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Binding Symmetry of External Divalent Cations to Cyclic Nucleotide-gated IonChannel Reveled by Channel Tandem Dimers

Ryuk-Jun Kwon* and Chul-Seung Park

Laboratory of Molecular Neurobiology, Department of Life Science, Kwangju Institute of Science and Technology, Kwangju, 500-712, Korea

Cyclic nucleotide-gated (CNG) channels are composed of homo or hetero tetramer of α and β subunits. The α subunits of these channels have a conserved glutamate residue within the pore-forming region. This residue determines the selectivity as well as the affinity for the extracellular divalent cations. Using the high affinity mutant (E363D) of bovine retinal CNG channel in which the Glu was replaced to Asp at position 363, we constructed tandem dimers and investigated the binding symmetry of divalent cation to the site. The gating and permeation characteristics of individual homomeric dimers are indistinguishable to those of homo-tetramers formed by parental monomers. The heteromeric dimers show the binding affinity for Sr^{2+} agrees perfectly with the geometric mean of the affinities for two parent channels indicating the energy additive and thus the symmetric nature of the interaction. The interaction with a smaller ion, Mg^{2+}, was asymmetrical and the apparent affinity was voltage-dependent. The simultaneous interaction between the four Glu residues and Sr^{2+} provide an important structural constraint to the outer vestibule of CNG channels.