Preparation of Pre- and Post-synaptic Density Fraction from Mouse Cortex

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[Abstract] The understanding of the organization of postsynaptic signaling systems at excitatory synapses has been aided by the identification of proteins in the postsynaptic density (PSD) fraction, a subcellular fraction enriched in structures with the morphology of PSDs. Here we described an efficient way to isolate the crude synaptosome, presynaptic fraction, and PSD fraction. It helps to identify the location of synaptic protein and find the potential synaptic complex.

Materials and Reagents

1. Sucrose
2. Protease inhibitor cocktail (1:100) (Sigma-Aldrich, catalog number: P8340-5ml)
3. 20 mM HEPES (pH 7.0)
4. Triton X-100
5. KCl
6. $NaHCO_3$
7. $MgCl_2$
8. $CaCl_2$
9. Tris-HCl
10. SDS
11. Glycerol
12. 2-mercaptoethanol
13. BPB
14. Solution A (see Recipes)
15. SDS-PAGE sample buffer (see Recipes)

Equipment

1. Eppendorf table centrifuge
2. Swinging bucket rotor (model: SW51Ti)
3. Fixed-angle rotor

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4. Dounce mini-homogenizer

Procedure

Note: PSD fraction of mouse cortex was prepared according to modified protocol.

1. Corticles are taken in the cold PBS under microscope. Could store at -80 °C if not using immediately. Brain is homogenized in Dounce mini-homogenizer, 50 strokes. Dissect 1 half cortex (or 2 Hipp), add 4 ml buffer to homogenize to looks milky and no obvious pieces of tissue.
   If not indicated below, all experiments are performed at 4 °C.
2. The homogenates were centrifuged at 470 *x g* for 2 min.
3. Resultant supernatants (S1 fraction) were centrifuged at 10,000 *x g* for 10 min to obtain mitochondria- and synaptosome-enriched pellets (P2) and supernatants (S2 fraction) containing soluble proteins.
4. P2 fractions were resuspended in 3.75 ml of 0.32 M sucrose, which was then layered onto 0.8 M sucrose. Centrifuge at 9,100 *x g* for 15 min in a swinging bucket rotor.
5. After centrifugation, synaptosomes (most of the loose pellets) were collected from 0.8 M sucrose layer (Figure 1) and resuspended with equal volume of 20 mM HEPES (pH 7.0), 2% Triton X-100 and 150 mM KCl.

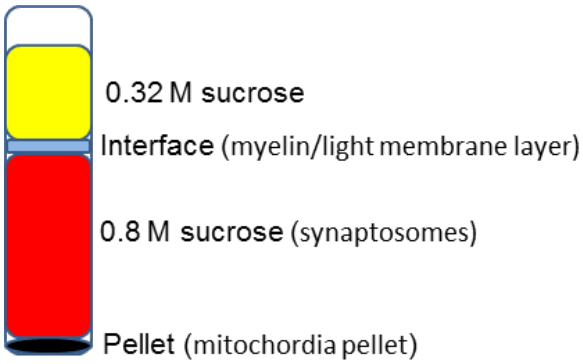

Figure 1. Sucrose ultracentrifugation of PSD fraction

6. Samples were centrifuged at 20,800 *x g* for 45 min using a fixed-angle rotor, and resulting supernatants were collected as presynaptic fraction.
7. Pellets were resuspended in a solution of 1% Triton X-100 and 75 mM KCl using a Dounce mini-homogenizer and centrifuged again at 20,800 *x g* for 30 min to yield final pellets (PSD fraction, which be identified by marker PSD95), which were washed with 20 mM HEPES and dissolved in 1x SDS-PAGE sample buffer.

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Recipes

1. Solution A
   0.32 M sucrose
   1 mM $NaHCO_3$
   1 mM $MgCl_2$
   0.5 mM $CaCl_2$
   1 mM PMSF and protease inhibitors
2. SDS-PAGE sample buffer
   0.125 M Tris-HCl (pH 6.8)
   4% SDS
   20% Glycerol
   10% 2-mercaptoethanol
   0.2% BPB

Acknowledgments

This work was supported in part by grants from American Heart Association (C.Y). Methods are previously simply described in Tao *et al*. (2013).

References

1. Tao, Y., Chen, Y. J., Shen, C., Luo, Z., Bates, C. R., Lee, D., Marchetto, S., Gao, T. M., Borg, J. P., Xiong, W. C. and Mei, L. (2013). Erbin interacts with TARP gamma-2 for surface expression of AMPA receptors in cortical interneurons. *Nat Neurosci* 16(3): 290-299.

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