Dissecting movement of the transmembrane segments of non-gastric proton pump mutants with voltage-clamp fluorometry

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The Na^+,K^+-ATPase (NKA) extrudes 3 Na^+ and imports 2 K^+ across the plas- malemma of every animal cell. The non-gastric H^+,K^+- ATPase (ngHKA) ex- ports one H^+ and imports one K^+ in the apical membrane of several epithelia. Both P-type pumps have ~65% identity and nearly identical catalytic cycles driven by binding of different ions to two major conformations (E1, high affinity for Na^+ or H^+ and E2 higher affinity for K^+). Recently, we reported the structures of the wild type (WT) ngHKA and its electrogenic mutant K794A and the NKA-like K794S/A797P/W940/R949C (SPWC) mutant, in the K^+- occluded E2 state. We also reported SPWC's AMPCP-bound E1 Cryo-EM structure, which is nearly identical to that of the E1($3Na^+$) NKA. We obtained the WT (Na^+ and AlF-ADP) and K794A (Na^+ and AMPPCP) structures. While the WT structure was identical to E2P state, the AMPPCP-bound K794A structure with (presumably) 2 Na^+ bound has a mixed conformation. The P and N domains as well as cytoplasmic portion of transmembrane segment TM4 shows E1-like conformation, but the A domain, TM1-TM3 and the luminal portion of TM4 took an E2-like conformation. To evaluate displacements of the moving TM segments in all three constructs, we introduced a single Cys residue in the loops between TM1-TM2, TM3-4, or TM5-6, labeled them with tetramethylrhodamine maleimide (TMRM) and evaluated currents and fluorescence changes under voltage clamp fluorometry in *Xenopus* oocytes. Consistent with the stable E2 state in WT, a TMRM introduced in TM1-TM2 showed minimal voltage-dependent changes in fluorescence, while the voltage dependence of the TM1-TM2 fluorescent signals observed with K794A and SPWC mutants were progressively closer to signals observed in NKA. Studies with TMRM introduced at TM3-4 and TM5-6 positions are underway. Funded by NSF MCB-2003251.