Bacterial Expression and Purification of an Engineered Cannabinoid 1 Receptor
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Abstract –
Cannabinoid 1 receptor (CB1) is a type A G-protein coupled receptor (GPCR), known to be involved in the modulation of a number of key neurotransmitter signaling pathways in the central nervous system (CNS). It is therefore considered to be a key therapeutic target in a number of disease conditions such as neuropathic pain, obesity, drug abuse, etc. Understanding the structural organization of the human CB1 receptor (hCB1R) is vital for the development of ligands with high affinity and selectivity.

GPCRs are membrane proteins and expressed in different systems at low level with poor stability as well as functional activity. To obtain a large quantity of the receptor suitable for structural studies we engineered an hCB1R by deletion of N-terminal 102 amino-acid residues and replaced the intracellular loop 3 (ICL3) with T4 lysozyme (T4L). To assist in hCB1R expression and purification N-terminal flag- and C-terminal his- affinity tags were added to protein. The recombinant hCB1R (FlagΔhCB1-His6) was overexpressed in Escherichia coli (E. coli) BL21 cells under optimized conditions with a yield of ~4 micrograms of functional receptor per 1 L of bacterial culture. A modified radioligand binding assay was used to evaluate expression and functional activity (ligand binding) of the hCB1R.

Successful expression and purification of the hCB1R using a bacterial system enhances our efforts in structural and ligand-binding site characterization of the receptor.

Aims –
• Design a novel construct for E. coli based expression of functional recombinant human cannabinoid 1 receptor (hCB1).
• E. coli strain selection and condition optimization to improve receptor expression.
• Optimization of E. coli membrane preparation for cannabinergic radioligand binding assay.
• Purification of recombinant receptor using affinity based chromatography.

Background –
• Full length hCB1 receptor expression in E. coli is extremely poor with no functional activity.
• 102 amino acid N-terminal truncated receptor was found to express predominantly into the membrane and was able to bind cannabinergic ligands. However, the expressed receptor had very poor stability and lost over 50% of ligand binding activity within a day of storage at ~80°C (unpublished results).
• It has been shown previously for other GPCRs that the replacement of the flexible intracellular loop 3 with T4L significantly improves the receptors’ stability, without any major alterations in the overall protein structure.
• Using this strategy, we engineered the hCB1 receptor by combining the N-terminal truncation and the T4L insertion to generate a stable and functionally active protein.

Methods –
• DNA construct for expression of recombinant FlagΔhCB1-His6 receptor was assembled in pET26 vector.
• Protein expression: A single E. coli colony of BL21(DE3) cells transformed with the pET26-FlagΔhCB1-His6 construct was grown in 5ml of LB-kanamycin (25 μg/ml) media at 37°C for 18 hours. The next day, the entire overnight culture was added to 500 ml of LB-kanamycin and grown another 18 hours at 33°C. Protein expression was induced at OD ~3 with 0.3 mM IPTG for 4 hours at 25°C.
• Saturation binding assay: E. coli spheroplasts and membrane fractions were prepared using previously published protocols. The membranes were used to perform saturation binding experiments with radiolabeled CP-55940 in 0.5% PEl-treated GF/B plates.
• Affinity purification: The membrane preparation was solubilized in buffer containing 1% dodecyl maltoside. The FlagΔhCB1-His6 receptor was purified using either immobilized metal-affinity chromatography (IMAC) or Flag affinity M2 resin.

Results and Discussion –

![Figure 1. Snake Diagram of Engineered Human Cannabinoid 1 Receptor](image1)

- **Bacterial expression of FlagΔhCB1-His6 receptor was optimized using different conditions of E. coli strains, incubation temperatures and times (Fig. 2), cell densities, and IPTG concentrations.**
- **A saturation binding experiment using cannabinergic ligand CP-55940 with the spheroplast membrane preparation confirmed the formation of functional receptor (Fig. 3) with a Bmax of 813 pmol/g and a Kd of 17.7 nM.**
- **Recombinant receptor was purified with IMAC Talon resin or anti-Flag M2 affinity resin.** However, Flag-tag affinity purified samples were better quality (Fig.4).

Conclusions –
• Recombinant human cannabinoid 1 receptor was expressed in E. coli cells under optimized conditions.
• The functional activity (ligand binding) of the expressed receptor was confirmed by a radioligand binding assay.
• The presence of T4L in intracellular loop 3 significantly improved receptor stability.
• N-terminal Flag or C-terminal His6 tags are suitable for purification of recombinant receptor.

References –

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