Deoxycholic Acid Blocks *Vibrio cholerae* OmpT but Not OmpU Porin*

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OmpT and OmpU are general diffusion porins of the human intestinal pathogen *Vibrio cholerae*. The sole presence of OmpT in the outer membrane sensitizes cells to the bile component deoxycholic acid, and the repression of OmpT in the intestine may play an important role in the adaptation of cells to the host environment. Here we report a novel important functional difference between the two porins, namely the sensitivity to deoxycholic acid. Single channel recordings show that submicellar concentrations of sodium deoxycholate induce time-resolved blocking events of OmpT but are devoid of any effect on OmpU. The effects are dose-, voltage-, and pH-dependent. They are elicited by deoxycholate applied to either side of the membrane, with some asymmetry in the sensitivity. The voltage dependence remains even when deoxycholate is applied symmetrically, indicating that it is intrinsic to the binding site. The pH dependence suggests that the active form is the neutral deoxycholic acid and not the negatively charged species. The results are interpreted as deoxycholic acid acting as an open-channel blocker, which may relate to deoxycholic acid permeation.

*Vibrio cholerae* is an enteric pathogen responsible for cholera. After ingestion of contaminated water by the human host, the bacteria will encounter various micro-environments before colonizing the gut and producing an infection (1, 2). Important aspects of the physiology and pathogenesis of the bacterial cell rely on detection of and response to the extracellular environment (3). The flow of hydrophilic solutes through the outer membrane of this Gram-negative bacterium is largely controlled by the general diffusion porins OmpU and OmpT. The pore-forming ability of these proteins has been demonstrated by liposome swelling assays (4), antibiotic flux assays in live cells (5), and electrophysiology (6). Recently, we have demonstrated that the two porins are functionally distinct on the basis of their kinetics, which are controlled by the general diffusion porins OmpU and OmpT. The conductance of the monomers is similar for OmpU (every ~4 s, comparable with that of the *Escherichia coli* porin OmpF) but much higher for OmpT (every ~0.3 s), indicating that OmpT may be a much more dynamic pore. General diffusion porins are characterized by their tendency to inactivate at high transmembrane voltage, a phenomenon whose functional relevance and molecular mechanism remain elusive. OmpT was found to be more voltage-sensitive than OmpU and inactivates at a threshold voltage of ~90 mV, a voltage that is about half the voltage required to inactivate OmpU and that might be in the range attainable by Donnan potentials in physiological conditions. Finally, OmpT was shown to be relatively non-selective with a ratio of permeability for potassium to chloride (**P**<sub>k</sub>/**P**<sub>Cl</sub>) of ~4 (similar to *E. coli* OmpF), while OmpU is a much more cation-selective channel (**P**<sub>k</sub>/**P**<sub>Cl</sub> ~14). The conductance of the monomers is similar for the two porins (~350 picosiemens for OmpT and ~300 picosiemens for OmpU in 150 mM KCl), but OmpU has a tendency to display transitions to various states of lower conductance (6).

OmpU allows the passage of larger sugars than OmpT does (4) but, however, displays a smaller rate of β-lactam antibiotic flux (5). Since the three-dimensional structure of OmpU and OmpT is unknown, and as the transport rate of a pore depends on both size and the thermodynamics of the interactions between permeating solutes and the channel wall, it remains difficult at this point to assess whether the pore of OmpU has larger dimensions than that of OmpT or vice versa.

The two porins also appear to confer different physiological properties to bacterial cells. In particular, the sensitivity of cells to bile and bile components is an important factor for this intestinal pathogen. One of the major components of bile is deoxycholic acid, a cholesterol derivative with detergent-like properties. Previous work has shown that mutant *V. cholerae* cells that express exclusively OmpT (and no OmpU) grow more poorly in presence of deoxycholate than cells that express exclusively OmpU (7). This phenotype is similar to the behavior of *E. coli* bile acid-sensitive ompC mutants versus *E. coli* cells that express only OmpC (8) and has been attributed to the fact that some porins (*V. cholerae* OmpT and *E. coli* OmpF) allow a better permeation of deoxycholate than others (*V. cholerae* OmpU and *E. coli* OmpC). The outer membranes of Gram-negative bacteria are intrinsically more resilient than phospholipid bilayers to the action of detergents, such as bile acids, due to the presence of lipopolysaccharides in the outer leaflet (9). Permeation of bile acids does occur though, as several promoters are regulated by these bile components (10–13) and multidrug resistance pumps play important roles in detergent extrusion (8, 14). Although general diffusion porins are poorly permeable to hydrophobic compounds (9), it is anticipated that amphi-

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paths such as deoxycholate have a finite flux rate through these pores. Once in the periplasm, detergents molecules can partition in the cytoplasmic membrane, thus compromising cellular integrity and growth. It is important to note, however, that many studies have used submicellar concentrations of sodium deoxycholate or other bile salts and have shown a variety of effects, from pump-mediated efflux to biofilm formation (8, 12–15).

The expression of the ompU and ompT genes is under the control of the transcriptional regulator ToxR, a membrane bound environmental sensor that plays a major role in regulating expression of virulence factors, such as cholera toxin and toxin-coregulated pilus (16). ToxR was shown to be an activator of ompU expression and a repressor of ompT expression (17, 18). The activity of ToxR is itself modulated by a variety of external factors, such as pH, bile salts, osmolarity, and temperature (3, 10, 13). Once inside the host, activation of ToxR would turn on ompU expression and turn off ompT expression. Presumably this switch in porin expression has an important role in the adaptive success of the cells in the host environment. Interestingly, Klose and collaborators (19) have also shown that engineered cells that express solely ompT from a ToxR-activated ompU promoter have attenuated virulence properties, as they become defective in colonization and virulence factor production. Although these porins are not required for virulence (7), it appears that the type of porin present in the outer membrane may have an impact on the ability of V. cholerae cells to cause disease.

To continue our characterization of functional differences between OmpU and OmpT that may have important physiological consequences, we have investigated the effect of the bile salt sodium deoxycholate (DC)\(^2\) on the channel properties of OmpU and OmpT. Our results demonstrate that OmpT is reversibly blocked by protonated deoxycholate, presumably as it transits through the pore, while OmpU is impervious to the presence of DC. To our knowledge, this is the first report of block of a pore-forming protein by deoxycholic acid.

**EXPERIMENTAL PROCEDURES**

**Chemicals, Media, and Buffer Composition**—The OmpU and OmpT porin samples were prepared from the porin-deficient V. cholerae strain KKv884 (7) expressing the ompU or ompT gene cloned into a PBAD30 plasmid. Cells were grown in Luria-Bertani broth (1% tryptone, 1% NaCl, and 0.5% yeast extract) with appropriate antibiotics (0.1 mg/ml ampicillin, 0.1 mg/ml streptomycin) and 1-arabinose (0.01% for ompT expression, 0.05% for ompU expression). Tryptone and yeast extract were from Difco Laboratories. N-Octyl-oligo-oxyethylene (octyl-POE) was purchased from Axxora. Other chemicals were from Sigma. For electrophysiology, the following buffers were used: buffer A (150 mM KCl, 10 μM CaCl\(_2\), 0.1 mM K-EDTA, 5 mM HEPES (pH 7.2)), buffer B (= buffer A + 20 mM MgCl\(_2\)), buffer K (= buffer A where HEPES is substituted by 5 mM MES and the pH is 5.2), buffer L (600 mM KCl, 10 μM CaCl\(_2\), 0.1 mM K-EDTA, 5 mM HEPES (pH 7.2)), and buffer M (= buffer A where HEPES is substituted by 5 mM CHES, and the pH is 9.2). A 25 mM stock solution of sodium deoxycholate was made in the appropriate buffer in a plastic tube. The solution was diluted to the desired concentration and immediately perfused into the patch clamp chamber. The solutions were stable for at least 1 h, while each individual experiment with fresh DC sample lasted for 10–15 min.

**Protein Purification**—Purification of OmpU and OmpT protein was essentially done as described (6). Protein extraction with the detergent octyl-POE was done at 4 °C. A first column chromatography was done on an anion exchange column (Mono Q HR10/10, Amersham Biosciences), and the protein eluted between 130 and 210 mM NaCl (in 1% octyl-POE, 10 mM sodium phosphate buffer (pH 7.6)). Subsequently, OmpT- or OmpU-containing fractions were further purified by size exclusion chromatography on a HiLoad 26/20 Superdex 200 prep grade column (Amersham Biosciences) in 1% octyl-POE, 50 mM NaCl, 10 mM sodium phosphate buffer (pH 7.6). Proteins were identified by Western blot. Protein visualization and purity were assessed by silver staining after SDS-PAGE. Pure protein was kept at −80 °C in 1% octyl-POE, 10 mM sodium phosphate buffer (pH 7.6), and 50 mM NaCl, prior to use in electrophysiology. Protein concentration was determined with the bicinchoninic assays (Pierce).

**Reconstitution into Liposomes and Patch Clamp Electrophysiology**—Pure protein was reconstituted into soybean phospholipids (Azolectin, from Sigma) at protein-to-lipid ratio of 1:3,000 to 1:5,000 (w/w), and patch clamp experiments were performed on unilamellar blisters emerging from liposomes when placed in buffer B, as described (6, 20). Patch pipettes of ∼10 megaohm resistance were filled with buffer A or buffer A + DC and brought into contact with the blister membrane to generate seals of 0.5–1.0 gigaohm. All experiments were conducted on excised patches produced by brief air exposure. After excision, the bath solution was exchanged for buffer A or other buffers, as dictated by the experiment. An Axopatch 1D amplifier (Axon Instrument) was used to monitor currents under voltage clamp conditions. The current was filtered at 1 KHz, digitized at 1.25-ms sampling intervals (ITC-18, Instrutech), and stored on a PC computer using the Acquire software (Bruxton).

**Data Analysis**—Analysis of patch clamp traces was done with a program specifically developed in the laboratory and written by Arnaud Baslé using Microsoft Visual Studio C. Amplitude histograms were constructed by scanning current records and counting the number of sample points at each current value. The open probability (Po) was calculated as the ratio of the observed integrated current obtained over a 1-min-long recording to the total current expected for the same duration if the current value remained at the fully open level. The dose-response curve was fitted to the Hill equation below using SigmaPlot (Marquardt-Levenberg algorithm),

\[
P_o/(1-P_o) = 1/[1 + ([DC]/IC_{50})^n]
\]

where \(P_o(DC)\) and \(P_o(CON)\) are the open probabilities in presence and absence of DC, respectively, \(IC_{50}\) is the inhibitory concentration at which \(P_o\) is reduced by 50% relative to control, and \(n\) is the Hill coefficient.

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\(^2\) The abbreviations used are: DC, sodium deoxycholate; octyl-POE, N-octyl-oligo-oxyethylene; MES, 2-(N-morpholino)ethanesulfonic acid; CHES, 2-(cyclohexylamino)ethanesulfonic acid.

\(^3\) In solution, deoxycholate exists as a mixture of ionized and protonated deoxycholate.
RESULTS

Based on the fact that cells expressing solely OmpU or OmpT have different sensitivities to DC in growth and that antibiotic flux through OmpU or OmpT are differently affected by crude bile, we hypothesized that DC might show differential effects on the pore properties of OmpU and OmpT. To test this hypothesis, we recorded the electrophysiological behavior of purified OmpU or OmpT pores in the absence or the presence of DC with the use of the patch clamp technique. Fig. 1 shows traces of the activity of a single trimer of OmpT in symmetric buffer A (see “Experimental Procedures” for composition) before and after applying 0.008% DC to the bath of the same patch. In all experiments, the voltages indicated on the figures correspond to the pipette voltage. The effect was reversible upon application of respective channels, the effect of DC on OmpT was found to be voltage-dependent and much less pronounced at negative voltages. The expanded trace below trace B shows the clean square pulse pattern of closures and openings that suggests that each monomer is fully and reversibly blocked by DC. Indeed, the conductance of OmpT was measured by voltage-current plots and was found to be unaffected by DC (data not shown). This kinetic pattern is similar to the transient block of OmpF observed in the presence of ampicillin (21) or of LamB observed in the presence of maltodextrins (22, 23), as these solutes translocate through the channels. As for these solutes and their respective channels, the effect of DC on OmpT was found to be voltage-dependent and much less pronounced at negative pipette potentials. The effect was reversible upon application of buffer without DC.

In contrast, OmpU was completely insensitive to DC in this concentration range (Fig. 2). As was done for Fig. 1, a patch containing a single trimer of OmpU was sequentially studied in the absence and the presence of 0.008% DC, and the traces obtained at + and −50 mV pipette voltages are represented. The kinetic pattern of the channel was unchanged in the presence of DC, and no other effect was detected. This is not due to the artificial lack of access of DC to the patch, as the subsequent perfusion of 0.1 mM spermine in the same patch was fully effective at inhibiting OmpU, in a manner similar to the inhibition of OmpF by this polyamine (24). This insensitivity of OmpU-containing patches to DC also serves as a control for unspecific effects that this amphipathic molecule might have on pores and membranes. We have found that patches were
stable for at least 1 h at concentrations less than 0.01% (~250 μM). Higher concentrations lead to membrane breakdown. These low concentrations are well below the critical micellar concentrations for DC at room temperature (2–6 mM (25)) and do not introduce noise or other artifacts into the electrophysiological traces.

To quantify the effect of DC on OmpT, we have measured the \( P_0 \) of a single full trimer at various voltages and various concentrations. Fig. 3 shows the concentration dependence of the ratio of \( P_0 \) with and without DC obtained from traces of either an OmpU (closed symbols) or an OmpT (open symbols) trimer at +50 mV. The open probability of OmpU is so reproducibly maintained close to 1.0 that the error bars are within the thickness of the symbols. The solid line is a linear regression fit yielding a horizontal line. For OmpT, a concentration dependence is evident in the range of 0.001% to 0.01% DC. The solid line is a fit to the Hill equation (Equation 1) given under “Experimental Procedures,” where the IC_{50} is 0.008% DC, and the Hill coefficient is 2.5. These data are supportive of a model where the minimum number of binding sites is 3, and each monomer is blocked by a single molecule of DC, with, surprisingly, some amount of cooperativity. Cooperativity has been postulated for the simultaneous activation of porin trimers reconstituted in bilayers as clusters (26) but not for substrate binding or modulation nor between monomers of the same trimer.

The effect of bath-applied DC on OmpT in symmetric buffer A is also voltage-dependent, as illustrated in Figs. 1 and 4. In the absence of DC, the open probability of OmpT remains close to 1.0 in the voltage range of −50 mV to +70 mV (Fig. 4, filled circles). At higher positive or negative voltages, the channels inactivate, leading to a decreased \( P_0 \) even in the absence of DC (6). For this reason, the investigation was limited to a voltage range where \( P_0 \) remains constant, so the analysis of DC effect would not be complicated by channel inactivation. Bath-applied DC is quasi-ineffective in the negative voltage range, but the block becomes increasingly stronger as the transmembrane voltage increases in the positive range (Fig. 4, open circles). We initially thought that the voltage dependence of bath-applied DC was due to negatively charged deoxycholate molecules being driven from the bath into the pore at pipette positive potentials. If this scenario is correct, we would expect to see the opposite voltage dependence when DC is present in the pipette and absent in the bath. We did observe an inhibitory effect of DC when applied from the pipette side, supporting the model that the site of DC action is within the pore. Surprisingly, we found that the trend of the voltage dependence was the same regardless of where DC is applied, i.e. the effect is always more pronounced at positive pipette voltages than at negative ones. There is some sidedness to the efficacy of DC, though, as the reduction of \( P_0 \) is more pronounced when DC is in the pipette (Fig. 4, closed squares) than when it is in the bath (Fig. 4, open circles). These results suggest that the asymmetric voltage dependence of the DC effect is intrinsic to the channel, and indeed this asymmetric voltage dependence is still observed when the same concentration of DC is applied to both sides of the membrane (Fig. 4, open squares). Since there is no voltage dependence of \( P_0 \) in this voltage range, the voltage dependence of the DC effect is not due to the increased availability of open pores at positive pipette voltages. Thus it seems that the configuration of the DC binding site within the pore displays voltage dependence.

These results also suggest that the active molecular species of DC is not the negatively charged molecules. The pK_a of deoxycholic acid being 6.2, the ratio of unprotonated to protonated molecules would be 10:1 at the pH of buffer A (pH 7.2). Therefore, there is a non-negligible amount of protonated deoxy-
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Figure 5. Effect of pH on DC-induced block. Representative current traces of OmpT activity at a pipette voltage of +50 mV were obtained in the presence of 0.006% bath-applied DC at the indicated pH values. All three traces were obtained from the same patch. Tick marks on the left side of the traces show the current levels corresponding to the closed trimer (c) and one, two, or three open monomers (1o, 2o, 3o).

Cholate molecules present at pH 7.2. If indeed the neutral protonated deoxycholate is the active species, its effect should be decreased at higher pH and increased at lower pH. The results of Fig. 5 and Fig. 6 show that it is indeed the case. Fig. 5 shows traces obtained from the same patch at +50 mV pipette voltage in the presence of 0.006% DC at three different pH values, where the ratios R of negatively charged to protonated deoxycholate are widely different: pH 9.2 (R = 1000), pH 7.2 (R = 10), and pH 5.2 (R = 0.1). At pH 9.2, there is only 0.1% of protonated deoxycholate, and no effect on the channel is observed (top trace). At pH 5.2 (bottom trace), there is 90% of protonated deoxycholate, and the effect is much more pronounced than at pH 7.2 (~9% protonated deoxycholate). It is important to note that the perfusion sequence for this experiment was buffer A + DC (pH 7.2), then buffer M + DC (pH 9.2), and finally buffer K + DC (pH 5.2). Therefore, the observed effect is not due to a time dependence. These results also re-emphasize the reversibility of the phenomenon, since the channel completely recovers from block once buffer A + DC (pH 7.2) is exchanged for buffer M + DC (pH 9.2). The enhancement of the effect of DC with increasingly acidic pH is illustrated in Fig. 6, which compares the values of P_o at these three different pH values. The top part shows that by themselves, the pH 5.2 and pH 9.2 buffers have no effect on the open probability.

We also attempted similar experiments on OmpU to investigate whether the 10-fold increase in concentration of protonated deoxycholate might induce some inhibition. Unfortunately, OmpU is sensitive to acidic pH, and its open probability is decreased to ~10% at pH 5.2 in the absence of DC. Perfusion of 0.006% DC at pH 5.2 had no additional effect, but of course these experiments are more difficult to interpret since the channel is already mostly in the closed state.

DISCUSSION

Here we report that the general diffusion porins OmpU and OmpT of the outer membrane of V. cholerae are differentially sensitive to channel block by deoxycholic acid in a physiological range. As bile and the bile component deoxycholic acid are known to affect a variety of physiological responses of this intestinal pathogen (10, 11, 13, 15, 27, 28), this distinct sensitivity of the two channels is likely to have an important cellular consequence. Deoxycholic acid constitutes about 20% of the bile acids present in bile. Bile acids are secreted as glycine or taurine conjugates but deconjugation by enzymes of the bacterial flora occurs to produce the unconjugated form used here. Bile acid concentrations are on the order of 40 mM in bile (29), but much of it is re-absorbed by the intestinal epithelium. Levels of deoxycholate of up ~0.8 mM have been measured in colonic water of human subjects consuming a high fat diet (30), and thus the DC concentrations that are effective on OmpT (0.02–0.2 mM) are within the range of physiological concentrations.

Bile salts, such as sodium cholate and sodium deoxycholate are used as detergents at concentrations above their critical micellar concentrations of ~6–12 and 2–6 mM, respectively (25). In eukaryotes, submicellar concentrations of these compounds, such as used in this study, have subtle effects on membrane properties, including activation of membrane-bound enzymes and signaling pathways without membrane disruption (31, 32). Activation of cationic and anionic channels by 0.5 mM DC has also been reported in a human colon adenocarcinoma.

FIGURE 6. Effect of pH on the OmpT open probability in presence of DC. The P_o of a single trimer of Ompt in the absence (A) or the presence (B) of 0.006% bath-applied DC is plotted against the pipette potential. Symbols represent the averages of P_n (± S.E.) from four separate patches, except for the cases of buffer M in the bath (n = 3) and of buffer A + DC in the bath (n = 5). When not seen on the graph, the error bars lie within the thickness of the symbols. In all cases, the pipette buffer was buffer A. The pH values of bath solutions are as follows: pH 9.2 (●, ○), pH 7.2 (▲, △), and pH 5.2 (■, □).
cell line (Caco2 cells) (33). We found that the maximum concentration we could use on our patches without membrane dissolution was −0.2 mM (0.01%), a somewhat lower value than in the aforementioned electrophysiological study, probably due to different lipid composition of the membranes under study. In prokaryotes, DC in the concentration range of 0.004 to 0.04% has been implicated in the regulation of a variety of biological phenomena such as gene expression (11–13, 15), toxin production (13), protein conformational changes (12), and biofilm formation (15). The activity of deoxycholic acid as an ion channel blocker has not been reported previously to our knowledge.

The pattern of OmpT channel kinetics in the presence of DC is reminiscent of those observed when the maltoporin LamB is blocked by maltodextrins (23) and OmpF by ampicillin (21). In all cases, well defined full closures of monomers are observed, along with a concentration-dependent decrease in the number of active channels. Interestingly both maltodextrins and ampicillin are known to use LamB and OmpF, respectively, to cross the membrane. Thus, it has been argued that the transient block of the open pores may represent permeation of these solutes through their respective channel and not just binding to and release from a blocking site. Indeed, Bezrukov and colleagues (23) estimated that a blocking event of LamB by maltohexaose may eventually lead to translocation 20–50% of the time. Although we cannot completely rule out membrane mediated effects, the simplest interpretation for the effect of deoxycholic acid on OmpT is that the observed channels closures also correspond to transient blockages of the open pores, possibly linked to permeation. At submicellar concentrations, deoxycholic acid exists as a monomer. The molecular weight of deoxycholic acid is 392, within the size exclusion limit of typical porins (9), and thus the molecule may be able to access the pore. The main arguments in favor of this model are the reversibility, the asymmetry of the effect depending on the site of DC application, and the lack of effect of DC on OmpU, a similar porin to OmpT. Membrane-mediated effects are anticipated to be relatively unspecific. Even if we were to evoke the presence of a specific binding site for deoxycholic acid at the protein-lipid boundary of OmpT, it would be surprising that accessibility to this site be side-dependent. Asymmetrical rates of maltose entry into LamB have been observed and attributed to the inherent asymmetric structure of the pore vestibules and access to the so-called “greasy slide” where binding events occur (23). An asymmetry in the sensitivity of OmpF to spermine, which binds to the constriction zone, has also been reported (24). In fact, asymmetries in other parameters, such as conductance and selectivity, have been documented for OmpF (34, 35) and are attributed to the asymmetry of the channel and of the distribution of charges in the lumen. We anticipate that asymmetries in the pore structure of OmpT, and/or in the vestibules leading to the constriction zone, are also at the root of the side-dependent effect of deoxycholic acid on OmpT.

Since OmpU is not affected by DC and is much more cation-selective than OmpT, a straightforward interpretation is that the blocking events observed in OmpT are mediated by negatively charged deoxycholate molecules, which are excluded from OmpU because of its selectivity. A surprising result of this work is the observation that acidic pH, instead of annihilating the effect, as expected for the negatively charged deoxycholate being the active form, actually enhances the block of OmpT by DC and thus suggests that the active form is the protonated deoxycholate, i.e., deoxycholic acid. We cannot completely rule out that protonation of pore residues at acidic pH also participate in the enhancement of the effect, but the lack of blocking events at pH 9.2, where less than 1% of deoxycholate is in the protonated form, strongly suggests that the negatively charged species is ineffective. This interpretation is also supported by our finding that raising the ionic strength of the buffer by using a 600 mM KCl solution instead of 150 mM KCl (with no change in pH) had no influence on the DC-induced blocking events (data not shown). In other words, ionic interactions between a charged blocker and the pore are not likely to be taking place, since they would be sensitive to salt concentrations. The fact that neutral protonated deoxycholate is the active form raises the possibility that even the conjugated form of the acid might be effective, if it is not too large.

The voltage dependence of inhibition also strengthens our conclusions that the active species is the neutral protonated form of deoxycholate. If the negatively charged deoxycholate molecules were the effective blocker, they would be driven to their site of action by a positive potential applied to the membrane side opposite to the side of DC application. The asymmetric voltage dependencies in the presence of bath-applied DC or pipette-applied DC would be a mirror image of each other. This is not the case. The asymmetric voltage dependence is unchanged regardless of the side of DC addition and is manifested even in presence of DC on both sides of the membrane. An asymmetric voltage dependence has also been documented for the transport of uncharged maltodextrins across LamB (22, 23). A theoretical model describing the kinetic parameters for maltohexaose permeation, including a voltage-dependent conformational change, has been presented (22). Similarly, the kinetic parameters of the transient blockade of OmpF observed in presence of ampicillin are also voltage-dependent (21). Therefore there is precedence voltage-sensitive binding of uncharged or zwitterionic solutes in the constriction zone of other porin channels. These observations are interpreted in terms of a voltage-induced change in the architecture of the pore, which may be subtle enough not to lead to any effect on the ionic conductance but significant enough to alter the fit of the blocking solute in the constriction zone.

Why do OmpU and OmpT show such distinct sensitivities to deoxycholic acid? A recent study by Bezrukov’s group highlights the molecular requirements for optimal block of OmpF by various β-lactam antibiotics and establishes strong correlations between the induction of time-resolved blocking events, the existence of a binding site at the constriction zone, and the efficiency of translocation (36). Their results emphasize the importance of sterically and electrostatically favorable interactions between the pore and the solutes to bring forth the documented effects on pore blockage. For example, ampicillin, one of the antibiotics with the highest permeation rates, induces the largest number of blocked events at a pH where the molecule is zwitterionic and establishes the strongest interactions with the charged constellation of the OmpF pore (21, 36). Other antibiotics with poor diffusion rates
through OmpF, such as the dianionic carbencillin, do not bind to the narrowest part of the channel with high affinity and do not induce time-resolved blocking events. These observations prompt us to propose by analogy that the lack of effect of deoxycholic acid on OmpU in the concentration range investigated is due to a poorer fit of the solute molecule in this channel. Since the active deoxycholate species is the neutral protonated form, we need to dismiss the greater cation selectivity of OmpU relative to OmpT as the main reason for the lack of binding and rather evoke steric considerations as being the prime determinant in deoxycholic acid sensitivity. In principle, a pore may be insensitive to an open-channel blocker because it is too narrow and physically restricts blocker entry or because it is too large to permit strong interactions between the blocker and pore residues. We favor the former model, since the conductance of OmpU is about ~15% smaller than the conductance of OmpT (the conductance values of OmpU and OmpT monomers in 150 mM KCl are 300 and 350 pico siemens, respectively). Although conductances are not a sole function of pore diameter, it seems likely that OmpU may form a slightly smaller pore than OmpT, in a manner similar to the relationship of pore size observed between OmpC and OmpF of E. coli. In addition, if OmpU were a larger pore than OmpT, it would be unlikely to provide the documented protective role with respect to growth and survival of cells in presence of DC.

Our current thinking is that the blocking events represent occupancy of the OmpT pore, which, by analogy with maltodextrins and ampicillin, may lead to permeation, with a certain probability. Calculating this probability requires the determination of the “on” rate constants obtained from the average times between successive blocking events, upon application of DC from one or the other side of the membrane (23). Because of the high level of spontaneous closures even in the absence of DC, successive blocking events per se cannot be readily discerned, and thus the time intervals between successive closures reflect both the spontaneous closing activity and block. We attempted to obtain the time constant for block from the exponentials fits of dwell time distributions, but the analysis was inconclusive. Nevertheless, because the presence of OmpT confers a deoxycholic acid-sensitive phenotype to V. cholerae cells that express solely this porin (7), it is reasonable to propose that deoxycholic acid is able to flux through OmpT and that the blocking events that we document here can indeed be resolved by translocation with a certain probability. This property would allow deoxycholic acid to access the cytoplasmic membrane, where, at high enough concentrations, integrity may be compromised, leading to growth deficiency and cell death. Conversely, the lack of effect of deoxycholic acid on OmpU may correlate with a decreased permeation efficiency (as seen for some β-lactam antibiotics and OmpF (36)) and provides an explanation for the relative DC insensitivity of cells expressing solely OmpU (7). It has been suggested that the relative resistance to DC provided by OmpU may play an important role in the ability of V. cholerae cells to survive in the intestinal environment. It will be interesting to verify that OmpU mutants that show DC-induced channel block in patch clamp experiments also display an increased sensitivity in physiological assays.

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REFERENCES

1. Krukonis, E. S., and DiRita, V. J. (2003) Curr. Opin. Microbiol. 6, 186–190
2. Lee, S. H., Hava, D. L., Waldor, M. K., and Camilli, A. (1999) Cell 99, 625–634
3. Skorupski, K., and Taylor, R. K. (1997) Mol. Microbiol. 25, 1003–1009
4. Chakrabarti, S. R., Chaudhuri, K., Sen, K., and Das, J. (1996) J. Bacteriol. 178, 524–530
5. Wibfenmeyer, J. A., Provenzano, D., Landry, C. F., Klose, K. E., and Delcour, A. H. (2002) Infect. Immun. 70, 121–126
6. Simonet, V. C., Basé, A., Klose, K. E., and Delcour, A. H. (2003) J. Biol. Chem. 278, 17539–17545
7. Provenzano, D., Lauriano, C. M., and Klose, K. E. (2001) J. Bacteriol. 183, 3652–3662
8. Thanassi, D. G., Cheng, L. W., and Nikaio, H. (1997) J. Bacteriol. 179, 2512–2518
9. Nikaio, H. (2003) Microbiol. Mol. Biol. Rev. 67, 593–656
10. Gupta, S., and Chowdhury, R. (1997) Infect. Immun. 65, 1131–1134
11. Provenzano, D., Schuhmacher, D. A., Barker, J. L., and Klose, K. E. (2000) Infect. Immun. 68, 1491–1497
12. Rosenberg, E. Y., Bertenthal, D., Nilles, M. L., Bertrand, K. P., and Nikaio, H. (2003) Mol. Microbiol. 48, 1609–1619
13. Hung, D. T., and Mekalanos, J. J. (2005) Proc. Natl. Acad. Sci. U. S. A. 102, 3028–3033
14. Bina, J. E., and Mekalanos, J. J. (2001) Infect. Immun. 69, 4681–4685
15. Hung, D. T., Zhu, J., Sturtevant, D., and Mekalanos, J. J. (2006) Mol. Microbiol. 59, 193–201
16. Klose, K. E. (2001) Int. J. Med. Microbiol. 291, 81–88
17. Crawford, J. A., Kaper, J. B., and DiRita, V. J. (1998) Mol. Microbiol. 29, 233–246
18. Li, C. C., Crawford, J. A., DiRita, V. J., and Kaper, J. B. (2000) Mol. Microbiol. 35, 189–203
19. Provenzano, D., and Klose, K. E. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10220–10224
20. Delcour, A. H., Martinac, B., Adler, J., and Kung, C. (1989) Biophys. J. 56, 631–636
21. Nestorovich, E. M., Danelon, C., Winterhalter, M., and Bezrukov, S. M. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 9789–9794
22. Schwarz, G., Danelon, C., and Winterhalter, M. (2003) Biophys. J. 84, 2990–2998
23. Kullman, L., Winterhalter, M., and Bezzrakov, S. M. (2002) Biophys. J. 82, 803–812
24. Iyer, R., and Delcour, A. H. (1997) J. Biol. Chem. 272, 18595–18601
25. Matsuoka, K., and Moroi, Y. (2002) Biochim. Biophys. Acta. 1580, 189–199
26. Schindler, H., and Rosenbusch, J. P. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3751–3755
27. Schuhmacher, D. A., and Klose, K. E. (1999) J. Bacteriol. 181, 1508–1514
28. Chatterjee, A., Chaudhuri, S., Saha, G., Gupta, S., and Chowdhury, R. (2004) J. Bacteriol. 186, 6809–6814
29. Begley, M., Gahan, C. G., and Hill, C. (2005) FEMS Microbiol. Rev. 29, 625–651
30. Slader, J., Stern, H. S., Yeung, K. S., McGuire, V., Furrer, R., Marcon, N., and Bruce, W. R. (1988) Gut 29, 1326–1331
31. Im, E., and Martinez, J. D. (2004) J. Nutr. 134, 483–486
32. Akare, S., and Martinez, J. D. (2005) Biochim. Biophys. Acta. 1735, 59–67
33. Mauricio, A. C., and Ferreira, K. T. (1999) Exp. Physiol. 84, 489–499
34. Nestorovich, E. M., Rostovtsvea, T. K., and Bezzrakov, S. M. (2003) Biophys. J. 85, 3718–3729
35. Alcaraz, A., Nestorovich, E. M., Aguilera-Arrozo, M., Aguilera, V. M., and Bezzrakov, S. M. (2004) Biophys. J. 87, 943–957
36. Danelon, C., Nestorovich, E. M., Winterhalter, M., Ceccarelli, M., and Bezzrakov, S. M. (2006) Biophys. J. 90, 1617–1627