Complex Inhibition of OmpF and OmpC Bacterial Porins by Polyamines*

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The effects of four polyamines (putrescine, cadaverine, spermidine, and spermine) on the activity of bacterial porins OmpC and OmpF were investigated by electrophysiology. Membrane vesicles made from the outer membrane of Escherichia coli strains expressing only OmpC or OmpF were reconstituted into liposomes probed by patch clamp. The channel activity was recorded in control solutions and in the presence of increasing concentrations of a specific polyamine. In all cases, concentration- and voltage-dependent inhibitory effects were observed. They include both the suppression of channel openings and the enhancement of channel closures as well as the promotion of blocked or inactivated states. OmpF and OmpC, although highly homologous, have distinct sensitivities to modulation, especially by spermine. This compound inhibits OmpF in the nanomolar range, which is in agreement with its potency on eukaryotic channels. Putrescine was the least effective (upper millimolar range) and also had inhibitory effects qualitatively distinct from those exerted by the other polyamines. The compounds appear to bind to at least two distinct binding sites, one of which resides within the pore. The potencies to this site are lower when the polyamines are applied from the extracellular side than from the periplasmic side, suggesting an asymmetric binding site.

Polyamines are a class of naturally occurring polycationic molecules produced through complex pathways involving de-carboxylations of ornithine, arginine, or lysine (1, 2). The most ubiquitous are spermine, spermidine, cadaverine and putrescine. With the exception of spermine, which is associated exclusively with eukaryotes, the other three are endogenous to both eukaryotic and prokaryotic cell types. Polyamines have been implicated in a wide range of biological phenomena (1, 2). Polyamines are associated with the outer membrane of E. coli, possibly through their interactions with the lipopolysaccharides (11). Although polyamines have not been measured directly in the periplasmic space between the outer and cytoplasmic membranes, they are likely to accumulate in this compartment during their synthesis and transport (12–14). An arginine decarboxylase involved in the production of putrescine is located in the inner periplasmic space (12), and a lysine-cadaverine exchanger of the cytoplasmic membrane participates in the extrusion of cadaverine (13). Thus, polyamines appear to reside in the vicinity of the major pore-forming proteins of the outer membrane, the porins. Porins are trimeric channels characterized extensively at the biochemical, structural, and genetic levels (15). They are the only ion channels whose structure is known at atomic resolution (16). Each monomer is a 16 β-stranded barrel with a pore that allows the passage of water-soluble compounds of molecular mass up to 600 Da. Two major porins, OmpC and OmpF, are slightly cation-selective and share a high degree of homology with each other (~60%).

Porins are traditionally believed to be permanently open pores. Playing the main role of molecular filter, they are largely responsible for the overall permeability of the outer membrane. However, the regulation of porin function by potentially physiologically relevant factors is still poorly understood. We showed recently that polyamines inhibit chemotaxis and flux of β-lactam antibiotics through porins and thus decrease the permeability of the outer membrane (17). In electrophysiological experiments, we also demonstrated that cadaverine induces closures of porins (18). This study was limited to the investigation of a single polyamine on heterotrimers of OmpF and OmpC. In this report, we have extended our investigations to the modulation of homotrimers of OmpF or OmpC by all four polyamines (spermine, spermidine, cadaverine, and putrescine) and showed that the effects are complex. In conjunction with our previous work on polyamine inhibition of site-directed OmpC mutants (19), the present study allows us to present a model for the possible molecular interactions between porins and polyamines.

MATERIALS AND METHODS

Strains and Chemicals—E. coli K12 strains AW738 (expressing OmpF only) and AW739 (expressing OmpC only) (20) were used. Tryptone growth medium (T-broth) contained 1% tryptone (Difco Laboratories) and 0.5% NaCl. Cadaverine, putrescine, spermidine, and spermine were purchased from Sigma as the hydrochloride forms and thus did not affect the pH of the solutions in which they were dissolved. Azolectin (phosphatidylcholine) was from Sigma, and all other chemicals were from Fisher Scientific.

Preparation of Reconstituted Liposomes—Cells were grown to mid-log phase in T-broth at 37 °C, harvested, and lysed by two passages through a French press at 16,000 p.s.i. Outer membrane fractions were purified by sucrose gradient centrifugation as described (21) and stored at −80 °C. The biocinchoninic acid method (Pierce) was used for determination of protein concentrations.

An aliquot of native membrane was mixed with sonicated azolectin (10 mg/ml) at a protein:lipid ratio of ~1:1.700 (w/w) and reconstituted according to a dehydration-rehydration protocol (21). Electrophysiologically experiments were performed on liposome blisters, as described (21).

Electrophysiological Recording—Current measurements were made...
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using standard patch-clamp techniques (22) with an Axopatch 1D amplifier (Axon Instruments). Pipettes were filled with 150 mM KCl, 5 mM Hepes, 0.1 mM K-EDTA, 10 mM CaCl₂ (pH 7.2) and had an initial resistance of 10 megohms. Seals of 0.5–1.0 gigahms were typically obtained on blisters because of the presence of a large number of predominantly open porins in the patches. After patch excision by air exposure, recordings were made in symmetric solutions. The data were filtered at 2 kHz with an eight-pole Bessel filter (Frequency Devices) and stored on VCR tapes (Instrutech). For data analysis, specific segments of data were filtered at 1 kHz and digitized at a 100-μs sampling interval. Data acquisition and analysis were performed with personally developed software using Axodac (Axon Instruments).

Data Analysis—Because of the clustering and high open probability of porins, patches typically contain a large (20–80) number of predominantly open porin monomers that lead to seal currents of the order of 40–150 pA at pipette voltages of −60 mV. In control conditions, the current dwells at the preferred leak current level, arbitrarily chosen as a base line (BL) in Fig. 1. The values of single channel amplitudes were obtained from inspection of individual closures of single or multiple channels and plotted against voltage. A single channel conductance of ~30 picoSiemens was deduced from the best fit through all points, on the assumption that larger current amplitudes are integral multiples of the single channel amplitude. Our working hypothesis is that the smallest unit of conductance represents a porin monomer (for further discussion, see Refs. 19 and 23).

Opening transitions from the base line are typically so rapid that they rarely show as canonical square top events. Thus, kinetic analysis was done only for the closures with an algorithm that uses the half-amplitude criterion (19) to classify events lasting for more than 300 μs as closures of 1, 2, . . . n channels. The numbers and average durations of such events are computed, as well as the average time spent at the preferred base-line level, <tBL>. In control conditions, the average length of closures is on the order of 0.5–1 ms, whereas <tBL> varies from ~20 to 150 ms, depending on the number of open channels in the patch.

Polyamines promote prolonged closures where one or a few channels remain in a long lived nonconducting state while additional channels show fast closing kinetics. We define as a “long closure of at least n channels” an event that (i) lasts longer than three times the S.D. of the mean closed time; (ii) has at least n closures during its entire duration; (iii) can be interrupted by closures of additional channels regardless of their durations; and hence (iv) is terminated only by return to a current level corresponding to a larger number of open channels (i.e., an opening transition that also lasts at least three times the S.D. of the mean closed time). In the diagram representing these events in idealized form in Fig. 4A, long closures of at least one channel include events a and c; long closures of at least three channels include events b and c; and long closures of four channels include event b. Note that event b is not counted as a long closure of at least one channel because it is already counted as part of event a. Regular closures are represented as the unlabeled short lived upward deflections.

RESULTS

Kinetic Signature of Modulated Channels—Representative traces of porin activity in control conditions are shown in Fig. 1, A and C. The current dwells at a preferred level, labeled BL (base line), which represents the total amount of current flowing through a large number (20–80) of predominantly open channels. The occasional departures of these channels into closed states lead to upward deflections of the current trace. Each tick on the right of the traces indicates the current level reached after the closure of one or multiple porin monomers. Downward deflections from the base line are frequent, rapid, and often unresolved openings from an unknown number of additional, predominantly closed, channels. These two types of activities most likely represent channels that occupy different stable conformational states that are interconvertible. We know that they originate from a single type of channel because both the closing and opening kinetics from the base-line level can be affected by single amino acid mutations of the OmpC protein (19, 24). In addition, opening transitions are always more clearly observable at positive pipette potentials than negative ones and more so in OmpC than in OmpF.

The perfusion of spermine to the bath side of the membrane leads to drastic changes in the kinetics of both opening and closing events (Fig. 1, B and D). The polyamine promotes closures of both OmpC and OmpF porins. In addition, closures tend to be prolonged and to involve a larger number of channels. The long lasting closure of many channels sometimes makes the current dwell at a new level, from which closures and reopenings of additional channels can be observed. In most cases, multiple channels close and reopen in concert. Similar kinetic effects have been observed when spermidine or cadaverine is applied, but not in the case of putrescine. Channels, however, are not completely resistant to putrescine, since inhibition of openings and apparent instantaneous block or inactivation have been observed (see below).

Suppression of Openings—All polyamines reduce the occurrence of openings (downward deflections) from the base-line level at negative pipette potentials (Fig. 1). This effect is further documented in Fig. 2 for putrescine and spermine. Amplitude histograms were generated from 20-s recordings of OmpC (panels A and B) or OmpF (panels C and D) activity in control (solid lines) and modulated conditions (dotted lines) at a pipette voltage of −60 mV. They show a large peak centered around 0 pA, which corresponds to the base-line level. In Fig. 2, only the foot of this large base-line peak is displayed. We have arbitrarily chosen to represent as negative values the amplitudes that correspond to openings, whereas those of positive values are from closures. Because the gating of porins is so fast, closures and openings tend not to show as well defined peaks on amplitude histograms but rather as shoulders of the base-line peak. The decreased occurrence of openings in the presence of polyamines is reflected in the amplitude histograms by the disappearance of the left shoulder and the concomitant narrowing of the base-line peak in the negative range. This effect can

1 The abbreviation used is: <tBL>, average time spent at the preferred base-line level.
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Fig. 2. Amplitude histograms generated from 20-s recordings of OmpC channels (panels A and B) or OmpF channels (panels C and D). Solid lines are from data obtained in control conditions. Dotted lines are from data obtained in the presence of bath-applied putrescine (30 mM, panels A and C) or spermine (1 mM in panel B; 0.1 mM in panel D). Only the foot of the base-line peak centered at 0 pA is shown. Negative values represent openings; positive values represent closures. Bins are 0.1 pA.

The experiment of panel A yields an I_{500} of −1.5 in control solutions and −0.5 in the presence of 30 mM putrescine; in the histograms of panel B, I_{500} is −1.5 in control solution and −0.8 in the presence of 1 mM spermine. Thus, at the 500 points/bin mark there is a shift of about 0.7 to 1.0 pA of the left shoulder of the histograms when the channels are switched from control to polyamine-containing solutions. Shifts of this magnitude have been seen consistently for OmpC in 17 experiments and are significantly different from zero (paired t test, \( p < 0.005 \)). Their values do not seem to be strongly correlated with the nature or the concentration of the polyamines. It appears that the effect is already profound at the lowest concentrations that elicit modulation of the closing kinetics. At low concentrations, the suppression of the openings has the same voltage dependence as the modulation of the closing kinetics (see below), but high concentrations of polyamines can inhibit openings even at positive pipette potentials.

In the case of OmpF, shifts in the I_{500} values are of lesser magnitude (in Fig. 2C, I_{500} is −1.2 in control and −0.8 in 30 mM putrescine). They average 0.4 pA for putrescine, cadaverine, and spermidine \( n = 10 \) but are not observed in the presence of spermine. In panel D, I_{500} are −1.3 and −1.4 for control and modulated conditions, respectively. This lack of effect on the opening kinetics is observed even though the channel closing kinetics are clearly modulated, as indicated by the decrease in peak size at 0 pA and the drastic broadening of the base-line foot in the positive range. The lack of inhibition of OmpF openings by spermine has been seen in five independent patches. This distinct behavior of spermine and OmpF suggests that the binding site controlling suppression of the openings is distinct from the site involved in closing kinetics modulation.

Stabilization of Closed States—Fig. 2B and D shows that spermine promotes also a widening of the foot of the base line in the positive range because of an increase in the number of closing transitions involving one or two channels. When the number and duration of closures become large enough, distinct peaks of positive values can be seen on the amplitude histograms (19). These effects are observed also with spermidine and cadaverine (but not putrescine) and are better documented with the use of an analysis algorithm based on event detection, as described below.

We have defined an operational parameter that allows us to compare the effects of spermine, spermidine, and cadaverine on OmpC and OmpF across patches. This parameter, \(<f_{BL}>\), is the average time that the current dwells at the base-line level, between successive closures, irrespective of whether the closures involved one or multiple channels and ignoring openings of additional channels from this base line. The ratio of this parameter in the presence and the absence of polyamine is plotted against polyamine concentration in Fig. 3. The increased frequency of closures in the presence of polyamines is reflected as a decrease in this ratio. The most potent inhibitor, spermine, is effective in the submillimolar range for OmpC and in the submicromolar range for OmpF. Cadaverine is the least potent and requires tens of millimolar to exert its effect; spermidine is intermediate. Both porins are equally sensitive to cadaverine and spermidine, but OmpF is more sensitive to spermine than OmpC. Nanomolar concentrations of spermine have been seen (17) or OmpF channels (but not putrescine) and are better documented with the use of an analysis algorithm based on event detection, as described below.

A hallmark of porin modulation by polyamines is the appearance of prolonged closures that involve the simultaneous, often cooperative, transitions of many channels (see diagram in Fig. 4A). In Figs. 4, B and C, we have plotted the number of such events in a 20-s recording versus the minimum number of channels that are closed during such events. Only non-zero values are represented by symbols. The essential features illustrated with this figure are (i) that the number of closures is increased with increasing concentrations of polyamines; (ii) that the number of channels involved in such closures is increased with polyamine concentrations; and (iii)
that long closures involving many channels are seen only in the presence of polyamines and are absent in control conditions. The average time of such closures ranges from a few milliseconds to a few seconds, about 1–4 orders of magnitude longer than the average time of regular closures. Prolonged closures are seen more frequently at high polyamine concentrations and may represent the occupancy of temporarily inactivated states. They are most prominent in the presence of spermine.

On a few occasions, we have observed sudden bursts of flickering activity of OmpF channels in the presence of spermidine or spermine (even at concentrations as low as 10 mM spermine). These bursts often interrupt the typical modulated activity, such as described above, and are clearly distinct from it because of the high frequency of flickers between a large number of conductance levels and the concomitant loss of prolonged closures such as depicted in Fig. 4A. These bursts resemble the type of flickering activity which might be expected from a true ion channel block (25). But it may also be that they represent other, yet rarely visited, modulated states. They occurred in less than 10% of the patches.

The kinetic effects described in Figs. 3 and 4 are seen even though there is a decrease in the number of active channels after polyamine perfusion because of the apparent instantaneous loss of some of the open pores. At this point, we cannot distinguish whether this phenomenon is due to pure block or to the stabilization of deep closed states that represent a quasi-irreversible channel inactivation. We quantify this effect by measuring the current flowing through all of the open porins upon stepping the pipette voltage from 0 to −60 mV (seal current). The extent of the instantaneous block or inactivation is dependent on the rate of bath perfusion of the polyamine, as shown in Fig. 5A. Slow perfusion (8 min) of identical polyamine concentrations will induce the loss of more channels than fast perfusion (2 min). This phenomenon does not appear to be at equilibrium because the same amount of seal current inhibition cannot be achieved after a 6–10-min incubation at 0 mV following a 2-min perfusion. This hysteresis and the variability in the time dependence of onset of this instantaneous inhibition can create some variability in the kinetic effects observed from the population of remaining channels. Thus, for the plots of Fig. 3, we have used only patches that showed a limited reduction in the number of channels during the course of the experiment. Occasionally, kinetic effects are not evident after polyamine perfusion, simply because the number of “lost” channels is so large that even the modulated activity of the few remaining channels still has slower kinetics than that of the large number of channels in control solutions. Such experiments have not been used in the analysis of $t_{95}$ even though inhibition by polyamines was evident.

This instantaneous and irreversible inhibition of channels is induced by all polyamines, in concentration ranges similar to...
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...those observed for the kinetic effects (Fig. 5B). It is noteworthy that putrescine can produce such inhibition but no measurable decrease in $t_{\text{int}}$ (actually, the $t_{\text{int}}$ values are sometimes slightly increased because of the smaller number of active channels). Most likely, the increased closing frequency intrinsically induced by putrescine is not sufficient enough to override the decreased closing frequency caused by the loss of active channels. This observation highlights the fact that $t_{\text{int}}$ should be used only to document the increased gating frequency and not to derive closing rate constants. If channels were not permanently inactivated or blocked, the observed $t_{\text{int}}$ would be even much smaller.

Voltage Dependence of Polyamine Inhibition—The apparent instantaneous block or inactivation by polyamines is observed only at negative pipette potentials when the compounds are applied to the bath side of the patch (Fig. 5C). From previous work, we know that the periplasmic side of the porin faces the bath solution in our reconstituted system (26). This asymmetric voltage dependence is also observed in the case of the kinetic effects.

In Fig. 6, the fold increase in the total number of closures in a 20-s recording is plotted against four voltages in control solutions and in the presence of a representative concentration of spermine, spermidine, and cadaverine. For both porin types, an increase in the frequency of closures and stabilization of closed states are more pronounced with higher negative pipette potentials. In the positive pipette range, the porin behavior is reversed, and modulation is seen only at positive pipette potentials, whereas spermine increases this number by −14 (0.1 mM spermine, OmpF) or −20 (1 mM spermine, OmpC) at −60 mV. No significant changes in the number of closures are observed at positive pipette potentials.

To investigate the effects of polyamines applied from the pipette side, we have compared groups of independent experiments which were carried out with either control or polyamine-containing solutions in the pipette. From previous work, we know that the pipette side corresponds to the natural extracellular side (23, 26). Fig. 7, C and D, plots the average total number of closures in 20-s recordings obtained from many patches in control solutions (hatched bars), or with 30 mM cadaverine in the pipette (open bars), or with 1 mM spermine in the pipette (gray bars). Significant effects were observed only with spermine, but not cadaverine. (Significance levels are given in the legend.) In addition, the voltage dependence is reversed, and modulation is seen only at positive pipette potentials. Thus, the polyamine appears attracted to its binding site by a negative voltage on the membrane side opposite to where it is applied.

If modulation was simply due to surface charge effects or accumulation of charged compounds in the channel mouth, we would anticipate that the extent of inhibition would be independent of the site of polyamine application. Even at positive pipette potentials, pipette-applied spermine produces only a −6-fold (OmpF) or −3-fold (OmpC) increase in the total number of closures. These numbers are much smaller than those obtained with bath-applied spermine, suggesting that spermi-
ine is more effective from the periplasmic side than the extracellular side. In addition, the effectiveness of the polyamines is even more porin-dependent than in the case of bath-applied polyamines. Thus, the extent of the effects can be varied by a combination of three parameters: the porin type, the polyamine type, and the side of application. Stronger effects are seen with OmpF than OmpC, spermine than cadaverine, periplasmic exposure than extracellular one.

Openings are also suppressed when cadaverine or spermine is present in the pipette. The \( I_{500} \) values obtained from 20-s recordings of OmpC activity in control solutions averaged \(-2.17 \) pA (\( n = 6 \)) but were decreased to \(-1.00 \) pA in the presence of 1 mM spermine (\( n = 4 \)) and \(-0.85 \) pA in the presence of 30 mM cadaverine (\( n = 4 \)). Two-sample \( t \) tests show that the difference between the values in the absence and the presence of either polyamine is statistically significant (\( p < 0.005 \)).

**DISCUSSION**

Polyamines have recently been discovered to regulate the activity of eukaryotic channels (3–10). In the present study, we demonstrate that this role can be extended to prokaryotic channels, since polyamines can inhibit homotrimers of the \( E. coli \) major porins, OmpC and OmpF. The potencies of the compounds on porins are similar to those found with eukaryotic channels, but the molecular mechanisms of inhibition appear very different. In the case of porins, the potency follows the series: spermine > spermidine > cadaverine > putrescine. In both prokaryotes and eukaryotes, spermine is an effective compound eliciting inhibition of channels even at submicromolar concentrations. The estimated concentration of free spermine in eukaryotic cells is on the order of a few tens of micromolar, high enough for this compound to play a role as a natural regulator of some channels (27). However, spermine is not synthesized by bacteria (1) and thus is not an endogenous modulator of channels in this system. This is not the case for the other three polyamines investigated. The calculated whole cell concentrations range from \(-0.05 \) to \(10 \) mM for spermidine (11, 28) and are on the order of 10–50 mM for cadaverine and putrescine (11). These values are within the effective range for porin modulation (Fig. 3). Although not known, the periplasmic concentrations of polyamines may be higher because of the presence in the cell envelope of enzymes and transporters involved in their synthesis and export (12, 13). Polyamines also seemingly associate with the negatively charged lipopolysaccharides of the outer membrane (11) and could reach high amounts in the vicinity of the porins. In addition, the excretion of cellular putrescine is promoted by a sudden increase in medium osmolarity (29), and that of cadaverine is induced by a drop in external pH or oxygen tension (13). Thus, the changes in polyamine concentrations in the cells envelope in response to environmental conditions may influence the activity state of porins and provide an avenue for modulation of outer membrane permeability. The modulation of porins by polyamines would then constitute a physiologically relevant phenomenon, in particular in stressful situations. Preliminary experiments suggest that \( \beta \)-lactam fluxes through porins are indeed inhibited when cells are grown at low pH, a condition of increased cadaverine synthesis.\(^2\)

The inhibition of porin channels by compounds in the submillimolar range has not been shown previously. At this point, it is not clear whether the higher potency of spermine with respect to other polyamines is the result of its longer chain or its increased positive charge. Molecular length may be a determinant factor, since cadaverine shows a slightly higher potency that the shorter, but still divalent, putrescine. But ionic interactions between porins and polyamines also play an important role, as evidenced by the voltage and pH dependence of inhibition (18). The replacement of aspartate residues on the L3 loop of OmpC by glutamines yields channels with severely compromised modulation by polyamine (19). The involvement of acidic residues has also been demonstrated in polyamine binding to eukaryotic channels (3, 4, 30–32) and in the association of biogenic amines with their receptors (33).

It has been proposed that polyamines plug the long \( K^+ \)-selective pore of heart inward rectifier channels by binding to multiple sequential sites (34). The overall effect is that of a voltage-dependent block. We propose that the molecular mechanism for inhibition of porins is different. In patches containing a few channels, open channel block typically leads to one of the following three patterns, depending on the relative residence time of the drug inside the channel with respect to the channel's intrinsic gating kinetics (25). In mechanism 1, slowly dissociating blockers lead to a quasi-irreversible loss in the number of channels. In mechanism 2, fast dissociating blockers leave the total number of active channels in the patch intact but decrease the single channel conductance. In mechanism 3, blockers with intermediate dissociation kinetics lead to bursts of fast flickering activity. The most consistently observed behavior of porins in the presence of polyamines does not include a reduced single channel conductance or fast flickering activity, ruling out mechanisms 2 and 3. Bursts of flickering activity have been seen rarely, and slight decreases (<20%) in single channel conductance are only observed occasionally at high polyamine concentrations (mostly in the case of spermidine). But the observed increased closing frequency and prolonged closed times of the modulated channels are indicative of an effect of the polyamines on the intrinsic rate constants controlling channel opening and closure. In addition, the channels remain highly cooperative: simultaneous closing (followed by simultaneous reopening) of a large number of channels is observed frequently and is unlikely to represent the block and unblock of many channels at exactly the same time. The suppression of openings from a population of mostly closed channels would also not be expected in an open channel block mechanism. Therefore, we propose that the main mechanism for modulation of porin channels involves an alteration in the intrinsic rate constants for gating, leading to stabilized closed states.

We cannot rule out that block by a slowly dissociating molecule (mechanism 1) is responsible for the instantaneous loss of channels after polyamine perfusion. Such a mechanism would require that the polyamine binds with very high affinity to the open channel. Loss of channels is seen even in the presence of cadaverine and putrescine, which both have low binding affinities. This effect still occurs in the L3 mutants that have lost the modulation of closing kinetics,\(^3\) indicating that it does not necessitate negative residues present inside the pore. This result would be surprising if open channel block did take place. For these reasons, we believe that the polyamine-induced instantaneous channel loss is more likely to be caused by the rapid inactivation of some channels.

At this point, the simplest mechanism that is supported by all of our data is that polyamines exert allosteric effects on kinetic rate constants such that closed states are stabilized. Allosteric mechanisms are also proposed for the \( N \)-methyl-D-aspartate and acetylcholine receptors (6, 8). A related phenomenon was described for the modulation of mitochondrial VDAC channels by polyanions (35). In this case, the accumulation of

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\(^2\) H. Samartzidou and A. H. Delcour, unpublished data.

\(^3\) N. Liu and A. H. Delcour, unpublished data.
impermeant and highly charged compounds in the channel mouth was held responsible for the increased voltage dependence of closure. We do not believe that such an unspecific effect is responsible for polyamine modulation of porins because the extent of inhibition is clearly dependent on the site of application of the chemicals. This asymmetry, the difference in sensitivity between the two homologous porins, and the high potency of spermine are suggestive of specific binding. The lack of effect of cadaverine from the pipette side is not surprising, considering that this compound is already much less potent than spermine when bath-applied.

What is the potential location of the binding site(s)? From our previous work with mutants (19) and the experiments presented here, we propose the following. The increased closing kinetics observed from a population of predominantly open channels is controlled by binding of the polyamines to an asymmetric site within the pore that in OmpC involves Asp-105 and Asp-118 (19), but not Glu-109. Access to this site is possible from both periplasmic and extracellular sides, but potency is higher from the periplasmic side. The suppression of openings from predominantly closed channels is due to polyamine binding to a different site because (i) it is not affected by mutations on the L3 loop (19), and (ii) it is not induced by spermine binding to OmpF even while a strong modulation of closing kinetics observed from predominantly open channels is due to polyamine modulation of porins because the extent of inhibition is clearly dependent on the site of application of the chemicals. This asymmetry, the difference in sensitivity between the two homologous porins, and the high potency of spermine are suggestive of specific binding. The lack of effect of cadaverine from the pipette side is not surprising, considering that this compound is already much less potent than spermine when bath-applied.

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