protein spans the mem-

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[1] *Abbreviations used:* DDM, *n*-dodecyl-β-D-maltoside; OG, *n*-octyl-β-D-glucopyranoside; IPTG, isopropyl β-D-thiogalactoside; RND, resistance-nodulation-cell division; TMS, transmembrane segment; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis, PMSF, phenylmethylsulfonyl fluoride.

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brane 12 times leaving amino- and carboxyl termini at the cytoplasmic side of the inner membrane and forms two large hydrophilic domains extending towards the periplasmic space [10–12]. These large loops might interact with the periplasmic subunit, MexA, and the outer membrane subunit, OprM [13–17]. Among five charged amino acid residues found in the transmembrane domains, three were highly conserved in the RND family efflux proteins. Specific localization of the highly conserved charged residues in the transmembrane domains suggested that they might play an important role in proton conduction [11].

Purification of MexB from natural sources is difficult due to low yield (about 0.1 mg/L of culture (Nakae et al., unpublished data)). Heterologous MexB overexpression in *E. coli* cells offers an alternative to overcome this problem [18]. A protein produced using an overexpression system may be more homogeneous than that from natural sources, and therefore may yield crystals more readily. In addition, DNA cloning techniques facilitate the addition of affinity tags to the recombinant proteins to enhance their purification. Introduction of a small affinity tag, such as a hexa-histidine-tail, may not interfere with protein structure or activity and therefore may not need to be removed after purification [19]. Here, we report on the hexahistidine tagging, overexpression in *E. coli*, and purification of the detergent-solubilized form of the MexB. Homogeneously purified MexB has been used for experimental determination of the quaternary structure.

Materials and methods

Strains and Plasmids

Bacterial strains and plasmids used in this study are listed in Table 1.

Detergents

To choose the optimal surfactant, the following detergents have been used: *n*-octyl-β-D-glucopyranoside (OG) (Anatrace), *n*-octyl-oligo-oxyethylene (octyl-POE) (Alexis), β-D-fructopyranosyl-α-D-glucopyranoside monododecanoate (sucrose monolaurate) (Dojindo), and *n*-dodecyl-β-D-maltoside (DDM) (Anatrace).

Construction of the pMexB plasmid

To clone *mexB* with the hexa-histidine tag added to its C-terminus on the pET22b+ (Novagen) vector, the 3151 bp fragment of *mexB* was amplified with Pfu-turbo DNA-polymerase (Stratagen) and primers MexBF (5′-T**catatg**TCGAAGTTTTTCATTGATAG-3′) and MexBR (5′-T**ctcgag**TTGCCCTTTTCGACGGA CG-3′). The *Nde*I and *Xho*I restriction sites are shown in bold. PCR mixtures (100 μl) contained 20 ng of genomic DNA of *Pseudomonas aeruginosa* (PAO4090 strain), 1 pM of each primer, 2.5 mM of each nucleotide, 2 mM $MgSO_4$, 10% (v/v) dimethyl sulfoxide, and 1 U Pfu-turbo (Stratagene) polymerase in 1× reaction buffer. Mixtures were heated at 94 °C for 1 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 3.5 min at 72 °C before finishing with 5 min at 72 °C. The PCR product was then purified with the Qiaquick PCR purification kit (Qiagen), digested with *Nde*I and *Xho*I, and cloned into *Nde*I–*Xho*I-restricted pET22b+. The plasmid with the MexB protein containing six extra histidine residues encoded at the carboxyl terminal end was designated as pMexB.

MexB expression and purification

Escherichia coli C43(DE3) [20] cells were transformed with pMexB, plated on solid 2× YT agar (1.6% (w/v) tryptone (Difco), 1.0% (w/v) yeast extract (Difco), 0.5% (w/v) NaCl, and 2.0% (w/v) agar (Difco), pH 7.2) containing 100 μg/ml ampicillin, and incubated at 37 °C. An individual bacterial colony was inoculated in 5 ml of 2× YT medium with 100 μg/ml of ampicillin. This pre-culture was grown overnight at 37 °C with continuous shaking at 200 rpm and then used to inoculate 1 L of 2× YT (1.6% (w/v) tryptone (Difco), 1.0% (w/v) yeast extract (Difco), and 0.5% (w/v) NaCl, pH 7.2). The culture was grown for about 2.5 h to $A_{600}^{1\,cm} = 0.6$, and then cooled down on

Table 1
Strains and plasmids

Strains or plasmids	Description	Source
Pseudomonas aeruginosa		
PAO4290	*leu-10*, *argF10*, *aph-9004*; FP	Matsumoto collection
Escherichia coli		
Top10	F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lacZ*ΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G	Invitrogen
BL21(DE3)	F$^-$ *ompT hsd*SB (rB$^-$mB$^-$) *gal dcm* (DE3)	Novagen
C43(DE3)	BL21(DE3) derivative with uncharacterized mutations	Avidis
pET22b+	expression vector Ap^r	Novagen
pMexB	pET22b+ derivative with MexB gene	This work