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SHORT PROTOCOL No. 52

Purification of Detergent-Resistant Membrane Rafts Using the Ultracentrifuge CP-NX Series with the Rotor P40ST

Kohji Kasahara[1], **Shuichi Kani**[2], **Jan Knop**[3], **Martin Armbrecht**[3]
[1]Tokyo Metropolitan Institute of Mecical Science, Tokyo, Japan; [2]Eppendorf Himac Technologies, Tokyo, Japan; [3]Eppendorf SE, Hamburg, Germany

Introduction

In this article, we present a method to isolate a specific region of the cell membrane known as lipid rafts using density gradient ultracentrifugation (Figure 1). Lipid rafts differ from the rest of the membrane by having an altered lipid and protein composition. They play a special role in signal transduction as well as regulatory processes. Lipid rafts may also serve as entry points for bacterial and viral infections.[1,2]

The term "lipid raft" derives from the image of a raft floating on the surface of water. The entire membrane can be compared to a water surface.

These surfactant-insoluble rafts can be removed from the membrane by treating them with a nonionic surfactant and centrifuging them in a density gradient solution of sucrose. The rafts can then be extracted from the medium- and low-density interfaces.

Ultracentrifugation is one of the standard methods for localizing target molecules in microdomains of the cell membrane, which includes the previously mentioned lipid rafts.

In this protocol, we demonstrate how a ultracentrifuge of the CP-NX series is used with the rotor P40ST (Figure 2) to purify detergent-resistant membrane (DRM) raft fractions from developing cerebellum through density gradient centrifugation.[3,4]

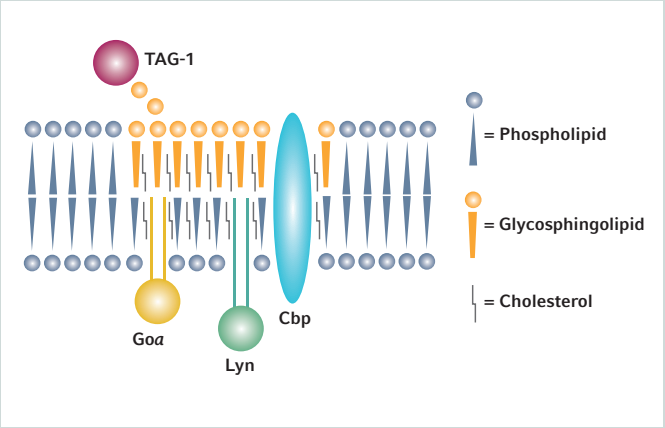

Figure 1: Overview of a lipid raft with its typical components in the membrane[5] (TAG-1 = transient axonal glycoprotein; Goα = subunit family of GTP-binding proteins; Lyn = Src family kinase; Cbp = CREB-binding protein).

A

B

Figure 2: Ultracentrifuge of the CP-NX series (A) with the P40ST swinging bucket rotor (B).

Method

Separating the membrane raft fraction is achieved in two steps: The first step is to prepare the sample for sucrose density gradient centrifugation:

Sample pre Solubilization of the

Applied reagents	Protocol steps
> TNE buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, pH 7.5) > TNE buffer containing 1% (w/v) of Triton X-100 > TNE buffer containing 80% (w/v) of sucrose	1. Suspend *rat cerebellar granule cells in 2 mL of TNE buffer containing 1% (w/v) of Triton X-100 2. Homogenize the solution 3. Add 2 mL TNE buffer containing 80% (w/v) of sucrose 4. Mix the complete solution thoroughly by pipetting the mixture up- and downward Total sample volume: 4 mL

*Primary Culture of rat cerebellar granule neurons / dissected from 7-day-old rats.

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Membrane raft extraction using sucrose density gradient centrifugation

Centrifuge and accessories:

> Ultracentrifuge of the CP-NX series
> P40ST rotor with the 13PP tube

Parameters:

> Speed: 40,000 rpm (RCF: 284,000 x *g* max.)
> Time: 17 h
> Temperature: 4°C
> Acceleration mode: 8
> Deceleration mode: 8
> Sample volume: 4 mL
> Density gradient solution: 6 mL

Applied reagents:

> TNE buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, pH 7.5)
> 5% (w/v) sucrose in TNE buffer
> 30% (w/v) sucrose in TNE buffer

Protocol:

> Put 4 mL of the sample in the bottom of the 13PP tube
> Add a 6 mL layer of continuous sucrose density gradient solution (5%–30% [w/v]) containing TNE buffer to the sample
> Set the tube in the P40ST rotor and centrifuge at 40,000 rpm (284,000 x *g* max.) at 4°C for 17 hours
> Following centrifugal separation, perform fractionation as follows: Harvest 1 mL of each fraction from the top to the bottom and allocate to fractions No. 1 through 10
> Note that the lipid raft fractions DRM (detergent-resistant membrane) are in fractions No. 3–5 (see Figure 3)
> Check fractionation using SDS-PAGE/western blot analysis

Complete raft isolation method

Figure 3 below provides an overview of the complete raft isolation method:
solubilization > centrifugation > fractionation.

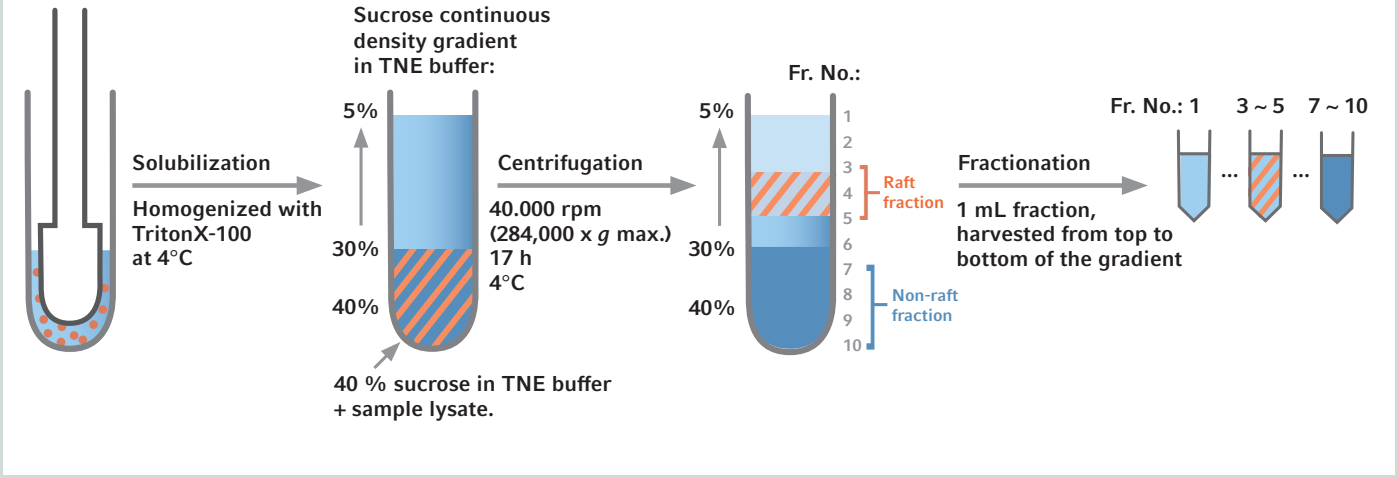

Figure 3: Isolation of raft fractions through a sequence of discontinuous sucrose gradient centrifugation steps.

Checking results

The purification success of the isolated fractions (fig.3) can be checked by SDS-PAGE and subsequent western blots using the following protein markers as it was done in a previous publication[4]:

1) Csk-binding protein (Cbp) and Flotillin (Flt): raft fraction (fraction 3-5)
2) Calnexin (Cnx) and Transferrin receptor (TfR): non-raft fraction (fraction 7-10).

Conclusion

The protocol presented here for fractionation of membrane rafts by density gradient centrifugation can be followed for CP-NX series centrifuges with the rotor P40ST using the elongated shape of the 13 PP tube.

The method presented here is also published in the article "Involvement of Gangliosides in the Process of Cbp/PAG Phosphorylation by Lyn in Developing Cerebellar Growth Cones."[4]

Notes

[1] Hartlova et al., "Membrane Rafts: A potential Gateway for Bacterial Entry into Host Cells, " Microbiol Immunol 54 (2010): 237–245.
[2] Chazal et al., "Virus Entry, Assembly, Budding, and Membrane Rafts," Microbiology and Molecular Biology Reviews 67, no. 2 (June 2003): 226–237.
[3] Kohei Yuyama et al., "Translocation of Activated Heterotrimeric G Protein Gαo to Ganglioside-Enriched Detergent-Resistant Membrane Rafts in Developing Cerebellum," Journal of Biological Chemistry 282, no. 36 (2007): 26392–26400.
[4] Naoko Sekino-Suzuki et al., "Involvement of Gangliosides in the Process of Cbp/PAG Phosphorylation by Lyn in Developing Cerebellar Growth Cones," Journal of Neurochemistry 124 (2013): 514–522.
[5] Kohji Kasahara, "Physiological Functions of Glycosphingolipids in Transmembrane Signaling," Tokyo Metropolitan Institute of Medical Science website: https://www.igakuken.or.jp/biomembrane/english.html.

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