Simple molecular model for the binding of antibiotic molecules to bacterial ion channels

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A molecular model aimed at explaining recent experimental data by Nestorovich *et al.* [Proc. Natl. Acad. Sci. USA **99**, 9789 (2002)] on the interaction of ampicillin molecules with the constriction zone in a channel of the general bacterial porin, OmpF (outer membrane protein F), is presented. The model extends T. L. Hill's theory for intermolecular interactions in a pair of binding sites [J. Am. Chem. Soc. **78**, 3330 (1956)] by incorporating two binding ions and two pairs of interacting sites. The results provide new physical insights on the role of the complementary pattern of the charge distributions in the ampicillin molecule and the narrowest part of the channel pore. Charge matching of interacting sites facilitates drug binding. The dependence of the number of ampicillin binding events per second with the solution pH and salt concentration is explained qualitatively using a reduced number of fundamental concepts. © *2003 American Institute of Physics.*
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I. INTRODUCTION

One of the factors influencing the bacterial resistance to antibiotics is the outer membrane permeability. Recently, Nestorovich *et al.*[1] have been able to resolve single ampicillin molecules moving through a channel of the general bacterial porin (OmpF, outer membrane protein F) reconstituted in a planar lipid bilayer. OmpF porins facilitate the translocation of many hidrophilic solutes and are believed to be the principal pathway for the permeation of β-lactam antibiotics. High resolution conductance recordings show that ampicillin, together with other zwitterionic penicillins as amoxicillin, strongly interact with the residues at the constriction zone of the OmpF channel, and this specific interaction appears to facilitate drug binding and penetration through the pore. Molecular modeling suggests that the charge distribution of the ampicillin molecule complements the charge distribution at the narrowest part of the bacterial pore.[1–3] In this paper, we study theoretically the equilibrium binding (translocation across the channel pore is not considered) and provide qualitative explanations for the dependence of the number of ampicillin binding events per second on the solution pH and salt concentration.[1] This experimental magnitude gives information on the antibiotic docking in the pore.[2] The theoretical model is based on Hill's approach for the matching pairs of interacting sites on two large molecules,[4,5] and has the advantage of simplicity at the price of introducing a limited amount of structural information. The importance of the complementary pattern of the functional groups in the ampicillin molecule and the monomeric pore is clearly emphasized using a reduced number of fundamental concepts.

II. PHYSICAL MODEL

Figure 1 shows a schematic construction for the ampicillin molecule at the constriction zone of the ion channel pore. We consider that the three OmpF channels forming the trimer behave independently in the ampicillin blockage.[1] Also, it is assumed that the interactions between the charged groups of ampicillin and the charged residues at the pore constriction are principally of electrostatic origin.[1–3] The distribution of negative and positive charges attached to the narrowest part of the pore surface[1,3] is simulated by one effective functional group with a pK value typical[6] of the acid residue in the Glu side chain ($pK_{P1}=4.2$) and one effective functional group with a pK value typical of the basic residue in the Arg side chain ($pK_{P2}=12.5$), respectively.[6] The pore center is blocked by an ampicillin molecule[7] having a carboxylate group with $pK_{A1}=2.5$ and an ammonium group with $pK_{A2}=7.3$. Hydrophobic interactions between the channel pore and the ampicillin molecule may also be important (the phenyl group of ampicillin could be stabilized by the local hydrophobic environment in the pore[1,2]) but they are out of the scope of the present model. However, the possibility that small salt ions compete with ampicillin for binding to the fixed charge groups in the pore will be considered to account for the decrease in the number of ampicillin blockages with the salt concentration.[1] Although this experimental observation might be ascribed to the Debye screening of the fixed charge distribution at the constriction zone, the application of continuous models over spatial regions less than 1

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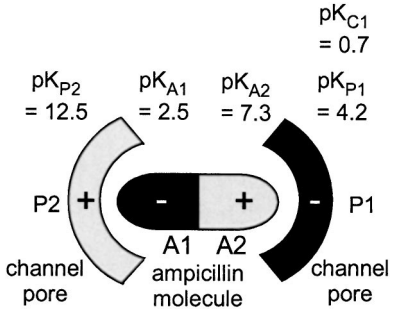

FIG. 1. Schematic description of the pore blockage by ampicillin showing the complementary pattern of functional groups. The distribution of negative and positive charges attached to the narrowest part of the pore surface is simulated by only one effective functional group with a pK typical of the acid residue in the Glu side chain ($pK_{P1}=4.2$) and only one effective functional group with a pK typical of the basic residue in the Arg side chain ($pK_{P2}=12.5$), respectively. The pore center is blocked by the ampicillin molecule having a carboxylate group with $pK_{A1}=2.5$ and an ammonium group with $pK_{PA2}=7.3$. For the salt cation binding to the acid group in the pore, we assume $pK_{C1}=0.7$.

nm thick could be questionable.[8–10] We have therefore introduced an effective binding constant in the model to account for the limited translation of a small mobile ion in the vicinity of a fixed group of opposite charge at the pore constriction. Since the aqueous pore formed by the ion channel contains more salt cations than anions,[2] we will consider as a first approximation that, for high enough salt concentrations, the proximity of the cation to the negative fixed charge at the pore constriction effectively screens this charge, making thus less likely the ampicillin blockage. (The pore is only slightly selective to the cation,[8–10] and it would then be possible that both the cation and the anion contribute to the screening of their respective oppositely charged groups at the pore. However, we will show later that including the effect of only one salt ion suffices to explain qualitatively the observed phenomena.)

Transmembrane carboxylic amino acids have been considered as relatively strong cation coordinating residues in the literature.[11] Also, adsorption of small inorganic cations (lithium, sodium, potassium) to acidic lipid membranes is well documented,[12] giving intrinsic binding constants in the range 0.1–2.0 M^{-1}. These values would give dissociation constants in the range 0.5–10 M. On the other hand, dissociation constants of sodium bound to carboxyl groups in macromolecules distribute over a lower range of values, 0.01–1 M.[13] We will assume $pK_{C1}=0.7$ for the potassium binding in the calculations, which corresponds to a dissociation constant $10^{-pK_{C1}}=0.2$ M lower than those found in acidic lipid membranes but in the midrange of the values reported for macromolecules. The effect of increasing the dissociation constant up to 1 M will also be considered.

Figure 2 shows the hydrogen and potassium occupation of the relevant sites participating in the ampicillin binding to the pore. The interacting sites are treated as two independent pairs (P2-A1 and A2-P1 or A2-C1; see Fig. 1) of binding sites. The complementary pattern of functional groups is clearly shown. In principle, the system has a total number of 2 (P2)×2 (A1)×2 (A2)×3 (P1)=24 states (see Figs. 1 and 2). However, the requirements imposed by the pK series $pK_{C1}<pK_{A1}<pK_{P1}<pK_{A2}<pK_{P2}$ on the hydrogen and potassium occupation states of the binding sites, together with the experimental pH and salt concentration ranges employed,[1] reduce the number of available states down to only nine in practice (for example, the states with site A2 unprotonated but site P1 protonated can be neglected because $pK_{P1}=4.2<7.3=pK_{A2}$; similar arguments apply also to the other excluded states). The nine states of Fig. 2 correspond to the different manners of occupation of the sites by H^+ and K^+ and can now be averaged over to obtain the properties of the system.

To obtain the relevant theoretical equations from the idealized construction of Figs. 1 and 2, we consider each pair of sites as a system in a grand canonical ensemble.[4,5] Figure 2 shows the contributions of the different occupation states to the grand partition function of the system formed by the two ion pairs (see, e.g., Refs. 14–16 for relevant applications of the grand canonical formalism to biochemical systems). The matching charge distributions of Fig. 1 are favored with respect to other manners of H^+ and K^+ occupation of the sites: note the factor $e^{u/kT}>1$ in the terms of the grand partition function corresponding to oppositely charged binding sites, where u is the interaction energy between matching sites on a pair, T is the temperature and k is the Boltzmann constant.

Note that the free, unblocked pore is not included in Fig. 2. The experimental data for ampicillin modulation of the electric current[1] show that the equilibrium probability of

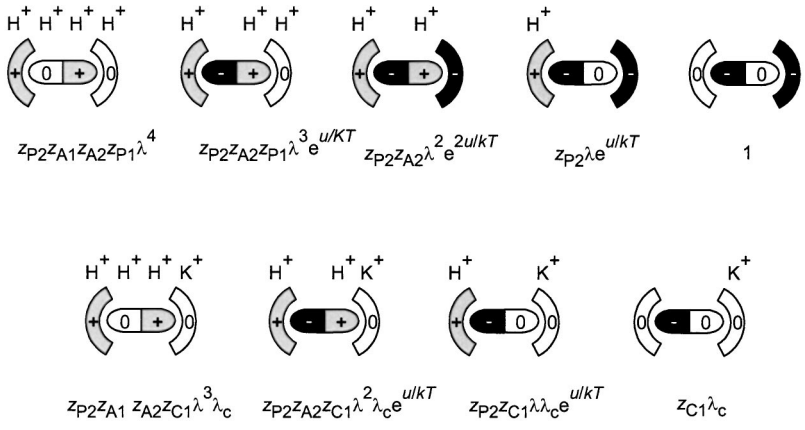

FIG. 2. States for hydrogen and potassium occupation of the binding sites, together with the respective contributions to the grand partition function. The interacting sites are treated as two independent pairs (P2-A1 and A2-P1 or A2-C1) of binding sites. The attractive force between the matching charge distributions in the pairs of Fig. 1 is energetically favored ($u>0$) respect to other manners of hydrogen and potassium occupation of the sites.

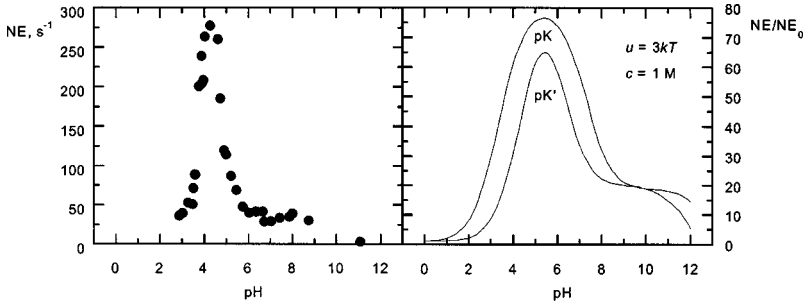

FIG. 3. The ratio NE/NE_0 vs. the solution pH for $c=1$ M with $u=3kT$ and $pK_{C1}=0.7$. The reference value for the blocking event is $NE_0=NE(pH=0,c=1\ M)$. The experimental points (absolute values of NE) reported in Fig. 4(B) of Ref. 1 are shown on the left for comparison. The pK curve results from Eq. (3) with the literature pK values in Fig. 1 while the modified pK′ curve is obtained with the shifted values $pK'_{P2}=pK_{P2}-1$, $pK'_{A1}=pK_{A1}+1$, $pK'_{A2}=pK_{A2}-1$ and $pK'_{P1}=pK_{P1}+1$. When compared to the pK values, the pK′ values correspond to the acid residues becoming more easily protonated when the solution pH decreases and the basic residues deprotonating more rapidly when increasing the pH.

finding a given monomer of the three OmpF pores in the blocked state is always much smaller than unity. Therefore, if we compare two different experimental conditions for ampicillin binding, the ratio of the respective blocking probabilities will be given by $P_1/P_2=e^{-(W_1-W_2)/kT}$ approximately, where W is the isothermal reversible work required to bring the molecule from the solution to the pore. Therefore, to calculate W we must consider the different states for the charge distributions at the ampicillin molecule and the pore constriction zone. This is shown in Fig. 2, where z_i (i=P2, A1, A2, P1, and C1) are the partition functions (including the binding energy) of an ion bound to site i and λ and λ_c are magnitudes proportional to the absolute activities of the hydrogen and the salt cation, respectively, defined[14–16] as $z_i\lambda=10^{pK_i-pH}$ (i=P2, A1, A2 and P1) and $z_{C1}\lambda_C=10^{pK_{C1}-pC}$, with $c(M)=10^{-pC}$ the KCl concentration in M units. The grand partition function of the system in Fig. 2 is

$$\begin{aligned}q=&1+z_{C1}\lambda_c+z_{P2}z_{A1}z_{A2}z_{C1}\lambda^3\lambda_c+z_{P2}z_{A1}z_{A2}z_{P1}\lambda^4\\&+(z_{P2}\lambda+z_{P2}z_{C1}\lambda\lambda_c+z_{P2}z_{A2}z_{P1}\lambda^3\\&+z_{P2}z_{A2}z_{C1}\lambda^2\lambda_c)e^{u/kT}+z_{P2}z_{A2}\lambda^2e^{2u/kT}\\=&1+10^{pK_{P2}+pK_{A1}+pK_{A2}+pK_{P1}-4pH}+10^{pK_{C1}}c(M)\\&+10^{pK_{P2}+pK_{A1}+pK_{A2}+pK_{C1}-3pH}c(M)+[10^{pK_{P2}-pH}\\&+10^{pK_{P2}+pK_{C1}-pH}c(M)+10^{pK_{P2}+pK_{A2}+pK_{P1}-3pH}\\&+10^{pK_{P2}+pK_{A2}+pK_{C1}-2pH}c(M)]e^{u/kT}\\&+10^{pK_{P2}+pK_{A2}-2pH}e^{2u/kT}.\end{aligned}\tag{1}$$

The potential of the average force (averaged over the different manners of occupation in Fig. 2) between the interacting sites P2-A1 and A2-P1 or A2-C1 can be obtained from Eq. (1) as[4,5]

$$W=-kT\ln[q/q(u=0)].\tag{2}$$

W is also the isothermal work required to bring the two pairs of sites from an infinite distance at which $u=0$ up to the finite separation in Fig. 1. Let us denote by NE the number of ampicillin binding events per second (the rate for the molecule blockage of the pore reported in Ref. 1). If we assume that the ratio (NE/NE_0) for two rates measured in different experimental conditions is proportional to the ratio of the respective blocking probabilities, then

$$NE/NE_0=e^{-(W-W_0)/kT},\tag{3}$$

where NE_0 and W_0 are reference values for the number of ampicillin binding events and the potential of the average force corresponding to fixed values of the pH and salt solution concentration. Note that $e^{-W/kT}$ is only one of the factors influencing the ampicillin distribution between the pore and the external solution. In addition to this energy contribution, other factors such as the entropy change due to ampicillin confination to the pore can be important, although these factors will cancel out in Eq. (3) if they are common to both NE and NE_0. It should be kept in mind that the model provides relative rather than absolute values of NE. Finally, the ratio in Eq. (3) does not depend on the ampicillin concentration in the vicinity of the pore mouth because we assume that this concentration does not change significantly with the pH and salt concentration of the external solution.

III. RESULTS AND DISCUSSION

The results obtained from Eqs. (1)–(3) with the literature[6,7] pK values of Fig. 1 are given in Figs. 3–5. Figure 3 corresponds to the ratio NE/NE_0 versus the solution pH for a KCl bathing solution concentration $c=1$ M with $u=3kT$. The reference value for the ampicillin blocking event

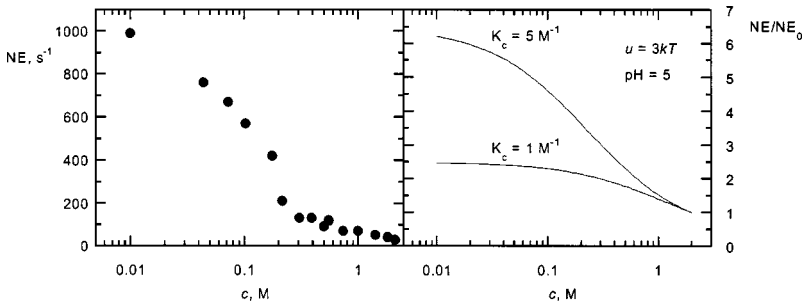

FIG. 4. The ratio NE/NE_0 vs. the KCl concentration c for pH=5 with $u=3kT$. The reference value for the blocking event is $NE_0=NE(pH=5,c=2\ M)$. The experimental points (absolute values of NE) reported in Fig. 5(A) of Ref. 1 are shown on the left for comparison. The pK values are those in Fig. 1. Two values for the binding constant of the salt cation to the acid group in the pore, $K_c=5\ M^{-1}$ ($pK_{C1}=0.7$) and $K_c=1\ M^{-1}$, are considered in the theoretical curves for the sake of comparison.

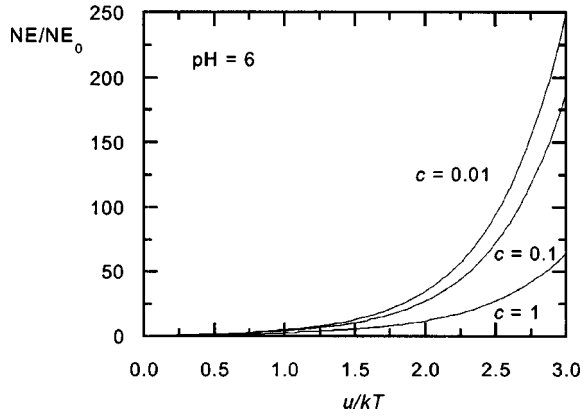

FIG. 5. The ratio NE/NE_0 vs the interaction energy u (in kT units) between matching charged sites for pH=6 and different values of c (in M units) with the pK values of Fig. 1. The reference value for the blocking event is $NE_0 = NE(pH=6, u=kT)$.

is $NE_0 = NE(pH=0, c=1\ M)$. The experimental points for NE reported in Fig. 4(B) of Ref. 1 are shown on the left for comparison. The two theoretical curves on the right are obtained with the pK values in Fig. 1 (curve pK) and with the modified values resulting from the shifts $pK'_{P2} = pK_{P2} - 1$, $pK'_{A1} = pK_{A1} + 1$, $pK'_{A2} = pK_{A2} - 1$ and $pK'_{P1} = pK_{P1} + 1$ (curve pK′). The theoretical results of Fig. 3 reproduce some of the experimental trends: NE/NE_0 is a strong function of pH, and shows a clear peak at a central pH. However, the agreement is not quantitative, as it could be expected for such a simple modeling. The theoretical ratio NE/NE_0 peaks at approximately pH=5.7 while the experimental data peak at a external solution pH about 4.3. (The ampicillin isoelectric point[7] is close to 5, just in the midrange of the above pH values.) Although these differences might indicate that the pH of the internal solution in the pore can be different to the pH in the external solution (an effect characteristic of systems having ionizable fixed groups: charged phospholipid monolayers,[17] ion exchange membranes,[18] conducting polymers,[19] and ionic gels[20]), it is more likely that the discrepancies between theory and experimental data result from the oversimplified model employed. Note also that the theoretical results in Fig. 3 are not so sharply peaked as the experimental data, although they can be made considerably sharper by shifting the literature pK values in Fig. 1 (see the pK′ curve). In the pK′ curve, the acid residues become more easily protonated when the solution pH decreases and the basic residues deprotonate more rapidly when increasing the pH, with respect to the pK curve. This pK shift might perhaps be due to hydrogen bonding effects between the functional groups in the side chains at the constriction zone as well as to the decreased hydrophilic environment provided by this zone[3] compared to the case of the external aqueous solution. The charged amino and carboxyl groups are solvated by water, a highly favorable interaction,[21] and the relative low water content together with the hydrophobic nature of some of the residues inside the channel pore[1–3] could make the protonation of the amino groups and the deprotonation of the acid groups more difficult. It is well known that interacting side chains confined to small volumes can show effective pK values significantly different from those of the individual amino acids in solution[3,22,23] because of electrostatic interactions and solvation effects. (In particular, unusual titration behavior for the basic and acid residues at the pore constriction has been reported for porins.[3]) By trial and error, we might find those effective pK values giving a much better fit of the theoretical curve to the experimental data in Fig. 3, but we did not attempt it because we consider the model to be useful only for qualitative purposes (note that the distribution of negative and positive charges attached to the narrowest part of the pore surface[1,3] is simulated here by the idealized construction in Figs. 1 and 2). In any case, the nature of the peak sharpness in Fig. 3 is not still clear, and cooperativity effects between the simultaneous titration of the fixed charge in the pore channel and the functional groups in the ampicillin molecule have been invoked[1] (see also Ref. 24). It is likely that the simultaneous consideration of all ionic interactions between the constellation of charges at the constriction zone and those in the ampicillin molecule could lead to the sharpening of the theoretical curve, but this question could only be resolved using computer-based molecular models.[2,8–10,23]

Figure 4 gives the ratio NE/NE_0 versus the KCl concentration c for a solution pH=5 with $u=3kT$. The reference value for the blocking event is now $NE_0 = NE(pH=5, c=2\ M)$. As in Fig. 3, the experimental points for NE reported in Fig. 5(A) of Ref. 1 are shown on the left for comparison. Two values for the intrinsic binding constant K_c of the salt cation to the acid group in the pore are considered: $K_c = 5\ M^{-1}$, which corresponds to $pK_{C1} = 0.7$, and $K_c = 1\ M^{-1}$, which corresponds to $pK_{C1} = 0$. Again, the theoretical results appear to reproduce the experimental trends: Figure 5(A) of Ref. 1 shows a pronounced increase in the number of ampicillin blockages when the salt concentration is reduced from 2 to 0.01 M, although a quantitative agreement would require higher effective binding constants (the consideration in the model of simultaneous salt anion binding to the positive fixed charge at the constriction zone would also enhance the salt concentration effect in Fig. 4). Note that the salient feature of the model is that NE/NE_0 can reach high values only when the pattern of charged groups in the molecule and the pore is complementary and the effective binding of the salt ions to the charged groups in the pore destroys the required charge matching (perfect charge matching would only be possible for low enough salt concentrations). The decrease in the number of ampicillin blockages with increasing salt concentration is ascribed here to a oppositely charged salt ion located in the immediate vicinity of a fixed charge group. This effective fixed charge neutralization produces a conversion between the respective manners of site occupation shown in Fig. 2, which the concomitant decrease in the potential W seen by the ampicillin molecule [see Eq. (2)]. Note finally that although the model introduces an effective binding constant for the ion pair formed by the salt ion and the fixed charge group in Fig. 2, no particular molecular mechanism is invoked for this pair. It is likely that contact ion pairs between the fixed and mobile charges do not exist, and thus every salt ion will have some limited translation over the region close to the oppositely charged

group. What the model assumes implicitly is that the increase of the average number of salt ions in the channel with the salt concentration contributes to the effective neutralization of the fixed charge groups.[25] No continuous Debye screening is invoked because the average number of salt ions in the constriction zone is close to one.[8–10,26]

Figure 5 shows the ratio NE/NE_0 versus the interaction energy u (in kT units) between matching charged sites for pH=6 and the pK values of Fig. 1. The reference value for the blocking event is $NE_0 = NE(pH=6, u=kT)$. The curves are parametric in the salt concentration c and show that NE/NE_0 decreases dramatically when decreasing the interaction energy between the charged groups of the ampicillin and the charged residues in the pore constriction. The experimental results in Fig. 3(B) of Ref. 1 show that NE is a strong function of the voltage applied to the channel pore, V, and it is argued[1] that small field-induced changes in the channel pore geometry may cause large changes in NE because of the required exact fit between the ampicillin and pore charges. We might hypothesize that changes in V modifying the ampicillin docking in the pore may also change the interaction energy u between charged sites (the distance between sites may change), causing the dramatic changes in Fig. 5. However, it is difficult to analyze the experimental results in Fig. 3(B) of Ref. 1 because the results in Fig. 4(B) of Ref. 1 suggest that at pH=6 a nonzero fraction of ampicillin molecules may be negatively charged. If that were the case, the observed changes of NE with V might reflect both field-induced changes in the energy u and the effect of the applied electric field on the axial transport of the fraction of charged molecules. Also, future work should address the influence of voltage and pH on channel closure[27] as well as the effects due to the orientation of dipolar ampicillin in the electric field created by the pore charge distribution.[2,3,28]

A final question concerns the conditions of applicability of the present thermodynamic approach. The model assumes that all three pores of the OmpF trimer behave independently[1,29] and concentrates on the problem of ampicillin binding to the pore. To obtain drug translocation rates, a kinetic approach would be necessary. Our approach does not consider absolute values for the number of binding events per unit time but only relative ones [see Eq. (3) and Figs. 3–5 for the theoretical ratios NE/NE_0 for two rates measured under different experimental conditions]. These theoretical ratios are obtained with the assumption that the equilibrium probability of finding a given monomer of the three OmpF pores in the blocked state is always much smaller than unity and comparing two different experimental conditions for ampicillin binding. We are, therefore, implicitly assuming that for a given ampicillin concentration, the number of binding events is given by a kinetic factor common to both NE and NE_0 multiplied by an equilibrium blocking probability that depends on the external pH and salt concentration. Omission of kinetic factors in the ratio NE/NE_0 should be reasonable if the kinetic factor does not change with the external conditions as much as the blocking probability does. This could be the case here since the experimental data in Figs. 5(A) and 5(B) of Ref. 1 show that while NE changes by almost two orders of magnitude over the range of KCl concentrations employed, the ampicillin residence time in the pore changes only by a factor three over this range. In any case, it must be noted that absolute values of NE could not be obtained with the present model because of the absence of kinetic considerations. A theory for maltodextrin translocation through maltoporin channels has recently been presented[30] and the relationship between equilibrium constants and translocation rates was discussed. Kinetics concepts were also introduced in a recent study dealing with the effects of voltage on the ampicillin residence time.[31]

IV. CONCLUSION

A molecular model aimed at explaining the increased binding of zwiterionic ampicillin to charged residues inside the channel pore has been presented. We assume that the interacting sites in Fig. 1 represent the electrostatic interaction between the ampicillin molecule and the charge distribution at the pore constriction, and consider the potential of the average force between sites as the crucial parameter determining the changes in the ampicillin binding with the pH and salt concentration of the external solution. The results provide qualitative explanations to some of the observed phenomena and can be useful for more elaborated treatments.

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