The *Vibrio cholerae* Porins OmpU and OmpT Have Distinct Channel Properties*

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Valérie C. Simonet†, Arnaud Baslé‡, Karl E. Klose§, and Anne H. Delcour¶

From the †Department of Biology and of Biochemistry, University of Houston, Houston, Texas 77204-5001 and the §Department of Microbiology and Immunology, University of Texas Health Science Center, San Antonio, Texas 78229-3900

Numerous environmental signals regulate the production of virulence factors and the composition of the outer membrane of *Vibrio cholerae*. In particular, bile promotes the ToxR-dependent expression of the porin OmpU. Strains expressing solely OmpU are more resistant to bile, are better able to colonize the intestine, and produce more cholera toxin than strains expressing solely the OmpT porin. To gain some understanding in the physiological relevance and the molecular mechanism underlying these porin-dependent phenotypes, we have undertaken a thorough electrophysiological characterization of the channel properties of the two porins. Purified OmpU or OmpT was reconstituted in liposomes suitable for patch clamp and in planar lipid bilayers. The high resolution of the patch clamp technique allowed us to analyze in detail the behavior of single OmpU and OmpT channels. Both channels exhibit closing transitions to various conductance states. OmpT is a much more dynamic channel than OmpU, displaying frequent and prolonged closures, even at low transmembrane potentials. With a critical voltage for closure of approximately ±90 mV, OmpT is much more voltage-sensitive than OmpU (with a V₀ of approximately ±160 mV), a feature that is also readily apparent in the voltage dependence of closing probability observed in patch clamp in the ±100 mV range. OmpT has low ionic selectivity (P₃/K₄ = 10⁻⁴), whereas OmpU is more cation-selective (P₃/K₄ ≈ 10⁻¹⁴). The distinct functional properties of the two porins are likely to play an integrated role with environmental regulation of their expression. For example, the higher selectivity of OmpU for cations provides a possible explanation for the protective role played by this porin in a bile-containing environment, because this type of selectivity would restrict the flux of anionic bile salts through the outer membrane and thus would reduce the exposure of the cytoplasmic membrane to this natural detergent.

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*Vibrio cholerae* is a Gram-negative bacterium causing cholera, a severe diarrheal disease in humans. After ingestion and passage through the gastric acid barrier, this bacterium colonizes the small intestine, where it produces virulence factors such as cholera toxin. There is much evidence that the production of virulence factors is under a complex array of environmental stimuli that influence the activity of regulators, such as the transmembrane protein ToxR (1). Examples of factors that modulate expression of the ToxR regulon include pH, salt, amino acids, and bile. As bacteria progress through different microenvironments during the infection process, it is likely that they sense and respond to these, and maybe some yet unknown, stimuli to maximize survival, colonization, and virulence. Little is known about the importance of the outer membrane composition in these processes. Because the expression of outer membrane proteins is itself regulated by many of the environmental conditions that also influence virulence factor production (1–4), it is clear that there is a need for a thorough characterization of the function of outer membrane proteins to elucidate the reason for this parallel regulation. In particular, much would be learned from investigating the role that outer membrane proteins may play in allowing specific chemical environmental signals to reach ToxR and other cytoplasmic membrane regulators, at the appropriate time and in the appropriate circumstances.

Six major outer membrane proteins have been reported in *V. cholerae* (5). The 45-kDa protein OmpS is a maltoporin induced upon growth on maltose and similar to the LamB porin of *Escherichia coli* (6). OmpV (25 kDa) is a heat-induced, highly immunogenic protein associated with peptidoglycan (7). The 35-kDa outer membrane protein (OmpA) is heat modifiable and reminiscent of OmpA of *E. coli* (8). The OmpX protein (27 kDa), although osmoregulated and trypsin-resistant, does not have pore-forming properties and is not classified as a porin (5). Finally, OmpU (38 kDa) and OmpT (40 kDa) have been shown to allow the transport of hydrophilic solutes in liposome swelling assays (5) and are considered general diffusion porins. Similar proteins have also been described in other *Vibrio* species and shown to have expression patterns that are sensitive to medium composition or external conditions (4, 9–11).

The expression of the ompU and ompT porin genes is regulated by ToxR in opposing ways; ToxR activates ompU transcription but represses ompT transcription (2, 12, 13). Levels of OmpU and OmpT porins are also affected by changes in osmolarity and by other environmental signals (2, 5). Of particular interest is the stimulation by bile of the ToxR-dependent transcription of *ompU* (4), because it has also been shown that cells expressing only OmpU porin are more resistant to bile than cells expressing solely OmpT (14). These types of OmpU-expressing cells also have a greater ability to colonize the small intestine and to express virulence factors than cells expressing solely OmpT (14). Interestingly, the OmpU-mediated flux of β-lactam antibiotics is also impervious to the presence of bile in the external solution, whereas permeability through OmpT is reduced by bile in a concentration-dependent manner (15).

With the long-term goal of identifying the molecular basis for this difference in bile sensitivity between the two porins and in the spirit of establishing correlations between porin function

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† To whom correspondence should be addressed: Dept. of Biology and of Biochemistry, 369 Science & Research Bldg. 2, University of Houston, Houston, TX 77204-5001. Tel.: 713-743-2884; Fax: 713-743-2836; E-mail: adelcour@uh.edu.

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Electrophysiology of OmpU and OmpT

and the ability of external stimuli to activate cytoplasmic membrane sensors, we have undertaken a detailed biophysical study of the pore properties of the OmpU and OmpT porins by electrophysiology. Comparative studies between these two porins have been so far limited to a description of their sugar and antibiotic permeability (5, 15). This type of data only provides some very general characterization of pore properties. Electrophysiological techniques can provide more precise and more extensive knowledge on parameters such as selectivity, modulation, probability of being in the open state, etc. A previous electrophysiological analysis was performed on OmpU reconstituted in planar lipid bilayers, but a comparison with OmpT was not provided (16). The 40-kDa MOMP (major outer membrane protein) of Vibrio anguillarum was investigated with a similar technique (17). The work presented here provides the first detailed characterization of purified OmpU and OmpT by two complementary electrophysiological techniques, namely patch clamp and planar lipid bilayers. The former approach allows the detection of single channels and provides high resolution analysis of channel behavior (18); the latter allows the investigation of population of channels and is particularly useful in the study of voltage dependence (19). Together, these two techniques have allowed us to highlight a number of distinctive functional features of the OmpU and OmpT porins, which may shed some light on the impact of regulation of outer membrane composition on cell survival and pathogenesis.

EXPERIMENTAL PROCEDURES

Strains, Chemicals, and Media—The strains used were V. cholerae KK1107 and KK1108, expressing only ompU or ompT, respectively (15). The cells were grown in LB broth (1% tryptone, 0.5% NaCl, and 0.5% yeast extract) with 100 μg/ml streptomycin. Tryptone and yeast extract were from Difco Laboratories. N-Octyl-oligo-oxyethylene (Octyl-POE)1 was purchased from Alexis Biochemicals. Other chemicals were from Sigma or Fisher.

Protein Purification—The cells were grown in LB broth to an OD600 of ~0.8, harvested by centrifugation at 6,000 × g, washed with cold 10 mM Hepes (pH 7.6), and resuspended at a concentration of 20 g of wet weight/100 ml of 10 mM Hepes (pH 7.6) containing 0.4 mg/ml DNase. The cells were subsequently broken with a French press at 16,000 p.s.i. The unbroken cells were removed by centrifugation at 8,000 × g for 45 min at 4 °C, and a total membrane fraction was obtained after ultra-centrifugation of the supernatant at 350,000 × g for 15 min at 4 °C. The membrane pellet was resuspended with a glass-Teflon homogenizer in 20 mM sodium phosphate buffer (pH 7.6) containing 10 mM NaCl and 1% Octyl-POE and stirred for 30 min at 4 °C (first extraction). The suspension was then ultracentrifuged at 350,000 × g for 15 min at 4 °C. Another extraction at 1% Octyl-POE, followed by two extractions at 3% Octyl-POE, was performed on the samples. OmpU and OmpT porins were specifically extracted at 3% Octyl-POE. The 3% extracts were subjected to dialysis (Spectra/Por 7, VWR) against 10 mM sodium phosphate buffer (pH 7.6), 50 mM NaCl, 0.5% Octyl-POE, and the proteins were quantified with the bicinchoninic acid assay (Pierce). Five mg of proteins were applied to an anion exchange column (Mono Q HR/5; Pharmacia Corp.) equilibrated with 10 mM sodium phosphate buffer (pH 7.6), 50 mM NaCl, 0.5% Octyl-POE. The proteins were eluted with a salt gradient (final concentration, 1 M NaCl). In some cases, fractions containing the protein of interest were concentrated on a Centriprep or Microcon (Amicon) unit (10,000 molecular weight cut-off), and subsequently purified by size exclusion chromatography on a Superdex 75 HR 10/30 column (Pharmacia Corp.). Protein visualization and purity were assessed by silver staining after SDS-PAGE. The samples were either left at room temperature or heated at 96 °C for 10 min prior to electrophoresis. The OmpU or OmpT bands were identified by Western blot using rabbit polyclonal antibodies for N-Octyl-oligo-oxyethylene; they were transferred to a polyvinylidene difluoride membrane, and sequenced at the Protein Chemistry Core Laboratory of Baylor College of Medicine (Houston, TX).

Reconstitution in Planar Lipid Bilayers—Planar lipid bilayers were formed with azolectin, a lipid preparation containing essentially phosphatidylcholine (Sigma), as described (20). Reconstitution of channels was performed by adding 1–2 μg of pure porin into ~4 ml of 1 x KCl, 10 mM Hepes (pH 7.4) in each compartment. Voltage ramps were applied with an Agilent function generator at a rate of 1.6 mV/s. The critical voltage for voltage dependence (Vc) was defined as the highest voltage at which the slope of the curve tangent reverses sign.

Reconstitution in Liposomes—Patch clamp experiments were performed on blisters induced from giant liposomes containing the reconstituted pure porin (21). Reconstitution into azolectin multi-lamellar liposomes was performed by a 1-h incubation of the lipids and pure protein at room temperature followed by a 3-h incubation of this mixture in the presence of 40 mg/ml BioBeads (Bio-Rad) (22). Protein/lipid ratios of 1:6,000 to 1:14,000 (w/w) were typically used. Following this step, the beads were removed by sedimentation, and blister formation was obtained according to previously published methods (21). The patches were obtained as described with 10-megohm pipettes (21). Patch clamp experiments were performed with the following buffers in the pipette and/or the bath, as dictated by the experimental protocol: Buffer A (150 mM KCl, 5 mM Hepes, 0.1 mM K-EDTA, 0.01 mM CaCl2, pH 7.2) or Buffer C (50 mM KCl, 5 mM Hepes, 0.1 mM K-EDTA, 0.01 mM CaCl2, pH 7.2).

Data Recording and Analysis—The currents were recorded with an Axopatch-1D amplifier (Axon Instruments), using the CV-4 headstage for patch clamp experiments and the CV-4B headstage for bilayer experiments. The current was first filtered at 1 kHz. Continuous recordings were digitized (VR-100, Instrutech), and data acquisition was done with the Acquire program (Bruxton) at 84.75-μs sampling intervals for patch clamp experiments or at 1.356-ms sampling intervals for bilayer experiments. Analysis was done with a Windows-based program developed in the laboratory. For OmpT, single channel currents were measured from the Gaussian fit of amplitude histograms (Igor, WaveMetrics). The peaks were well defined and gave current values that were identical to those measured by fitting lines directly on the computer screen through the open and closed current levels of individual events. Because the gating transitions are too infrequent and short-lived in OmpU to give reliable amplitude histograms, single channel currents were obtained from individual events and averaged. Measurement of average closed times at the base line and calculation of closing probability were obtained from kinetic analysis of the closures (23). Closing probability was defined as the ratio of the total time spent at any closed level to the total duration of the analyzed trace.

RESULTS

Purification of OmpU and OmpT—The solubilization of OmpU and OmpT from V. cholerae outer membranes was performed by using Octyl-POE, a detergent successfully used for the crystallization and functional studies on E. coli and V. anguillarum porins (17, 24). After solubilization and dialysis (see “Experimental Procedures”), a single anion exchange chromatography step was sufficient to obtain OmpU in a pure form. Although the protein elutes over a wide range of salt concentrations (from 150 to 230 mM NaCl), it was typically found as a single protein species in two or three fractions eluting at 150 mM NaCl in the elution buffer. These fractions were pooled and analyzed by SDS-PAGE as shown in Fig. 1. The sample heated at 96 °C ran as a single band in a gel containing 0.1% SDS (Fig. 1A, lane 96), as revealed by silver staining. This single band migrates at a molecular mass of 38 kDa and represents the denatured monomer of OmpU. If not heated, the same sample shows the expected trimeric form, running as a ladder of bands at 97 kDa and above (labeled trimer in Fig. 1A, lane RT). The ladder is due to the association of the protein with various amounts of tightly bound lipopolysaccharide molecules, as seen for OmpF (25). Surprisingly, two other bands of lower molecular masses also appear at 66 and 31 kDa. Because the same heated sample contains no other protein except OmpU, the 66- and 31-kDa bands represent other forms of OmpU, namely the dimer and a monomeric form with a distinct migration pattern relative to the denatured monomer (labeled folded monomer in Fig. 1A, because it is likely to have retained some secondary structure at room temperature). In addition, these bands were also revealed in a Western blot with an anti-OmpU antibody.

1 The abbreviations used are: Octyl-POE, N-octyl-oligo-oxyethylene; pS, picosiemens.
The nonheated sample was collected (sample TQS3) and analyzed by size exclusion chromatography followed by size exclusion chromatography (70-kDa cut-off). OmpT eluted in the void volume together with OmpV and OmpA (identified by N-terminal sequencing). Two consecutive anion exchange chromatography steps yield a pure sample of OmpT (TQQ3). OmpT extracted at 1% Octyl-POE is fully purified with anion exchange chromatography followed by single anion exchange chromatography (TQI). For all three panels, M indicates the molecular mass marker, and RT indicates the temperature (96 °C or room temperature, respectively) at which proteins in sample buffer were treated prior to electrophoresis. All of the gels are silver-stained.

Electrophysiology of OmpU and OmpT

A similar anion exchange protocol was applied to the purification of OmpT, extracted with 3% Octyl-POE, and subsequently diluted to 0.5% Octyl-POE. The protein eluted at NaCl concentrations ranging from 180 to 210 mM but was never found in a pure form. Fractions containing OmpT associated with the least number of contaminants were pooled and subjected to size exclusion chromatography (70 kDa cut-off). The void volume was collected (sample TQS3) and analyzed by SDS-PAGE (Fig. 1C). The nonheated sample (Fig. 1C, lane RT) shows some high molecular mass bands that disappear upon denaturation at 96 °C, revealing the presence of trimeric and dimeric forms of OmpT. The heated sample (lane 96) contains three polypeptides identified by N-terminal sequencing as OmpT, OmpV, and the heat-modifiable OmpA, indicating that these proteins migrated as a complex on the size exclusion column. Nevertheless, we were able to obtain pure samples of OmpT, either by performing two consecutive anion exchange chromatography steps (Fig. 1C, sample TQQ3) or by extraction of OmpT with 1% Octyl-POE followed by single anion exchange chromatography (Fig. 1C, sample TQI). Samples TQQ3 and TQI were used for electrophysiology.

Single Channel Recordings—For patch clamp experiments, the purified porins in 0.5% Octyl-POE were reconstituted into liposomes. Control experiments were performed where the same volume of a protein-free 0.5% Octyl-POE solution was added to liposomes, and no channel activity was detected. In the presence of OmpU or OmpT, current fluctuations are readily observed. The electrophysiological signatures of OmpU and OmpT are quite different. Both display a typical porin pattern, i.e. a predominance of channels in the open state, but vary in the frequency and average dwell time of the closing transitions. The traces of OmpU activity shown in Fig. 2 are similar to typical OmpF or OmpC traces. The current level labeled BL (base line) corresponds to the total current flowing through all the channels of the patch (macroscopic current). Departures from the base line, seen as downward or upward deflections at positive or negative pipette potentials, respectively, represent transient closing events. The closing events typically average a duration in the range of 0.5–1.0 ms, irrespective of the membrane voltage. A slight increase in the frequency of these transitions is observed at higher potentials, leading to a decrease in the time spent at the base-line level (more on this below). For the patch shown in Fig. 2, for example, the average time spent at the base-line level in between two consecutive closing events went from 52 ms at −30 mV to 36 ms at −80 mV. This type of activity has been seen reproducibly in 27 individual patches.

OmpT is characterized by a much more active gating pattern (Fig. 3). At small potentials, the predominant current level is also that corresponding to the current flowing through all the channels, but the average duration at the base line is much shorter than for OmpU (for example, ~16 ms at −30 mV for the illustrated patch, which has a comparable number of channels to the OmpU patch above). Notably, the average duration of the closing events ranges from 2 to 30 ms, depending on the voltage used. These values are much larger than the 0.5–1.0-ms average closing duration found for OmpU. As seen in Fig. 3, OmpT displays frequent prolonged closures, which are never seen.
with OmpU. The combination of more frequent and more prolonged closures makes OmpT a more active and less frequently open channel than OmpU. This gating pattern has been consistently observed in 31 patches. Occasionally (in <10% of the traces), the channel temporarily switches to an even more active gating pattern characterized by an intense flickering activity (not shown).

A close inspection of the gating patterns also reveals differences between the two porins. Representative traces are shown on an expanded time scale in Fig. 4 to highlight the details of the closing events. The most frequently observed events in OmpU patches are closures of small amplitudes, corresponding to a conductance of 51 pS. On occasion, such events are interrupted by closures of larger amplitude that are usually five or six times the small event amplitude. Examples of such transitions are shown in Fig. 4A, where the dashed lines mark the small event current level and its multiples. It is unclear whether the 51-pS conductance represents the monomeric conductance or that of a substate.

For OmpT, the most frequent transition, as clearly seen in the traces of Fig. 3, has a conductance of 354 pS. Events of conductances that are 1/3 or 2/3 of this value are also often seen (although not consistently in all patches). Occasionally, a conductance level of 50–80 pS appears mixed in with the other levels. This presumed substate is marked by an asterisk in the traces of Fig. 4 (C and D), where it is seen to be of smaller size than the multiples of the monomer conductance marked by the dashed lines.

Current-Voltage Plots and Selectivity—The selectivity of the OmpU and OmpT channels was investigated by measuring current amplitudes at various voltages in symmetric and asymmetric ionic conditions. The slope of the obtained current-voltage plot yields the conductance, whereas the x axis intercept represents the potential at which there is not net current (reversal potential, $E_{\text{rev}}$). In symmetric conditions (same ionic composition of the buffer in the pipette and the bath, here 150 mM KCl), $E_{\text{rev}}$ is 0 mV, as expected in the absence of any driving force for ion movement. In asymmetric conditions (150 mM KCl in the pipette buffer; 50 mM KCl in the bath buffer), the current is null at a potential that precisely counterbalances the driving force originating from the concentration gradient. In this condition, $E_{\text{rev}}$ depends on the relative permeabilities of the channel for anions and cations. Such permeability ratio can be calculated from $E_{\text{rev}}$ by using the Goldman-Hodgkin-Katz equation (30).

As observed for OmpF and OmpC, the frequency and duration of closing events being so low, it is not possible to obtain amplitude histograms for OmpU. Thus, we measured the amplitude of individual events for three separate patches. The data points plotted in Fig. 5A are the averages of current amplitudes obtained for the most frequent transition (the small conductance seen in Fig. 4A). The slope conductances obtained from the linear regressions were identical in symmetric (closed symbols) and asymmetric (open symbols) conditions (51 pS). The reversal potential was calculated to be $-23.7$ mV, indicating a preference for cations over anions, with a relative permeability ratio $P_K/P_C$ of 13.8. In the case of OmpT, amplitude histograms are easily obtained because the closures are frequent and long-lived. The peaks of the amplitudes were fitted with Gaussian curves, and the current values obtained from such fits were averaged across four individual experiments and plotted in Fig. 5B. The linear regressions produced slope conductances of 354 and 248 pS in symmetric (closed symbols) and asymmetric (open symbols) conditions, respectively. The non-parallel shift of the current-voltage plots indicates an effect of ionic conditions on the conductance, a hint that the ionic occupancy of single channels has not yet reached saturation in 50 mM KCl. The calculated reversal potential was $-15$ mV. Thus, OmpT is much less cation-selective channel than OmpU, with a relative permeability ratio $P_K/P_C$ of 3.6.

Voltage Dependence—Although the voltage-induced inactivation of channels has not been demonstrated in vivo and may not have a physiological relevance (31), it is a well known electrophysiological property of porins that can be used as a comparative tool (19, 29, 32). We have used the two complementary approaches of patch clamp and planar lipid bilayers to investigate this phenomenon with the V. cholerae porins. By performing voltage ramps on planar lipid bilayers containing a large number of reconstituted porins, it is possible to easily measure the so-called critical voltage ($V_c$), i.e. the threshold voltage at which channel inactivation is detected. Typical figure-eight-shaped hysteresis loops were found for both OmpU and OmpT in planar lipid bilayers where purified proteins had been added to both sides of the membrane (Fig. 6). The graphs clearly show that $V_c$ is much larger for OmpU than for OmpT, and thus that OmpT is more voltage-sensitive. The values of $V_c$ were found to be $163 \pm 9$ mV and $-170 \pm 10$ mV for OmpU ($n = 10$) and $92 \pm 11$ mV and $-92 \pm 11$ mV for OmpT ($n = 11$), for the positive and negative voltage ranges, respectively. Fig. 6B was obtained from a bilayer with a large macroscopic current, and thus, the curve is rather smooth. The inset illustrates an experiment where the macroscopic current was comparable to that of the experiment in Fig. 6A and shows that in this case, as in Fig. 6A, it is also possible to observe sharp deflections in the current because of the abrupt closing of one or a few channels.

Patch clamp experiments allowed us to observe the detailed
behavior of individual channels. For OmpU, increasing the membrane voltage causes a small increase in the frequency of closing transitions and has no effect on the average closed time. However, because the effect is mild and the closures are still extremely short-lived, there is no appreciable increase in overall closing probability (Fig. 7) in the voltage range of $-110$ to $+110$ mV (it is hard to obtain stable recordings at voltages greater than this). A similar analysis was performed with OmpT channels. As indicated above, OmpT patches can show some variability in the extent of gating to conductance levels that are fractions of the trimeric conductance. To ensure consistency, we have restricted our kinetic analysis to OmpT patches that mostly display closing transitions to the trimeric conductance level over the whole voltage range investigated and have a limited gating to lower conductance levels. Fig. 7 shows the averaged results obtained from seven such patches. As seen in planar bilayer experiments, OmpT reveals a more voltage-sensitive behavior than OmpU, with increased closing probability at voltages greater than $+50$ mV. There is a hint of some asymmetry in this behavior, because negative pipette voltages tend to be more effective than positive ones. As pointed out earlier, the increase in closing probability originates from a combination of increased closing frequency, more prolonged closed times, and gating to current levels that correspond to the simultaneous closure of multiple trimers.

**DISCUSSION**

The outer membrane of *V. cholerae* contains six major proteins, of which only OmpT and OmpU appear to have properties that are similar to those of the *E. coli* general diffusion porins OmpF and OmpC (5). Much work has been performed on the purification and biochemical analysis of the OmpU porin. However, functional characterization of OmpU and OmpT has been limited. The experiments presented here provide a detailed and more complete comparative study of these major outer membrane proteins.

Different procedures have been documented in the literature for the purification of major outer membrane proteins from various *Vibrio* species. Detergents such as Triton X-100 (5), sodium deoxycholate (10), Genapol (16), and Octyl-POE (17) have been used. The use of Triton X-100 was justified by Chakrabarti et al. (5) on the basis of the irreversible denaturation of OmpU by SDS, although no data on this phenomenon were presented. Here, we have confirmed that the presence of SDS in electrophoresis gels is sufficient to promote a breakdown of OmpU tertiary structure, a behavior that is not seen
Electrophysiology of OmpU and OmpT

with *E. coli* porins (27) but has been documented for porins of other organisms (26), including other *Vibrios* (10). It is likely that the 31-kDa band that we detect in nonheated samples corresponds to the 32-kDa form reported for OmpU when heated at 50 °C (33, 34). Our initial purification of OmpT following an extraction with 3% Octyl-POE yielded a complex of OmpT with OmpA and OmpV, as evidenced by the co-migration of these polypeptides on a size exclusion column. This complex was not documented by Chakrabarti *et al.* (5) in their OmpT purification, but it is likely that this is due to their use of Triton X-100 (and not Octyl-POE) for solubilization. Indeed, we also found that the complex could not be isolated from samples first solubilized with 1% Octyl-POE, and thus the ability of the three proteins to remain in close association is, not surprisingly, dependent on solubilization conditions. Whether the existence of the complex has functional implications remains to be shown.

The electrophysiological demonstration of pore activity has been documented for *V. cholerae* OmpU (16) and for a major outer membrane protein MOMP of *V. anguillarum* (17). The porins described in these reports have similarly functional properties as those presented here, such as large conductance, cation selectivity, and oscillations between open and closed states. Only liposome swelling and antibiotic flux assays were performed with OmpT (5, 15). The patch clamp analysis presented here allows a detailed comparison of the activity of these two pore-forming proteins. The salient distinctive features that this work has revealed can be summarized as follows: 1) OmpT exhibits more frequent and more prolonged closures than OmpU and thus appears to be a more dynamic channel; 2) OmpT is much less specific for cations than OmpU; and 3) OmpT is more voltage-sensitive than OmpU. Much of the previous literature on these proteins attempts to draw analogies between these proteins and the *E. coli* porins OmpF and OmpC. We believe that some caution has to be exercised in making such comparisons. For example, OmpT has more resemblance to OmpF than to OmpC on the basis of poor cation selectivity and the pattern of environment-regulated gene expression (27). However, its pore properties based on permeability to sugars (5) put it closer to the smaller OmpC than to OmpF. Despite this, the most common gating steps of OmpT correspond to a conductance that is greater than that of OmpU, even if we take into consideration the conductance of the less frequent, albeit large, transitions of OmpU. This mixture of features is reminiscent of *V. anguillarum* 40-kDa MOMP, which shares some aspects (but not all) of expression regulation by the environment with OmpC but whose pore properties suggest a functional similarity to OmpF (10). Thus, it seems prudent to accept that OmpU and OmpT have their own distinctive features, some of them shared with OmpC and others with OmpF. OmpT also displays unique characteristics, such as a much lower threshold voltage for inactivation and a higher propensity for gating than typically documented for porins. This uniqueness may correlate with a somewhat different structure, because no significant homology is found between OmpT and OmpF or OmpC, whereas OmpU shares ~40% homology with OmpF or OmpC.

It is anticipated that differences in the functional properties of OmpU and OmpT will provide insights into the physiological significance of the complex regulation of the expression of these proteins by environmental conditions. The expression of the *ompT* and *ompU* genes is under the control of the transmembrane regulator ToxR, whose activity is influenced by environmental stimuli (1). In particular, ToxR mediates the stimulation of *ompU* transcription by external bile or deoxycholate and thus appears sensitive to these agents (4). The increased level of OmpU in the outer membrane, although not a prerequisite, appears to favor virulence factor expression and intestinal colonization in *vivo*. A previous report has documented that *toxR*− strains, which express *ompT* exclusively, are more bile-sensitive (35). In addition, the mere swapping of porin expression (OmpU instead of OmpT, and vice versa) in the absence of

![Fig. 6. Critical voltage for OmpU and OmpT inactivation.](image)

**Fig. 6. Critical voltage for OmpU and OmpT inactivation.** Triangular voltage ramps were applied at a rate of 1.6 mV/sec to planar lipid bilayers containing purified OmpU (A) or OmpT (B) channels. Each plot shows the current obtained in response to the applied voltage for a single cycle of the following sequence: 0 mV → maximum positive voltage → 0 mV → maximum negative voltage → 0 mV (sequence shown by small arrows near the OmpU trace). The maximum voltages were ±200 mV for OmpU and ±130 mV for OmpT. The inset in B is obtained from an OmpT bilayer with comparable macroscopic current to the OmpU bilayer of A. The inset, only the 0 mV → ±130 mV and the 0 mV → −130 mV ramps are shown for sake of clarity. See “Experimental Procedures” for definition of the critical voltage.

![Fig. 7. Voltage dependence of channel gating.](image)

**Fig. 7. Voltage dependence of channel gating.** The closing probability was obtained in patch clamp experiments as described under “Experimental Procedures” and averaged across three OmpU patches (○) and seven OmpT patches (●) at the indicated voltages, in symmetric buffer A. The errors bars are S.D. and may lie within the thickness of the symbols for some points.
ToxR has profound effects on pathogenic properties, with the presence of OmpU positively correlating with increased bile resistance, expression of cholera toxin and co-regulated pilus, and intestinal colonization (14). Some results presented here provide a possible explanation for the increased bile resistance of OmpU-expressing strains relative to those expressing solely OmpT. Because OmpU is much more cation-selective than OmpT, and bile salts are negatively charged, it is anticipated that the flux of such salts through OmpU will be lower than through OmpT. Because bile is essentially deleterious to cells because of the detergent-induced damage caused to the inner membrane, the reduced accessibility of the inner membrane to bile in OmpU-containing strains will provide protection and thus will impart such strains with the ability to better survive bile-containing environments. This relatively simple explanation need experimental testing with mutants impaired in their selectivity properties, which is underway. It remains possible that the selectivity of the porins plays only a part in conferring bile resistance, because it has been shown that OmpU and OmpT also have different sensitivities to functional modulation by bile (15). The molecular relationship between channel selectivity and inhibition by bile has yet to be examined.

Interestingly, the nonselective nature of OmpT would also provide bile with the ability to penetrate inside the periplasm, come into contact with the cytoplasmic membrane-bound ToxR, and thus activate this protein. A possible scenario can thus be imagined where initial ingestion of contaminated water or food supply would infect the host with V. cholerae cells that essentially express OmpT. Upon reaching the intestine, this nonselective porin would allow some bile to enter the periplasm and activate ToxR, thus promoting the switch from an OmpT-containing outer membrane to an OmpU-containing outer membrane, in addition to expression of virulence factors. The presence of OmpU would then impart the surviving cells with the ability to withstand the bile-containing environment and to establish colonization. Whether some of the other distinct properties of OmpU and OmpT that we have reported here play a role in this scenario can also be examined with the help of specific mutants.

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Electrophysiology of OmpU and OmpT 17545

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Valérie C. Simonet, Arnaud Baslé, Karl E. Klose and Anne H. Delcour

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