

Mimicry of a Host Anion Channel by a *Helicobacter pylori* Pore-Forming Toxin

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ABSTRACT Bacterial pore-forming toxins have traditionally been thought to function either by causing an essentially unrestricted flux of ions and molecules across a membrane or by effecting the transmembrane transport of an enzymatically active bacterial peptide. However, the *Helicobacter pylori* pore-forming toxin, VacA, does not appear to function by either of these mechanisms, even though at least some of its effects in cells are dependent on its pore-forming ability. Here we show that the VacA channel exhibits two of the most characteristic electrophysiological properties of a specific family of cellular channels, the CIC channels: an open probability dependent on the molar ratio of permeable ions and single channel events resolvable as two independent, voltage-dependent transitions. The sharing of such peculiar properties by VacA and host CIC channels, together with their similar magnitudes of conductance, ion selectivities, and localization within eukaryotic cells, suggests a novel mechanism of toxin action in which the VacA pore largely mimics the electrophysiological behavior of a host channel, differing only in the membrane potential at which it closes. As a result, VacA can perturb, but not necessarily abolish, the homeostatic ionic imbalance across a membrane and so change cellular physiology without necessarily jeopardizing vitality.

INTRODUCTION

Many bacterial pathogens secrete water-soluble protein toxins that can insert into the membranes of eukaryotic cells and form pores (1). A number of different families of these toxins have been identified, but they are usually classified as belonging to one of two groups: those for which the pore is used as a conduit through which an enzymatically active peptide is transported (2) and those for which the pore permits a relatively unrestricted flux of various ions and small molecules across the membrane, ultimately leading to lysis of the cell or the cellular compartment (3). Examples of the former group include diphtheria, botulinum, and anthrax toxins (4–6), and those of the latter include hemolysins from *Staphylococcus aureus*, colicins from *Escherichia coli*, and cholesterol-dependent cytolysins from Gram-positive bacteria (7–9).

Helicobacter pylori is a Gram-negative bacterium that colonizes the human stomach and contributes to the development of peptic ulcer disease and gastric adenocarcinoma (10). An important virulence factor secreted by this bacterium is a water-soluble toxin, VacA, that, like the aforementioned toxins, has been found to form pores in the membranes of eukaryotic cells (11–13). However, VacA does not share any obvious homology with other known pore-forming toxins and,

moreover, does not appear to belong to either of the two groups described above: there is no evidence to indicate that VacA transports a peptide across a membrane, and VacA does not cause lysis of cells or organelles. Instead, among a range of other effects (13,14), this toxin induces a massive osmotic swelling, or vacuolation, of acidic cellular compartments that are derived from late endosomes and lysosomes (11,12). Although the mechanism by which VacA causes this vacuolation is not yet completely understood, a large body of evidence indicates that this phenomenon is dependent on the formation of VacA pores (15–21). Inasmuch as this vacuolation is a relatively fine-tuned toxic effect, in contrast to the cell lysis that results from intoxication with many other pore-forming toxins, it seems likely that the cellular alterations caused by VacA are a consequence of specialized electrophysiological features of its pore, which is usually not the case for a pore-forming toxin.

In our previous study (22), we found that the conductance of the VacA pore is surprisingly low (~12 pS in 1 M salt), ~40–400 times lower than that of typical bacterial cytolysins (23–27). In this respect then, the VacA pore seems to resemble a typical cellular channel (28). This earlier work also showed that the VacA channel is anion selective and remains anion selective over a range of pH values. This latter property differs from that observed with other bacterial toxins (29) but is shared by a number of cellular anion channels (30–32).

Thus, the subtle manner by which the VacA channel causes its cellular effects is likely to involve a change in the homeostatic transmembrane distribution of anions, which is a property usually determined by the characteristics of, among other membrane constituents, the host anion channels. Of these, some of the most thoroughly studied are members of the CIC family of

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chloride channels (33). In eukaryotic cells, these channels have been found to participate in a number of different cellular functions, including the regulation of membrane excitability and electrical stability (34), transepithelial transport (35), cell volume (36), and the size, trafficking, and acidification of endosomal and lysosomal compartments (37–41).

Here, we show that VacA channels exhibit two striking features that are characteristic of the CIC family of anion channels: an open probability that depends on the molar ratio of permeable anions, as well as two different voltage-dependent gating transitions. Moreover, VacA and CIC channels are similar in a number of other properties, including the magnitude of their conductance, a dependence on voltage for opening, and the degree of selectivity for anions over cations. Furthermore, CIC channels and internalized VacA have both been localized to the membranes of endocytic compartments (37,38,41–44). We therefore suggest that the cellular effects caused by VacA are in part attributable to the remarkable similarity in the electrophysiological properties of VacA and CIC channels. Our results further identify a property, the membrane potential at which the channels close, that is slightly different between these channels, and this may be the most significant difference in their channel characteristics that enables VacA, but not usually CIC channels, to cause vacuolation. Although a number of bacterial water-soluble toxins have previously been suggested to function as mimics of eukaryotic host proteins (45), this is the first mimicry of a host ion channel by a bacterial pore-forming toxin.

MATERIALS AND METHODS

Materials and electrophysiology

VacA was purified from the broth supernatant of *H. pylori* 60190 as previously described (46). Planar lipid bilayers were formed across an aperture separating two compartments in a Teflon chamber (22). The buffer in each compartment consisted of (buffer A) 2 mM EDTA, 5 mM citric acid, pH 4.0, and the salt composition described in the figure captions. VacA was then added to the *cis* chamber at a monomer concentration of 3 nM. The setup to measure membrane current was as described previously (22), and voltages are given as the potential of the *cis* compartment, defined as the side to which VacA was added, with respect to that of the opposite *trans* compartment.

Data analysis

The lifetime of an open or closed state of a channel can usually be determined from a histogram plot of the open or closed times measured from single channel recordings using a program such as Transit (Baylor College of Medicine). However, the VacA transitions at many voltages proved to be too rapid for this program to identify many of the open or closed states, and so the lifetimes calculated in this way were significantly different than those suggested by a direct inspection of the recordings.

An objective measure of the lifetimes of these states was therefore obtained by a power spectral analysis of the recordings. In this treatment (47), the number of transitions (and their lifetimes) can be determined from a Fourier transform of the autocovariance function, called the spectral density, $G(f)$, of the single channel recordings. For the two independent gating mechanisms described below, this is

$$G(f) = \frac{G_{o1}}{1 + (f/f_{o1})^2} + \frac{G_{o2}}{1 + (f/f_{o2})^2}, \quad (1)$$

where $G_{oi} = (4I_{sc}^2\alpha_i\beta_i)/(\alpha_i + \beta_i)^3$, $f_{oi} = (\alpha_i + \beta_i)/2\pi$, I_{sc} is the single channel current, and α_i , β_i are the closing and opening rate constants, respectively. Fitting the spectral density to the sum of two Lorentzians (using Origin (Microcal Software, Taejon, Korea)) yields two amplitudes, G_{o1} , G_{o2} , and the corner frequencies, f_{o1} , f_{o2} , from which each of the rate constants can be determined. The open probability curves for each of the transitions is then given by

$$P_o^i(V) = \frac{\alpha_i}{\alpha_i + \beta_i}. \quad (2)$$

Model for ion translocation through the VacA channel

In the rate theory approach, ion translocation through the channel is characterized by the progression of the ion through a series of energy barriers and wells (28,48–50). For reasons described in the Results, a minimal rate theory model of the VacA channel should include two binding sites within the pore, and so a three-barrier, two-site model was investigated. Because of the large number of independent parameters in such a model, a simplified, symmetric barrier diagram was considered first. The values obtained in this simpler case were then used as the initial parameters in a model in which the parameters may attain any value.

The rate theory approach is frequently employed to determine whether the association of different ion occupational patterns (occurring at different concentrations of ions) with states differing in current can describe the observed ion dependencies on current. In a similar way, we tested whether a scheme in which a singly occupied channel identified as blocked could describe the concentration dependence of the faster VacA transition (described in the Results). The sum of the occupational probabilities of these states in this model is the closed probability of the channel, P_{cl} . The open probabilities (equaling $1 - P_{cl}$) at three concentrations of Br^- (0.5 M, 1.1 M, 2.0 M) were separately fit, yielding the electrical distances, the relative strengths of energy barriers and wells, and a measure of the ion-ion repulsion. Each fit produced roughly identical values of the parameters, except for the values of the parameter associated with the ion concentration (K), which changed in the expected proportion, as shown in Table 1. The value of the affinity constant for the lower affinity site obtained from these fits (~ 0.4 M) agrees with the value obtained from the relationship between the conductance and activity (H. Iwamoto, unpublished observations).

TABLE 1 Parameters obtained from the fit of the faster transition open probability to a rate theory model obtained at different activities of NaBr

	0.5 M	1.1 M	2.0 M
$D1$	0.002	0.003	0.002
$D2$	0.300	0.313	0.325
$D3$	0.198	0.184	0.173
ΔG_{12-34}	9.4	9.2	8.4
ΔG_{23-34}	11.0	10.8	10.7
K	1.5	2.8	5.6
Q	141	137	135
F	1.11	1.11	1.39

$D1$, $D2$, and $D3$ are the electrical distances of the three energy barriers from left (*cis*-side) to right (see *inset* to Fig. 6), ΔG_{12-34} and ΔG_{23-34} are the relative energy differences in units of RT (R , the gas constant and T , the temperature) of the two barriers, G_{12} and G_{23} , with respect to the barrier, G_{34} , K is the ratio between the ion concentration and the affinity constant of site B , Q is the ratio between the affinity constants between site B and site A , and F is the measure of ion-ion repulsion (48–50).

RESULTS

Mole fraction dependence of the VacA conductance and open probability

Our previous study of the VacA channel suggested that it may be a useful model system to investigate fundamental aspects of chloride channel behavior (22). One common but still not well understood property of many chloride channels is an anomalous mole fraction effect (AMFE) in conductance, where the conductance exhibits a minimum as the molar ratio of two permeant ions is varied. Since the conductance of a single ion pore is expected to vary linearly with this mole ratio, observation of an AMFE is often taken to indicate that the pore of the channel may be simultaneously occupied by at least two ions and that repulsive interactions between these ions influence conductance (28,51,52). As Fig. 1 illustrates, VacA exhibits an AMFE in conductance in solutions of SCN^- and Br^- , showing a clear minimum at a SCN^- molar ratio of 0.15.

However, these channel recordings also show that the degree to which the channel is open is also dependent on the mole ratio of permeant anions (Fig. 1). The VacA channel thus also exhibits a mole fraction effect in its open probability. To our knowledge, the only other channels which have shown an AMFE in conductance and a mole fraction effect in the open probability are members of the CIC family of channels (53).

Voltage dependence of the open probability

The similarity of the features of VacA and CIC gating processes described above suggest that VacA channels may also exhibit other properties of the CIC gating process. Many CIC channels are voltage dependent (33), and in our previous study, we found that the VacA channel is also voltage dependent, although only weakly (22). However, measured over a greater range of membrane potentials than used in this earlier work, the open probability of the VacA channel is actually found to change significantly at large potentials of either polarity (Fig. 2). Since the open probability curve for a simple two-state (open-closed) mechanism of channel opening is sigmoidal (28), it is apparent from this figure that a more complicated process is occurring within the VacA channel.

An explanation for this behavior emerges from a direct inspection of the single channel recordings (Fig. 3). At a voltage of -300 mV, the channel is seen to open in bursts, separated by long closures. Within the burst (see the higher time resolution data in Fig. 3 A), the channel is almost always open. This behavior, transitions into a burst and transitions within a burst, has also been observed in CIC-0 channels (54). In these, it has been suggested that there are two different, independent gating processes: a slower one operating to open the channel into the burst state and a faster one that operates within the burst. We therefore suggest that, like CIC-0 (and CIC-1 (30)) channels, VacA also exhibits two

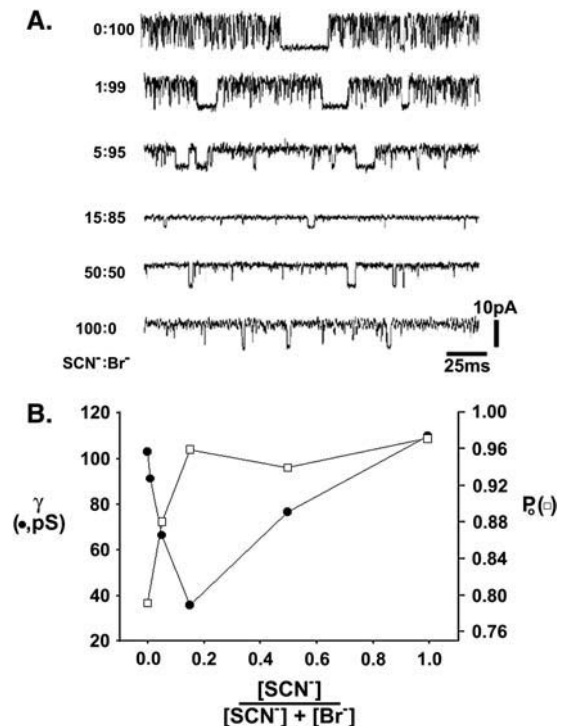


FIGURE 1 Dependence of the VacA conductance and open probability on the molar ratio of permeant anions. (A) Representative single channel recordings of the VacA channel in planar lipid bilayers at different molar ratios of SCN^- and Br^- . The total ionic concentration of the Na^+ salts of these anions was 1.5 M, and the holding potential was -100 mV. Like other Cl channels, VacA exhibits an AMFE in its conductance, reaching a minimum at an SCN^- mol ratio of 0.15. Also evident in these recordings is a significant increase in the channel open probability as the molar ratio of SCN^- increases from 0 to 0.15. A strong dependence of the open probability on the permeant ion is not a common feature of many ion channels but has been observed in CIC channels (53). (B) The open probability of VacA reaches its maximal value at the same molar ratio of SCN^- (0.15) at which the conductance exhibits a minimum, suggesting that these two unusual features of the VacA channel may both be caused by the simultaneous occupation of the pore by two or more anions.

different gating processes that function independently of each other. At a voltage of -300 mV, the gating process associated with the slower transition is more frequently closed whereas the process associated with the faster transition is more frequently open.

It should be mentioned that a number of CIC channels are dimeric, and in these, the slow gating process operates on both pores simultaneously whereas the faster one operates on each pore independently (33). Thus, each individual pore in these CIC complexes exhibits two independent transitions (a slow one and a fast one), and it is this property of the CIC pore that is similar to the observation described here of the VacA channel (which has only a single pore).

As shown in Fig. 3 B, at a potential of -100 mV, the VacA channel transitions are also seen to occur in bursts, but, compared with those observed at -300 mV, the bursts occur much more frequently, and within the burst, the channel is

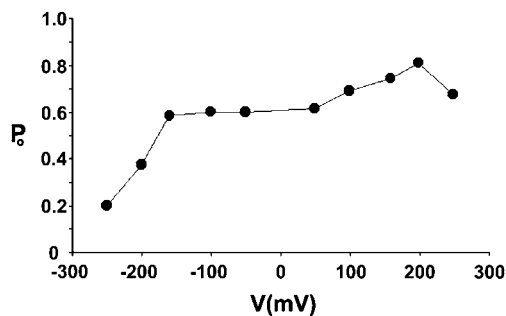


FIGURE 2 Voltage dependence of the open probability of the VacA channel. The open probability changes significantly at larger magnitude potentials of either polarity but only changes slightly at lower magnitude potentials. This dependence contrasts with the more common sigmoidal voltage dependence, suggesting a simple two-state gating process observed with many other voltage-dependent channels (28). The open probability was determined from single channel recordings obtained from channels in buffer A with 1.5 M NaBr.

more often closed. Hence, at this voltage, the slower gate is more frequently open but the faster one is more frequently closed. At a voltage of +250 mV (Fig. 3 C), again the channel is seen to open in bursts, but here, the transitions into the burst and within the burst resemble those which occur at -300 mV. Thus, at this voltage, the slower gate is again more frequently closed and the faster gate is almost always open.

Taken together, the single channel events of VacA appear to be owing to two different, independent gating transitions, each with a different voltage dependence: a slower gating process that is more often open at lower potentials of either

polarity and a faster gating process that is more frequently open at greater potentials of either polarity.

Spectral analysis of the gating transitions

To obtain a more quantitative measure of these transitions, we determined the power spectral density of the single channel recordings, which is a particularly useful treatment to evaluate rapid transitions (47). As shown in Fig. 3, D–F, the results of this analysis show that, indeed, there are two types of transitions in each recording: a slow transition that occurs with a frequency of $\sim 10\text{ s}^{-1}$ and a faster transition that occurs at $\sim 1000\text{ s}^{-1}$. Assuming that each transition corresponds to a two-state process, a value of the open probability for each type of transition can be calculated, as described in the Methods section. Fig. 4 shows the open probability curves determined in this way.

Consistent with the direct observations, the voltage dependence of the slower transition is bell shaped (Fig. 4 A), with the open state almost completely occupied for all voltages between -150 mV and +200 mV and then less occupied at larger potentials of either polarity. In contrast, the voltage dependence of the faster transition is “U”-shaped, centered at 0 mV, with the open state more frequently occupied at large potentials of either polarity (Fig. 4 B).

Ionic dependence of the faster transition open probability

Many channels exhibit a bell-shaped open probability. The “U”-shaped dependence, though, is more rare and in fact,

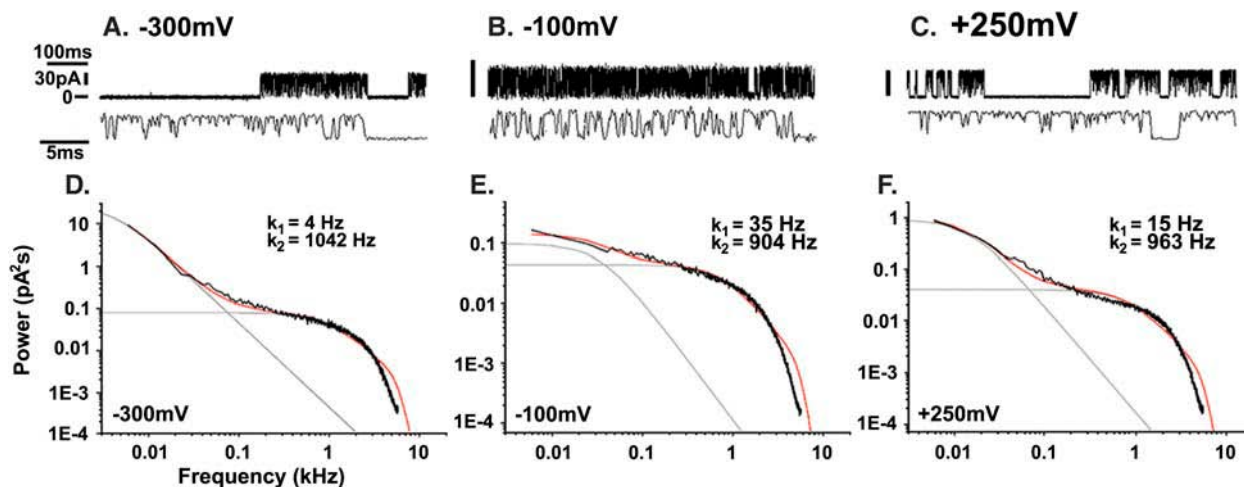


FIGURE 3 VacA exhibits two independent transitions, each with a different voltage dependence. (A–C) Direct inspection of the single channel recordings reveals two different transitions: a slower transition that opens the channel into a “burst” state and a faster transition that operates within the burst state. The slower transition into the burst occurs less frequently at larger magnitude potentials of either polarity. In contrast, as the higher time resolution recordings show, the gating process associated with the faster transition is more frequently open at the larger magnitude potentials of either polarity. (D–F) The presence of two independent transitions in the single channel measurements is verified by a power spectral analysis of the recordings. Each curve is well described by the sum of two Lorentzians, consistent with the presence of two independent transitions in the recordings (47). The frequencies obtained from these fits are roughly the same at each voltage and correspond to the slower and faster transitions observed in the single channel recordings. The buffer conditions were the same as described in Fig. 2.

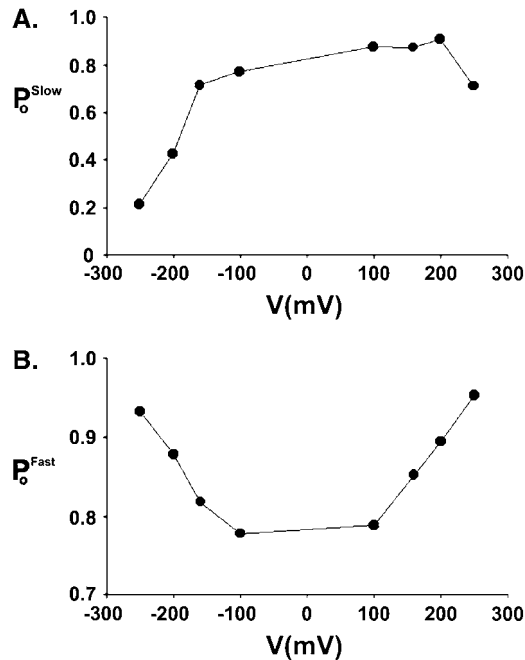


FIGURE 4 Voltage dependence of the open probabilities of the two different VacA transitions. For each transition, the opening and closing rate constants at each voltage were determined from the fits of the power spectral (Fig. 3) according to Eq. 1, from which the open probability was calculated. (A) The open probability associated with the slower transition exhibits a bell-shaped voltage dependence, achieving a maximal value at lower magnitude potentials and then decreasing at larger magnitude potentials. This relationship has been found only in channels that have a β -barrel transmembrane region (62), suggesting that the VacA channel may have a similar structure. (B) In contrast, the open probability associated with the faster transition increases with the magnitude of the holding potential, regardless of the polarity. This dependence is reminiscent of the high voltage relief of the block by a permeant ion observed in other ion channels (30,55,56).

seems to resemble the high voltage relief of the block by a weakly permeable ion that has been observed in squid axon and KcsA K^+ -channels (55,56) and in the channels formed by the anthrax toxin, PA₆₃ (57). Owing to the rapidity and the voltage dependence of these fast transitions, we hypothesize that this faster gating mechanism in VacA actually reflects a channel block by the permeant anion. In this view, at low electrical driving forces (near 0 mV), the ion does not have sufficient energy to overcome the energy barriers to exit and the channel frequently appears closed. At higher voltages, because of the possibility for ion-ion effects influencing conductance, two scenarios may occur: the larger electrical driving force may either reduce the barrier for exit and thereby directly reduce the time that the channel appears closed, or it could reduce the barrier for entry and promote a more multiply occupied channel, which would enhance the rate of exit of the ions as a consequence of repulsive ion-ion interactions (28,58,59).

In either event, in this scheme, the open probability curve for the faster transition would be expected to depend on the concentration and type of permeant anion but not on the type

of cation. If ion-ion interactions play a more dominant role, greater concentrations of anions would be expected to enhance the rate of exit since the channel would more likely be multiply occupied.

As shown in Fig. 5 A, the open probability curve for the faster transition is indeed different for different anions but the same for different cations. Moreover, there is a marked dependence of the open probability on the concentration of anions (Fig. 5 B), with greater concentrations of anions increasing the open probability as expected for a dominant influence of ion-ion interactions.

DISCUSSION

An understanding of the mechanism by which VacA causes its effects on cells likely requires a detailed knowledge of the electrophysiological properties of the pore it forms. Previous work demonstrated that VacA forms a voltage-dependent, anion-selective channel that exhibits a number of properties in common with known eukaryotic chloride channels (22). This study demonstrates that VacA channels in fact exhibit two of the most characteristic features of a specific group of these channels, those of the CIC family.

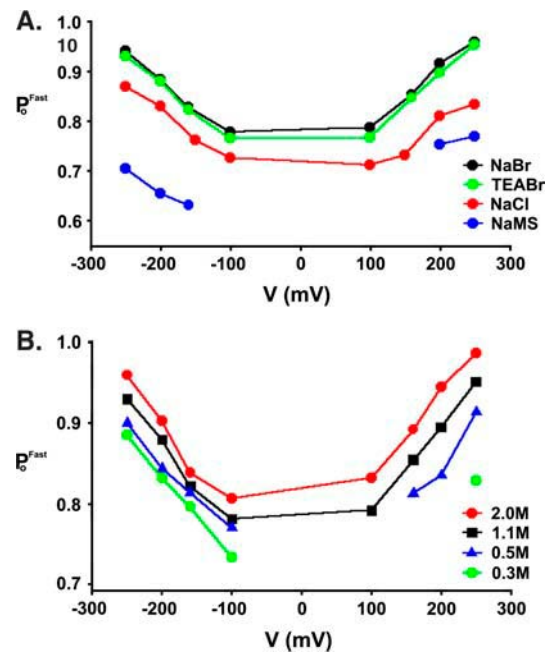


FIGURE 5 Dependence of the fast transition open probability on the permeant anion type and concentration. (A) The open probability of the faster transition is similar in NaBr as in TEABr but changes significantly in NaCl and NaMS. The ionic concentration for each measurement was 1.5 M. (B) This open probability also changes with the concentration of NaBr, showing lower values at lower concentrations. The concentrations indicated have been adjusted with the corresponding activity coefficients (76). With some of the ionic compositions, the currents through single channels at lower magnitude potentials could not be clearly differentiated from the noise of a closed channel and so these values are not included.

First, VacA exhibits an AMFE in its conductance and a mole fraction effect in its open probability, the latter of which has been, we believe, only observed previously in CIC channels (53). The molecular mechanisms underlying these properties in the CIC channels are not yet known, although the recently determined crystal structures of two members of the CIC family will likely prove useful in this regard (60). However, the fact that, in VacA channels, the maximum value of the open probability first occurs at the same molar ratio of anions at which the conduction is at a minimum (Fig. 1) suggests that, as is believed with the AMFE (28), the mole ratio dependence of the open probability in VacA may also have its origins in the simultaneous occupation of the pore by two or more permeant anions.

Second, like CIC channels (30,54), VacA exhibits two independent transitions, each with a unique voltage dependence. It should be clear that dimeric CIC channels contain two independent pores (61), whereas the VacA channel has only one. It is the pair of independent transitions that each individual CIC pore exhibits that is similar to what was observed here with the VacA channel. With the VacA channel, there is a slower gate operating over the timescales of tens to hundreds of milliseconds, showing a bell-shaped open probability voltage dependence, and a faster one operating over the timescales of hundreds of microseconds that shows a “U”-shaped voltage dependence. The complete open probability curve (Fig. 2) is the product of these two curves.

The bell-shaped open probability curve observed here with the slower VacA transition resembles the curves observed with a number of other pore-forming toxins and cellular channels, most notably the bacterial porins, all of which have β -barrel transmembrane pores (62). Although it is not yet clear whether a bell-shaped open probability curve is an exclusive property of β -barrel channels, this similarity suggests that at least a portion of the VacA pore may resemble a β -barrel structure. In this regard, it should also be mentioned that there is recent data to suggest that the VacA channel also has a transmembrane α -helical region (18,63, 64). Hence, if both predictions should prove correct, the pore structure of VacA would be unique among known ion channels in consisting of both a β -barrel and an α -helical bundle along its channel lumen.

In contrast to the slower transition though, the faster transition described here is suggested to be the result of a block by the permeant anion. This is based on the rapidity of the transition, the more frequent “opening” at larger voltages of either polarity (also found in other “blocked” channels (55–57)), and the dependence of these transitions on the permeant anion type and concentration (Fig. 5). To further investigate this hypothesis, we tested whether a rate theory model could explain this behavior. This theory has been frequently used to test for whether particular patterns of ion occupation within the channel that could occur at different concentrations of ions could be associated with a greater current (28,48–50). In a similar way, we associated

a singly occupied channel with the “closed” state and determined whether there was an energy profile that could account for the voltage dependence of the open probability. Fig. 6 shows that this rate theory approach, with the depicted energy profile (see Table 1 in the Methods section for the values of the fitted parameters), can indeed account for the observed voltage dependence. Moreover, increasing the concentration of ions in the model changes the calculated curve in the same manner as is experimentally observed (Fig. 6). Hence, the salient experimentally observed voltage and ion concentration dependencies of the open probability can be accounted for in this model, providing further support for the notion that there is likely only a single “proteinaceous” gating mechanism in the VacA channel, that which corresponds to the slower transition.

The properties of the VacA channel described here provide insights into the process by which VacA causes cell vacuolation. It is remarkable that, of all the properties that VacA could possibly have, it exhibits those that are uniquely characteristic of CIC channels. In addition to the shared properties described in this study, there are also a number of other properties that VacA and CIC channels both exhibit. For example, as noted above, both VacA and CIC channels are voltage dependent, remain anion selective under acidic conditions, and have a similar small conductance (~ 12 pS in 1 M salt for VacA channels (22) and 0.7 pS–8 pS in ~ 0.1 M salt for CIC channels (61,65–67)). In addition, the degree to which CIC channels select anions over cations (from 5:1 to 20:1 (68–70)) is, as found with VacA channels (5:1 (17)), only moderate (28), particularly when compared with the 10,000-fold selectivity of K^+ -channels for K^+ over Na^+ (71). The permeability sequence for CIC channels (though slightly different for different members (69)) is consistent with a “weak field strength” binding site for the permeant anion (69), also a feature of VacA channels (22). Finally, in the cell, VacA has been localized to the plasma membrane and membranes of the endosomes and lysosomes (42–44),

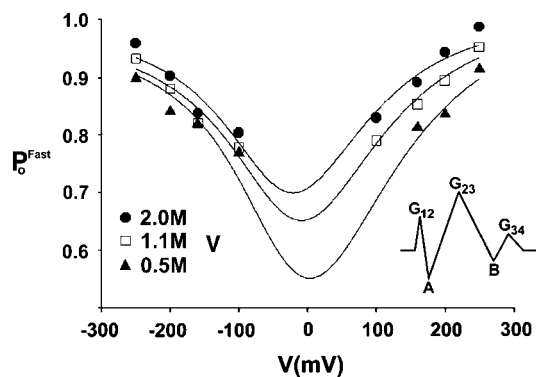


FIGURE 6 A rate theory model for the faster transition in which the closed state is associated with a singly occupied channel describes well the voltage and anion concentration dependencies of the open probability. The fit to each curve produced the same energy profile (shown in the inset and see Table 1). The labels in the energy profile are described in the legend to Table 1.

which are also the sites where CIC channels are localized (37,38,41). In fact, overexpression of one member of the CIC family, CIC-3, has been found to cause a massive vacuolation of acidic compartments, which are probably derived from lysosomes (37). To our knowledge, no other bacterial pore-forming toxin characterized to date exhibits this degree of similarity in behavior with an endogenous host ion channel. Thus, VacA, unlike any other known bacterial toxin, may function in cells by mimicking the behavior of a host ion channel, namely, a member of the CIC family of chloride channels.

Several examples are known in which the activity of a water-soluble bacterial protein mimics the activity of a host cell protein, including the tyrosine phosphatases, YopH and SptP, from *Yersina* spp. and *Salmonella* spp., respectively, and the GTPase-activating proteins, SptP and ExoS, from *Salmonella* spp. and *Pseudomonas aeruginosa*, respectively (45). The latter are believed to have become mimics of host enzymes through convergent evolution. The effects caused by any foreign protein in a host cell are clearly dependent both on the properties it exhibits and the response of the endogenous cellular components. In this context, the cellular response to formation of VacA channels, in mimicking endogenous CIC channels, may thus involve activation of the repertoire of cellular components that are usually involved in maintaining a proper level of endosomal acidity.

The common model of the mechanism by which VacA causes vacuolation (which has some evidentiary support (19, 20,42,44,72–75)) posits the formation of the VacA channel in the endosomal/lysosomal membrane, which results in an influx of anions into the lumen and a reduction of the membrane potential. This would activate the electrogenic proton ATPase, which would increase the luminal acidity and lead to an accumulation of exogenous weak bases inside the compartment, resulting in an increase in the osmotic pressure. The constancy of the anion selectivity of the VacA channels at acidic conditions would ensure a continually increasing pressure as the intraluminal pH decreases. This proposed capacity of VacA to change luminal acidity, in fact, parallels a proposal for the mechanism by which CIC channels regulate endosomal acidity (33). The salient difference in the models for the two channels is the point at which they are proposed to close, with the VacA channel remaining constitutively open under conditions at which the CIC channels would close. Where known, the voltage dependence of the open probability (of the slower transition) of the CIC channels is sigmoidal, with a typical $V_{1/2}$ magnitude of ~ 50 mV. Since VacA likely remains open for all voltages between +150 mV and –150 mV, there is a large range of potentials at which the VacA channels remain open but the CIC channels would be closed. This model for VacA activity is therefore consistent with the voltage dependency for the gating of the VacA channel described here as well as the suggested mimicry in its behavior. This mimicry by VacA may thus be another example of a microbial organism adapting to life in a eukaryotic host through a process that

involves imitating the functional properties of a key regulatory component.

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