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Molecular modeling of the bacterial outer membrane receptor energizer, ExbBD/TonB, based on homology with the flagellar motor, MotAB

Yu Feng Zhai, Wilbert Heijne, Milton H. Saier Jr.*

Division of Biological Sciences 0116, University of California at San Diego, 9500 Gilam Drive, La Jolla, CA 92093-0116, USA

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Abstract

The MotA/MotB proteins serve as the motor that drives bacterial flagellar rotation in response to the proton motive force (pmf). They have been shown to comprise a transmembrane proton pathway. The ExbB/ExbD/TonB protein complex serves to energize transport of iron siderophores and vitamin B_{12} across the outer membrane of the Gram-negative bacterial cell using the pmf. These two protein complexes have the same topology and are homologous. Based on molecular data for the MotA/MotB proteins, we propose simple three-dimensional channel structures for both MotA/MotB and ExbB/ExbD/TonB using modeling methods. Features of the derived channels are discussed, and two possible proton transfer pathways for the ExbBD/TonB system are proposed. These analyses provide a guide for molecular studies aimed at elucidating the mechanism by which chemiosmotic energy can be transferred either between two adjacent membranes to energize outer membrane transport or to the bacterial flagellum to generate torque.

Keywords: Bacterium; Proton channel; Motility; Outer membrane transport; Energization; Molecular modeling

1. Introduction

Many species of bacteria are propelled by flagella, thin helical filaments that are energized by a reversible rotary motor [1–3]. Unlike eukaryotic flagella, which are powered by nucleoside triphosphate hydrolysis, bacterial flagellar motors are powered by the electrochemical gradients of protons [4–9] or sodium ions [10–15] across the cytoplasmic membrane of the cell. Numerous mechanisms have been proposed for the flagellar motor over the years, but there is still too little information to decide among them [2,9,16,17]. The proteins most closely involved are MotA, MotB and FliG.

MotA and MotB (PomA and PomB for the Na^+-driven motor in *Vibrio alginolyticus*) [18] are integral membrane proteins that are believed to comprise the torque generator and form the proton (or sodium) channel [8,17,19–23]. MotA (PomA) has four hydrophobic segments, each traversing the membrane [24]. The three cytoplasmic domains of MotA contain about 190 of the nearly 300 residues of this protein, whereas the periplasmic loops are short, each of about 10 residues. Mutations in the transmembrane regions severely impair H^+ or Na^+ conduction [25]. MotB (PomB) is also part of the transmembrane proton (sodium ion) channel but traverses the membrane only once [17,26]. Unlike MotA, the bulk of MotB is periplasmic and is believed to anchor the motor to the peptidoglycan layer [27,28]. MotA and MotB and their homologues have together been classified as H^+- or Na^+-translocating channel-forming constituents that comprise the bacterial flagellar motor (Mot) family (TC #1.A.30.1) [29–31]. The MotA/B heterooligomeric complex, with a possible stoichiometry of $(MotA)_4(MotB)_2$ [32,33], is distantly related to the TonB-ExbB-ExbD and the TolA-TolQ-TolR complexes of outer membrane transport energizers (the Exb family; TC #1.A.30.2) ([34–36], and see below). Homology between the MotAB, ExbBD and TolQR systems is well established [30,34–36].

Homologues of TonB protein complexes have been found widely distributed among Gram-negative bacteria. They energize the active uptake of iron obtained from mammalian iron-binding proteins [37–40], iron chelated to heme [41,42], iron siderophores [43] and vitamin B_{12} [44,45] across the outer membranes of Gram-negative bacteria. They also mediate drug and solvent tolerance [46,47] and have

* Corresponding author. Tel.: +1-858-534-4084; fax: +1-858-534-7108.
E-mail address: msaier@ucsd.edu (M.H. Saier).

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been parasitized for the import of toxic type B colicins [48]. TonB, ExbB and ExbD are three components of the multimeric TonB-dependent energy-transducing system [49–51], present in stoichiometries of about 1:7:2 [52]. According to the available experimental evidence, the energized TonB protein physically contacts a ligand-bound outer membrane receptor and transduces energy from ExbBD, inducing a conformational change in the outer membrane receptor [38,53–55]. This process results in release of the ligand into the periplasm of the cell and causes accumulation against large concentration gradients, up to at least 1000-fold [56–59]. ExbB is predicted to span the cytoplasmic membrane three times with its N terminus and a short loop located to the periplasm [35]. Most of the protein is localized to the cytoplasm [60]. ExbD has a single N-terminal transmembrane domain consisting of its signal anchor, but most of the protein is in the periplasm [61].

ExbB and ExbD of *E. coli* are encoded within the *exbBD* operon. Mutations in either gene produce the same phenotype, namely, loss of about 90% of the energy-transducing activity of the TonB-dependent system [62–65]. Mutation of D25 in ExbD completely eliminates activity of the complex [65] while an H10R mutation in TonB substantially reduces activity [66]. The two Exb proteins together contribute to the stability of TonB [62,64,67] and, therefore, these three proteins are presumed to interact directly.

TolA/TolQ/TolR systems are homologous to the TonB/ExbB/ExbD systems, but they energize a different set of putative outer membrane receptor porins. They promote detergent and bile salt resistance as well as outer membrane stability, and they have been parasitized for the translocation of group A colicins and filamentous phage DNA into the cell [68–70]. Their mechanism of action is undoubtedly comparable to that of the TonB–ExbBD systems although little experimental data bear on this postulate [71]. Additional proteins may play important roles in the energy transduction process [68,70].

Arriving at a molecular model for the ExbB/ExbD/TonB energy transduction complex and understanding the mechanism by which outer membrane receptor activities are regulated are important research goals [38,49]. Establishment of homology between the MotA/MotB system, the ExbB/ExbD/TonB system and the TolA/TolQ/TolR system provides a reference point for designing a model and proposing common features. Although neither an X-ray nor an NMR structure of the MotA/MotB complex is available, molecular genetic and biochemical experiments have provided evidence for essential features of the channel structure [19,20,31,72,73]. For example, because of the large size and moderately hydrophobic character of the tryptophan side chain, Sharp et al. [72,73] used systematic tryptophan mutagenesis of the five membrane-spanning segments of MotA/B to gain evidence for their interactions. Tryptophan residues were introduced at consecutive positions in each of the hydrophobic transmembrane helices, and the effects on function were measured. Using this approach, these investigators identified the lipid-facing parts of each helix.

As noted above, some evidence suggests that the MotA/MotB (PomA/PomB) complex might be a heterohexamer of $(MotA)_4(MotB)_2$ composition [32,33,74]. Evidence for two channels in the complex has been presented [32] suggesting that the functional H^+ transporting unit might have a $(MotA)_2(MotB)_1$ stoichiometry. Both MotA subunits proved to be required for the functional association with MotB [33], but the basis for the requirement for both MotA subunits has not been established.

Three possibilities can be considered: (1) Both MotA subunits together with one MotB subunit contribute to the formation of a single transmembrane proton pathway (the H^+ channel). (2) Only one MotA subunit is required with a MotB subunit to form the H^+-translocating channel, and the second MotA subunit served a structural role to maintain the stability and/or functional conformation of the first MotA subunit. (3) Both MotA subunits contribute to the H^+ flux but in an alternating site type mechanism [75] whereby the two MotA subunits functionally interact with the MotB subunit sequentially but not simultaneously. Either possibility (2) or (3) requires that the H^+ pathway be formed at the interface of a single MotA subunit and a single MotB subunit. This is the simplest model currently available, and the one we have adopted for molecular modeling purposes.

Based in part on the published results and the considerations cited above, we have proposed a simple structure for the functional unit of MotA/MotB using modeling methods. We have assumed that the ion channel is formed at the interface of one MotA subunit and one MotB subunit. Our theoretical structure was then used as the template for designing a structural model for ExbB/ExbD/TonB. Using this model, we have proposed general features of two potential cation channels through the ExbBD/TonB complex.

2. Methods

Pair-wise sequence alignments were generated using the Genetics Computer Group (GCG) GAP program [76]. Comparison scores, expressed in standard deviations (S.D.), were calculated using this program with 500 random shuffles. Multiple sequence alignments were generated using the Clustal X program [77] and confirmed with other programs [78,79]. Similarity, searches against the NCBI database were performed using the PSI-BLAST [80] program with a cutoff point of 0.001 and with iterations to convergence.

The initial backbone structure of MotA/MotB, that is, the arrangement of the five helices in the helical bundle, was based largely on the results of Sharp et al. [72,73]. The structure was refined by energy minimization (ME) and molecular dynamics (MD) using the InsightII program, Discover (version 98.0, Molecular Simulation Inc. (MSI)). This procedure allowed us to define the best possible orientation of the helices relative to each other as well as

the preferred conformations of the residue side chains. Simulation was performed in vacuo with a dielectric constant of 4 using the consistent valence force field (CVFF). The protein was kept neutral during the structural refinement procedure. All of the ionizable amino acyl residues were maintained in their unionized forms as outlined by Lin and Yan [81]. Structural refinement procedures were as follows: (1) Poor contacts were eliminated by a 100-step steepest descent ME followed by a 1000-step conjugated gradient ME. (2) MD was performed at 600 K for 10 ps, and a local minimum structure was selected near the end of the trajectory for optimization by 1000-step conjugated ME. (3) Steps (1) and (2) were repeated at lower temperature intervals of 100 K to a final value of 300 K. (4) The final structures were optimized with conjugated gradient ME and a convergence criterion of 0.05 kcal/mol [81,82]. The structure of ExbB/ExbD/TonB was based on that obtained for MotA/MotB with structural refinement procedures similar to those used for MotA/MotB.

Without constraints, the structure would be destined for denaturation because the ME and MD simulations were not conducted under native conditions in the presence of a lipid bilayer, an aqueous solution or other potentially important factors. To prevent the helix bundle from disintegrating at high temperatures, the polypeptide backbone atoms were subjected to a harmonic force field during the entire refinement procedure. The force constant was reduced gradually from the first to the last MD run. A hydrogen bond restraint was also used in the modeling calculations. Relatively strong distance restraints were imposed to maintain proper geometry for i, $i+4$ hydrogen bonds. The distances between the carbonyl oxygens of residues i and the backbone nitrogens of the amide protons of residues $i+4$ were constrained to a range of 2.7–3.2 and 1.8–2.3 Å, respectively, for the segment of an α-helix.

3. Results and discussion

3.1. Homology between MotA/MotB and ExbB/ExbD/TonB

Although the MotA/MotB proteins and the ExbB/ExbD/TonB proteins have very different biological functions, they nevertheless have several functional and structural characteristics in common. First, these two systems are integral membrane proteins that presumably comprise a proton channel. The energy generated by transmembrane proton flux is used by MotA/MotB to rotate the flagellum or by ExbB/ExbD/TonB to import iron siderophores and vitamin B_{12} (see Introduction). Second, although the sequences of these two systems are strikingly divergent, they exhibit sequence similarity and are probably homologous [34–36, see below]. Of particular note, the most conserved and functionally important transmembrane helices 3 and 4 in MotA are similar to helices 2 and 3 of ExbB. Third, the largely conserved residues in MotA (P173, G176, G183, A205, T209 and G212) are also conserved in ExbB. Fourth, binary comparison scores obtained when these two transmembrane helices were compared using the GAP program [76] with 500 random shuffles gave comparison scores of about 6 standard deviations (S.D.). When the entire regions of homology for the *E. coli* MotA and the *Pseudomonas syringae* ExbB proteins (encompassing the three common TMSs and the intervening loops) were compared using the same method, a comparison score of 9.5 S.D. was obtained. These results show that MotA and ExbB are homologous to each other and therefore share a common evolutionary origin. Fifth, the only transmembrane helix in MotB exhibits sequence similarity to that of ExbD. The fully conserved aspartate at the N terminus of the MotB transmembrane helix is also fully conserved in the corresponding region of ExbD. Sixth, in addition to the sequence similarity between the transmembrane helices of MotA/MotB and ExbB/ExbD, the membrane topology of MotA/MotB is the same as that of TonB/ExbB/ExbD (Fig. 1), and the extramembrane domains exhibit size similarities. For example, the bulky C terminus and the loop region between helices 2 and 3 in MotA are localized to the cytoplasmic side of the membrane while the short loop between helices 1 and 2, as well as that between helices 3 and 4, are localized to the periplasmic side of the membrane. Although there are only three transmembrane helices in ExbB, the membrane topology is similar to that of helices 2–4 of MotA. Although there is no direct evidence in favor of the postulate, the single transmembrane helix of TonB may replace transmembrane segment 1 (TMS1) in MotA. The large loop between helices 1 and 2 of ExbB, the C terminus localized to the cytoplasmic side of the membrane, and the short loop between helices 2 and 3 in the periplasm all resemble these features in MotA. The TMSs in MotB and ExbD both have their N termini localized in the cytoplasm, with the bulk of the proteins localized to the periplasmic side. Finally, both complexes have a total of five TMSs with the same orientation in the membrane. It is therefore reasonable to suggest that MotA/MotB and ExbB/ExbD are similar in biochemical function.

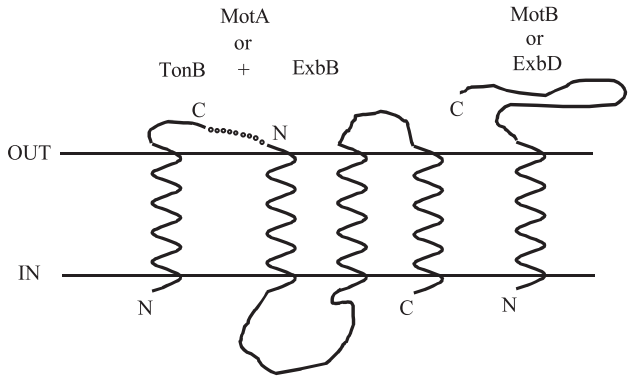

Fig. 1. Membrane topology of the MotA/MotB and ExbB/ExbD/TonB proteins. OUT and IN stand for the periplasm and cytoplasm, respectively. The dotted line shows the portion of MotA which is absent in TonB plus ExbB. N, N terminus; C, C terminus.

Partial multiple sequence alignments for the transmembrane α-helices of MotA/MotB and of ExbB/ExbD are presented in Fig. 2. Conserved residues are presented in bold print. Helix 3 of MotA and helix 2 of ExbB as well as Helix 4 of MotA and Helix 3 of ExbB show significant sequence similarity. The largely conserved P173, G176, G183, A205, T209 and G212 in MotA (amino acyl residues are indicated according to their sequence number in MotA from *E. coli*) are conserved in ExbB. Using these two helices in any one of these proteins, all of the others can be retrieved with a single PSI-BLAST iteration [80]. The sequence similarities between the transmembrane helices of the MotB and ExbD proteins are also significant. The fully conserved, functionally important Asp residue at the N-terminal region in all MotB and ExbD proteins is particularly worthy of note. F34 is conserved in most of the ExbD proteins, although it is replaced by other hydrophobic residues in some homologues. The similarity between helix 2 of MotA and helix 1 of ExbB is minimal, with only three conserved hydrophobic positions being found. We therefore conclude that this transmembrane helix is of little importance to the proton-conducting function of either complex; it may serve a structural role.

There is no evident sequence similarity between the only transmembrane α-helix in TonB and the first TMS of MotA. Conserved residues in this helix of MotA are all Gly residues. The well-conserved residues in the single TMS of TonB are S16 and H20, both on the same side of the presumed transmembrane α-helix. They are probably adjacent to each other in the intact protein complex and may comprise part of the proton pathway.

3.2. General features of the transmembrane bundle of MotA/MotB

Fig. 3 shows the general shape of the five transmembrane α-helices of MotA/MotB predicted using the modeling methods described in Methods. The channel structure is asymmetric. The MotB TMS is associated with the TMSs of MotA but is tilted relative to them with its cytoplasmic end embedded in the complex. This asymmetry may be important to the torque-generating function of the MotA/MotB channel,

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Helix 1          10                  29
TONB_ECOLI         PWPTLLSVCIHGAVVAGLLY
TONB_KLEPN         PWPTLLSVAIHGAVVAGLLY
TONB_HAEDU         RIGLISSVFIHIVLFASFIS
TONB_HAEIN         LLGLLISLIAHGIVIGFILW
TONB_ENTAE         PWPTLLSVAIHGAVVAGLLY
TONB_YEREN         TWPLAFSVGIHGSVIAALLY
TONB_SALTY         PWPTLLSVGIHGAVVAGLLY
TONB_SERMA         SVPFVLSVGLHSALVAGLLY
                         *:  *  :.. ::

Helix 2          23                  42
MotA_ECOLI         PAELVIIAGAGIGSFIVGNN
MotA_VPARA         PAEFLIIIGAAAGSLIIGNP
MotA_BSUBT         PAAILIIIAGTISAVVIAFP
MotA_BMEGA         VPSILIVLGGVFGTLCVSFP
ExbB_ECOLI         VKCVMIGLILASVVTWAIFF
ExbB_PSEPU         VKIVMIGLAIASIITWTIWI
ExbB_NEIME         LIGVFVLMLLMSIVTWCLVV
ExbB_XANCP         SWVVLITLIAMSAASWYWTV
                      ..:

Helix 3         141                 159
MotA_ECOLI         PAFGIVAAVMGVVHALGSA
MotA_VPARA         PGFGILAAVGGIIITMQAI
MotA_BSUBT         PTLGVLGAVIGLIAALSHM
MotA_BMEGA         PAWGMIGTLVGLVLMLKSL
ExbB_ECOLI         PFVGLFGTVWGIMNSFIGI
ExbB_PSEPU         PFVGLFGTVWGIMNSFIGI
ExbB_NEIME         PFIGLFGTVWGIYHALINI
ExbB_XANCP         PFVGLLGTVWGIYGALIKI
                   *  *:.:: *:    :

Helix 4         172                 194
MotA_ECOLI         ALIAHAMVGTFLGILLAYGFISP
MotA_VPARA         YHVAAALVGTFIGIFGCYCGLDP
MotA_BSUBT         HAISAAFVATLLGIFTGYVLWHP
MotA_BMEGA         PDMAIALLTTFYGALLSNLFFQP
ExbB_ECOLI         PGIAEALLATAIGLVAAIPAVVI
ExbB_PSEPU         PGIAEALLATALGLVAAIPAVVI
ExbB_NEIME         GPIGEALVATAAGLFVAIPAVLA
ExbB_XANCP         GPVGEALIMTAIGLFVAIPAVFA
                     :. *:: *  * .

Helix 5          24                  44
MotB_ECOLI         ADFMTAMMAFFLVMWLISISS
MotB_BSUBT         ADILTLLLALFIVLYASSSID
MotB_BMEGA         ADLVTLILVFFILLFSMSSVD
MotB_VPARA         ADFMIALMALFLVLWVMQVVD
ExbD_ECOLI         IDVMLVLLIIFMVAAPLATVD
ExbD_PSEPU         IDVMLVLLIIFMVAAPLATVD
ExbD_NEIME         VDVMLVLLIVFMITMPVLTHS
ExbD_XANCP         VDVMLVLLIIFIITAPLMSHK
                    *.:  :: .*::       .

ECOLI:             Escherichia coli
BSUBT:             Bacillus subtilis
BMEGA:             Bacillus megaterium
VPARA:             Vibrio parahaemolyticus
PSEPU:             Pseudomonas putida
NEIME:             Neisseria meningitidis
XANCP:             Xanthomonas campestris
KLEPN:             Klebsiella pneumoniae
HAEDU:             Haemophilus ducreyi
HAEIN:             Haemophilus influenzae
ENTAE:             Enterobacter aerogenes
YEREN:             Yersinia enterocolitica
SALTY:             Salmonella typhimurium
SERMA:             Serratia marcescens
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Fig. 2. Partial multiple sequence alignments for the transmembrane α-helices of MotA/MotB and TonB/ExbB/ExbD. The shaded sequences (from the ExbB/ExbD/TonB complex of *E. coli*) are derived from the proteins we chose for modeling. Well-conserved residues are displayed in bold print. The numbers above the top line of each alignment are the residue numbers in ExbB/ExbD/TonB of *E. coli* (the shaded sequences). The organisms from which the proteins were chosen are displayed in the right-bottom cell.

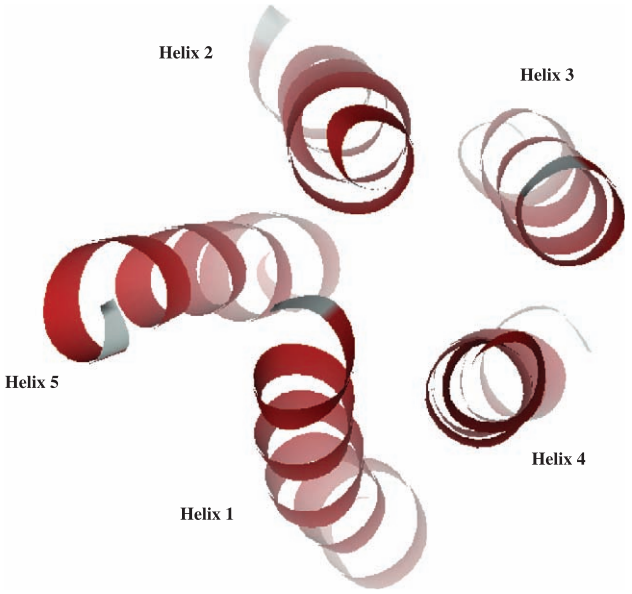

Fig. 3. The general shape of the five transmembrane α-helices of MotA/MotB. Helices 1–5 correspond to the four MotA TMSs and one MotB TMS, respectively.

through which protons flow to the cytoplasmic face of the membrane, inducing conformational changes in the protein complex as Asp-32 in MotB is sequentially protonated and deprotonated [36,83]. The inward tilt of this segment might form a constriction that controls ion conductance and provides a basis for ion (H^+ or Na^+) selectivity [10,15,25].

Helices 1 and 2 of MotA tilt slightly inward on the periplasmic side of the bundle, whereas helices 3 and 4 tilt slightly inward on the cytoplasmic side of the bundle. Helix 1 is located relatively far from the other four helices; this is in good agreement with experimentation [72] showing that the protein is still functional when several of the residues in this helix are mutated.

All of the side chains of the conserved residues marked in Fig. 1 face inward except for Phe41 of MotB. Phe41 is only partially conserved in the MotB and ExbD proteins and is sometimes replaced by other hydrophobic residues [84]. This residue may not be important for proton conduction, but it might play a structural role.

Most of the polar amino acyl side chains face the center of the helical bundle. These residues may act as H^+ donors or acceptors, and almost all of these residues are located at the upper or bottom portion of the channel (Fig. 4). Among them, only D32 and T209 are fully conserved. There are many small-sized amino acyl residues such as Gly and Ala in the middle region of the protein, and most of these face inward. They may provide space for water molecules that could facilitate the passage of protons through the membrane.

3.3. General features of the TM bundle of ExbB/ExbD/TonB

Fig. 5 reveals the general shape of the modeled channel of the ExbB/ExbD/TonB protein complex. As for the MotA/MotB protein complex, the only helix of ExbD tilts inward on the cytoplasmic side of the channel. Fig. 6 shows the conserved amino acyl residues in the ExbB/ExbD/TonB complex. In ExbB, these residues are P141, G144 and G151 in helix 3 and A177, T181 and G184 in helix 4. In ExbD, these residues are D25 and F34. In TonB, the conserved residues are H20 and S16 (see Fig. 2). All of these residues except F34 in ExbD face inward and may be important to the function of the channel. F34 and other hydrophobic residues may be important for structure. T181 and D25 are the only two polar residues that could serve as proton donor/acceptors. In ExbB/ExbD/TonB and MotA/MotB, T181 and T209, respectively, are localized to the upper middle of the channel. Experiments have shown that this conserved residue is important for H^+ conduction in MotA and for Na^+ conduction in PomA [31]. D25 in ExbD (D32 in the *E. coli* MotB) is located on the cytoplasmic side of the channel and is fully conserved. It is essential for the function of MotA/MotB [85]. For example, mutation of D32 to N32 in MotB abolished function altogether. This aspartyl residue might serve as a donor in proton transfer to a neighboring residue on the cytoplasmic side of the membrane in both of MotA/MotB and ExbB/ExbD/TonB. Alternatively, if the inward tilt of ExbD forms a constriction needed to make the channel specific for protons, the aspartyl residue would be a good candidate for the group that in part

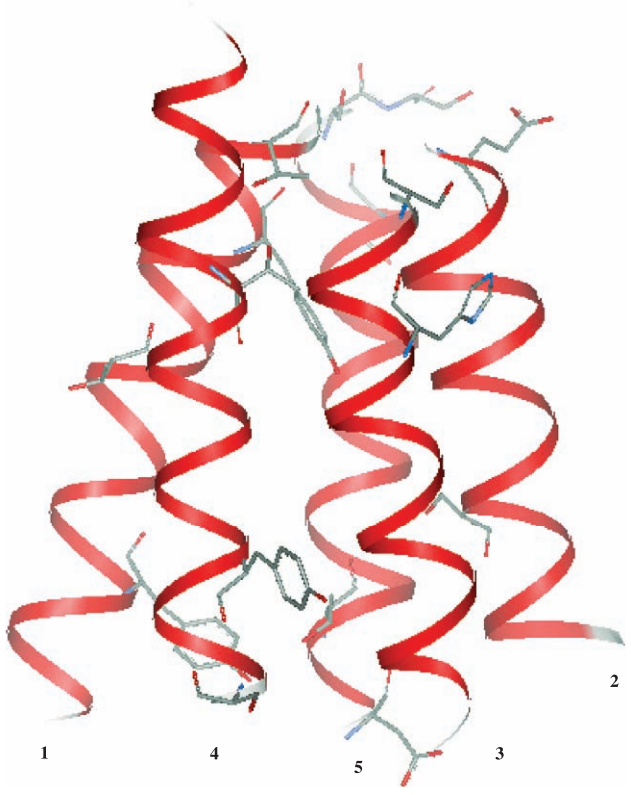

Fig. 4. Polar amino acyl residue side chains in the five TMSs of MotA/MotB. Most of these residues are located in the upper or bottom portion of the channel.

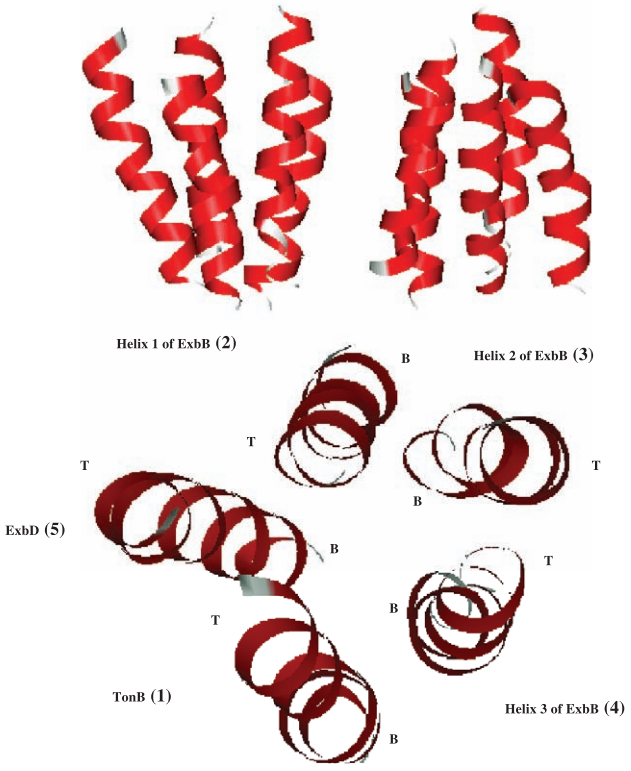

Fig. 5. The general shape of the modeled channel of the ExbB/ExbD/TonB proteins. The upper two figures are the channel seen from two different viewpoints. The lower figure is the channel viewed from above. The numbers in parentheses correspond to the helix numbers indicated for the MotA/B complex in Figs. 3 and 4. T, top; B, bottom.

confers proton specificity. Possibly relevant to the mechanism of action of the motor, protonation of D32 in MotB has been shown to generate a conformational change in the cytoplasmic loop between helix 2 and helix 3 of MotA [36].

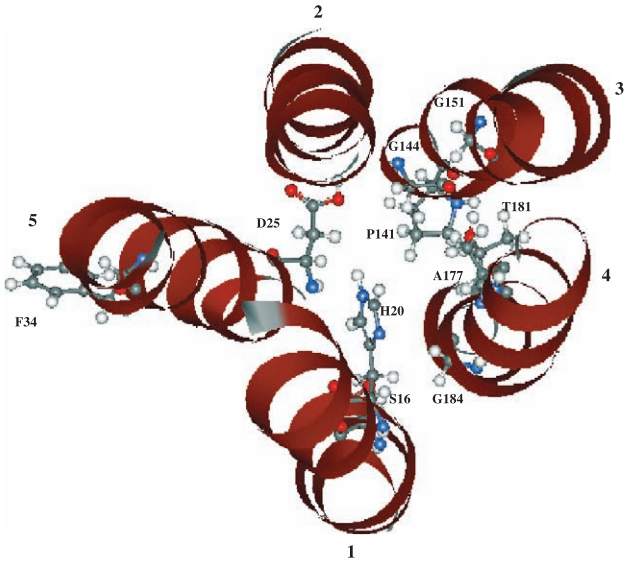

Fig. 6. Conserved residues in the TMSs of the ExbB/ExbD/TonB complex. These residues are P141, G144 and G151 on helix 3, A177, T181 and G184 on helix 4, D25 and F34 on helix 5, and H20 and S16 associated with helix 1.

3.4. Polar residues in ExbB/ExbD/TonB

Fig. 7 shows the residues that have polar hydrogens in the five TMSs of the ExbB/ExbD/TonB complex. Among these residues, 8 out of 15 have side chains facing inward. These residues are S16, H20, K24, T148, S155, T181, D25 and D44. The functionally important T181 and D25 are included. Some of these polar residues may be important for translocating protons from the periplasm of a Gram-negative bacterial cell to the cytoplasm.

3.5. Proposed pathway for H^+ transport in ExbB/ExbD/TonB

Integral outer membrane receptors for iron chelates and vitamin B_{12} mediate accumulation against large concentration gradients in the periplasm [38,45]. The proton motive force (pmf) across the inner membrane provides the energy for periplasmic accumulation, and energy transduction is mediated by the ExbB/ExbD/TonB complex (see Introduction). From the analysis of its distant homologue, the MotA/MotB complex, and the presence of conserved small amino acyl residues, we suspect that water molecules located inside the channel are necessary for proton transport.

Fig. 8 provides one proposed pathway for H^+ transport in ExbB/ExbD. The important residue side chains are all localized to three TMSs, namely helix 3 (the second TMS of ExbB), helix 4 (the third TMS of ExbB) and helix 5 (the only TMS of ExbD). These three TMSs exhibit high similarity to their counterparts in the MotA/MotB system.

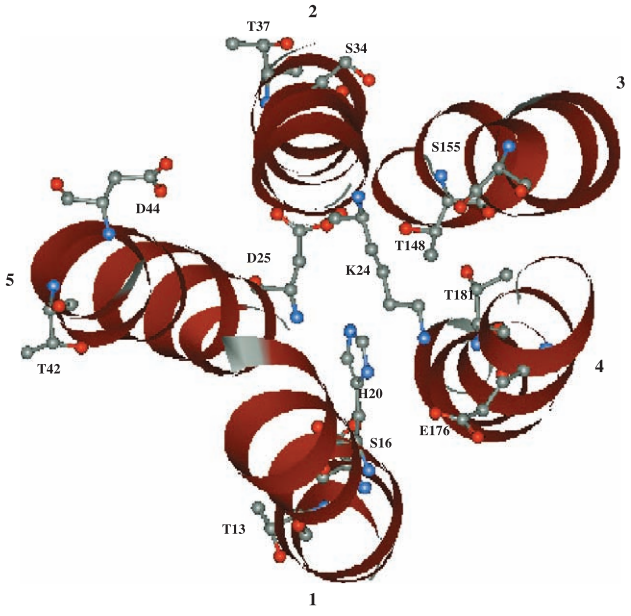

Fig. 7. Polar residues in the TMSs of the ExbB/ExbD/TonB complex. These residues are T13, S16 and H20 in helix 1; K24, S34 and T37 in helix 2; T148 and S155 in helix 3; E176 and T181 in helix 4; and D25, T42 and D44 in helix 5.

The proposed proton transport pathway is T181 → S155 → H_20 (near G151) → T148 → H_20 (near G144) → D25. T181 is fully conserved in both ExbB/ExbD/TonB and MotA/MotB. Consequently, the hydroxyl group of its side chain renders it appropriate for accepting or donating a proton. The next residue to comprise the pathway is S155 in helix 3. There are no large hydrophobic residues at this position in the various MotA and ExbB proteins from other bacterial species. For example, in MotA from *Vibrio parahaemolyticus*, the corresponding residue is Thr. In the *Bacillus megaterium* MotA, the correspondent residue is Met, which might form a weak hydrogen bond with an appropriate H-donating atom. In other species, Ala is present. Ala may permit inclusion of a nearby water molecule for participation in proton transfer. The remaining proposed residues in the pathway are: (1) The fully conserved G151; water near this residue could act as hydrogen donor/acceptor. (2) T148; this residue could accept the H^+ from water and pass it to the next residue. It is well conserved in all ExbB proteins, and its counterpart in MotA is either Thr or Ala. The latter residue could allow accommodation of a water molecule. (3) Near the fully conserved G144, a water molecule could then accept the H^+. Finally, (4) the proton could be transferred to D25 in ExbD before being passed into the cytoplasm.

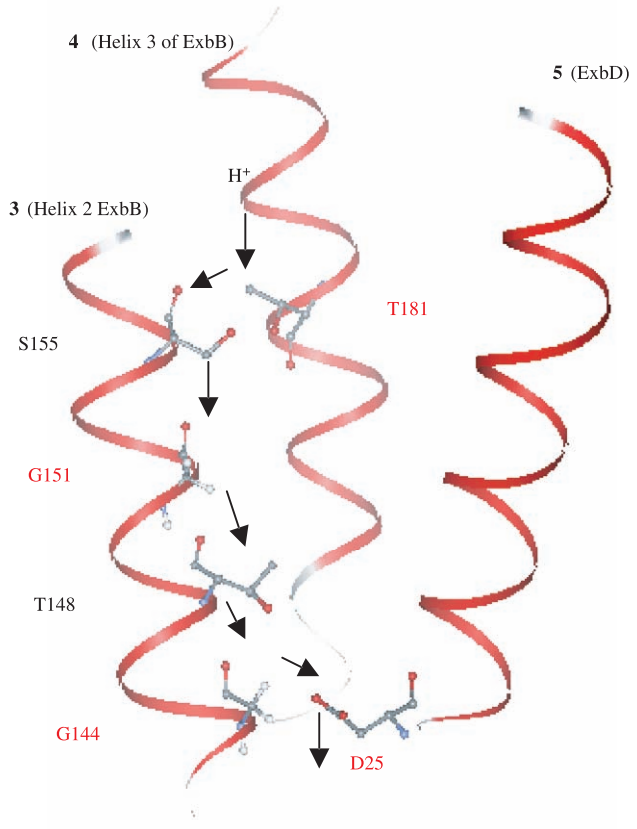

Fig. 8. One proposed pathway for H^+ transport in the ExbB/ExbD complex. Residues indicated in red are conserved.

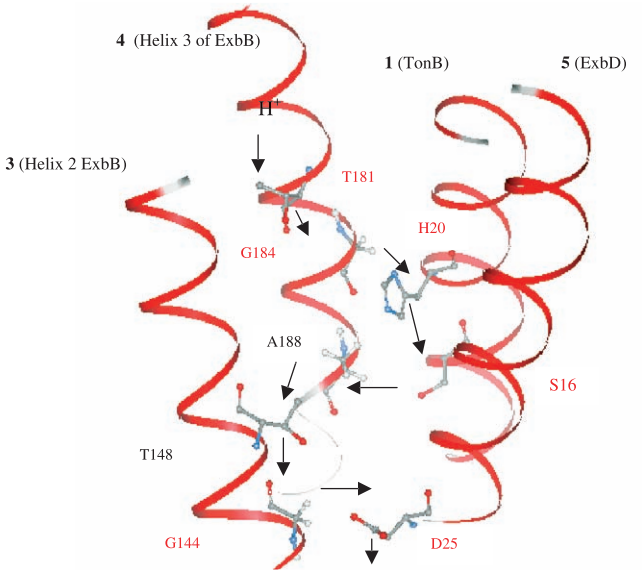

Fig. 9. An alternative H^+ transport pathway involving TonB as well as ExbB/ExbD. Residues shown in red are conserved.

3.6. An alternative pathway involving TonB

The proton transfer pathway described above utilizes residues in three α-helices, all of which are well conserved in both ExbB/ExbD and MotA/MotB. The following model includes residues in the transmembrane helix of TonB in the proposed H^+ transfer pathway. This pathway is: T181 (helix 3 of ExbB) → H_2O (near G184 in helix 3 of ExbB) → H20 in TonB → S16 in TonB → H_2O (near A188 in helix 3 of ExbB) → T148 in helix 2 of ExbB → H_2O (near G144 in helix 2 of ExbB) → D25 in ExbD → cytoplasm (Fig. 9). All of the residues that are suggested to comprise the pathway are conserved in the TonB/ExbB/ExbD complex. Although A188 is not conserved in MotA, its counterpart is the small-sized Ala, Gly, Thr or Cys that can either act as a component of a hydrogen-bonding network or accommodate water. This pathway is unique in that it incorporates the TonB protein. TonB may thus become energized as a constituent of the channel due to a conformational change that is transmitted to the outer membrane receptor where opening of the outer membrane channel allows for molecular import.

4. Conclusions

The stator of the flagellar motor is a complex composed in part of MotA/MotB, and this complex, which translocates protons in response to the pmf, is used to rotate the flagellum. A second complex of membrane proteins, ExbB/ExbD/TonB, must similarly act as a proton transfer channel to energize transport of iron siderophores, vitamin B_{12} and other molecules across the outer membrane (see Introduction). We noted that the putative channels within these two systems exhibit certain similarities. Of particular note were residues in the

second and third TMSs of ExbB and the single TMS in ExbD. They resemble the third and fourth TMSs of MotA and the only TMS of MotB. A 3-D structural model for the five transmembrane helices of ExbB/ExbD/TonB was obtained based on experimental data for the MotA and MotB protein complex. The theoretical model shows well-defined tilt angles for the five transmembrane α-helices. Residues that probably play functional roles were described, and two cation-translocating pathways were proposed. These analyses should serve as guides for the design of further experiments aimed at defining the mechanism of action of the energizing complex for outer membrane transport.

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