Toetab TÜ ja TTÜ doktorikool
"Funktsionaalsed materjalid ja tehnoloogiad" (FMTDK)

ESF projekt 1.2.0401.09-0079

PRODUCTION OF A CYCLIC AMP FLUORESCENCE BIOSENSOR

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Cyclic adenosine monophosphate (cAMP) is a ubiquitous second messenger regulating a variety of cellular responses. Measuring changes in the level of cAMP may give valuable information about the action and efficacy of certain drugs or potential drug candidates under development. Nikolaev et al. have engineered a cAMP fluorescence sensor (Epac-camps), which utilizes the cAMP-binding domain of a guanine-nucleotide-exchange factor, Epac (exchange protein directly activated by cAMP), being fused with cyan and yellow fluorescent proteins (CFP and YFP) [1]. Change in cAMP concentration is detected by measuring the change in fluorescence emission ratio of YFP and CFP.

To ease the purification of Epac-camps we have introduced a nucleotide sequence, which encodes for a short 8 amino acid peptide (Streptag: WSHPQFEK) at the N-terminus of its gene. Further, the gene was subcloned into a baculovirus genome. A highly efficient viral replication mechanism enables fast and bulk production of a recombinant protein in different hosts like insect cells (i.e. *Spodoptera frugiperda*, Sf9 cells) that are easy to culture in high volume suspensions.

The Streptagged Epac1-camps was overexpressed in Sf9 cells and purified in one step by Streptactin-Sepharose gravity flow column chromatography. The Epac1-camps eluted in one band, confirmed and visualized by SDS-PAGE, having a MW around its theoretical value (77 kDa). The protein fraction was analyzed by spectrophotometer and an emission spectrum typical for CFP and YFP was observed. A cAMP response curve was blotted showing that the protein was active with an expected affinity for cAMP (EC50=4 μM).

References

1. V. Nikolaev et al., *JBC*, **279,** (2004), 34215.