Kinetic Analysis of Single Molecule Electrodiffusion in a Biological Nanopore with Two Binding Sites

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When single short adenine oligonucleotides (d.p. 3-5) block the pore formed by the bacterial toxin aerolysin, the resultant resistive pulses are complex with two levels (short and deep vs. long and shallow) in a variety of configurations, the probability of which depends on voltage. We account for this by a four-state kinetic mechanism with two undistinguishable open states i,o and two blocked states m1 (short-deep) and m2 (long-shallow) linked as o<->m1<->m2->i. M1 is directly accessible from o which designates the presence of the nucleotide at the pore0s cis-side mouth, while m2 is inaccessible from state i, as nucleotides are unable to enter from the trans-side irrespective of voltage. In this framework, several experimentally accessible statistical quantities such as the frequency or probability of returns to m1 from m2, the fraction of restive pulses ending in m1and the mean dwell times in m2 as well as the mean total duration of resistive pulses acquire mechanistic significance and allow direct kinetic predictions using a Q-matrix approach. For A3, the data are consistent with a charged particle moving through a one-dimensional energy landscape with two minimain an electrically biased random walk. For longer nucleotides (e.g. A5) the success rate for translocation is higher than predicted by the rate constants determined from the other observables. It appears likely that this is due to an ability of the longer oligomers to simultaneously link with the two binding sites, producing an excess of returns from m2 to m1, which, however, does not entail a propensity to result in translocation failures. Such double tethering of DNA might promote translocation of longer chains by producing an extended conformation and may also contribute to the strong rectification of transport observed for aerolysin.