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Abstract: 86O

Structural mechanisms of human Aquaporin-1 ion channel gating and block by divalent cations, analysed by histidine scanning mutagenesis.

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Many classes of aquaporins (AQPs) have recently been recognised as gated multifunctional channels needed for enabling fluxes of diverse signalling and metabolic agents, well beyond the permeability to water which was originally thought to define the AQP channel superfamily. Water- and glycerol-selective pores are located individually in each subunit of AQP tetramers. The central pore in the middle of the tetramer serves as an ion channel in human AQP1 and a subset of other AQP classes. In hAQP1, the non-selective monovalent cation conductance is activated by binding of intracellular cGMP at a cytoplasmic gating domain. Prior work using molecular dynamic simulations [(1)] identified hydrophobic barrier residues within the central pore thought to limit ion conduction in the closed state. Here, we tested the idea that hAQP1 ion channel opening involves substantial conformational reorganisation of the pore-lining domains and flanking loops. The effects of central pore residues located in the 2nd and 5th transmembrane domains (M2, M5) and adjacent residues were tested by single introductions of histidines via site-directed mutagenesis of human AQP1 (a single mutation alters all four subunits of the homomeric channel). Expressed in Xenopus oocytes, wild type and mutant hAQP1 constructs were analysed by voltage clamp and imaging assays to evaluate expression, channel conductance, selectivity, and sensitivity to divalent cation modulators. None of the histidine mutations disrupted protein assembly or membrane expression as confirmed by the retention of normal osmotic water permeability, but did differentially affect ion channel properties. Mutations in the second transmembrane domain M2 (V50H, K51H, L58H, Q65H) conferred pH-sensitive relief of ion channel block by Cd^{2+}; other His mutant constructs showed block by Cd^{2+} comparable to that of wild type, which is pH-insensitive. Nickel (Ni^{2+}) had no effect on wild type hAQP1 channel properties and did not block ionic conductances in any AQP1 mutant constructs; however, at pH 8.4 it dramatically potentiated current amplitude specifically in A61H, an effect that required the presence of a second endogenous histidine residue in loop B. Results are consistent with a gain-of-function stabilisation of open conformation by creating a site for nickel coordination, of possible interest as a molecular calliper for estimating distances between residues during channel activation (Fig 1). Results here provide insights into structural mechanisms involved in hAQP1 channel activation. Ion channel activity of AQP1 has diverse roles in pathologies ranging from cancer cell metastasis to sickle cell disease, but the full range of functions and the signature features of ion channel AQPs in this broad and ancient class of membrane proteins are still being discovered.

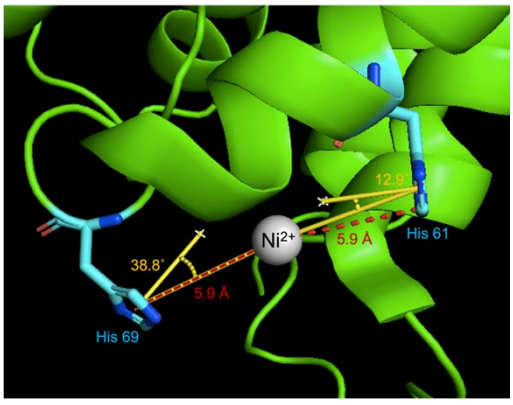

Figure 1: Modelled interaction between histidine residues and Ni^{2+} in human AQP1. View of proposed Ni^{2+} coordination between His 61 and His 74 (transmembrane domain M5 removed for clarity). Cation-π interactions are feasible between the imidazole rings of histidine sidechains (red) and a Ni^{2+} cation approaching at optimal angle ϑ (yellow).

[(1)] Yu J, Yool AJ, Schulten K, Tajkhorshid E. 2006 Mechanism of gating and ion conductivity of a possible tetrameric pore in aquaporin-1. Structure 14:1411-23. doi: 10.1016/j.str.2006.07.006.