

What You Can Learn From Force Spectroscopy on a Tetrameric Ion Channel

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Tetrameric potassium channels are highly specific nanopores, which mediate the flux of potassium ions across the hydrophobic core of lipid bilayers. Here we present the mechanical characterization by single molecule force spectroscopy (SMFS) [1] of the cyclic nucleotide modulated potassium channel from *Mesorhizobium loti*, mCNG [2-4]. The monomer of this protein consists of six transmembrane helices (S1-S6), two of which contribute to the channel pore (S5 and S6), and a C-terminal cytosolic domain that binds cyclic nucleotides, e.g. cAMP. Atomic force microscopy (AFM) and transmission electron microscopy (TEM) studies revealed that the proteins are arranged in an upside-down orientation when reconstituted into an artificial lipid bilayer. This is the preferential case for mechanical unfolding from the terminus of membrane proteins having an even number of membrane spanning elements. Furthermore, TEM analysis on two-dimensional crystals and single particles of mCNG [3] showed the four-fold symmetry of the channel, which agrees with the published X-ray structure of the transmembrane part [4].

A semi-automated SMFS procedure [5] was used to unfold the reconstituted channel from its C-terminal end. The unfolding of several channel constructs allowed locating the unfolding barriers in the protein. SMFS data revealed that many molecular interactions in the pore region (S5, pore loop, S6) are sequentially broken. This indicates that various unfolding barriers stabilize the three-dimensional arrangement of this protein region, which is an essential part of an ion channel. Consecutive unfolding of two mCNG monomers from the same tetramer resulted in a significant change in the observed force peaks in the pore region, which was ascribed to the loss of local interaction.

To investigate this observation in more detail two identical monomers were concatenated to form a tetramer made of a dimer of these concatemers. This allowed pulling two subunits out of the same tetramer in a single step. Such experiments have shown that the unfolding of the first monomer mainly affects mechanical properties of the pore region of the second, while having minor effects on the remaining part of the protein (S1-S4). The molecular assembly of the concatenated monomers within the tetramer (“dimer of a dimer”) was assessed by cysteine scanning mutagenesis. These experiments have shown that the loops that connect the subunits do not cross, which is a prerequisite for successful SMFS experiments. Furthermore, single particle analysis of detergent solubilized proteins was used demonstrate that the concatenated construct had the similar dimensions than the native mlCNG.

- [1] Kedrov, A., Janovjak, H., Sapra, K., and Müller, D. (2007) *Ann. Rev. Biophys. Biomol. Struct.* **36**.
- [2] Nimigean, C., Shane, T., and Miller, C. (2004) *J. Gen. Physiol.* **124**, 203–210.
- [3] Chiu, P. L., Pagel, M. D., Evans, J., Chou, H. T., Zeng, X., Gipson, B., Stahlberg, H., and Nimigean, C. M. (2007) *Structure* **15**, 1053–1064.
- [4] Clayton, G. M., Altieri, S., Heginbotham, L., Unger, V. M., and Morais-Cabral, J. H. (2008) *PNAS* **105**, 1511–1515.
- [5] Bosshart, P. D., Casagrande, F., Frederix, P. L. T. M., Ratera, M., Bippes, C. A., Mueller, D. J., Palacin, M., Engel, A., and Fotiadis, D. (2008) *Nanotechnology* **19**, 384014–38028.