Determination of the Stoichiometry between alpha- and gamma 1 Subunits of the BK Channel Using LRET

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BIOPHYSICAL JOURNAL
Volumen: 114
Número: 11
Páginas: 2493-2497
DOI: 10.1016/j.bpj.2018.04.008
Fecha de publicación:JUN 5 2018
Tipo de documento: Article
Ver impacto de la revista

Abstract

Two families of accessory proteins, beta and gamma, modulate BK channel gating and pharmacology. Notably, in the absence of internal Ca2+, the gamma 1 subunit promotes a large shift of the BK conductance-voltage curve to more negative potentials. However, very little is known about how alpha- and gamma 1 subunits interact. In particular, the association stoichiometry between both subunits is unknown. Here, we propose a method to answer this question using lanthanide resonance energy transfer. The method assumes that the kinetics of lanthanide resonance energy transfer-sensitized emission of the donor double-labeled alpha/gamma 1 complex is the linear combination of the kinetics of the sensitized emission in single-labeled complexes. We used a lanthanide binding tag engineered either into the alpha- or the gamma 1 subunits to bind Tb+3 as the donor. The acceptor (BODIPY) was attached to the BK pore-blocker iberiotoxin. We determined that gamma 1 associates with the alpha-subunit with a maximal 1:1 stoichiometry. This method could be applied to determine the stoichiometry of association between proteins within heteromultimeric complexes.

Palabras clave

KeyWords Plus:CA2+-ACTIVATED K+ CHANNEL; LUMINESCENCE ENERGY-TRANSFER; BETA-SUBUNITS; VOLTAGE; ACTIVATION; MOVEMENT; PROTEINS; CALCIUM; CA2+

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Financiación

Entidad financiadora	Número de concesión
National Fund for Scientific and Technological Development (FONDECYT)	1150273
US Air Force Office of Scientific Research (AFOSR)	FA9550-16-1-0384
Anillo Grant	ACT-1107
National Institutes of Health	GM030376 U54GM087519
Millennium Scientific Initiative of the Chilean Ministry of Economy, Development, and Tourism	P029-022-F

Ver texto de financiación

Editorial

CELL PRESS, 50 HAMPSHIRE ST, FLOOR 5, CAMBRIDGE, MA 02139 USA

Información de la revista

- **Impact Factor:** Journal Citation Reports

Categorías / Clasificación

Áreas de investigación: Biophysics

Categorías de Web of Science: Biophysics

Información del documento

Idioma: English

Número de acceso: WOS:000434787900003

ID de PubMed: 29705199

ISSN: 0006-3495

eISSN: 1542-0086