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Single-Molecule Electrophoresis of β-Hairpin Peptides by Electrical Recordings and Langevin Dynamics Simulations

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We used single-channel electrical recordings and Langevin molecular dynamics simulations to explore the electrophoretic translocation of various β-hairpin peptides across the staphylococcal α-hemolysin (αHL) protein pore at single-molecule resolution. The β-hairpin peptides, which varied in their folding properties, corresponded to the C terminal residues of the B1 domain of protein G. The translocation time was strongly dependent on the electric force and was correlated with the folding features of the β-hairpin peptides. Highly unfolded peptides entered the pore in an extended conformation, resulting in fast single-file translocation events. In contrast, the translocation of the folded β-hairpin peptides occurred more slowly. In this case, the β-hairpin peptides traversed the αHL pore in a misfolded or fully folded conformation. This study demonstrates that the interaction between a polypeptide and a β-barrel protein pore is dependent on the folding features of the polypeptide.

Introduction

A major goal of bionanotechnology is the study of biomolecules at single-molecule resolution. In this way, both the kinetics and thermodynamics of structural fluctuations and self-assembly can be illuminated. In this work, single-channel electrical recordings in planar lipid bilayers and Langevin molecular dynamics simulations were used to probe the translocation of single β-hairpin peptides[1] of the B1 domain of protein G through an α-hemolysin (αHL) protein pore.[2] The αHL pore is a heptameric mushroom-shaped protein of known crystal structure[2] and remains open for long periods in an applied transmembrane potential.[3,4] Three β-hairpin peptides (Table 1) were selected for this preliminary study: (i) the wild-type peptide (G41) has the sequence GEWTYDDATKTFT-VTE (residues 41−56),[1] (ii) Ac-G40 has acetylated Gly at the N-terminus (residues 40−56), and (iii) K41 has a Lys residue in position 41 (residues 41−56).

It has been suggested by molecular dynamics simulations,[5] and experimentally confirmed by NMR and circular dichroism studies,[6] that simple replacement of the first residue or acetylation of the N-terminus can alter the folding properties of the β-hairpin peptides. Indeed, the three β-hairpins explored in this work, which varied by the number of terminal ion pairs, had different stabilities in the aqueous phase (Table 1).[6]

TABLE 1: The Three β-Hairpin Peptides Used in Single-Channel Electrical Recordings

peptide	ion pairs	charge[a]	MW (kDa)	fraction of folded peptides[b]	τ_{off}[c] (μs)
Ac-G40	0	−4	1.91	<5%	730 ± 90
G41	2	−3	1.86	∼30%	1150 ± 50
K41	4	−2	1.94	∼50%	1210 ± 40

[a] The charge is estimated at pH 7.0. [b] As determined previously by circular dichroism spectroscopy.[6] The number represents the percentage of folded β-hairpin peptides out of the total amount of molecules present in the aqueous phase.[6] The fraction of folded peptides is related to the free energy of folding, assuming the two-state folding behavior.[6] [c] The average dwell time of long-lived events (τ_{off}) that corresponds to a force of 3.8 pN (see Figure 2).

Single-Channel Electrical Recordings

The interaction of the negatively charged β-hairpin peptides with the wild-type αHL protein pore was examined by single-channel electrical recordings.[7−9] The β-hairpin peptides, when added to the trans side of the bilayer at low micromolar concentrations, produced transient current blockades (Figure 1 and the Supporting Information), the nature of which depended on the features of the β-hairpins (Table 1). The frequency of the current blockades increased with the applied transmembrane potential (Supporting Information Figure S2), similar to the findings of a previous study with α-helical peptides.[4]

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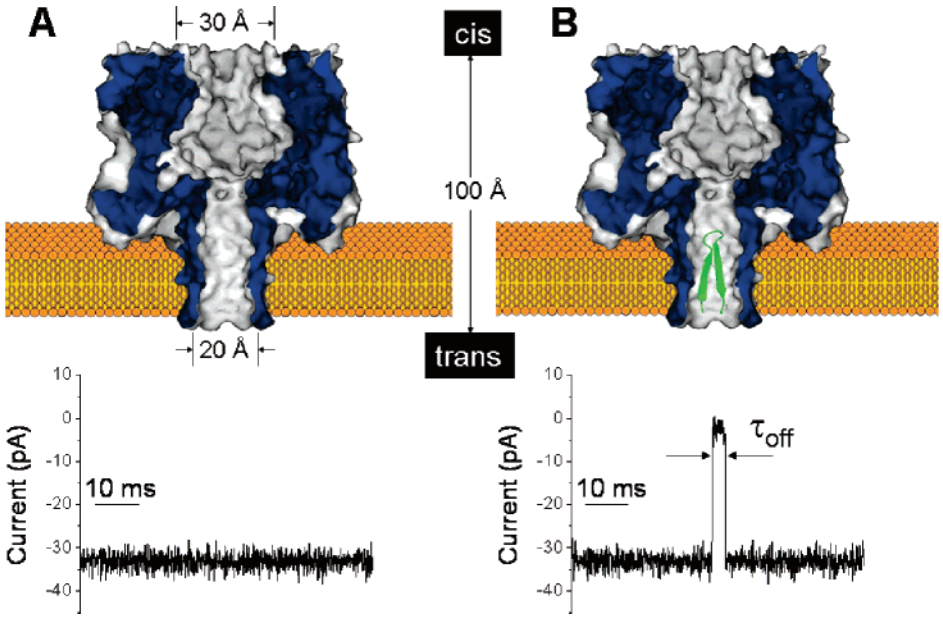

Figure 1. Interaction of β-hairpin peptides with an αHL pore: (A) αHL forms a channel that remains open for long periods. (B) The translocation of a β-hairpin peptide through an αHL pore produces a transient current blockade.

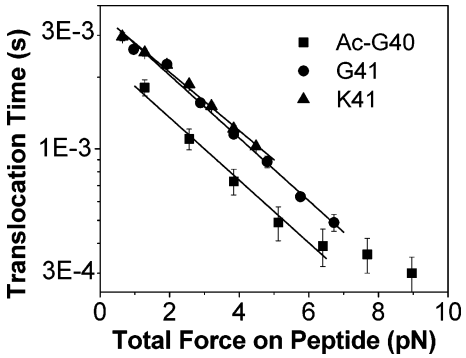

Figure 2. Dependence of the dwell time of the long-lived current blockades produced by the β-hairpin peptides on the electric force. The electric force was derived as $F = qV/l$, where the effective charge (q) is half of the peptide charge,[4] V is the transmembrane potential, and $l = 50$ Å is the length of the β-barrel lumen. The concentration of the β-hairpins added to the trans side was 40 μM. The buffer solution in the chambers was 1 M KCl, 10 mM potassium phosphate, pH 7.5.

The log likelihood ratio (LLR) test[3,4,10] of dwell time histograms indicated two species of current blockades: 85−95% were very short-lived current spikes, with a duration of several tens of microseconds, and 5−15% were long-lived current blockades, with a duration of several hundreds of microseconds to milliseconds. We interpret that the very short-lived spikes are either collisions of β-hairpin peptides with the trans opening of the pore[4] or fast translocations of the β-hairpin peptides in extended conformation (see the Langevin dynamics simulations below). Interestingly, the voltage dependence of the long-lived current blockades revealed an exponential decrease in the event duration with the applied transmembrane potential (Figure 2). This finding suggests that the long-lived events represent translocations of the β-hairpin peptides through the αHL pore, because an increase in the driving force produced a decrease in the translocation time (Figure 2). Importantly, for similar electric forces, the long-lived events produced by the unfolded Ac-G40 peptide were shorter than those values measured for the folded G41 and K41 peptides (Figure 2).

Langevin Molecular Dynamics Simulations

In parallel with these single-channel studies, we have performed Langevin dynamics simulations of peptide translocation using a minimalist off-lattice model[11−17] for both the β-hairpins and the β-barrel part of the αHL pore. In this model, each residue was treated as a united atom that could be hydrophobic, hydrophilic, or neutral.[11] While more detailed, all-atom simulations of peptides including explicit solvent molecules are desirable, they remain computationally prohibitive for processes occurring at sub-microsecond time scales, forcing us to use such coarse grained models. We have designed sequences of four β-hairpin peptides, all of which show two-state folding but differ in their thermodynamic stability and their hydrophobic content. The hydrophobic residue content is 56, 37, 37, and 37%, and the fraction of folded molecules in the equilibrium ensemble is 82, 70, 54, and 32% for peptides 1−4, respectively. Residues in each of the peptides were assigned charges corresponding to those in the K41 peptide. The simulation results are summarized in Figure 3.

The decrease in translocation time with an increase in the electric force, observed both in the experiments (Figure 2) and in the simulation data (Figure 3), can be qualitatively understood within the framework of Bell's model, where escape from the pore involves crossing a free energy barrier that is lowered by the electric force.[16,18] In this picture, the slope of the curves plotted in Figures 2 and 3 is directly related to the location of the barrier inside the pore; we find a reasonably good agreement between the experimental slope and that found from simulations at low forces (Supporting Information). The Bell picture does not apply to translocation at high forces, where the barrier vanishes. The translocation time is correlated with the equilibrium fraction of the folded molecules and decreases monotonically from peptide 1 to peptide 4. The somewhat counterintuitive observation that translocation is faster for the peptides that are more likely to be unfolded can be understood if one considers the three typical translocation trajectories observed in the simulations (Figure 3B). In the trajectories of type 1, the peptide enters the pore in an extended conformation and the residues are threaded through the pore in a single file. This type of

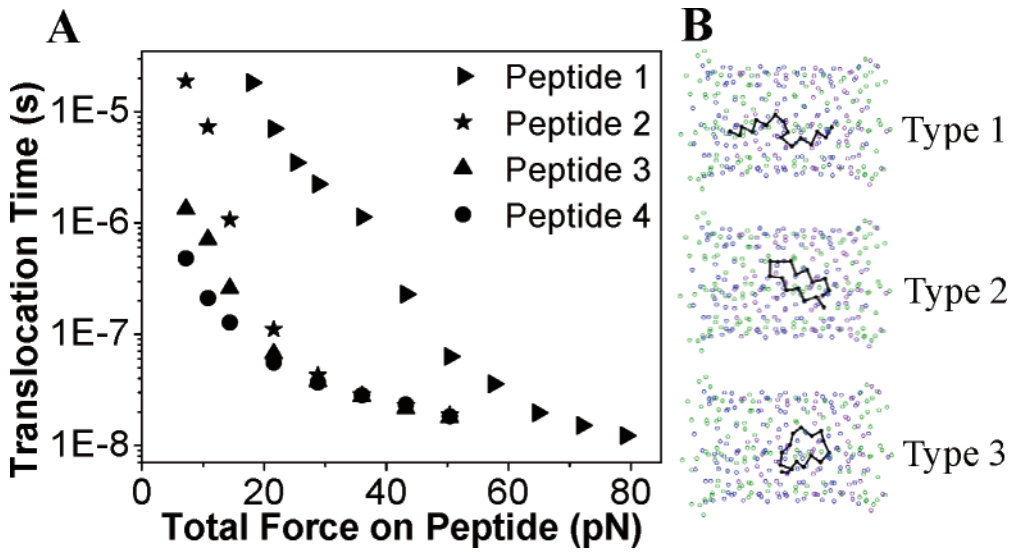

Figure 3. (A) Computed mean translocation time as a function of the total force on the β-hairpin. (B) Typical conformations of the peptide inside the β-barrel part of the αHL pore corresponding to three types of observed translocation trajectories. See the Supporting Information for the animations of the entire trajectories. Only the positions of the C_α atoms are shown in the pore structure.

trajectory corresponds to the fastest observed translocation times. In type 2 trajectories, corresponding to longer dwell times, the peptide remains folded as it goes through the pore. Type 3 trajectories, which were only observed for the most hydrophobic peptide 1, correspond to the slowest translocation events, in which a peptide−pore complex is formed and the peptide remains trapped inside the pore in a misfolded conformation, until it either refolds or attains an extended conformation to exit the pore. Less stable hairpins are more likely to enter the pore in an open conformation, resulting in a fast type 1 translocation event. In contrast, translocation of the more stable peptides is more likely to occur via the type 2 or 3 trajectories.

Discussion

Minimalist peptide models of the type used here are not realistic enough to allow a direct quantitative comparison with experimental data for a number of reasons. Since the translocation mechanism here involves activated barrier crossing, the translocation time is exponentially sensitive to the height of the free energy barrier, which cannot be reliably estimated with a model that treats interaction energies in a very crude way and ignores the molecular structure of the solvent. In addition, CPU limitations commonly necessitate using electric forces that are higher than those employed experimentally, although the use of a minimalist model has enabled us to achieve an overlap between the simulated and experimental force ranges. Despite the above caveats, the simulated translocation times and the experimental translocation times extrapolated to higher forces are within an order of magnitude from one another (Supporting Information Figure S5) and show similar sensitivity to the force (i.e., the slope in the curves plotted in Figures 2 and 3 and also in Supporting Information Figure S5), suggesting that our model may correctly capture the translocation mechanism, even though no attempt was made to adjust the parameters of the model to fit the experimental data. The translocation trajectories observed in the simulations provide a plausible explanation of the experimental correlation between the equilibrium fraction of folded hairpins and the translocation time.

Furthermore, turning off the hydrophobic/hydrophilic interactions between the peptide and the pore in the simulation has resulted in translocation times being ∼2−3 orders of magnitude shorter. This suggests that geometric confinement effects alone cannot explain the experimentally observed translocation time scales. On the other hand, strong attractive interactions between a peptide and a pore may lead to a non-monotonic force dependence of the translocation time,[4] which is not observed.

These observations, combined with recent single-channel studies on the translocation of α-helical peptides through the αHL pore,[4,19] reveal the complexity of the translocation mechanism and its sensitivity to the details of the peptide/pore interactions as well as to the secondary structure of the peptide. It is also conceivable that the kinetics of the translocation of polypeptides through protein pores is dependent on the features of the pore lumen.[20] More experimentation and computation, to reveal changes in the translocation kinetics with dramatic alterations of these two factors, is underway in our groups. It is expected that these efforts will help us to obtain a mechanistic understanding of how a polypeptide translocates though a protein channel, which is both fundamental and ubiquitous in biology.[14−16,21]

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Supporting Information Available: Materials and Methods section, details of single-channel recordings, Langevin dynamics simulations, and supplementary figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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