Title: PI-(3,5)P_2 mediated oligomerization of the endosomal sodium/proton exchanger NHE9

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Abstract

NHE9, a crucial component found in all cells, plays a pivotal role in maintaining intracellular pH, sodium levels, and cell volume regulation.NHE9 (SLC9A9), intricately fine-tunes the pH within the endosomes of hippocampal neurons. Its activity has been closely associated with a range of health conditions, including glioblastoma, autism spectrum disorders, epilepsy, and attention deficit hyperactivity disorder (ADHD). We present cryo-electron microscopy (cryo-EM) structures of the NHE9 homodimer at impressive resolutions of 3.2 Å and 3.6 Å. These newly revealed structures uncover previously unknown features of NHE9. Notably, we unveil a loop domain, comprising two β-hairpin strands, positioned approximately 15 Å above the dimerization interface. This domain interacts with the endosome-specific lipid PI-(3,5)P2. Our findings are substantiated through a combination of thermal-shift assays, solid-state membrane (SSM) electrophysiology, and molecular dynamics (MD) simulations, all of which confirm the specific binding of PI-(3,5)P2 to NHE9. Furthermore, we establish that this lipid enhances sodium binding.In light of our findings, we propose a model in which the activity of the late endosomal exchanger NHE9 is tightly regulated by the PI-(3,5)P2 lipid. This model suggests an 'activation-upon-arrival' mechanism driven by lipids, aligning seamlessly with the well-established requirement for both NHE9 and PI-(3,5)P2 in ensuring the correct trafficking of epidermal growth factor receptor (EGFR).