REPLY TO YAMINI AND NESTOROVICH:

Alternate clamped states of the anthrax toxin protective antigen channel

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Yamini and Nestorovich (1) recently commented on our article in PNAS (2). In their letter, they weigh in on two issues with the previously reported (3, 4) alternate conductance substate of the protective antigen (PA) translocase channel.

The first point they raised was whether the conductance substate was a clamped state of the PA channel's phenylalanine clamp (ϕ-clamp) site. We believe that such is the case because when the ϕ-clamp was mutated from phenylalanine to alanine, the current difference between the two substates of the channel increased from 0.7 pA for wild type to 2.1 pA for the PA F427A mutant. Because the ϕ-clamp is the major conductance bottleneck in the channel, dilation of this structure may explain the current difference. Because the ϕ-clamp is critical to the mechanism of peptide translocation, we named the two states unclamped empty dilated and clamped empty, where empty denotes that the channel is peptide-free.

The second point raised was related to a fast-flickering closure of the PA channel. We did not study this process directly; however, we attributed some of the slower time-scale closures of the PA channel to contaminants in the buffer or bilayer setup. This attribution should have included a spurious gating process in the channel itself as well, and we thank Yamini and Nestorovich (1) for heroically showing that these closures occur in highly purified water. Nonetheless, this point is relatively minor and has no bearing on the central arguments of the paper.

Yamini and Nestorovich (1) also suggest the time scales of the two conductance substates were relevant in the absence of peptide. They cited a study where the lower conductance state was populated only "on occasion." We contend that this time scale is not relevant to the system containing the peptide, because the peptide allosterically alters the system >300-fold in terms of the equilibrium dissociation constants for the relaxed and taut states of the system. A relatively rare appearance of the subconductance state is in line with this large change in binding dissociation constants. They additionally cited that the binding of β-cyclodextrins to the two subconductance states of the system was indistinguishable in terms of their equilibrium dissociation constants. What may be true for β-cyclodextrins is not necessarily the case for peptides. We contend that the allosteric effect measured for peptides is unique for peptides that can adopt an α-helix, where the shape of a β-cyclodextrin is much different from a peptide α-helix.

Acknowledgments

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3 Nestorovich EM, Karginov VA, Berezhkovskii AM, Bezrukov SM (2010) Blockage of anthrax PA63 pore by a multicharged high-affinity toxin inhibitor. *Biophys J* 99(1):134–143.
4 Blaustein RO, Lea EJ, Finkelstein A (1990) Voltage-dependent block of anthrax toxin channels in planar phospholipid bilayer membranes by symmetric tetraalkylammonium ions. Single-channel analysis. *J Gen Physiol* 96(5):921–942.

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