Секция «Биофизика и бионанотехнологии»

Purification and crystallization of a thermostable LOV protein

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Developing of fluorescent proteins has become a breakthrough in biological studies. Used for protein tagging and in monitoring of gene expression they uncover dynamic cellular events with high spatial and temporal resolution. Scientists Roger Y. Tsien, Osamu Shimomura, and Martin Chalfie were awarded the 2008 Nobel Prize in Chemistry for their discovery and development of the green fluorescent protein (GFP). However, usage of GFP has some significant limitations such as restrictions of its utility under low-oxygen conditions, slow maturation of the fluorophore, temperature and pH stability. The point is, that a study of thermophilic microbes, which can be used in industry as efficient high value compounds producers and waste into biofuel recyclers, demands appropriate *in vivo* reporters to observe protein production processes and to visualize protein localization in living cells [1]. Therefore, we are interested in construction of an alternative genetically encoded fluorescent sensor, and light, oxygen, and voltage (LOV) sensing proteins may provide us with such a sophisticated tool not only for cell imaging [2], but also many more novel techniques of usage of fluorescent proteins [3].

Flavin mononucleotide (FMN) is bound within LOV domains [4]. It is an UV/blue light-absorbing chromophore molecule, which causes reversible modification: formation of a bond between the FMN chromophore and a conserved cysteine residue within the protein [5]. The LOV domain loses its fluorescence after the FMN-cysteinyl adduct formation and regenerates it after being incubated in darkness. Therefore, fluorescent properties of the protein can be improved by abolishing its photochemical activity, i.e. replacement of the active cysteine residue.

In the project we cloned the genes of interest of a novel thermostable LOV-based FP with encoded mutations, where the conserved cysteine residue is replaced with alanine. After the step of protein expression and cells disruption, we are purifying the proteins of interest using metal affinity chromatography in Ni^{2+}-NTA matrix and size exclusion chromatography. Purification was followed by crystallization trials. Almond-shaped 100-200 um long crystals were obtained. We believe, the structure of the protein of interest will provide us with crucial information for further modifications, what will lead to development of innovative photo- and thermostable fluorescent proteins.

Источники и литература

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