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ARTICLE

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Protein electrostriction: a possibility of elastic deformation of the α-hemolysin channel by the applied field

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Abstract While conformational flexibility of proteins is widely recognized as one of their functionally crucial features and enjoys proper attention for this reason, their elastic properties are rarely discussed. In ion channel studies, where the voltage-induced or ligand-induced conformational transitions, gating, are the leading topic of research, the elastic structural deformation by the applied electric field has never been addressed at all. Here we examine elasticity using a model channel of known crystal structure—*Staphylococcus aureus* α-hemolysin. Working with single channels reconstituted into planar lipid bilayers, we first show that their ionic conductance is asymmetric with voltage even at the highest salt concentration used where the static charges in the channel interior are maximally shielded. Second, choosing 18-crown-6 as a molecular probe whose size is close to the size of the narrowest part of the α-hemolysin pore, we analyze the blockage of the channel by the crown/K^+ complex. Analysis of the blockage within the framework of the Woodhull model in its generalized form demonstrates that the model is able to correctly describe the crown effect only if the parameters of the model are considered to be voltage-dependent. Specifically, one has to include either a voltage-dependent barrier for crown release to the *cis* side of the channel or voltage-dependent interactions between the binding site and the crown. We suggest that the voltage sensitivity of both the ionic conductance of the channel seen at the highest salt concentration and its blockage by the crown reflects a field-induced deformation of the pore.

Keywords Crowns · Blockage · Generalized Woodhull model · van der Waals interactions · Bottleneck barriers

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Introduction

Ion channels are ubiquitous in both prokaryotic and eukaryotic cells, playing important roles in supporting homeostasis, signaling, and producing and maintaining membrane potential. The conformational flexibility of ion channel proteins is widely recognized to be essential for their regulatory role. It is known that channels respond to changes in applied voltage, ligands, temperature, mechanical tension, etc., by changing their conformation. However, the elasticity of a given conformation and its possible consequences for channel function have never been discussed or analyzed. Accounting for channel elasticity and, therefore, possible electrostriction effects may prove important in interpreting functional properties of the channels.

The geometrical features of the channel lumen in the fully open state are usually assumed to be voltage-independent. Although this assumption generally works, it is desirable to realize its limitations. Voltage-induced elastic deformations may manifest themselves in the changes of the channel transport properties that are especially important in the case of molecules with a close fit to the channel pore dimensions. It is clear that channels with an expressed nonlinearity of their small-ion current–voltage dependences are likely candidates for the electrostriction effects, though indeed, the nonlinearity per se does not necessarily mean electrostriction.

To approach this problem one needs a single ion channel with a well-known structure and appropriate molecular tools. For this purpose we chose the channel

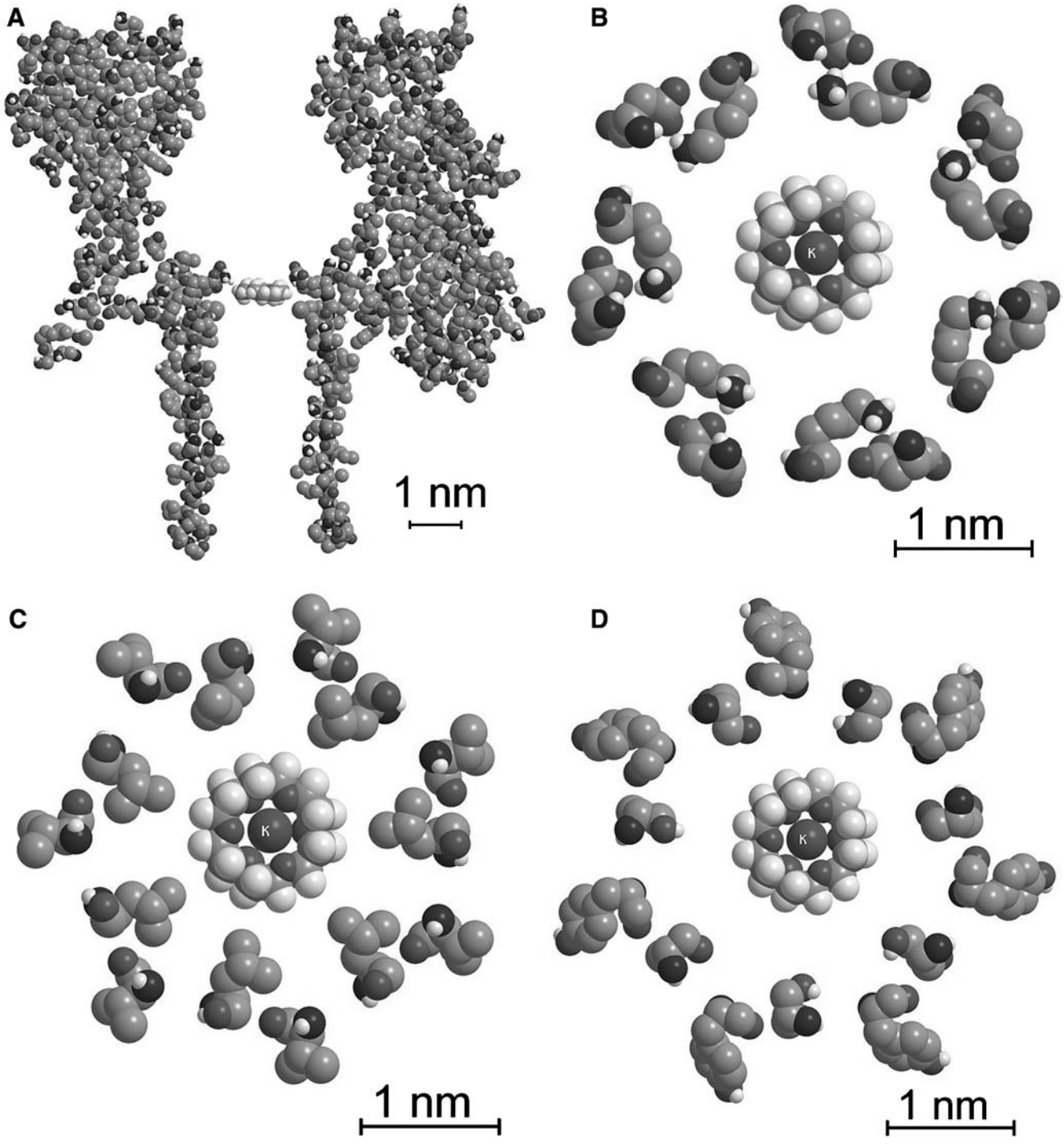

Fig. 1 Space-filling model presentation of 1,4,7,10,13,16-hexaoxacyclooctane (*18-crown-6*) inside the stem region of the α-hemolysin channel. **a** A longitudinal slice of the α-hemolysin pore and the 18-crown-6 close to the constriction region of the α-hemolysin pore—the hypothetical binding site. **b**–**f** Cross-sections (slices) of the α-hemolysin channel stem region at the level of Lys131, Leu135, Ser141, Met113, and Lys147, which are about 0.3, about 1.4, about 3.1, about 4.4, and about 4.7 nm from the *trans* opening and 18-crown-6. The atoms are represented using the following color codes: O in *dark gray*, C in *gray*, H in *white*, N in *black*, and S in *light gray*. The comparison between 18-crown-6 and the internal pore sizes at the different distances from the *trans* opening of the channel gives an idea of the areas occupied by the molecules and the extent of channel block that might be expected

formed by *Staphylococcus aureus* α-hemolysin in planar lipid bilayers (Krasilnikov et al. 1980, 1981). α-Hemolysin is a protein with molecular mass of 33.2 kDa (Gray and Kehoe 1984). According to crystallographic data it forms a channel that is composed of seven monomers (Gouaux et al. 1994; Song et al. 1996). The stoichiom-

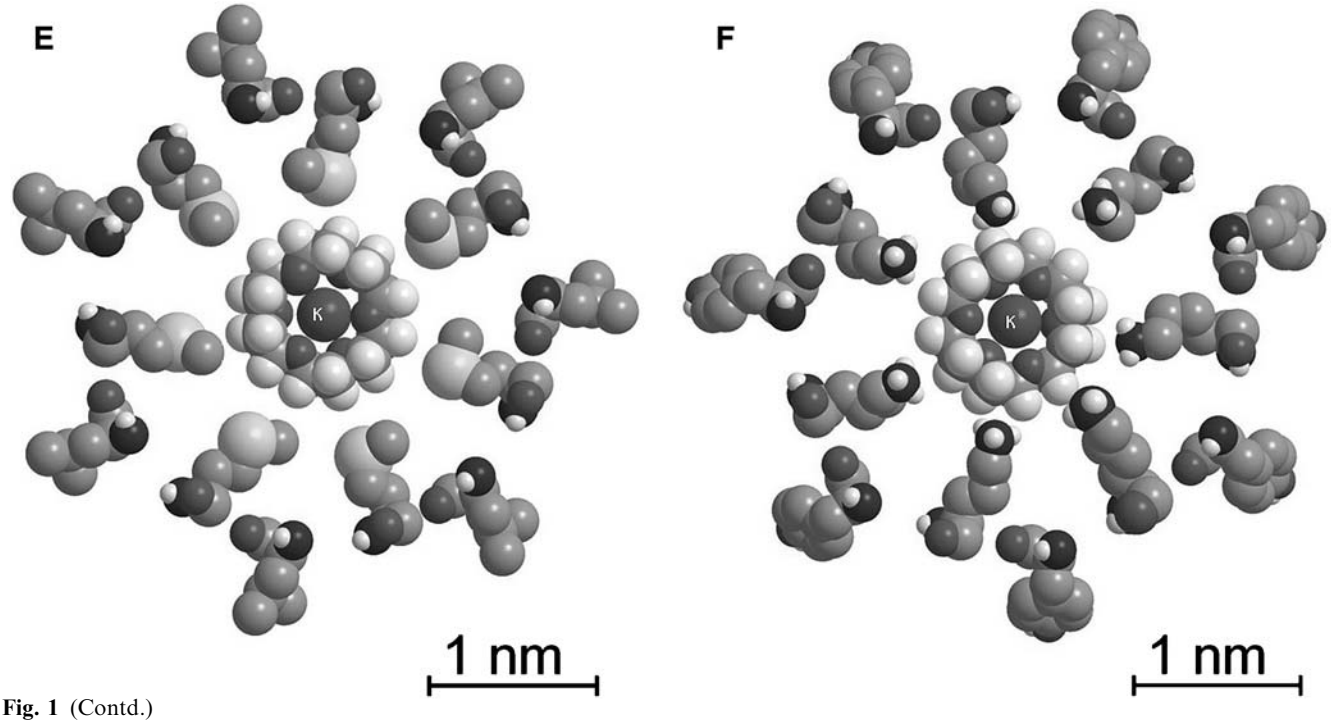

Fig. 1 (Contd.)

etry and the geometry of the channel lumen were also confirmed in reconstitution experiments with planar lipid bilayers (Merzlyak et al. 1999; Krasilnikov et al. 2000; Movileanu et al. 2001). A representative of a large family of crown ethers, 1,4,7,10,13,16-hexaoxacyclooctane (18-crown-6), was chosen as a tool to evaluate a possible change in the α-hemolysin pore because its molecular size (about 1.15 nm in diameter) is very close to the size of the narrowest part of the pore (diameter of about 1.2 nm, Fig. 1).

The crown consists of six ethylene oxide (–O–CH_2–CH_2–) units, joined covalently into a relatively rigid macrocyclic ring. It does not possess ionogenic groups and belongs to nonelectrolytes. However, the crown is characterized by a central hydrophilic cavity surrounded by an external hydrophobic ring. These structural features enable it to form stable complexes with alkaline metal ions (Pedersen 1967, 1968, 1988; Ozutsumi and Ishiguro 1992).

In this study, we attempted to probe the voltage-induced elastic deformation of the α-hemolysin channel by analyzing the effect of crown addition to the bathing solutions on channel conductance. To analyze the data obtained in the −200 to +200-mV range, we used the Woodhull model (Woodhull 1973; Hille 1992) in its generalized form (Tikhonov and Magazanik 1998). We found that the model is able to correctly describe the crown effect only if the parameters of the model are considered to be voltage-dependent. We suggest that this dependence as well as the residual (at 4 M KCl) asymmetry in the G/V curve is due to an elastic deformation of the channel structure in its fully open conformation by the applied field.

Materials and methods

Diphytanoylphosphatidylcholine (DPhPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). The wild type of *S. aureus* α-hemolysin was a generous gift of Hagan Bayley (Texas A & M University, USA). The other chemicals were analytical grade.

Planar lipid bilayer membranes were formed as described elsewhere (e.g., Merzlyak et al. 1999 after Montal and Mueller 1972). The two compartments of the cell were separated by a 20-μm-thick Teflon partition with an orifice for bilayer formation of 70–100-μm diameter, which was pretreated with a 1% solution of hexadecane in *n*-pentane. The DPhPC was dissolved at 5 mg/ml in pentane or hexane and layered onto the surface of aqueous buffered solutions on both sides of the partition. Bilayers were formed on the partition's orifice by raising the level of the solutions.

The protein was added to the *cis* side of the chamber. The applied potential difference was considered to be positive when the potential was higher at the side of protein addition. Milli-Q plus treated water (Millipore, USA) with a resistivity of 18 MΩ cm was used to prepare all buffer solutions. Unless stated otherwise, the crown was added to both sides of the membrane in equal concentrations; the standard solution contained 1 M KCl and 5 mM tris(hydroxymethyl)aminomethane adjusted to pH 7.5 with citric acid.

Experiments were performed at room temperature (23 ± 2°C) using an Axopatch 200 B amplifier (Axon Instruments, Foster City, CA, USA) in the voltage clamp mode. Currents were filtered by a low-pass eight-

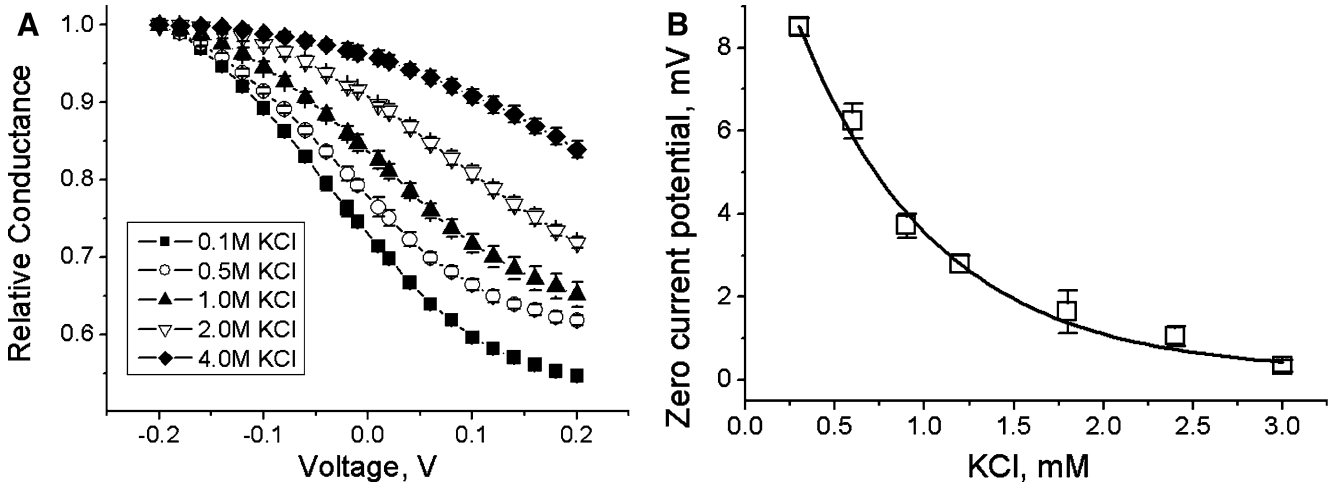

Fig. 2 **a** Relative conductance of a single α-hemolysin channel at different KCl concentrations normalized to the value at −200 mV. The channel conductance was decreased by positive voltages in a salt-dependent manner. The higher is the salt concentration, the smaller is the relative voltage-induced decrease. The KCl solutions were buffered with 5 mM tris(hydroxymethyl)aminomethane (*Tris*) and adjusted to pH 7.5 with citric acid. Each *data point* represents the mean from seven to nine separate experiments ± the standard deviation (*SD*). **b** Effect of KCl concentration on the zero current potential of α-hemolysin channels. The data were obtained in the presence of a threefold KCl concentration gradient. The zero-current potential was defined as the potential that must be applied to the experimental cell to reach a virtual zero transmembrane current equal to that of a symmetrical system with 0-mV applied potential. A positive sign of the zero-current potential indicates anion selectivity of the channel. The data presented are referred to the larger values of KCl concentration (*abscissa*). Reversal potentials were corrected for liquid junction potential effects. Liquid junction potentials were calculated using the Henderson equation (Barry and Lynch 1991). The selectivity in a symmetrical salt is expected to be smaller than in this asymmetrical configuration. The KCl solutions were buffered with 5 mM Tris and adjusted to pH 7.5 with citric acid. Each *data point* represents the mean from three to five separate experiments ± the SD

pole Butterworth filter (model 9002, Frequency Devices, Haverhill, MA, USA) at 15 kHz and recorded simultaneously by a video-cassette recorder operated in a digital mode and directly saved into the computer memory with a sampling frequency of 50 kHz.

The profile of the electrical distance along the α-hemolysin channel axis was calculated based on the crystallography and nonelectrolyte exclusion studies (Gouaux et al. 1994; Song et al. 1996; Merzlyak et al. 1999) of the channel geometry. The electrical distance can be determined in terms of the transmembrane potential drop across the channel lumen or in terms of the resistance, taking into account that the transmembrane potential drops along the channel discontinuously but is piecewise continuous according to the channel lumen geometry. This can be written in the form $R_{total} = \sum_{i=n}^{i=n} R_i$, where $R_i = l_{\text{slice}}/\chi\pi r_i^2$, l_{slice} is the thickness of a slice, r is the average radius of the channel for the slice with correction for the hydration water layer, and χ is the specific conductivity of the buffer solution. Then for the potential drop on each slice we have $\Delta V_i = R_i/R_{\text{total}}$. The drop of potential in relative units, the so-called "electrical distance", is $\Delta V_i / \sum_{i=1}^{i=n} \Delta V_i = \Delta V_{rel}$, where $\sum_{i=1}^{i=n} \Delta V_i = V_{total}$ is the total voltage drop along the lumen. The electrical distance varies from 0 at one opening (*trans*) to 1 at the other (*cis*). The calculated electrical distance was brought into correlation with the physical length of the channel. In this way we found that the constriction is at around 0.2 units from the *cis* opening.

The cross-sections of the transmembrane β-barrel domain (residues 110–148) and the longitudinal section of the α-hemolysin channel (Protein Data Bank 7AHL.pdb) were visualized with Swiss-PdbViewer version 3.7 (Guex and Peitsch 1997). The desired amino acid residues were when displayed and exported as

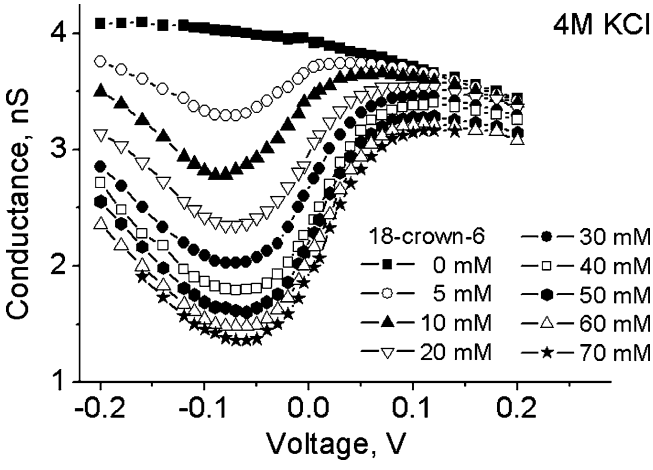

Fig. 3 Effect of symmetrical (to both sides of the membrane) addition of the crown to 4 M KCl membrane-bathing solutions on channel conductance. The blockage of ion currents by the crown is highly voltage dependent and is most pronounced at voltages close to −70 mV. The blockage saturates with crown concentration: the initial effect corresponding to addition of 5 mM crown in the membrane-bathing solution exceeds the effect of a crown concentration increase from 40 to 70 mM. The result of a typical experiment with the same single channel is presented

image (TGA) or PDB files. CS Chem3D Pro (Cambridge Soft Corporation, MA, USA) was used to build the 18-crown-6/K^+ complex and combine it with the "slices" of the α-hemolysin channel. The final representations were produced with Adobe Photoshop.

Results and discussion

The dependence of single channel conductance on the applied voltage V at different KCl concentrations in the membrane-bathing solution is shown in Fig. 2a. To facilitate comparison, all conductance curves were normalized to the conductance value at −200 mV. It is seen that conductance of the α-hemolysin channel in its fully open conformation has different sensitivity to voltage at different salt concentrations, higher salt concentrations promoting lower sensitivity. As a result, the G/V curves became less asymmetrical as the salt concentration increased. This indicates the screening of charges asymmetrically distributed at both ends of the pore. At 4 M KCl the screening is complete as the weakly anion selective (at low salt concentrations) α-hemolysin channel became completely nonselective at 3 M KCl (Fig. 2b). Hence, the residual asymmetry in the G/V curves (seen at 4 M) may indeed represent the elastic change in the pore geometry with voltage, while the static asymmetry of the internal shape of the pore has no significant effect on the asymmetric conduction in the α-hemolysin channel (Noskov et al. 2004). On the basis of this assumption all the results presented in this report were obtained at 4 M KCl. To get additional insight into the possibility of electrostrictive effects, we probed the channel with 18-crown-6, whose molecular size is very close to the size of the narrowest part of the α-hemolysin pore (Fig. 1).

Figure 3 shows that at 4 M KCl for symmetric crown application the major blockage is observed when the applied voltage is close to −70 mV. The blocking effect is very asymmetric. When the crown is present in the *trans* compartment only, the effect is practically indistinguishable from that of symmetrical addition. In contrast, the presence of the crown on the *cis* side is felt slightly and at high positive potentials only (data not shown). This behavior indicates that the blockage is due to the charged crown/K^+ complexes, which easily reach the binding site from the *trans* side. The complex is driven onto the binding site by the applied potential, most of which drops in the area of the channel stem.

Highly asymmetric blockage was found recently for β-cyclodextrin binding to a site within the α-hemolysin pore (Gu et al. 2003). The difference between the 18-crown-6 and β-cyclodextrin effects is that the latter does not show the maximum in the blockage versus applied voltage. Also, its binding reaction is slower by about 3 orders of magnitude. The characteristic lifetime of β-cyclodextrin on the binding site is close to 1 ms (Gu et al. 2003); the characteristic lifetime of the crown is close 1 μs (Bezrukov et al. 2004).

The crown appears to interact with the channel lumen in a simple dose-dependent manner. The data points at −70 mV (maximum of crown blockage) in Fig. 4a can be approximated by a first-order binding isotherm with the limiting conductance of about one fifth the initial channel conductance in crown-free solution. This suggests that a single crown molecule participates in the blockage of the channel, and when the molecule is bound, it reduces the channel conductance by a factor of about 5. The half-blocking concentration was found to be dependent on voltage (Fig. 4b). It suggests that the depth of the well and the barrier heights change substantially with the applied voltage.

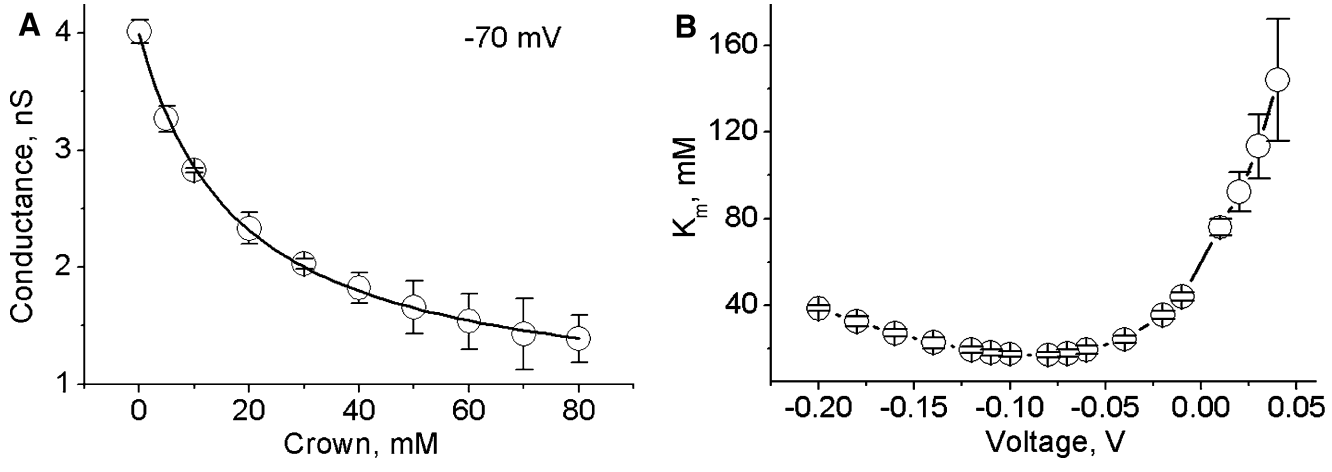

Fig. 4 **a** Channel conductance (g_i) in 4 M KCl solution as a function of crown concentration (C) at −70 mV. The effect is described by a first-order binding isotherm $g_i = g_{max} - (g_{max} - g_{min})C/(C_{0.5} + C)$ with g_{min} of about 0.7 nS. $C_{0.5}$ is the half-blocking concentration. Each *data point* represents the mean from four separate experiments ± the SD. **b** Dependence of half-blocking concentration on the applied voltage obtained from data like those presented in **a** and Fig. 3. Each *data point* represents the mean from four separate experiments ± the SD. The *line* was drawn as a guide for the eye

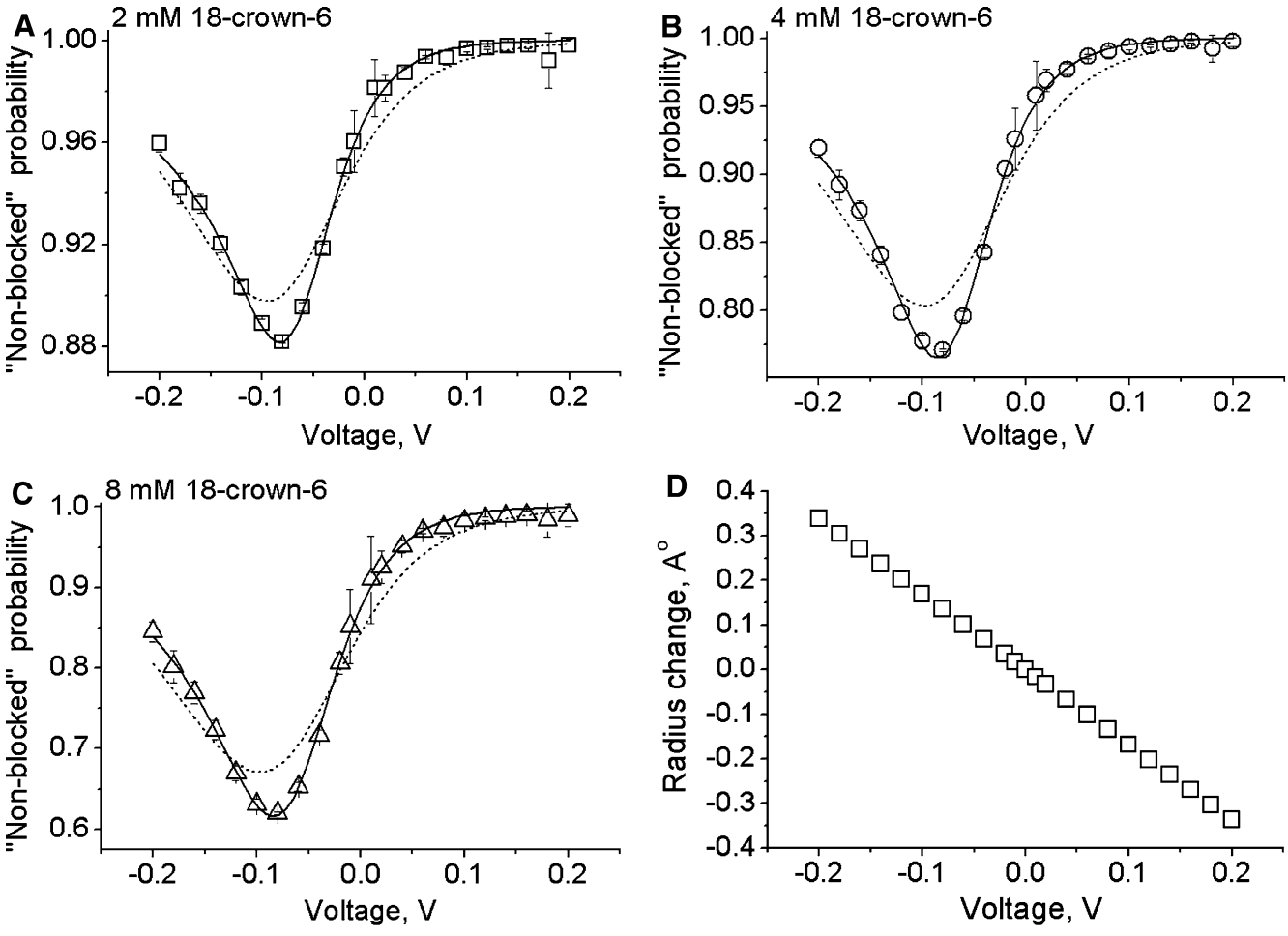

Fig. 5 **a** Probability that the channel is not blocked by the crown as a function of applied voltage at three crown concentrations. **b** *Dashed lines* are best fits by the Woodhull model (in its generalized form) for a blocker carrying a single elementary charge, Eq. 1, where parameter δ_m, A^0 or B^0 is allowed to change, while δ_c, in accord with Howorka and Bayley (2002) and our own estimation (see "Materials and methods"), was assumed to be 0.8 and $\delta_t = 0$. δ_m, A^0, and B^0/C (B^0 divided by the crown concentration) were found to be 0.52 ± 0.02, 0.087 ± 0.004, and 22.7 ± 0.5, respectively. **c** *Solid lines* are best fits of a modified model that included the elastic deformation with voltage. δ_m, A^0, and B^0/C were found to be 0.68 ± 0.02, 0.51 ± 0.03, and 16.7 ± 1.1, respectively. Numerical values of the parameters were obtained from the analysis of all G/V dependencies measured in the presence of different crown concentrations in three to five separate experiments. The voltage dependence of the parameter A reflects channel deformation by the applied field (see text). **d** Influence of the voltage on the apparent change in the radius of the ion channel lumen. The apparent change in the radius, Δl, was calculated on the basis of the modified Woodhull model

Figure 5 gives the voltage dependence of the probability that the binding site is not occupied by the crown for 4 M KCl solutions. The probability was calculated as $p_n = 1 - \langle \Delta I \rangle / \Delta i$,, where $\langle \Delta I \rangle$ is the crown-induced change in the average current through the channel and Δi is the reduction of the "instantaneous" current induced by binding of a single crown molecule. The error bars in Fig. 5 are mostly due to the error in Δi obtained in noise analysis (Bezrukov et al. 2004). Very close results were obtained by assuming that Δi is equal to the crown-induced change of the average current in the limit of high crown concentrations (data not shown). It is seen that the probability exhibits a rather sharp minimum at negative voltages close to −70 mV.

We applied the Woodhull model (Woodhull 1973; Hille 1992) in its generalized form (Tikhonov and Magazanik 1998) to analyze the voltage dependence of the crown block. Some of the main requirements for the validity of this approach are that the blockage of the conductance by a reagent is not a result of cooperative allosteric effects changing protein conformation and that the blocking molecules are independent of each other, except for their competition for the same binding site. Our recently published results (Bezrukov et al. 2004) and results such as those presented in Fig. 4a suggest that the data in Fig. 5 are in perfect agreement with these requirements.

The model is represented by Fig. 6. The restriction imposed by equilibrium thermodynamics on the rate constants ($k_1^0 k_2^0 = k_{-1}^0 k_{-2}^0$, where subscript 0 refers to zero applied voltage) is known as microscopic reversibility and detailed balance. Because of this restriction,

in the case of symmetrical application of a blocker carrying a single elementary charge, the probability that the channel is not blocked can be described as a function of dimensionless voltage $\psi = \varphi e/kT$(ϕ is the transmembrane potential, e is the elementary charge, k is the Boltzmann constant, T is the absolute temperature):

$$P = \frac{\exp(\delta_m\psi) + A^0\exp[(\delta_\mathrm{m} + \delta_\mathrm{t} - \delta_\mathrm{c})\psi]}{B^0 + A^0B^0\exp[(\delta_\mathrm{c} + \delta_\mathrm{t} - \delta_\mathrm{m})\psi] + \exp(\delta_m\psi) + A^0\exp[(\delta_\mathrm{m} + \delta_\mathrm{t} - \delta_\mathrm{c})\psi]}, \quad (1)$$

where $k_1 = Cb_1\exp(-\delta_\mathrm{t}\psi)$, $k_{-1} = b_{-1}\exp[(\delta_\mathrm{m} - \delta_\mathrm{t})\psi]$, $k_2 = b_2\exp[(\delta_\mathrm{m} - \delta_\mathrm{c})\psi]$, $k_{-2} = Cb_{-2}\exp[(\delta_\mathrm{c} - \delta_\mathrm{m})\psi]$, $A^0 = k_2^0/k_{-1}^0$, $B^0 = Ck_1^0/k_{-1}^0$, b_i is a voltage-independent constant, δ_c, δ_m, and δ_t are fractions of the membrane field which correspond to the location of the *cis* barrier, the minimum, and the *trans* barrier, C is the blocker concentration, and is the same on both sides of the channel.

Taking into consideration the inner channel geometry, data published by others (Howorka and Bayley 2002) and our own estimation, the location of the *cis* barrier, we took δ_c as 0.8. When the *trans*-barrier location (δ_t) was allowed to be free it took unrealistic negative values. So we need to assume δ_t is zero by placing the barrier exactly at the *trans* opening. As a result, this function contains only three free parameters: δ_m, the fraction of the transmembrane potential that is assumed to be felt by the blocker molecule, A, and B.

The best fit of Eq. 1 to the data in Fig. 5 is shown by the dashed lines. It is seen that the theoretical curves do not accurately describe the experimental points. Relatively large error bars at small and positive voltages do not jeopardize this conclusion. To obtain better agreement with the model one has to accept one (or both) of the two assumptions: (1) the charge of the crown/K^+ complex is larger than 1; (2) the applied field introduces structural deformations of the pore constituents, thus changing the crown–pore interactions in a way that is not accounted for by Eq. 1.

To choose between these two assumptions we first note that it is firmly established that a 1:1 complex is formed between the crown and a potassium ion (Rounaghi et al. 1977), so assuming one elementary charge per blocker molecule is well justified. An additional complication could come from the finite anionic selectivity of the α-hemolysin channel (Krasilnikov et al. 1981) easily seen at low salt concentrations (Fig. 2b). This selectivity may contribute to the effectiveness of the action of the blocker. Actually, this effect was recently observed in experiments with the α-hemolysin channel and a neutral blocker, β-cyclodextrin (Gu et al. 2003) at lower (NaCl) concentrations. In the case of the positively charged complex explored here, the force of such coupling has to act in the *opposite* direction, thus reducing the effective drag on the crown/K^+ complex. However, similar to other large channels (Alcaraz et al. 2004), the α-hemolysin channel becomes practically unselective at KCl concentrations above 3 M (Fig. 2b) so this property of the channel does not contribute to the effectiveness of the crown blockage. Additionally, as mentioned earlier, the possibility of some cooperative action involving two or more crown molecules does not agree with the data in Fig. 4a. The dependence of the blockage on the crown concentration obeys the behavior of a simple first-order binding isotherm. Moreover, the data in Fig. 5 represent rather small crown concentrations. Therefore, the first explanation can be safely ignored.

The elastic deformation of the channel structure by the applied electric field is thus the primary candidate to explain the pronounced discrepancies between the model predictions and the experiment. We explored two possibilities. First, we assumed that the strength of interaction between the binding site and the crown is voltage-dependent. In the model represented by Fig. 6 this

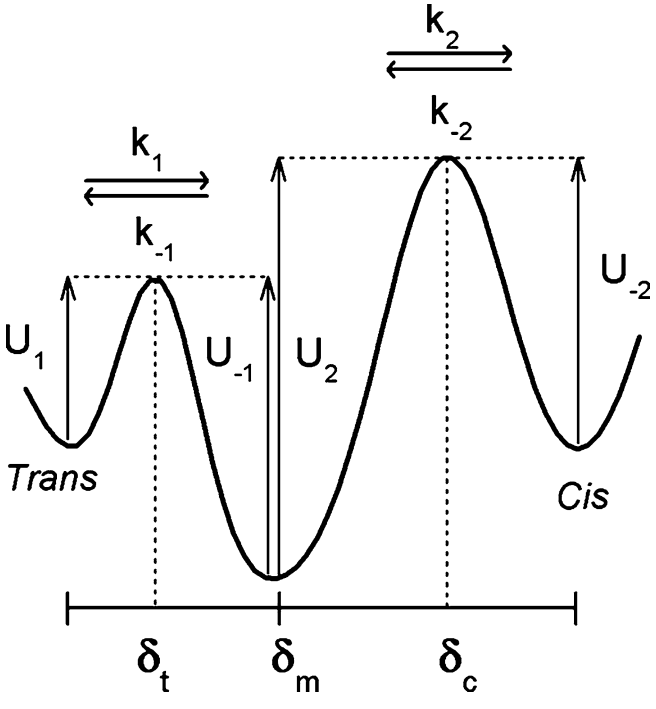

Fig. 6 Potential profile diagram for the Woodhull model together with schematic representation of the binding reaction. In the original model the voltage dependence of the blockage stems from the biasing of the profile by the external field (Tikhonov and Magazanik 1998; Woodhull 1973) and is reflected by the voltage-dependent exponential terms in Eq. 1. In the modified model accounting for electrostriction, either the depth of the central well or the height of the *cis* side barrier varies with the applied voltage because of the elastic deformation of the channel structure

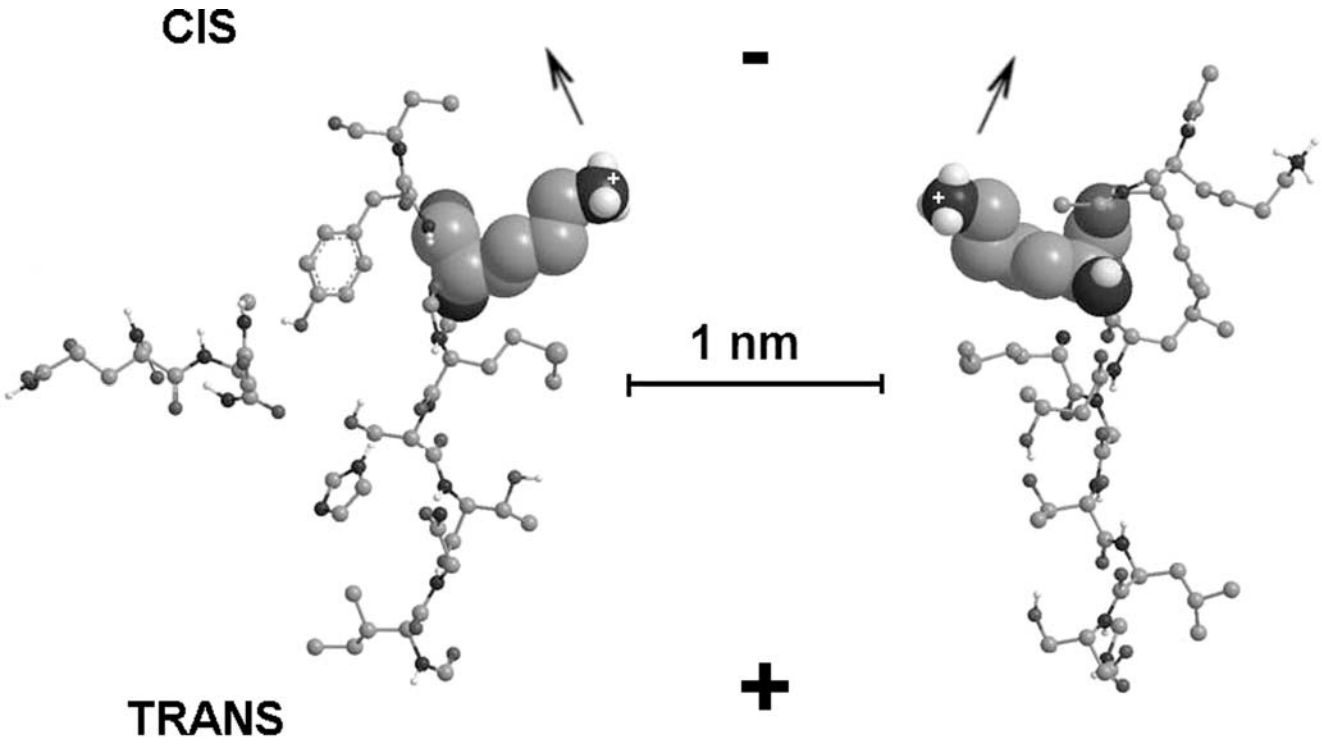

Fig. 7 A mechanistic model of the possible elastic deformation at the constriction zone of the α-hemolysin channel. A slice of the constriction zone of the channel. Lys147 are presented as a space-filling model. Neighboring residues are shown as ball-and-stick representations. *Arrows* point in the direction of the possible displacement that may increase the channel diameter at this position and facilitate crown exit when a negative voltage is applied on the *cis* side. The nitrogen of the NH_3 groups of Lys 147 is marked with *plus sign*. The atoms are represented using the following color codes: O in *dark gray*, C in *gray*, H in *white*, N in *black*, and S in *light gray*

means that the depth of the central potential well is now a function of the applied voltage. It is seen that the depth variation will change k_2 and k_{-1} proportionally to each other (either decreasing or increasing both of them by the same factor). Therefore, their ratio A will be constant. At the same time, B will be increased by the increase in the well depth and will decrease otherwise.

The second possibility is to assume that the negative voltage reduces the barrier for the crown exit to the *cis* side of the channel by increasing the size of the "geometric bottleneck" (Fig. 1a). Indeed, if the radius of the constriction is exactly equal to or smaller than the radius of the crown, the barrier is infinite. Crown molecules cannot cross the channel. As the constriction radius is increased by negative voltages, the height of the barrier quickly decreases, thus allowing crowns to exit the channel to the *cis* side. In this modification of the model B is constant, but A is now a function of voltage, increasing towards negative voltages.

As one of the possibilities to describe the depth and/or the barrier variation with the applied voltage, we chose the energy of the van der Waals interactions between the crown/K^+ complex and the pore wall. Then we expressed the radius-dependent interaction through the known constants of the Lennard–Jones potential for hydrogen–hydrogen pairs (Allen and Tildesley 1989). Assuming realistic dependences of the constriction radius on voltage that are qualitatively compatible with the channel conductance change shown in Fig. 2a, we arrived at a reasonable agreement between the model prediction and experiment. Using this approach, we found that the only the assumption about the barrier variation allows correlation of the binding site with the narrowest part of the channel pore ($\delta_m \sim 0.7$) and that a very small (~0.8 Å) field-induced elastic change in the radius of this part of the channel (Fig. 5d) is able to change the energy of binding by a couple of kTs and to remove the discrepancies between the model predictions and the experiment. The curves accounting for such changes in the crown–pore interactions are shown in Fig. 5a–c as the solid lines. Indeed, the actual number for the voltage-induced increase in the radius depends on the approximation that one uses for the van der Waals forces. For example, it was shown (Parsegian and Weiss 1974; Parsegian 2004) that for "cylinder in a cylinder" or "sphere in a sphere" geometry the attractive term of the interaction could be expressed as the inverse second power of the separation. Assuming this dependence, we can obtain larger radius changes. So, our hypothesis on the role of electrostriction in the α-hemolysin transport properties allows us to explain observations on the voltage dependence of both the asymmetry of the residual conduction (Fig. 2a) and the blocking by the crown (Figs. 4, 5) in a unified manner. In both cases the supposed elastic changes in the ion channel structure are gradual, continuous, and proportional to the applied force. This is principally different from the

well-known "gating" where the field-induced conformational changes are discrete and frequently mentioned as "all or nothing" processes.

In our study we assumed that the apparent change in the pore radius under the applied electric field is the product of the local electrostriction. This does not mean that the applied electrical field "squeezes" the α-hemolysin pore as an elastic microscopic object, since the dipoles and charges in different parts of the pore could be stretched or squeezed at the same time.

The total number of charges is of 525 for a whole heptameric channel. With the assumption that the additivity of the electric field influence, the total force and energy becomes about 1.9×10^{-19} J/m and about $16kT$, respectively. Both values are considerable. Dipoles are other targets of the electric field. To calculate the dipole moment of the ST channel structure, we used a Web server for protein dipole moment evaluation (http://bioinformatics.weizmann.ac.il/dipol/dipol.html), the algorithm of which is based on the Poisson–Boltzman method (Felder et al. 1997). The algorithm assumes that the total dipole moment of a protein molecule can be represented as the sum of two contributions: the first is due to the charges of all amino acids in the neutral state (summing the dipole moments of all of the α-hemolysin peptide bonds), and the second is due to formal charges of ionizable groups. Atomic coordinates for the α-hemolysin channel were taken from the Protein Data Bank (7AHL). This way, the computed effective dipole moment of a single molecule of α-hemolysin in the channel structure was estimated as about 260 D. The energy that this structure can possess in the electric field can be estimated as about $4kT$, which gives about $28kT$ for the whole structure of the heptameric channel (at $\Delta V = 0.2$ V). However, the different regions of the channel possess differently directed dipole vectors, which hinder the analysis of electric field effect on the α-hemolysin channel. Because several channel characteristics are mainly determined by the highly structured stem region (including the crown binding, as established in the present study), we made an attempt to estimate the energy of the peptide bond dipoles of this region of the channel. It was found that the estimated value already gives about $18kT$ (about $2.5kT$ for two β-stands of each α-hemolysin monomer). This region also contains the 35 charges of ionizable groups, whose interaction with the electric field can also add energy to protein deformation. Hence, the stem region looks like a principal element of the interaction of the α-hemolysin pore with the electric field where the transmembrane potential will try to displace the T129-K147 and T129-E111 strands in opposite directions because these two strands have differently charged groups in amino acid side chains. At the same time, T129-K147 strands should be squeezed at negative voltage (applied at the *cis* side) and stretched at positive voltages, while the opposite influences should be expected for T129-E111 strands because the dipole moments of the peptide bonds of these two strands are oppositely directed.

Generally speaking, the energetic basis of the proposed deformation of the protein structure by the applied field is common for electrostriction in any other material. The electrostatic forces acting on charges and dipoles are counterbalanced by the forces of elastic deformation. The electrostatic force acting on a single charged residue is $F = eE$, where e is the electron charge and E is the electric field. The counteracting force is $F_c = K\Delta l$, where K is the elastic force constant and Δl is the displacement. The voltage-induced displacement can be found from equating these two forces, $\Delta l = eE/K$. Substituting $E = 2\times10^7$ V/m (200 mV over 100 Å) and $K = 10$–30 pN/Å (measured by neutron scattering for different proteins, Zaccai 2000), we obtain $\Delta l = 0.1$–0.3 Å .

So, the theoretical estimation of a possible elastic displacement of a single charge embedded in a protein structure under the influence of the electrical field already gives numbers of the same order of magnitude as those estimated for the electrostictive change in the radius (up to 0.4 Å for a voltage variation from 0 to ±200 mV) of the α-hemolysin pore in our study. However, it is not easy to interpret this displacement in terms of the changes in the pore radii. As a case in point, we can suggest a possible mechanistic model of the elastic deformation at the constriction zone of the α-hemolysin channel (Fig. 7) where Lys147 could be displaced under a voltage increasing the channel diameter at this position and facilitating crown exit when a negative voltage is applied on the *cis* side or decreasing the channel diameter and make difficult crown transport when a positive voltage is applied. This is the only mechanistic model. Nevertheless, these estimates show that the electrostriction hypothesis put forward in this Letter is plausible.

Conclusions

We used a charged complex of 18-crown-6 with potassium cations as a molecular probe to detect possible electrostrictive deformation of the α-hemolysin channel. The choice of the probe was motivated by the closeness of its diameter to the diameter of the narrowest part of the α-hemolysin pore. The results and tentative conclusions are:

- The ion conductance of the α-hemolysin pore as a function of voltage is asymmetric even at the highest KCl concentrations where the static charges in the channel interior are maximally shielded.
- The crown blocks the channel in a strongly voltage dependent manner. We find that the blocking probability is a much sharper function of the applied voltage than predicted by the generalized Woodhull model for charged penetrating blockers.
- We suggest that both the residual asymmetry in the channel conductance and the discrepancy between the model predictions and the experimental observations of the crown blockage effect can be explained if one

assumes that the channel structure is deformed by the applied electric field. The supposed magnitude of the elastic deformation of the α-hemolysin pore is very small and our approach seems to be the only way to experimentally detect it at present.
– We discussed two possibilities, both referring to the increase in the pore diameter by negative potentials. One is the voltage-dependent binding interaction between the crown and a site in the channel pore; the other, more realistic, is the voltage-dependent barrier, a "geometric bottleneck," for the exit of the crown from the channel.

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Note added in proof

After this manuscript was accepted, Aksimentiev & Schulten published an article (Aksimentiev A. and Schulten K. Imaging a-Hemolysin with Molecular Dynamics: Ionic Conductance, Osmotic Permeability, and the Electrostatic Potential Map. *Biophys. J.* 88 June 2005 3745–3761) showing that residues, which are located at the outer surface of the beta-barrel could change their orientations without any large structural changes of the channel when altering the value of the transmembrane potential. Moreover, in the personal communication they revealed that switching the transmembrane potential from +120 to −120 mV (the sign is assigned to the cis opening of the channel) directs Lys147, on average, upward (to the cis vestibule of the channel), increasing the radius of the pore constriction by about 0.8 Å. Thus there is remarkable agreement between that theoretical prediction and our experimental data indicating the elastic deformation of the alpha-hemolysin channel under the electric field influence.

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