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Polyelectrolyte and unfolded protein pore entrance depends on the pore geometry

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ABSTRACT

We determined the ability of Maltose Binding Protein and the polyelectrolyte dextran sulfate to enter into and interact with channels formed by *Staphylococcus aureus* α-hemolysin. The entry of either macromolecule in the channel pore causes transient, but well-defined decreases in the single-channel ionic current. The protein and polyelectrolyte were more likely to enter the pore mouth at the channel's cap domain than at the stem side. When the cap domain was denatured in the presence of 4 M urea, the probability that either the denatured protein or polyelectrolyte entered the pore from the cap-domain side decreased. For channels in their native conformation, the polyelectrolyte-induced current blockades were characterized by two mean residence times that were independent of the side of entry. For channels with a denaturated cap domain, the mean polyelectrolyte residence times for relatively long-lived blockades decreased, while that for short-lived blockades were unchanged. For denatured protein, we also observed 2 characteristic residence times that were relatively fast. Only the relatively short-lived blockades were observed with native channels. When the α-hemolysin monomers in aqueous solution were incubated in 4 M urea before channel formation, the two characteristic residence times were greater than those for pre-formed pores that were subsequently perturbed by urea. These times might correspond to the interactions between the unfolded protein and the partially unfolded channel.

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1. Introduction

Transport of macromolecules through membrane channels plays an important role in many biological processes. Most experiments trying to recreate this phenomenon use the same protein as a model channel, α-hemolysin inserted into a bilayer lipid membrane. This pore is an asymmetric mushroom shaped protein of beta-structures [1]. At its widest and narrowest point, the pore diameter is 4.6 and 1.4 nm, the pore length is around 10 nm. The conductance of the pore is higher at positive voltage than negative voltage with a non linear transition around zero [2]. The pore is very stable as a function of environment, temperature [3], pH [4] or urea [5] and it remains open over extended periods. The first experiment on the passage of one single-stranded DNA through an α-hemolysin pore was performed in 1996 [6]. The single-channel ionic current decreased transiently because of the transit of individual molecules through the pore. The sensitivity of electrical detection of molecule transport through a single protein channel has been used in many research areas. Fundamental experiments of translocation have been performed to study i) the dynamics of confined neutral polymer [7] and confined charged polymer chains [8–15], ii) the peptide or polypeptide–pore interaction [16–18] and mutant protein translocation [19], iii), the translocation coupled to the protein unfolding [20], and iv) to measure DNA–protein interactions [21]. In addition, several applications of this technique are possible: the ultra-fast sequencing of DNA and RNA [22;23], and the development of chemical and biological sensors [24;25]. Recent developments also include the transformation of nanopores into manipulation tools and force apparatus by active control techniques [26–28]. Up to now few experiments have been performed with channels to study the entry of charged macromolecules into the pore as a function of the channel geometry [29;30]. In the study by Henrickson, et al., they found that, at a given concentration of polynucleotide and magnitude of the applied potential, the single-stranded DNA molecules were more likely to enter the α-hemolysin pore from the cap domain entrance than from the stem domain pore mouth. The authors of this work suggested several reasons to account for those results: polynucleotide entry from the cis side, large vestibule, may be favoured by a lower entropic barrier and or an electrostatic attraction [29]. In the study of Kullman et al., they show that the constant rates for sugar entering the maltoporin channel differ significantly for the cis and trans sides. The sugar residence time in the channel does not depend on which side the sugar is added [30].

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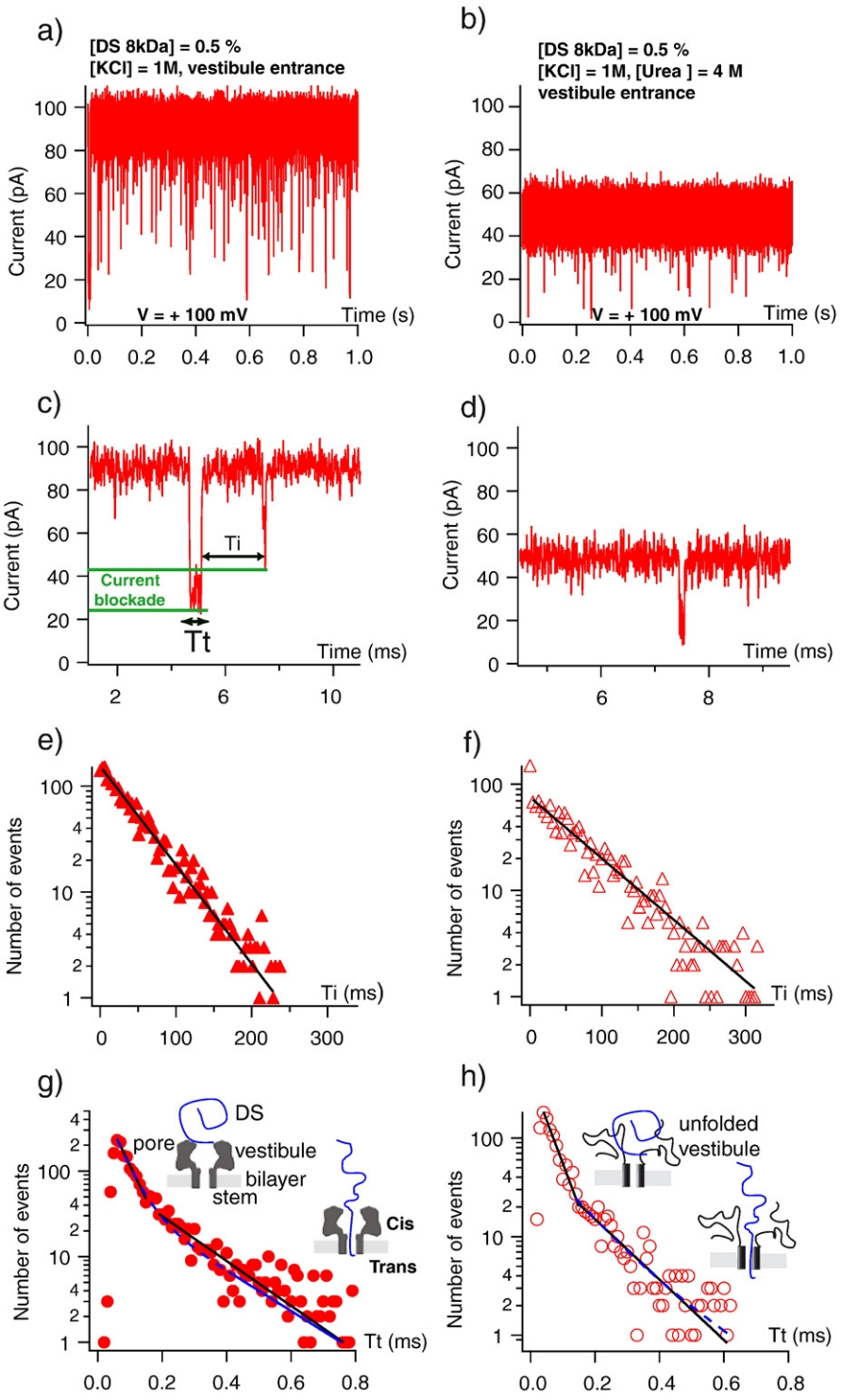

Fig. 1. Single molecule analysis of polyelectrolyte pore entrance through different channel geometries. Experiments performed with native protein pore (left, panels a, c, e, g) or with partially denaturated protein pore (right, panels b, d, f, h). Current traces through a protein pore inserted into a planar lipid bilayer in the presence of dextran sulfate (a, b, c, d), and statistical analysis of an example of the measured current trace (e, f, g, h). We define the times Tt: duration of the transient and Ti: time between two transients (c). Distribution of time intervals Ti (e, f). Distribution of blockade times Tt (g, h), continuous lines are single exponential fits (e, f, g, h), dashed line is a double exponential fit (g, h).

Notes to Table 1
Native: α-hemolysin channel without denaturing agent (#) or incubated with guanidium at 1.3 M (⊗).
Partially denaturated channel: the pore is formed before urea denaturation at 4 M (◊); the α-hemolysin is incubated in urea solution at 4 M before pore formation (*).
F (Hz): the frequency of blockade events.
t_1 (μs): mean short blockade time.
t_2 (μs): mean long blockade time.
A_1/A_2: is the ratio of the population of short events to the population of long events.
The applied voltage is $V = +100$ mV.

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