Ion Conductivity of the Bacterial Translocation Channel
SecYEG Engaged in Translocation*

Received for publication, June 10, 2014, and in revised form, July 1, 2014. Published, JBC Papers in Press, July 11, 2014, DOI 10.1074/jbc.M114.588491

Denis G. Knyazev, Lukas Winter, Benedikt W. Bauer1, Christine Siligan, and Peter Pohl2
From the Institute of Biophysics, Johannes Kepler University Linz, Gruberstrasse 40, A-4020 Linz, Austria

Background: How SecYEG excludes ions during protein translocation is not known.

Results: SecYEG gating is voltage-sensitive.

Conclusion: Voltage minimizes the ion leak that is induced by stalling a translocation intermediate.

Significance: Preservation of the proton motif force requires voltage-driven conformational changes.

While engaged in protein transport, the bacterial translocon SecYEG must maintain the membrane barrier to small ions. The preservation of the proton motif force was attributed to (i) cat i on exclusion, (ii) engulfment of the nascent chain by the hydrophobic pore ring, and (iii) a half-helix partly plugging the channel. In contrast, we show here that preservation of the proton motif force is due to a voltage-driven conformational change. Preprotein or signal peptide binding to the purified and reconstituted SecYEG results in large cation and anion conductivities only when the membrane potential is small. Physiological values of membrane potential close the activated channel. This voltage-dependent closure is not dependent on the presence of the plug domain and is not affected by mutation of 3 of the 6 con striction residues to glycines. Cellular ion homeostasis is not challenged by the small remaining leak conductance.

After being synthesized in the cytosol, many proteins need to be either (i) inserted into the plasma membrane or the mem brane of the endoplasmic reticulum or (ii) transported across them (1). A major pathway in bacteria is provided by the heterotrimeric protein translocation channel SecYEG (translocon or SecY complex) (2). Its structurally conserved family member, the eukaryotic Sec61 complex (3), is located in the membrane of the endoplasmic reticulum.

The complexes must prevent proton or calcium leakages because collapses of (i) the proton motif force across the inner bacterial membrane or (ii) the Ca2+ concentration gradient across the membrane of the endoplasmic reticulum are incompatible with life. The pore ring, a hydrophobic constriction zone in the middle of the channel, and the plug, a mobile reentrant loop in the periplasmic half of the funnel, seal the resting channel (4).

It is not known how the prokaryotic and eukaryotic translocons prevent H+ or Ca2+ leakages during protein transport. The plug is moved out of the pathway for secretory proteins so that ions may also permeate (5). The pore ring is envisioned to form a gasket-like seal around the translocating protein (6). However, the very flexible belt of six hydrophobic amino acids may adopt any diameter between 4 and 24 Å (7). It is thus a rather unorthodox ion selectivity filter. For example, cation and proton exclusion by water channels (8, 9) requires a strong electrostatic field in an extremely narrow restriction zone that is created by a charged amino acid and by dipoles of reentrant loops (10).

Here we present mechanistic insight into how SecYEG manages to maintain the barrier to ions and protons during protein translocation. We show that binding of a translocation intermediate opens a large SecYEG pore only at small transmembrane potentials. Physiological membrane potentials close the channel. With a stalled polypeptide chain, the unitary leak conductance of the closed channel is about 2 orders of magnitude smaller than the conductance of the open SecYEG.

MATERIALS AND METHODS

Signal Peptide—The signal peptide from the precursor form of outer membrane protein A (leader peptide (LP))3 contained a cysteine instead of an alanine in the last position (sequence: MKKTAIAIAVLAGFATVQ). This peptide was synthesized and purified to 98% purity by Peptide 2.0 Inc. (Chantilly, VA).

Translocation Intermediate—The preprotein construct (pOD) consisted of the precursor form of outer membrane protein A, proOmpA, which was truncated at position 69 (11, 12) and connected at its C terminus to the cytosolic enzyme dihydrofolate reductase. pOD was expressed in MM52 temperature-sensitive cells. 100 ml of 2× YT culture of transformed MM52 cells, supplemented with 100 μg/ml ampicillin, were grown at 30 °C to an optical density of 1 (measured at a wavelength of 600 nm). The culture was then added to 900 ml of prewarmed 2× YT medium, supplemented with 100 μg/ml ampicillin, and grown at 37 °C for another 30 min. Overexpression was induced by the addition of 2 g/liter arabinose, and cells were harvested after 2 h of growth at 37 °C. Cells were lysed in buffer containing 50 mM Tris, pH 7.5, 300 mM KCl, 10% glycerol, 1 mM tris(2-carboxyethyl)phosphine, and complete protease inhibitor mixture (EDTA-free, Roche Applied Science) using an Emulsi Flex C-5 (Avestin) at 20,000 p.s.i. The soluble fraction of the

* This work was supported by Grant P20872 from the Austrian Science Fund (FWF) (to P. P.).

† Author’s Choice—Final version full access.

1 Present address: Dept. of Cell Biology, Harvard Medical School, 240 Longwood Ave., Boston, MA 02115.

2 To whom correspondence should be addressed. Tel.: 43-732-2468-7562; E-mail: peter.pohl@jku.at.

3 The abbreviations used are: LP, leader peptide; proOmpA, outer membrane protein A; Mtx, methotrexate; pS, picosiemens; proOMPA-DHFR construct.
lysate was isolated by ultracentrifugation for 60 min at 40,000 rpm, 4 °C (Beckmann Ti90 rotor). The supernatant was incubated with nickel-nitrilotriacetic acid resin (Qiagen) for 60 min at 4 °C. Bound protein was washed in the presence of 20 mM imidazole, eluted with buffer containing 50 mM Tris, pH 7.5, 100 mM KCl, 10% glycerol, and 300 mM imidazole, and stored at −80 °C.

SecA Purification—SecA was overexpressed in Escherichia coli C43(DE3) from the pET30 SecA expression vector and induced by 1 mM isopropyl-1-thio-D-galactopyranoside at 37 °C (13). After 3 h, the cells were pelleted and lysed by a homogenizer in 0.5 M NaCl, 20 mM Tris (pH 7.5) using two cycles of 20,000 p.s.i. After centrifugation for 90 min at 40,000 rpm and 4 °C, the supernatant was incubated with equilibrated Ni²⁺-chelating beads for 1 h at 4 °C. The beads were loaded on a column and washed in the presence of 20 mM imidazole. SecA was eluted with 200 mM imidazole and then subjected to size exclusion chromatography using 100 mM NaCl, 20 mM Tris (pH 7.5), and 2 mM β-mercaptoethanol.

SecYEG Purification—Expression vectors for SecY complexes were based on the pBAD-SecYEG cysteine-less mutant containing an A204C substitution for labeling. In the plug-less SecY mutant (SecΔP), amino acids 60–74 have been replaced by amino acids GSGS, and the plug-less SecY triple ring mutant (SecΔP3G) additionally contained substitutions I86G, I191G, and I278G. All SecY complexes were purified from E. coli C43(DE3) cells after 4 h of induction with 0.2% arabinose at 37 °C as described (4). This included protein extraction by a homogenizer in 0.5 M NaCl, 20 mM Tris (pH 7.5), and 2 mM deoxy-Big-CHAP (Affymetrix Anatrace, Cleveland, OH) monolayers on top (14). Fusion of proteoliposomes was facilitated by a 450 mM:150 mM KCl gradient across the planar bilayer (15, 16). If a SecYEG channel was open in the vesicular membrane, the osmolyte entered the respective vesicle. Water from the hypertonic (trans) compartment followed when this vesicle adhered to the planar bilayer. Subsequent vesicle swelling resulted in fusion with the planar bilayer, thus allowing detection of current flow through open SecYEG channels.

To allow pOD to open the translocon, the cis compartment contained 650 nM E. coli SecA, 1 mM MgCl₂, and 0.8 mM ATP. The cis compartment also harbored the proteoliposomes. Both compartments were buffered by 50 mM K-HEPES at pH 7.5. To prevent aggregation of the signal peptide, 90 mM urea was also added to the cis compartment in the respective experiments.

Single Ion Channel Measurements—Single channel measurements were performed as described previously (17, 18). Ag/AgCl reference electrodes in the cis and trans compartiments were connected to command signal of the patch clamp amplifier to the command signal of the patch clamp amplifier (model EPC9, HEKA Electronics) and the ground, respectively. The recording filter for the transmembrane current was a 4-pole Bessel with −3 dB corner frequency of 0.1 kHz. The raw data were analyzed using the TAC software package (Bruxton Corp., Seattle, WA). Gaussian filters of 12 or 112 Hz were applied to reduce noise.

RESULTS

We formed planar bilayer lipid membranes and added SecYEG-containing proteoliposomes to the cis compartments. Subsequently, we raised the osmolarity in that compartment. If the SecYEG were open, the vesicles would fuse with the planar bilayer and, in turn, the thus inserted SecYEG would give rise to transmembrane current fluctuations. Because the resting SecYEG is closed (4), we did not detect channels, i.e. fusion did not occur and the membrane retained its low conductance state (Fig. 1A). Likewise, the addition of the dissolved 21-amino acid-long LP of proOmpA to the pure lipid bilayer did not induce channel activity, neither under isosmotic nor under hyperosmotic conditions.

However, the simultaneous presence of proteoliposomes and LP in the hyperosmotic compartment resulted in channel activity (Fig. 1B). That is, LP opened SecYEG in the vesicles, thereby enabling fusion of the vesicles to the planar bilayer. This resulted in current flow through open SecYEG channels. Because the vesicles contained several SecYEG copies, every fusion event was indicated by a large current step (Fig. 1B).

Subsequent closure of the SecYEG channels resulted in a stepwise decrease of the transmembrane current (Fig. 1, C and D). The LP from proOmpA opened the translocon for longer periods of time than was reported for the LamB LP bound to microsomal membranes (19).

We performed at least 20 more experiments and repeatedly observed the same picture; membrane current had its maximal value immediately after vesicle fusion. This conductance level was relatively stable at moderate membrane potentials (Fig. 1C) and declined at higher membrane potentials (Fig. 1D). We concluded that channel closure was driven by membrane potential.

When substituting the short LP for the longer pOD, the open probability of SecYEG significantly increased in the presence of SecA, as indicated by a 10-fold larger membrane current. To observe single channels (Fig. 1, F and G), we decreased the SecYEG concentration accordingly. Most interestingly, SecYEG now appeared to be more sensitive to external voltage. That is, we observed channel closure at smaller membrane potentials (Fig. 1G).
Complete translocation of pOD may explain the change in voltage sensitivity as it would be accompanied by dissociation of the leader sequence from its SecY binding site. To test this assumption, we repeated the experiment in the presence of methotrexate (Mtx). Its binding to dihydrofolate reductase prevents unfolding of the enzyme. As a result, pOD is unable to permeate the channel, and a true translocation intermediate is formed (11). Binding of the pOD-Mtx complex to SecYEG opened the channel. Mtx significantly accelerated closure, whereby the rate was voltage-dependent (Fig. 2C). A small leak current \( I_L \) remained (~65 pA in Fig. 2A) that could be attributed to the closed channels with a stalled translocation intermediate (as shown below). Moreover, the presence of Mtx rendered channel closure reversible (Fig. 2B). That is, when the bilayer lipid membrane was clamped to 0 mV, most of the channels reopened one after the other. As indicated by \( I_L \), the reconstituted SecYEG channels were still occupied by translocation intermediates. Consequently, these still bound intermediates must have triggered the reopenings.

On the contrary, without Mtx, pOD translocation into the trans compartment has most probably taken place because most of the channels lost the polypeptide during the transition from the open to the closed state, and therefore only a small fraction of them reopened under these conditions. The only alternative explanation that pOD has dissociated back into the cis compartment is not very plausible because the probability of this event should not depend on the presence of Mtx. That is, Mtx does not hamper the dissociation of pOD from the translocaton, but only its translocation.

Likewise, the response to voltage did not depend on Mtx. SecYEG closed at the same small potentials as if no Mtx was bound (Fig. 2A). We conclude that voltage sensitivity is not due to a voltage-driven release of the peptide from SecYEG.

In an attempt to identify the sensor, we (i) removed the plug (\( \Delta P \)) and (ii) substituted three isoleucines of the hydrophobic ring by hydrophilic glycines (\( \Delta P3G \)). If the plug was the voltage sensor, we expected \( \Delta P \) to remain open at large membrane potentials. If any other charged residue (i) in close vicinity to the pore ring or (ii) on the spontaneously forming new plug (20) was the sensor in \( \Delta P3G \), it must have been exposed to a decreased voltage drop per unit channel length. That is, instead of dropping in the very narrow region of the hydrophobic ring as in \( \Delta P \), the electric field drops over a longer distance in \( \Delta P3G \) because its constriction site is dilated and contains ions as indicated by spontaneous channel activity of pore ring mutants (4). However, the voltage sensor did not respond to the decreased strength of the electric field in the constriction zone. \( \Delta P3G \) and \( \Delta P \) closed with identical voltage sensitivities. Similar to the wild type channel (Fig. 2), both \( \Delta P \) and \( \Delta P3G \) reopened in the presence of Mtx when no external potential was applied (Fig. 3). We concluded that neither plug nor pore ring was primarily important for maintaining the membrane barrier to ions during...
translocation. Instead, the voltage-driven conformational change of SecYEG was crucial for blocking ion permeation. In the absence of an externally applied potential, the current through the channels was driven by the reversal potential, $\psi_{\text{rev}}$. Generation of $\psi_{\text{rev}}$ in a salt gradient (450:150 mM KCl) indicated that SecYEG must possess ion selectivity. It can be quantified in terms of the anion to cation permeability ratio, $P_{\text{Cl}^-}/P_{\text{K}^+}$. The voltage dependence of the single channel current of $\Delta P$ indicated a $\psi_{\text{rev}}$ of $-14.6$ mV (Fig. 4A, black line). According to the textbook equation for the resting membrane potential,

$$\psi_{\text{rev}} = \frac{RT}{F} \ln \left( \frac{P_{\text{Cl}^-}K_{\text{trans}}}{P_{\text{K}^+}K_{\text{cis}}} \right)$$  \hspace{1cm} (Eq. 1)

FIGURE 2. SecYEG closure is driven by application of a transmembrane voltage $\psi_a$. $\psi_a$ is shown on top of the experimental records. The upper trace shows consecutive channel closings for $\psi_a = 50$ mV. Histogram analysis and the zoom-in (right panel) show a single channel current of $27.5 \pm 2.4$ pA. The 450–150 mM KCl transmembrane concentration gradient and the anion selectivity of SecYEG gave rise to a reversal potential, $\psi_{\text{rev}}$. $\psi_{\text{rev}} - 7$ mV results in a total membrane potential $\psi = \psi_a - \psi_{\text{rev}} = 57$ mV and in $g$ of $-480 \pm 45$ pS, respectively. Doubling of $g$ (compare Fig. 1, C and D) corresponds well to the 2-fold increase in average salt concentration. B, zoom-in into the record of the upper panel. The current throughout the reopening SecYEG channels at $\psi_a = 0$ mV was driven by $\psi_{\text{rev}}$. The hypertonic compartment contained 100 nM pOD, 650 nM SecA, 1 mM MgCl$_2$, 0.8 mM ATP, and 9 $\mu$M Mtx. Both compartments were buffered with 50 mM K-HEPES (pH 7.5). C, the frequency of channel closure increased with voltage and the addition of Mtx. It remained unaltered by plug and ring mutations so that the combined data for SecYEG, $\Delta P$, and $\Delta P3G$ are shown (compare Fig. 3). Error bars indicate mean ± S.D.

FIGURE 3. Voltage-driven closure of $\Delta P3G$. The experimental conditions were as in Fig. 2. Assuming $\psi_{\text{rev}} = -3.5$ mV (compare Fig. 4) results in a total membrane potential $\psi = \psi_a + \psi_{\text{rev}}$ and $g$ of $53.4$ mV and $-476$ pS, respectively. The amplitude of channel reopenings at $\psi_a = 0$ mV was equal to $1.5 \pm 0.6$ pA.

Ion Conductivity of the SecYEG-Nascent Chain Complex
it corresponds to $P_{\text{Cl}^-}/P_{\text{K}^+} \approx 3.7$, where the subscripts cis and trans indicate the two sides of the membrane.

When measuring the voltage dependence of the current across the whole membrane, we found $\psi_{\text{rev}} \approx -12.6$, $-10.0$, or $-11.8$ mV (Fig. 4, B, C, and D, respectively, black curves) for membranes that contained the wild type channel, ΔP3G or ΔP, respectively. This indicated that the reconstituted bilayers possessed a smaller selectivity of potassium over chloride than the translocon proper. This observation suggested the presence of a leak current $I_L$ in addition to the current through the open SecYEG. Closed SecYEG channels with stalled pOD represented the most likely pathway for $I_L$. To test this hypothesis, we augmented the relative amount of closed channels with stalled translocation intermediates by allowing Mtx binding to pOD. The experiment confirmed our prediction; Mtx decreased $\psi_{\text{rev}}$ for membranes that were reconstituted with the wild type channel, with ΔP3G, or with ΔP (Fig. 4, B, C, and D, respectively, gray curves). In contrast, control single channel experiments revealed the same $\psi_{\text{rev}}$ in both the presence and the absence of Mtx (Fig. 4A). Thus, $I_L$ measurements corroborate the conclusion about pOD translocation in the absence of Mtx that was based on irreversible channel closure at elevated voltages.

We next proceeded to measure $I_L$. We obtained a current of 4.8 pA after closure of six wild type channels with stalled pOD-Mtx, i.e. $I_L$ amounted to 0.8 pA per SecYEG complex (Fig. 5A). For ΔP, $I_L$ per closed channel was equal to 3.5 pA, i.e. it was roughly four times larger than for the wild type channel (Fig. 5B). At about 4.3 pA, $I_L$ per channel was maximal for ΔP3G.

**DISCUSSION**

We have shown that the voltage-driven closure of the SecYEG pore preserves the membrane barrier to ions, and thus, the proton motive force. That is, at physiologically relevant values of ionic strength and the typical bacterial plasma membrane potential $\psi_{\text{Cl}^-} = -130$ mV (21), the total leakage current per translocon that contains a stalled translocation intermediate is negligibly small (~0.8 pA). The corresponding conductivity of 6 pS is 2 orders of magnitude smaller than the current through the ΔP mutant (4) or the open SecYEG (16).

The voltage dependence explains the viability of ΔP mutants (22) and prlA4 mutants. Both are known to provide a pathway to ions at small membrane potentials (4), but appear to be nearly closed at physiologically relevant values of the membrane potential (Fig. 3). Voltage-driven conformational changes also solve the apparent contradiction between the observation that ribosome binding opens SecYEG channels (16) at small membrane potentials and the lack of a significant ion or proton leak across the bacterial plasma membrane under physiological conditions.

Our conclusion that voltage-driven channel closure is responsible for the preservation of proton and ion gradients across the bacterial plasma membrane is in line with the reported lack of Cl− or xylitol leakage through wild type SecYEG that is occupied with a nascent chain, as measured with spheroplasts (6). The observation that ΔP mutants showed Cl− leakage only when treated with valinomycin (6) suggested anion selectivity (23, 24). Because we determined $P_{\text{Cl}^-}/P_{\text{K}^+} \ll 100$, we can now rule out that anion selectivity is crucial for maintaining the proton motive force. For example, a Cl− concentration increase of 3 mM would translate into a cytoplasmic pH drop of 1 unit assuming (i) $P_{\text{Cl}^-}/P_{\text{H}^+} = 3.4$ and (ii) characteristic values of buffer capacity and protein content per cell, −50 μmol/mg of protein (25), and ~20 mg (26), respectively.

The remaining question is how SecYEG closes in response to membrane potential, i.e. what exactly is the voltage-sensitive element? We probably can rule out the plug. It is true that plug removal is required for the observation of large ion conductivity (4). However, plug removal does not hamper the voltage-driven channel closure (Fig. 3). There is also no possibility that the
FIGURE 6. Putative scheme of voltage-driven channel closure. The resting channel is closed (left panel). LP binding opens the translocon (middle panel). It becomes permeable to the translocating peptide chain as well as to ions and water (represented by blue arrow) in the absence of $\psi_w$. Physiological values of $\psi_w$ close the channel (right panel). The stalled translocation intermediate is likely to be pushed to the lipid side of the lateral gate. The upper panel shows the channel from the cytoplasm. TM, transmembrane helix.

replacement plug formed upon plug deletion (20) takes over the role. It carries a positively charged residue (Arg-57) instead of two oppositely charged amino acids (Glu-62, Arg-74) in the original plug. If the replacement plug was the voltage sensor, the closure rates for the wild type and plug-less mutants should not have been similar (Fig. 2C).

Thus, unless the movements of the original and the replacement plugs are not accompanied by dramatic $pK_a$ shifts of the amino acid side chains, some other structural element must act as the voltage sensor. This sensor must respond to both positive and negative voltages with the same response: channel closure. The rotation of a helix involved in pore ring formation would be another possibility (compare Fig. 6). LP or the N terminus of pOD would reside on the lipid side of the lateral gate, reminiscent of what has been observed in crystal structures of the SecYEG-ribosome or Sec61-ribosomae complexes with a stalled translocation intermediate (27, 28). Further experiments are required to test these hypotheses.

We conclude that preservation of the bacterial proton motif force is due to voltage-driven closure of the translocation channel. Neither plug deletion nor pore ring mutations are able to significantly disturb the gating. Experiments devoted to the identification of the voltage-sensitive element are under way.

Acknowledgment—We thank Tom Rapoport for helpful comments.

REFERENCES

1. Blobel, G. (1980) Intracellular protein topogenesis. Proc. Natl. Acad. Sci. U.S.A. 77, 1496–1500
2. Van den Berg, B., Clemons, W. M., Jr., Collinson, I., Modis, Y., Hartmann, E., Harrison, S. C., and Rapoport, T. A. (2004) X-ray structure of a protein-conducting channel. Nature 427, 36–44
3. Becker, T., Bhushan, S., Jarasch, A., Armache, J. P., Funes, S., Jossinet, F., Gumbart, J., Mielke, T., Berninghausen, O., Schulten, K., Westhof, E., Gilmore, R., Mandon, E. C., and Beckmann, R. (2009) Structure of monomeric yeast and mammalian Sec61 complexes interacting with the translocating ribosome. Science 326, 1369–1373
4. Saparov, S. M., Erlandson, K., Cannon, K., Schaletzky, J., Schulman, S., Rapoport, T. A., and Pohl, P. (2007) Determining the conductance of the SecY protein translocation channel for small molecules. Mol. Cell 26, 501–509
5. Lycklama a Nijeholt, J. A., Wu, Z. C., and Driessen, A. J. M. (2011) Conformational dynamics of the plug domain of the SecYEG protein-conducting channel. J. Biol. Chem. 286, 43881–43890
6. Park, E., and Rapoport, T. A. (2011) Preserving the membrane barrier for small molecules during bacterial protein translocation. Nature 473, 239–242
7. Bonardi, F., Halza, E., Walko, M., Du Plessis, F., Nouwen, N., Feringa, B. L., and Driessen, A. J. M. (2011) Probing the SecYEG translocation pore size with preproteins conjugated with sizable rigid spherical molecules. Proc. Natl. Acad. Sci. U.S.A. 108, 7775–7780
8. Pohl, P., Saparov, S. M., Borgia, M. I., and Agre, P. (2001) High selectivity of water channel activity measured by voltage clamp: analysis of planar lipid bilayers reconstituted with purified AqpZ. Proc. Natl. Acad. Sci. U.S.A. 98, 9624–9629
9. Saparov, S. M., Tsunoda, S. P., and Pohl, P. (2005) Proton exclusion by an aquaglyceroporin: a voltage clamp study. Biol. Cell 97, 545–550
10. Wu, B., Steimbbron, C., Alsterfjord, M., Zethun, T., and Beitz, E. (2009) Concerted action of two cation filters in the aquaporin water channel. EMBO J. 28, 2188–2194
11. Arkowitz, R. A., Joly, J. C., and Wickner, W. (1993) Translocation can drive the unfolding of a preprotein domain. EMBO J. 12, 243–253
12. Bauer, B. W., and Rapoport, T. A. (2009) Mapping polypeptide interactions of the SecA ATPase during translocation. Proc. Natl. Acad. Sci. U.S.A. 106, 20800–20805
13. Or, E., Navon, A., and Rapoport, T. (2002) Dissociation of the dimeric SecA ATPase during protein translocation across the bacterial membrane. EMBO J. 21, 4470–4479
14. Montal, M., and Mueller, P. (1972) Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. Proc. Natl. Acad. Sci. U.S.A. 69, 3561–3566
15. Woodbury, D. J., and Hall, J. E. (1988) Role of channels in the fusion of vesicles with a planar bilayer. Biophys. J. 54, 1053–1063
16. Knyazev, D. G., Lents, A., Krause, E., Ollinger, N., Siligan, C., Papinski, D., Winter, L., Horner, A., and Pohl, P. (2013) The bacterial translocon SecYEG opens upon ribosome binding. J. Biol. Chem. 288, 17941–17946
17. Saparov, S. M., Antonenko, Y. N., Koepe, R. E., 2nd, and Pohl, P. (2000) Desformylgramicidin: a model channel with an extremely high water permeability. Biophys. J. 79, 2526–2534
18. Hoomann, T., Jahnke, N., Horner, A., Keller, S., and Pohl, P. (2013) Filter gate closure inhibits ion but not water transport through potassium channels. Proc. Natl. Acad. Sci. U.S.A. 110, 10842–10847
19. Simon, S. M., and Blobel, G. (1992) Signal peptides open protein-conducting channels in E. coli. Cell 69, 677–684
20. Li, W., Schulman, S., Boyd, D., Erlandson, K., Beckwith, J., and Rapoport, T. A. (2007) The plug domain of the SecY protein stabilizes the closed state of the translocation channel and maintains a membrane seal. Mol. Cell 26, 511–521
21. Felle, H., Porter, J. S., Slayman, C. L., and Kaback, H. R. (1980) Quantitative measurements of membrane potential in Escherichia coli. Biochemistry 19, 3585–3590
22. Junne, T., Schwede, T., Goder, V., and Spiess, M. (2006) The plug domain of yeast Sec61p is important for efficient protein translocation, but is not essential for cell viability. Mol. Biol. Cell 17, 4063–4068
23. Schiebel, E., and Wickner, W. (1992) Preprotein translocation creates a halide anion permeability in the Escherichia coli plasma membrane. J. Biol. Chem. 267, 7505–7510
24. Dalal, K., and Duong, F. (2009) The SecY complex forms a channel capable ofionic discrimination. Enno Rep. 10, 762–768
25. Kannan, P., Ignacimuthu, S., and Paulraj, M. G. (2009) Buffering capacity and membrane $H^+$ conductance of protease producing facultative alkaliphilic bacterium Bacillus flexus from mangrove soil. Indian J. Biochem. Biophys. 46, 261–265
26. Simon, M., and Azam, F. (1989) Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51, 201–213
27. Park, E., Ménétret, J. F., Gumbart, J. C., Ludtke, S. J., Li, W., Whynot, A., Rapoport, T. A., and Akey, C. W. (2014) Structure of the SecY channel during initiation of protein translocation. Nature 506, 102–106
28. Gogala, M., Becker, T., Beatrix, B., Armache, J. P., Barrio-Garcia, C., Berninghausen, O., and Beckmann, R. (2014) Structures of the Sec61 complex engaged in nascent peptide translocation or membrane insertion. Nature 506, 107–110