

FUNCTIONAL STUDY OF KCV POTASSIUM CHANNEL THROUGH MANIPULATION OF INDIVIDUAL SUBUNITS

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ABSTRACT

Potassium channels make up a ubiquitous, physiologically essential class of integral membrane proteins. Many K⁺ channels contain four identical symmetrically associated subunits. To explore the role of an individual subunit in the channel functions, i.e. functional stoichiometry, one can genetically manipulate individual subunits and characterize the variation of channel functions with subunit composition. The functional stoichiometry of ion channels can be studied through the co-expression and concatamer methods. In this report, we utilized Kcv as a potassium model for functional stoichiometric study. The chlorella virus-encoded Kcv can form a homo-tetrameric potassium (K⁺) channel in the lipid membrane. This miniature peptide can be synthesized *in vitro*, and the tetramer purified from the SDS polyacrylamide gel retains the K⁺ channel functionality. Based on this capability, here we propose a simple, straightforward method for detecting the contribution from individual subunits to the channel functions. By using this approach, several mechanisms for ion permeation and potassium channel blocker binding regulated by subunit composition were identified. For example, the structural change from only one subunit in the selectivity filter G65C is sufficient to cause permanent channel dysfunction (“all-or-none” mechanism), whereas the mutation near the extracellular entrance L70Y additively modifies the ion permeation with the

number of mutant subunits in the tetramer (“additive” mechanism). This study also demonstrates that four subunits of Kcv channel interact simultaneously with the potassium channel blocker tetraethylammonium (TEA) and each subunit contributes equally to the TEA binding free energy.