Research in Pharmaceutical Sciences, 2012;7(5)
School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences
Proceeding of 13th Iranian Pharmaceutical Sciences Congress

Overproduction bacteriorhodopsin in *E. coli* as pharmacological targets

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Background and Aims: Large-scale production of membrane proteins in their native form is crucial for understanding their mechanism of action and target-based drug design. G Protein coupled receptors (GPCRs) are represents approximately 3% of human proteome and the most prominent class of pharmacological targets. Indeed 60-70 % of drugs target membrane proteins. Bacteriorhodopsin (bR) is a model system for studying membrane protein folding, stability, function and structure. It can be used as biosensor in drug delivery.
Methods: Escherichia coli is the most widely used host for producing membrane protein. Using BL21 derived strain can reduce proteolyses of membrane protein. In this study we design a synthetic gene and clone in PET30a for over-expression of Bacteriorhodopsin gene. Over-expression of Bacteriorhodopsin was achieved by fusion to the mistic. The synthetic Bacteriorhodopsin was separated from mistic by trypsin cleavage at the factor Xa site between mistic and Bacteriorhodopsin. To express Bacteriorhodopsin from PET30a, transformed BL21 (DE3) and different medium like LB, TB and M9 with different temperatures such as 18, 15, 25 and 37°C was applied. The results of experiments were analyzed by SDS-PAGE, western blotting and cell growth and recombinant protein production kinetics.
Results: It was found that induction temperature and medium affect on quantity and quality of recombinant Bacteriorhodopsin production especially on purification yield. It was found that IPTG concentration can influence on productivity of recombinant IGF-I considerably. The best production was obtained from TB medium at 18 °C. The final yield was 20 % of all proteins which is the high value for Bacteriorhodopsin production in Escherichia coli.
Conclusions: In conclusion, the studies presented here establish that high-level expression of Bacteriorhodopsin was achieved by using mistic as a fusion protein. Using rich medium like TB and decrease temperatures can improve the amount of protein.

Keywords: Membrane protein; G protein coupled receptors; Over-expression; Bacteriorhodopsin-mistic