Effective Solubilization and Stabilization of Functional G Protein-Coupled Receptors

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PURPOSE

The requirement for stabilized G protein-coupled receptors (GPCRs) in their native, functional form presents significant challenges for in vitro functional and structural characterization. Efficient solubilization of the receptor most often requires stringent extraction that compromises structural integrity and activity. In addition, most activity assays must be performed immediately after solubilization of the receptor, with activity rapidly degrading over time. Our aim was to develop a simple method to efficiently solubilize active GPCRs that are fully stable outside of their native membrane environment.

METHODS

Protein Extraction

Membrane Protein Extraction: 1x10^6 cultured cells or 50-100 mg of tissue were washed in PBS, immersed in 1 mL of extraction buffer containing 1% Triton X-100, 1xPBS supplemented with 1x protease inhibitor cocktail, and incubated for 10 minutes at 4°C. Supernatant was collected and the membrane pellet was washed in PBS, resuspended in 1 mL of DPMC, and incubated for 30 minutes at 37°C in a sonicating water bath. After incubation, 1 mL of 2.5% polymer (SMAP) was added to sample and equilibrated at room temperature for 1 hour. Using a microcentrifuge, supernatant was collected at 16,000xg for 10 minutes. Protein in each fraction was determined using the BCA Assay Kit.

Western Blot

Membrane proteins were separated on a 4-12% Tris-glycine gel and transferred to nitrocellulose membrane at 150V for 1 hour. Membranes were then blocked, incubated in primary antibody for one hour at room temperature or overnight at 4°C, washed, incubated in secondary antibody for 30 minutes at room temperature, and visualized with enhanced chemiluminescence. Bands were quantified using densitometry.

RESULTS

When comparing different methods for extraction and stabilization of GPCRs, the following trends were observed:

- Improved solubilization enhances western blot results
- Receptor stabilization at the time of extraction is required to preserve >75% GPCR Activity
- Solubilization and stabilization of active receptor can be achieved using a whole cell lysis method.
- Receptor can be purified in its functional form

CONCLUSIONS

Here we report:

- Using optimized ratios of detergent and stabilizer for GPCR extraction not only efficiently solubilizes GPCRs better than other methods (i.e. membrane prep, SMALP), but also stabilizes the receptor.
- Receptor stability is preserved immediately after extraction as well as extended during cold storage, when using a non-denaturing detergent in conjunction with a stabilizer.
- Extraction using the GPCR Extraction and Stabilization Reagent allows for the assessment of receptor activity in both a whole cell lysate and a purified sample.
- With evolving techniques and technologies, such as native mass spectrometry and cryo-EM, extraction of a stable receptor will be essential. With the improved extraction efficiency, solubility and preserved activity obtained with this method, researchers will have a greater breadth and flexibility in their GPCR research and targeted therapeutic studies.

REFERENCES

