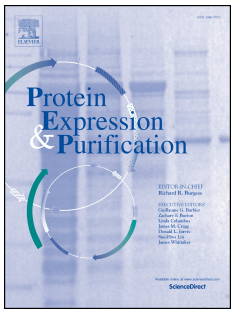
Accepted Manuscript



An improved method for the cost-effective expression and purification of large quantities of KcsA

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PII: S1046-5928(16)30129-2

DOI: 10.1016/j.pep.2016.07.002

Reference: YPREP 4960

To appear in: *Protein Expression and Purification*

Received Date: 26 April 2016

Revised Date: 22 June 2016

Accepted Date: 4 July 2016

Please cite this article as: C. Tilegenova, S. Vemulapally, D.M. Cortes, L.G. Cuello, An improved method for the cost-effective expression and purification of large quantities of KcsA, *Protein Expression and Purification* (2016), doi: 10.1016/j.pep.2016.07.002.

An improved method for the cost-effective expression and purification of large quantities of KcsA.

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ABSTRACT

KcsA, the bacterial K^+ channel from *Streptomyces lividans*, is the prototypical model system to study the functional and structural correlations of the pore domain of eukaryotic voltage-gated K^+ channels (K_v channels). It contains all the molecular elements responsible for ion conduction, activation, deactivation and inactivation gating[1]. KcsA's structural simplicity makes it highly amenable for structural studies. Therefore, it is methodological advantageous to produce large amount of functional and properly folded KcsA in a cost-effective manner. In the present study, we show an optimized protocol for the over-expression and purification of large amount of high-quality, fully functional and crystallizable KcsA using inexpensive detergents, which significantly lowered the cost of the purification process.

Keywords: ion channels, KcsA, biochemistry, over-expression, detergents, solubilization

1. Introduction

Potassium channels (K^+ channels) are specialized membrane proteins that form an aqueous pore through which potassium ions cross the cell membrane at near diffusion-limited rates (~10^7 ions per second)[2], regulating the potassium homeostasis within the cells. K^+ channels control the membrane potential of excitable cells such as neurons and muscle cells by opening and closing structural gates that regulate the flow of K^+ across the cell membrane. The opening of a K^+ channel gate (a process known as gating) is triggered by different type of stimuli (i.e., ligand binding, changes in the membrane voltage or mechanical deformation of the cell membrane). These multimeric membrane proteins have been classified by the number of transmembrane helices (TM) contained within their α-subunit (the pore forming subunit): among them the most common are the 2-transmembrane segments (2-TM) and the 6-TM subunits. Generally, four α-subunits assemble as a tetramer with four-fold symmetry around a