Pseudomonas aeruginosa Porin OprF

PROPERTIES OF THE CHANNEL

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Using ion channel reconstitution in planar lipid bilayers, we examined the channel-forming activity of subfractions of Pseudomonas aeruginosa OprF, which was shown to exist in two different conformations: a minority single domain conformer and a majority two-domain conformer (Sugawara, E., Nestorovich, E. M., Bezrukov, S. M., and Nikaido, H. (2006) J. Biol. Chem. 281, 16220–16229). With the fraction depleted for the single domain conformer, we were unable to detect formation of any channels with well defined conductance levels. With the unfraccionated OprF, we saw only rare channel formation. However, with the single domain-enriched fraction of OprF, we observed regular insertion of channels with highly reproducible conductances. Single OprF channels demonstrate rich kinetic behavior exhibiting spontaneous transitions between several subconformations that differ in ionic conductance and radius measured in polymer exclusion experiments. Although we showed that the effective radius of the most conductive conformation exceeds that of the general outer membrane porin of Escherichia coli, OmpF, we also found that a single OprF channel mainly exists in weakly conductive subconformations and switches to the fully open state for a short time only. Therefore, the low permeability of OprF reported earlier may be due to two factors: mainly to the paucity of the single domain conformer in the OprF population and secondly to the predominance of weakly conductive subconformations within the single domain conformer.

The channel properties of OprF, the major nonspecific porin of Pseudomonas aeruginosa, have been studied by several methods. Early studies (1, 2), utilizing the near equilibrium redistribution of radiolabeled solutes initially trapped in reconstituted liposomes, have suggested that the channel was large. OprF allows nearly complete outward diffusion of polysaccharides of 2,000–3,000 daltons in contrast to the Escherichia coli general porin channel that is permeable to sugars of only up to 600 daltons. Kinetic studies of solute diffusion (3, 4), using osmotic swelling of proteoliposomes, have shown that OprF has much lower permeability (i.e. allows much slower permeation of the same test solute) than the classical trimeric porins of E. coli but forms channels that are wider than the channels of E. coli porins because the diffusion rates are much less influenced by the size of the oligosaccharide solutes. Intact E. coli cells expressing OprF porin from plasmid-coded gene are capable of growing on raffinose (5), which is too large (505 daltons) to serve as an effective carbon source for the wild-type E. coli.

A number of studies of OprF have been carried out with planar bilayer systems. The first study by Benz and Hancock (6) has already shown that the addition of OprF produces channels of large single channel conductance (several nsiemens (nS) in 1 m KCl or NaCl) that are also large by other criteria, such as indiffERENCE to the nature of the permeating ions and proportionality of channel conductance to the concentration of salt solutions used. It has also been reported that OprF channel-forming activity is 100-fold lower than that of E. coli porins. However, the behavior of OprF channel is rather complex and probably sensitive to experimental details, so that quite different conclusions were reached in later studies. Thus Woodruff et al. (7) have reported that OprF produces mostly “small” channels whose conductances in 1 m KCl range from 0.1 to 1 nS with an average of 0.36 nS. The occurrence of rare larger channels was mentioned, but neither the actual current recordings nor conductance histograms illustrating this finding were given. In the most recent study by Brinkman et al. (8) the predominance of the small channels, with a broad distribution of conductances from 0.2 to 0.8 nS, has been confirmed. It has also been reported that channels with a similar conductance distribution are formed by the truncated OprF containing only its N-terminal domain.

Because most of the planar bilayer studies were performed on unfraccionated OprF, which is now known to be a mixture of two completely different conformers (9), we decided to examine the properties of channels formed by OprF in more detail using preparations enriched in different conformers. We also used the poly(ethylene glycol) partition assay (10–15) to gauge the pore size.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Their Cultivation—P. aeruginosa PA01 was used as the source of OprF. The plasmid for the expression of the N-terminal domain of OprF was constructed as follows. The portion of oprF gene coding for residues 1 through 170 of the mature OprF was cloned by PCR amplification by using a forward primer containing a PstI site and a backward primer containing a stop codon and a BamHI site. The ampiclon, after restriction endonuclease treatment, was ligated between the PstI and BamHI sites of the vector pBCKS(+) (Stratagene) previously modified by inserting a sequence coding for the signal sequence of E. coli OmpA protein followed by the hexahistidine tag (or the tag and the enterokinase cleavage site sequence) just in front of the PstI site (see Ref. 9). After confirming the correctness of the sequence, an EcoRI-NotI fragment was excised from the recombinant plasmid and was inserted into a medium copy number vector, pKY790 (obtained from K. Yoshida), digested with these two enzymes. This is a 5.10-kb plasmid with the pBR322 origin, chloramphenicol selection marker, lacI gene, and a tac promoter. The recombinant plasmid, pKY-OprFN, was transformed into E. coli host BLR (Novagen). Bacteria were grown in LB medium containing 0.5–1% glucose and (30 μg/ml chloramphenicol when needed for plasmid maintenance) with aeration by rotary shaking.
Preparation of the N-terminal Domain of OprF—Overnight culture (30 ml) of E. coli BLR containing pKY-OprF at 30 °C was diluted into 1 liter of fresh LB medium containing 30 µg/ml chloramphenicol, and the suspension was incubated at 30 °C with shaking until the A600 reached 0.6. At this time, 0.1 mM isopropyl 1-thio-β-D-galactopyranoside was added to initiate the expression of the truncated OprF, and the incubation was continued for a further 3 h. Crude cell envelope fraction was prepared by French pressure cell disruption, and it was then extracted with octyl β-D-glucoside as described for intact OprF (9). The truncated OprF, with the N-terminal hexahistidine tag, was purified by using a nickel-nitriolotriacetic acid Superflow column (Qiagen) according to the manufacturer’s instructions.

The fragment lacking the hexahistidine tag was made from a plasmid containing the tag in front of the enterokinase cleavage site. The protein was purified as above and treated with enterokinase, and the flow-through fraction from a nickel-nitriolotriacetic acid column was collected. The absence of the hexahistidine tag was further confirmed by SDS-PAGE.

Preparation of Unfractionated OprF Protein—P. aeruginosa OprF protein was purified as described in the preceding article (9) by selective detergent solubilization of the outer membrane followed by ion-exchange chromatography and gel filtration.

Isolation of Open Conformer-enriched and Open Conformer-depleted Fractions of OprF through High Resolution Size Fractionation—We utilized the tendency for the open conformers to form oligomers (especially at high concentrations) as follows. OprF samples were fractionated by gel filtration with a 1.5 × 90-cm column of high resolution medium with narrow particle size distribution (Toyo Pearl HW-50F, Tosoh Biosep, Montgomeryville, PA). The buffer used contained 0.1% SDS-PAGE.

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**P. aeruginosa OprF Channel: Properties**

**FIGURE 1.** The OprF N-terminal domain lacks pore-forming activity in the proteoliposome swelling assay. Different amounts of either the N-terminal domain or the complete OprF protein were reconstituted into proteoliposomes in 15% Dextran T-40, and the osmotic swelling was observed in isotonic solutions of various small solutes. The figure shows the behavior of complete OprF in L-arabinose (●) and N-terminal domain in L-arabinose (□), L-alanine (▲), glycine (■), and L-serine (○). Each data point represents the average of five experiments. Although there were marginal decreases in optical density with vesicles containing the N-terminal domain, these were not due to the true pore-forming activity as the swelling rate did not increase with increases in protein in contrast to the situation with the complete OprF.

**FIGURE 2.** Swelling rates of proteoliposomes containing either unfractionated or open conformer-enriched OprF in sugars of different sizes. Proteoliposomes were made either with 40 μg of the unfractionated OprF (●) or with 9.6 μg of the open conformer-enriched OprF (■), and osmotic swelling rates were measured in isotonic solutions of L-arabinose (M, 150), α-glucose (M, 180), N-acetyl-α-glucosamine (M, 221), and sucrose (M, 342). Each data point represents the average of three experiments. The results are expressed as relative rates with the swelling rates in L-arabinose taken as 100. The actual swelling rates in L-arabinose were 0.181 and 0.165 AOD/min with proteoliposomes containing unfractionated and enriched OprF proteins, respectively.

At low concentrations of about 0.02–0.1 μg/ml, the open channel-enriched fraction induced well-defined channels displaying several conductance substates and rich kinetic behavior. A typical current recording showing consecutive insertion of two OprF channels with amplitude of about 30–40 picosiemens (pS) under the applied voltage of −150 mV is given in Fig. 3 at 50-ms time resolution. In addition, intensive downward flickering to much higher currents was always seen. The amplitude of these flickering events measured at higher resolution (see below) was close to 1 nS. Although this recording shows definite “channel-like” behavior, its interpretation is rather complicated due to the presence of at least two levels of different conductance. One of the main questions is whether these conductance levels represent two types of OprF-induced channels as suggested before (8) or show two different substates of the same OprF channel.

To answer this question we performed most of the measurements at the level of minimal OprF-induced conductance. The current recordings in Fig. 4 illustrate one such experiments. Typically channel insertion was manifested by a small stepwise increase in the membrane conductance with the voltage-dependent amplitude corresponding to about 6 and 8 pA (40 and 55 pS) for −150 and 150 mV, respectively. These small current steps are marked as Llow levels for the uppermost (−150 mV) and lowermost (150 mV) tracks in Fig. 4.

**FIGURE 3.** Typical ion current recording obtained from a lipid bilayer after addition of ~50 ng of the single domain-enriched OprF fraction to 1.5 mL of aqueous phase in the cis compartment of the bilayer chamber. Applied voltage was −150 mV; aqueous solution of 1 M KCl was buffered at pH 7.4 with 5 mM Tris. Two consecutive insertions of independent single channels are shown. The time resolution for this recording was 50 ms, meaning that the signal from the amplifier output was filtered by averaging over this time interval.

**FIGURE 4.** Time-resolved flickering events of the same OprF channel. The topmost current track shows consecutive insertions of two channel-like events with amplitudes of about 6 and 8 pA (40 and 55 pS) for −150 and 150 mV, respectively. The lowermost current track illustrates a single channel-like event with an amplitude of about 100–150 pA (6–8 nS) at −150 mV. The time averaging for the topmost and lowermost current tracks was 10 ms.

**OprF in the Open Channel-enriched Preparations Has the Correct Pore Size—**Comparison of swelling rates with sugars of different sizes allows the estimation of pore size by the use of the Renkin approximation (19). This approach has been used for the E. coli OmpF channel (20) and has led to an estimate that is very close to the actual size of the constriction zone of the channel as defined by x-ray crystallography (21). The same approach earlier led to the estimation of 2-nm diameter for the OprF channel (3, 4). However, these studies were all performed with unfractionated OprF. Now that we have been able to obtain fractions enriched for the open channel conformers, with the specific activity much higher than that of the unfractionated protein, we compared these two preparations for the channel size. The results (Fig. 2) showed that the open channel-enriched fraction had a wide diameter very similar to that of the unfractionated OprF, thus confirming that the enrichment did not result from the purification of other contaminating pore-forming proteins.

**Open Channel-enriched Fraction in Bilayer Reconstitution Experiments—**Of the three different OprF fractions: open channel-enriched OprF sample, unfractionated OprF sample, and open channel-depleted OprF sample, we started with the channel-forming activity of the first one.
Our analysis suggests that the current recordings in Fig. 4 represent only one OprF channel and not a superposition of two or more different channels. Indeed, the channel always appeared as a step from zero to the low conductance level and only then reached the higher one. Moreover, at negative voltages and observation intervals of several seconds, we never observed channels that would exhibit only high ($L_{\text{high}}$) or only low ($L_{\text{low}}$) conductance levels. The behavior shown in Fig. 4 was typical. OprF channels spontaneously flickered between the two conductance levels, although the flickering frequency could be different from channel to channel and, for some channels, was time-dependent. In this case, flickering to the high conductance level was clustered in bursts of several seconds separated by shorter intervals of relative silence.

On closer examination it turned out that the lower conductance level ($L_{\text{low}}$) itself was also represented by two discrete conductances. In Fig. 5A we show that the noise of this level seen in Fig. 4 actually represents fast fluctuations between the two sublevels ($L_{\text{low}}^{(1)}$ and $L_{\text{low}}^{(2)}$) with a characteristic time at the submillisecond scale. The amplitude histogram of these fluctuations is given in Fig. 5B. The bold arrows in Fig. 5, A and B, indicate the average difference between the average currents of the sublevels (2.5 and 7.5 pA under the indicated conditions). The relative time the channel spends in the $L_{\text{low}}^{(1)}$ versus $L_{\text{low}}^{(2)}$ substate was quite reproducible in independent experiments and was close to 0.5 at −150 mV applied voltage. Again analysis of the amplitudes of the levels and their probability distributions demonstrates that these events belong to a single OprF channel.

Fig. 6A illustrates conductance dependence of the two major OprF channel levels ($L_{\text{low}}$ and $L_{\text{high}}$) on the transmembrane voltage. Because we could not observe any fast flickering events at positive voltages, data for the high conductance level are given for the negative potentials only. It is seen that OprF in the high conductance state is ohmic, i.e., shows voltage-independent conductance, whereas the conductance of the weakly conductive subconformation is a strong function of applied voltage. Close to ohmic behavior is characteristic of highly conductive $\beta$-barrel channels such as OmpF at high salt concentrations (e.g. see Ref. 17). On the other hand, weakly conductive $\beta$-barrel channels such as LamB exhibit a pronounced non-linearity: ionic current is superlinear in voltage (22, 23). The reason for this non-linearity is not clear at the moment.

Importantly, channel conductance from experiment to experiment was varying within the limits typical to those in other channel reconstitution experiments. Fig. 6B shows the histograms of channel conductances in $L_{\text{low}}$ and $L_{\text{high}}$ states collected from 60 independent channels. The solid lines through the data are Gaussian. The logarithmic scale was chosen because of an ~25-fold difference in average conductance of these states.

**Kinetic Properties of OprF Channels**—The results presented above demonstrate the dynamic behavior of single OprF channels. The chan-
P. aeruginosa OprF Channel: Properties

A

FIGURE 6. A conductance as a function of applied voltage for a single OprF channel in the weakly conductive $L_{low}$ (which is represented by $L_{low}^{(1)}$ and $L_{low}^{(2)}$, averaged by filtering over a time interval of 0.1 s, ( ) and highly conductive $L_{high}$ ( ) substates. The fast flickering events of OprF channel between the $L_{low}$ and $L_{high}$ states are observable only at negative voltages (see Fig. 4 for illustration), so the data for positive voltages are absent. The reproducibility of conductance measurements from channel to channel in the weakly conductive ($L_{low}$, left) and highly conductive states ($L_{high}$, right). Applied voltage was −150 mV.

B

FIGURE 7. Power spectral density of noise in the current through a single OprF channel. The background spectrum (curve 1) was measured for the membrane with a single OprF channel at 0 mV. Curve 2 represents current fluctuations within $L_{low}$ level, that is spontaneous transitions between sublevels $L_{low}^{(1)}$ and $L_{low}^{(2)}$ (see Fig. 5 for illustration). Transitions between $L_{low}$ and $L_{high}$ were excluded from this analysis. Curve 3 represents spectral analysis of a “raw” current recording that included transitions between $L_{low}$ and $L_{high}$ (see Fig. 4, the uppermost current recording).

$S(f) = \frac{S(0)}{1 + (2\pi f\tau)^2}$

(Eq. 1)

where $f$ is frequency, $S(0)$ is the low frequency spectral limit, and $\tau$ is a characteristic time of fluctuations. This kind of spectra suggests simple Markovian character of transitions between the states. Particularly the “$L_{low}^{(1)} - L_{low}^{(2)}$” noise spectrum (Fig. 7, curve 2) is well described by a single Lorentzian (solid line) with the characteristic time of about 0.1 ms. The “$L_{low} - L_{high}$” noise spectra (curve 3) at frequencies below 1,000 Hz also obey a Lorentzian dependence with a characteristic time of about 1 ms (solid line). The complete curve 3 is indeed a sum of these two fast and slow Lorentzians.

Results of noise measurements illustrated by Fig. 7 allowed us to calculate the kinetic parameters of OprF fluctuations as functions of voltage. The characteristic times and rate of $L_{low} - L_{high}$ flickering events are shown in Fig. 8 (A and B, filled circles). It is seen that the characteristic time of OprF fluctuations between $L_{low}^{(1)}$ and $L_{low}^{(2)}$ levels (Fig. 8A, open circles) stays virtually constant. Note also a double increase of the characteristic time for the second process: the $L_{low} - L_{high}$ transitions (Fig. 8A, filled circles) when voltage is shifted from −30 to −200 mV. The rate of flickering (number of events per second, $n$) can be calculated using the following equation (16),

$$n = \frac{S(0)}{4(\Delta f)^2\tau}$$

(Eq. 2)

where $\Delta f$ is the amplitude of the flickering events.

Fig. 8B shows this rate at different negative voltages. It is seen that the rate is a function of the applied voltage with a maximum at about −150 mV and a rapid decrease toward positive voltages. An important parameter, the probability of the highly conductive state, $L_{high}$, is shown in Fig. 8C. It is found as a product of the characteristic time (Fig. 8A) by the number of events per second (Fig. 8B). It is seen that this probability is at most $3 \times 10^{-3}$ at −150 mV and quickly decreases as the applied voltage approaches zero.
**Experiments with Other Fractions**—None of our experiments showed any channel activity for the open channel-depleted OprF fraction. Addition of this fraction at high concentrations exceeding 10 \( \mu \)g/ml to the membrane-bathing solution led to membrane instability and destruction. Usually we observed intensive irreproducible “leaky membrane” conductance noise that did not display any distinct levels even at the highest resolution of 15 \( \mu \)s.

With an unfraccionated OprF sample, we were able to obtain several successful single channel insertions at OprF concentrations above 5 \( \mu \)g/ml. Such events were rare, but the corresponding recordings looked just like the recordings taken for the open channel-enriched fraction described above. The leaky membrane conductance noise was also frequent. This hindered our ability to reliably establish the minimal channel-forming concentration of this fraction. It is reasonable to believe that the low activity of this sample was due to the low content of the open OprF fraction.

Therefore, OprF reconstitution into planar lipid bilayers qualitatively supports the main conclusions of the liposome swelling assay (Ref. 9 and Fig. 2 of the present study) about the differences in the pore-forming activity of different protein fractions. However, judging by the OprF sample concentrations necessary to obtain single channels, the planar lipid bilayer technique gives much higher ratios for these activities. The origin of the discrepancy is not clear at the moment. We speculate that it is due to the difference in conditions under which protein is inserted in these two methods. The tendency of OprF to aggregate (9) may also play a role.

In support of the proteoliposome swelling assay results (Fig. 1), our lipid bilayer experiments with OprF N-terminal domain also showed lack of any channel activity. This is not due to the presence of the N-terminal hexahistidine tag because a preparation without the hexahistidine tag, prepared as described under “Experimental Procedures,” also lacked the channel activity.

**Gauging Channel Size by Poly(ethylene Glycol) Partitioning**—The maximum single OprF channel conductance (~1 nS) (Fig. 6) is comparable with the conductance of a single monomer (~1.3 nS in 1 M KCl) in the OmpF trimer (13, 16, 17). This is consistent with the original concept (2–6) about the large size of the open OprF pore.

Recently we have shown (13) that the characteristic polymer cutoff size of poly(ethylene glycol) (PEG) partitioning into the pores of OmpF and \( \alpha \)-hemolysin correlates nicely with the effective pore radii calculated from the high resolution x-ray structures of these channels (21, 24). This finding further supports polymer partitioning as a tool for sizing channel pores in their functional states (10–15, 25, 26). To estimate the diameter of the OprF channel, here we studied partitioning of PEGs of different molecular weights into its aqueous pore.

The main results of polymer partitioning experiments are presented in Fig. 9. With 15% (w/w) PEG of different average molecular weights, we were able to measure the change of OprF conductance in the weakly conductive \( L_{\text{low}} \) (filled circles) and highly conductive \( L_{\text{high}} \) (open circles) substates. Here we show channel conductance in the presence of polymers normalized to corresponding conductances in polymer-free solutions as a function of the polymer average molecular weight. Also shown are the data for fully open OmpF channel (squares) obtained previously (13). It is seen that large polymers (with average molecular weight of 3,400 and higher) do not change OmpF conductance appreciably (thus do not penetrate into the pore), whereas smaller polymers decrease it significantly (penetrate into the pore). PEG with the characteristic average molecular weight of ~1000 separates these two regimes. Polymers with the average molecular weight of 400 and smaller decrease the channel conductance almost to the same extent as the bulk solution conductivity (dotted line, corresponds to the 0.6 ± 0.02 drop in bulk conductivity measured by taking solution from the cell compartments after the measurements were concluded). In the case of the highly conductive
OprF substate, the total curve is somewhat shifted toward polymers with higher molecular weights. Therefore, one can expect that the effective radius of state L_high is larger that that of E. coli OmpF.

The radius of the weakly conductive OprF substate L_low turns out to be significantly smaller that that of the highly conductive substate. It is seen (Fig. 9, filled circles) that starting with the average molecular weight of 600, polymers do not change the L_low conductance and therefore do not penetrate into the pore in this subconformation.

Protein-Protein Interaction—Most of the findings reported in this study were obtained on single OprF channels where protein concentration in the membrane-bathing solution was kept at the minimal level compatible with channel formation. At higher concentrations channels seem to interact with each other, which is manifested by substantial changes of their dynamic properties.

Fig. 10 illustrates membrane activity of the open channel-enriched fraction at its different concentrations in the membrane-bathing solution. At a relatively high concentration of 0.5 μg/ml, the ionic conductance induced by OprF corresponds to a multichannel system (Fig. 10A). At this concentration the stepwise changes in the current described above are difficult to resolve; large amplitude steps (arrows 2 and 3) similar to those described or mentioned by other researchers (6–8) are seen instead. These current steps most likely represent insertion of aggregates of OprF channels.

Using more diluted OprF stock solutions (30 μg/ml instead of 0.6–2 mg/ml) we reduced protein concentration in the membrane-bathing solution by a factor of 5, which allowed us to observe reproducible discrete conductance levels. Fig. 10B shows current tracks corresponding to only several (two or three) channels in the membrane. However, even at this relatively low concentration, the channels tend to interact with each other. Although the amplitudes of transitions between different states are very close to those described above (Fig. 4), the channel dynamics are changed. Indeed, the current tracks in Fig. 10B are not superpositions of the currents shown in Figs. 3–5. Quantitative statistical analysis of these data is difficult, but their visual examination suggests that the higher conductance states are somehow stabilized by protein aggregation.

The possibility of interaction between channels, which is seen as their changed dynamics in multichannel reconstitution experiments, is indirectly supported by the tendency of OprF to form aggregates (9). Modification of channel behavior in aggregates found in the present study may explain some of the existing discrepancies in the results reported by different laboratories, for example large OprF conductances reported by Benz and Hancock (6).

**DISCUSSION**

In our preceding study (9) we showed that the OprF outer membrane protein of *P. aeruginosa* occurs as a mixture of two conformers. The two-domain conformer, which corresponds to probably more than 95% of the population, was shown to have a conformation generally thought to be the conformation of OmpA, an OprF homolog. We could split the OprF protein by tobacco etch virus protease cleavage in between the two domains and show that the N-terminal domain was essentially a β-barrel, whereas the C-terminal domain was a hydrophilic globular protein rich in α-helices. The minority conformer, which produces channels allowing the diffusion of large solutes including sugars, could be enriched in different ways. First, it could be enriched by taking advantage of the tendency of this conformer to associate loosely to form an oligomeric structure. Second, it could be enriched by introduction of a cysteine residue close to the C terminus followed by the surface labeling of intact cells with a bulky biotinylation reagent. Finally it could be enriched by fractionation of proteoliposomes based on the permeability of the channel, although this method could not be used for the isolation of proteins in amounts sufficient for biochemical study. We have prepared open form-enriched preparations of OprF by the first method, and the main object of the present study was to investigate these conformers by using planar bilayer approaches.

The idea about the presence of a small fraction of open OprF conformers with presumably different folding was confirmed by our channel reconstitution experiments. With the open channel-enriched OprF fraction, we observed successful insertion of single channels with several levels of conductance (see Figs. 3–5). In contrast, with the open channel-depleted fraction coming from the tailing edge of the gel filtration peak, there was never any activity observed, and with the unfractonated OprF, formation of the channels identical to those seen for the open channel-enriched fraction was observed but only at higher protein concentrations.

**Channel Radius**—Although single channel conductance cannot be used directly to obtain the channel size in many cases (27), our results on polymer partitioning showed that in the highly conductive state the diameter of the OprF pore is significantly larger than in the weakly conductive state (Fig. 9). Under conditions discussed previously (13), the relationship between channel conductance and polymer partition coefficient $p(w)$ can be expressed as Equation 3,

$$g(w)/g(\infty) = 1 - \chi p(w)$$  \hspace{1cm} (Eq. 3)  

where $g(w)$ is channel conductance in the presence of polymer with the molecular weight $w$, $g(\infty)$ is channel conductance in the presence of large, completely excluded polymers; and parameter $\chi$ describes the relative amplitude of the channel conductance change between the regimes of completely excluded and completely penetrating polymers (for more details see Refs. 12 and 15).

To compare polymer partitioning into the OprF channel with partitioning into OmpF (13), we used a simple scaling law (12),

$$p(w) = \exp\left(-\left(w/w_0\right)^\alpha\right)$$  \hspace{1cm} (Eq. 4)  

with adjustable $\alpha$ and $w_0$. The first parameter characterizes the sharpness of transition between regimes of exclusion and penetration. Sharper transitions correspond to larger $\alpha$ values. The second parameter...
that the N-terminal eight-stranded β-barrel domain should be thought of as a completely closed channel despite the suggestions from other laboratories (8, 28) as well as from a computer modeling study of its homolog OmpA (30). In conclusion, our results as well as results from other laboratories suggest strongly that the large channel is produced by the folding of the entire OprF sequence to produce a β-barrel of many strands, certainly more than eight and perhaps close to 16, that are found in classical pores (see Fig. 10 of Ref. 9).

Finally, the open channel-enriched preparation of OprF was purified by using the tendency of this conformer to form oligomers. This tendency may explain the difference in OprF kinetic behavior at the single channel level compared with multichannel membranes as discussed under “Protein-Protein Interaction” under “Results.” In fact, in our multichannel experiments we repeatedly observed discrete conductance steps of high amplitude (up to 10 nS), which, depending on their size, could be interpreted as either insertion of a single OprF channel stabilized in its high conductance state by protein-protein interaction or simultaneous insertion of OprF oligomers. Three such events are marked by arrows 1, 2, and 3 in Fig. 10A. Their amplitudes are about 0.9, 2.1, and 8 nS, correspondingly. At low protein concentrations, the aggregates were unstable and tended to dissociate into monomers (9); this was precisely the reason why “pure” preparations of open conformers could not be prepared by repeated runs of gel filtration. Because the single channel assay necessitated the use of dilute samples of OprF, with the final protein concentration in the membrane-bathing solution smaller than 0.05 μg/ml, the oligomers were probably dissociated into monomers that produced reproducible single channels. It is not known yet whether the open channel conformers of OprF exist as oligomers in the intact cells of P. aeruginosa; however, the open channel-enriched OprF fraction isolated by gel filtration contained significant amounts of oligomers when analyzed by SDS-PAGE without the heat denaturation of the samples (see Fig. 5B of Ref. 9).

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