Conjugates of fluorescent proteins and animal toxins: molecular probes for ion channel visualization

P-03.1-21

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The problem of the ion channels detection and visualization seems to be relevant in the light of the high diversity of these proteins in different cells and tissues. At the present time, expression profile of ion channels is almost exclusively studied at the mRNA level (without information on expression at the functional protein level), and the detection and visualization of proteins is performed with antibodies (the most common limitations of their use include low discrimination between close channel isoforms, usage of intracellular epitopes, and high price). In our consideration, application of molecular probes based on natural polypeptide ligands that act on ion channels could serve a powerful approach to detect these target proteins. We have designed and produced dozen chimeric proteins which are comprised of several essential parts (modules). One of the modules is natural toxin serves for selective channel recognition, and other is presented by fluorescent protein for effective detection using fluorescent assays. Modules are separated by short amino acid linker to prevent interference between the module functions. We conjugated a number of known polypeptide ligands of different ion channels (acetylcholine receptors, potassium, sodium, and calcium channel) with several fluorescent proteins varying by spectral properties. Such toolkit of chimeric proteins could be further applied for ion channel detection on single cell membrane, visualization of ion channels in cell culture, and staining tissues slices. Also, these fusion proteins could be used as reporter instruments for observation of ion channel expression level during some diseases. Finally, toxins modified by fluorescent proteins are chip and effective compounds for the screening systems in pharmacological researches. This work was supported by the Russian Foundation for Basic Research [grant number 20-34-70031].