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NEUTRAL POLYMERS IN THE NANOPORES OF ALAMETHICIN AND ALPHA-HEMOLYSIN

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The ability of polymers to enter nanometer-scale pores can be probed by ionic channel conductance measurements because the movement of neutral polymer (e.g. poly(ethylene glycol), PEG) into the channel displaces ions and reduces their mobility in the pore. Both result in a reduction of the channel's conductance. Therefore, the state of occupancy of the pore by polymer is reflected by the conductance which we use to determine the polymer partition coefficient. We conclude that the available theoretical approaches to the entropic interaction between polymer and a pore (hard spheres, random flight model, and scaling theory) do not describe the partitioning of PEG into alamethicin and α-hemolysin. The empirically obtained partition coefficients for these two channels demonstrate a much sharper dependence on polymer molecular weight.

Many biological processes, including molecular interaction and recognition, molecular synthesis, ion transport, and polymer translocation take place at the nanometer-scale level. The last two use nanometer-scale pores in cell membranes; protein secretion [1–3], bacterial gene transduction, and viral infection [4] are prime examples. To reveal the mechanism by which polymers are transported through nanoscale pores, we study how differently sized molecules of a neutral polymer, poly(ethylene glycol), partition into a protein ion channel.

Water-soluble polymers have also been used as molecular probes of ion channel structure-function [5–14]. Interpretation of results obtained in those studies relied on either complete exclusion or partial partitioning of polymers between the bulk and the channel pore, a process that is dominated by the interaction between the polymer and the pore.

The changes in single channel conductance caused by polymer is a measure of pore occupancy by polymers. We deduce polymer partitioning as a function of polymer size for two chemically and structurally different channels, alamethicin and *Staphylococcus aureus* α-hemolysin (αHL). We compare the experimental results with the predictions of three theoretical approaches: hard spheres partitioning (e.g. [15]), random flight model [16], and scaling [17, 18]. We find that for both channels, the experimentally obtained dependence of polymer exclusion (or partitioning) on polymer weight is sharper than any of these models of pure entropic repulsion describes. We show that several possible complications that arise from either the deviation of pore shape from that of a regular cylinder, the polydispersity of PEG samples, or the non-ideality of concentrated polymer solutions do not solve the problem. In fact, each of them predict the opposite effect.

POLYMER PARTITIONING EQUILIBRIUM

In the case of a large pore (large with respect to the characteristic size of the polymer coil), one would expect a simple equi-partitioning of polymer between the bulk and the pore (Fig. 1, top). In this case, the action of polymer on the single pore conductance would be identical to that on the bulk conductivity because the average polymer density inside the pore is the same as that in the bulk. Neutral polymers (e.g. PEG) reduce the bulk conductivity of electrolyte solutions because their addition decreases the ionic concentration and increases the solution microviscosity. In addition, if ions bind to the polymer, this will further reduce the bulk conductivity.

For a small pore (small with respect to the characteristic size of the polymer coil), there is an entropic cost for confining a polymer in the pore because a number of possible polymer configurations are lost (Fig. 1, bottom). As a result, the average occupancy of the pore by polymer is decreased, and the polymer concentration in the pore will be less than that in the bulk. Correspondingly, the relative conductance reduction will be less than that for a large channel. In the case of very large, and therefore completely excluded polymers, there is no reduction in the conductance of the channel itself. In all cases, there is a small, and sometimes measurable, influence of polymer on the access resistance of the channel.

Several approaches are used to describe entropy-driven polymer exclusion. We discuss here three which provide analytical closed-form solutions: hard spheres partitioning [15], random flight model [16], and scaling [17, 18]. For the hard spheres model, we assume that the polymer radii r_b to scale as (molecular weight)$^{3/5}$. The corresponding partition coefficient between the

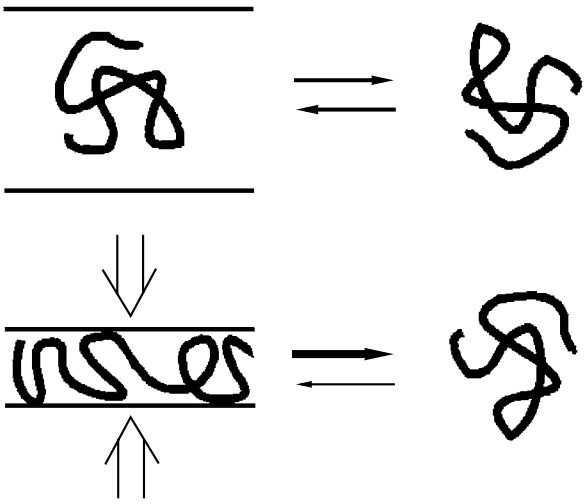

Fig. 1. Polymer partitioning into two idealized pores. Top: the polymer equilibrates between the bulk and a relatively large pore without distortion. Bottom: polymer entry into a relatively small pore is reduced because of entropic repulsion between the the polymer and the pore.

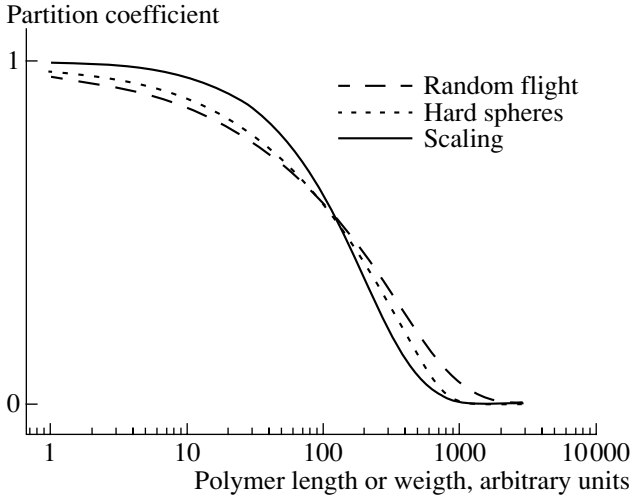

Fig. 2. A comparison between three theoretical descriptions of the entropic interaction between polymer and a pore: hard spheres (dotted line), random flight model (dashed line), and scaling theory (solid line). The polymer molecular weights are normalized to permit the midpoint of the three curves to coincide.

bulk and a cylindrical pore of radius R is given by [15]

$$p^{HS} = (1 - r_h/R)^2 \text{ for } r_b \le R \text{ and } 0 \text{ for } r_b > R. \quad (1)$$

Thus, even in the hard sphere model, the partition coefficient varies smoothly and monotonically with molecular weight and is zero for particles that are larger than the pore.

The random flight model [16] represents a polymer chain as a three dimensional random walk and accounts for the walks that are lost as a result of confinement. The partition coefficient is given by

$$p^{RW} = 4\sum_{m=1}^{\infty}\frac{1}{\beta_m^2}\exp[-(\beta_m r_i/R)^2] \quad (2)$$

where β_m are the roots of a zero order Bessel function of the first kind and r_i is the root-mean-square radius of an ideal chain ($r_i \sim$ (molecular weight)$^{1/2}$). Unlike the hard spheres model, this description predicts a finite value of the partition coefficient for particles that are larger than the pore.

The scaling theory approach considers the entropic cost of confining a large polymer chain in a long and narrow cylinder. Increasing the length of the polymer increases the number of "blobs" that are trapped in the cylindrical pore. The change in entropy is proportional to the polymer length and therefore the molecular weight [17, 18]. Because r_b scales as $\sim$ (molecular weight)$^{3/5}$, the partition coefficient is described by

$$p^{SC} = \exp[-\theta(r_h/R)^{5/3}] \quad (3)$$

where the parameter θ is not defined in scaling theory.

All three descriptions give rise to a smooth transition from equi-partitioning to complete exclusion (Fig. 2). Scaling theory gives the sharpest transition between these two regimes.

METHODS

The methods for measuring polymer partitioning are described elsewhere [7, 10, 19, 20]. Briefly, we measure the ionic current that flows through the pore at a constant applied potential in the presence of differently-sized poly(ethylene glycols), PEGs. Single channels were formed by adding either alamethicin or αHL to one side of a planar lipid bilayer membrane which was bathed by aqueous solutions containing 1 M NaCl, 2.5 mM MES (or HEPES) at pH 7.5 and 15% (w/w) of a given molecular weight PEG added to the salt solution. We typically used PEGs with molecular weights between 200 and 17000 Da.

RESULTS

The current through a fully open single αHL channel in the absence of polymer and in the presence of differently-sized PEGs is shown in Figure 3 [10]. The single channel current varies with PEG molecular weight in two ways. First, the mean current increases with increasing polymer weight. Second, there is a marked difference in the current noise of the pore's open state, depending on the polymer molecular weight. The noise corresponding to the current through the pore in the presence of PEG 2000 is much greater compared to that in the presence of higher and lower molecular weight polymers. Low molecular weight PEGs penetrate the pore and cause a significant decrease in the mean channel conductance. Intermediately-sized polymers cause a smaller decrease in the conductance but induce marked

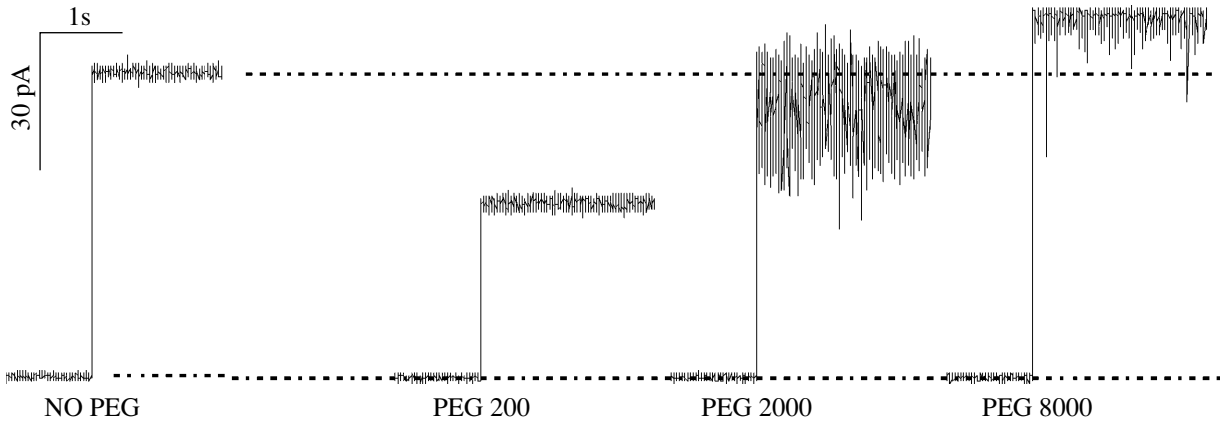

Fig. 3. The effect of different molecular weight PEGs on the current flowing through a single αHL channel. The current jump caused by the spontaneous formation of a pore in the absence of polymer is shown on the left. The other three recordings show the influence of differently sized polymers on the open channel current. The current noise varies non-monotonically in polymer molecular weight. The concentration of polymer was 15% for all PEGs and the applied potential was 100 mV.

fluctuations in the channel current. Large polymers, which are mostly excluded from the pore, increase the mean conductance. Qualitatively similar results were observed with single alamethicin ion channels [7, 19]. However, in this case, the low-frequency polymer-induced noise was not as pronounced.

A comparison of the steady-state mean conductance measurements for a single αHL and alamethicin channels in the presence of differently-sized PEG molecules is shown in Fig. 4. Level 1 of the multi-state alamethicin channel is chosen because its conductance, about 0.7 nS in 1M NaCl, in this state is close to that of the fully open αHL channel (~0.9 nS). We first consider the effect of PEG on the αHL channel conductance. Three features are clearly seen. First, PEGs with molecular weights ≤3400 partition into the pore and decrease the pore's conductance. Second, higher molecular weight PEGs, which apparently do not partition into the pore, increase the conductance; an effect caused by the water binding properties of PEG which increases the electrolyte activity [7]. Third, the lowest molecular weight PEGs (molecular weights ≤1000) decrease the pore conductance more effectively than they do the bulk solution conductivity (compare the data with the horizontal dotted line). The latter result suggests there is an attractive interaction between the polymer and pore. The mean conductance of a single alamethicin channel also decreases with decreasing PEG molecular weight. However, it does so less sharply. We discuss the significance of this difference below.

DISCUSSION

To determine the polymer partitioning into these two channels, we use the mean conductance data in Fig. 4 and assume that the polymer-induced conductance reduction is proportional to the polymer partition coefficient [10]. The results are shown in Fig. 5. Note that there is a significant difference between the polymer partition coefficients for αHL and alamethicin. For αHL, the slope of the partition coefficient dependence on polymer weight is much steeper. For alamethicin, the midpoint of the partition coefficient is shifted towards smaller polymer weights by at least two-fold.

The solid lines are the predictions for the partition coefficient using scaling theory. The fit is not good because it does not adequately describe the steepness of the molecular weight dependence. Although, as is shown in Fig. 2, scaling theory gives the sharpest transition between partitioning and exclusion compared to the hard-spheres and random-flight models, it is not sharp enough. The deviation is most pronounced for the αHL channel, but is also clearly seen for the alamethicin channel. We conclude that independent of the size, structural features, and chemical composition of these two pores, the three theoretical approaches do not quantitatively describe the empirically obtained polymer partitioning data.

The polymer partition coefficient is obtained here from conductance data. Implicit is the assumption that the effect of polymer on bulk conductivity and on channel conductance are caused by the same two primary mechanisms (an increase of the solution microviscosity and by dilution). This assumption is plausible because channel pores are large both with respect to the PEG monomer size and the Debye screening length in 1 M electrolytes. Also, for small PEGs that partition easily into the channel, the relative reduction in the pore conductance is close to the relative reduction in bulk solution conductivity (Fig. 4). Thus, there is no reason to expect a pronounced non-linearity between polymer partitioning and channel conductance reduction.

Using the above assumption, we consider three other possible complications that, at first glance, may account for deviations from ideal partitioning. They include: the pore's shape differs from that of a regular

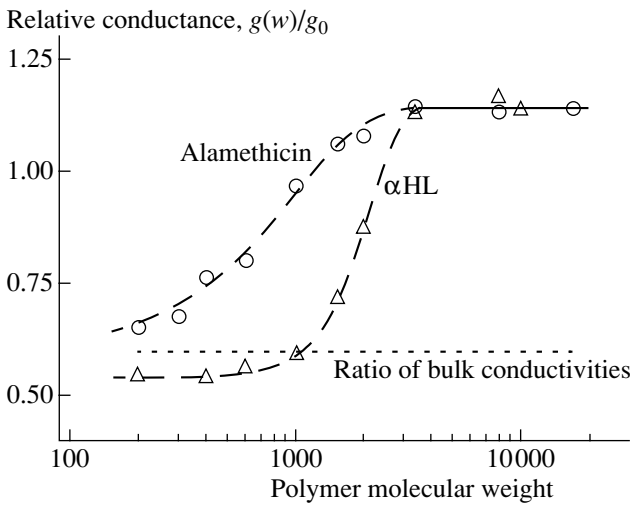

Fig. 4. The dependence of the mean conductance of single αHL and alamethicin ion channels on PEG molecular weight. The conductance values in the presence of PEG, $g(w)$, are normalized to that in the absence of polymer, g_0.

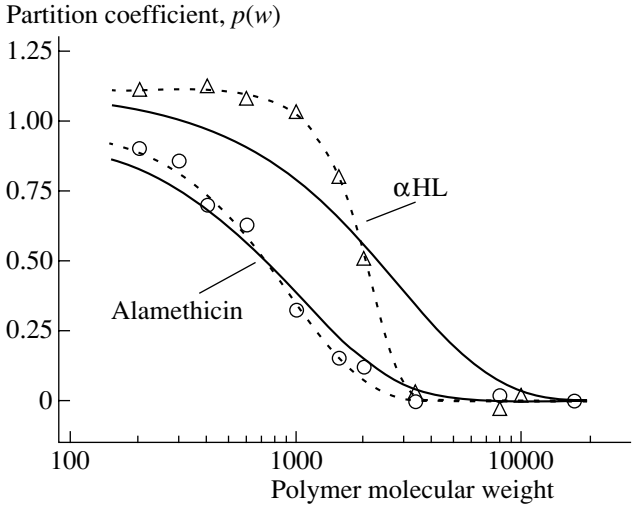

Fig. 5. Polymer partitioning into single αHL (triangles) and alamethicin (circles) ion channels as a function of PEG molecular weight. The solid lines are the least-squares best-fit predictions for the partition coefficient using scaling theory [17, 18]. The dashed lines are drawn to guide the eye.

cylinder, the PEG samples are poly-disperse mixtures with a number of polymer sizes present, the polymer solutions are not ideal at 15% (w/w) concentrations used in partitioning measurements.

Each of these complications results in the opposite effect, i.e. the flattening of the dependence of partitioning on polymer molecular weight. Deviations in the shape of the pore from a regular cylinder (e.g., to a conical shape) will lead to a wider transition range because the molecular weight cut-off will be converted from one value of polymer size to a spectrum of sizes.

Polymer size polydispersity also widens the transition range. Consider a polydisperse PEG sample with a primary size that is excluded from the pore. The low molecular weight components in the PEG sample will still partition into the pore and reduce the pore conductance, which would not occur if the PEG sample was monodisperse. In the other extreme of polymer partitioning (i.e. a low molecular weight PEG sample), high molecular weight components of the sample will be excluded from the pore. Thus, the pore conductance will decrease to a lesser extent compared to monodisperse low molecular weight PEG sample. A recent study addressed this question [21].

The effects of polymer solution non-ideality were discussed previously [14]. It was shown that only the highly artificial hard spheres model gives some sharpening of the transition. However, it is well known that PEG in water forms flexible coils with a Kuhn length of several Angstroms. Scaling arguments predict a shift in the partition coefficient curve to higher polymer molecular weights if polymer-polymer repulsion is included. Moreover, if we consider the increase in this repulsion as the polymer molecular weight is increased (solutions of higher weight PEGs are less ideal at the same weight/weight concentration [22]), the polymer solution non-ideality will broaden the transition between partitioning and exclusion. Larger polymers are driven into the pore with a stronger force of polymer-polymer repulsion.

CONCLUSIONS

The empirical study of polymer partitioning into nanometer-scale pores reveals a simple qualitative picture. Large polymers are excluded from the pores and thus do not significantly influence the pore conductance whereas small polymers equi-partition into the pore and reduce its conductance to the same degree as they decrease the bulk solution conductivity. The transition between complete exclusion and partitioning reveal the characteristic size of the pore. The larger the pore, the higher the polymer molecular weight at which this transition occurs. However, a rigorous quantitative analysis of the data clearly shows that three available theoretical models for entropic repulsion fail to describe the sharpness of the transition. Hard spheres, random flight model, and scaling theory predict a smoother dependence of polymer partition coefficient on molecular weight than is observed.Our analysis suggests that several possible complications caused by either the deviation of the pore shape from a regular cylinder, polydispersity of PEG samples, or non-ideality of concentrated polymer solutions all predict the opposite effect, i.e. a flattening of the dependence of partitioning on polymer molecular weight.

One might suggest that the deviation of the empirical polymer partitioning (Fig. 5) from the theoretical predictions discussed arises because of the finite size of the polymer. Polymers with small degrees of polymerization, e.g. PEGs with molecular weight less than

1000 Da, do not represent good random coils. The smallest polymers used in our study probably have an appearance more like a curved rod than a coil. Thus, the concepts of statistical polymer physics discussed above should be applied with caution. However, the results in Fig. 5 demonstrate that the larger channel (αHL) has a steeper dependence of the partition coefficient on polymer molecular weight than does the smaller channel (alamethicin). Thus, the deviation of the data from theoretical predictions is greater for the larger pore and therefore for larger polymers. Specifically, for αHL, the transition between partitioning and exclusion occurs over a PEG molecular weight range of 1000 and 4000 Da. This observation seems to disagree with the finite size argument discussed above, but does not necessarily exclude this possibility.

We conclude that more theoretical and experimental research is needed to reach a quantitative understanding of the mechanisms controlling flexible polymer partitioning at these biologically important length scales. New models for polymer partitioning must consider interactions between the polymer and the nanometer pore other than the purely entropic repulsion of polymer by an inert geometric constriction.

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REFERENCES

1. *Simon S.M., Blobel G.* // Cell. 1991. V. 65. P. 371–380.
2. *Walter P., Johnson A.E.* // Cell. 1994. V. 78. P. 461–471.
3. *Bustamante J.O., Hanover J.A., Leipins A.* // J. Membrane Biol. 1995. V. 146. P. 239–252.
4. *Miller R.V.* // Scientific American. 1998. V. 278. P. 66.
5. *Zimmerberg J., Parsegian V.A.* // Nature (London). 1986. V. 323. P. 36–39.
6. *Krasilnikov O.V., Sabirov R.Z., Ternovsky V.I., Merzliak P.G., Muratkodjaev J.N.* // FEMS Microbiol. Immun. 1992. V. 105, P. 93–100.
7. *Bezrukov S.M., Vodyanoy I.* // Biophys. J. 1993. V. 64. P. 16–25.
8. *Parsegian V.A., Bezrukov S.M., Vodyanoy I.* // Bioscience Repts. 1995. V. 15. P. 503–514.
9. *Korchev Y.E., Bashford C.L., Alder G.M., Kasianowicz J.J., Pasternak C.A.* // J. Membrane Biol. 1995. V. 147. P. 233–239.
10. *Bezrukov S.M., Vodyanoy I., Brutyan R.A., Kasianowicz J.J.* // Macromolecules. 1996. V. 29. P. 8517–8522.
11. *Desai S.A., Rosenberg R.L.* // Proc. Natl. Acad. Sci. USA. 1997. V. 94. P. 2045–2049.
12. *Bezrukov S.M., Kasianowicz J.J.* // Eur. Biophys. J. 1997. V. 26. P. 471–476.
13. *Kaulin Y.A., Schagina L.V., Bezrukov S.M., Malev V.V., Feigin A.M., Takemoto J.Y., Teeter J.H., Brand J.G.* // Biophys. J. 1998. V. 74. P. 2918–2925.
14. *Merzlyak P.G., Yuldasheva L.N., Rodrigues C.G., Carneiro C.M.M., Krasilnikov O.V., Bezrukov S.M.* // Biophys. J. 1999. V. 77. P. 3023–3033.
15. *Colton C.K., Satterfield C.N., Lai C.-J.* // AIChE J. 1975. V. 21. P. 289–298.
16. *Casassa E.F.* //Polymer Lett. 1967. V. 5. P. 773–778.
17. *de Gennes P.-G.* // Scaling Concepts in Polymer Physics. Ithaca, NY: Cornell University Press, 1979.
18. *Grosberg A.Yu., Khokhlov A.R.* // Statistical Physics of Macromolecules. New York, NY: AIP Press, 1994.
19. *Bezrukov S.M., Vodyanoy I., Parsegian V.A.* // Nature (London). 1994. V. 370. P. 279–281.
20. *Bezrukov S.M.* // J. Membrane Biol. 2000. V. 174. P. 1–13.
21. *Berestovskii G.N., Ternovskii V.I., Kataev A.A.* // Biofizika. 2000. V. 45. P. 69–78.
22. *Parsegian V.A., Rand R.P., Fuller N.L., Rau D.C.* // Methods Enzymol. 1986. V. 127. P. 400–416.

НЕЙТРАЛЬНЫЕ ПОЛИМЕРЫ В НАНОПОРАХ АЛАМЕТИЦИНА И α-ГЕМОЛИЗИНА

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Способность полимеров проникать в узкие поры размерами в несколько нанометров можно проверить, измеряя проводимость ионных каналов, поскольку нейтральный полимер (например, полиэтиленгликоль, ПЭГ) замещает внутри канала ионы и понижает их подвижность в поре. Оба эффекта приводят к понижению проводимости канала. Влияние полимера, проникающего в пору, на проводимость мы использовали для определения коэффициента распределения полимера. Мы пришли к выводу, что существующие теоретические подходы к оценке энтропийного взаимодействия полимера и поры (модель твердых сфер, случайного пролета и теория масштабов) не описывают распределение ПЭГ в аламетицине и α-гемолизине. Эмпирически найденные коэффициенты распределения для этих двух каналов имеют существенно более крутую зависимость от молекулярного веса полимера.