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Separation of G protein-coupled receptors using ultracentrifuge

CP-WX series ultracentrifuge / P40ST swinging bucket rotor

G protein-coupled receptors (GPCRs) are located in a cell membrane mainly, and receive many various information such as light, smell, neurotransmitters, hormones, and other external signals, and the signals are transduced into the cell via G protein. It is well-known that GPCRs and related proteins are closely linked to various kinds of diseases. They play one of the key roles as research targets for developing new drugs. They have also attracted considerable attention in association with Nobel Prizes twice (The Nobel Prize in Physiology or Medicine 1994 and The Nobel Prize in Chemistry 2012). The GPCRs are one of the proteins attracting the most global attention today.

Here, we take an example involving the separation of G proteins from lipid raft fractions. Since the lipid raft resists surfactants, we dissolved the lipid raft in a surfactant and separated it by isopycnic centrifugation using sucrose density gradient solution. The protein was obtained as a fraction unlike other membrane fractions using different degrees of density of the liquid. [*1, *2]

Details

1. Sample

   Primary culture of rat cerebellar granule cells

2. Sample preparation

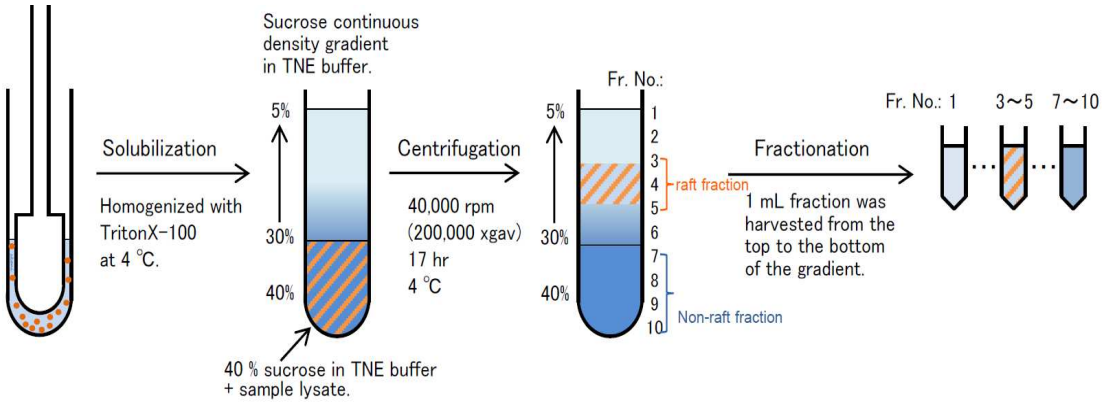
   We prepared a sample by suspending the rat cerebellar granule cells in 2 mL of TNE buffer containing 1% (w/v) of Triton X-100, homogenizing the solution, adding the same amount (2 mL) of TNE buffer containing 80% (w/v) of sucrose to the solution, and pipetting the mixture effectively.

3. Device and centrifugal conditions

Centrifuge :	Ultracentrifuge (Hitachi CP-WX series)
Rotor:	P40ST swinging bucket rotor (13 mL x 6tubes)
Centrifugal tube:	13PA tube
Speed:	40,000 rpm (Average RCF: 200,000 xg)
Time:	17 hours
Temperature:	4℃
Acceleration mode:	8 (slightly slow acceleration)
Deceleration mode:	8 (slightly slow deceleration)
Sample volume:	4 mL
Density gradient solution:	6 mL (5 to 30%(w/v) of continuous sucrose density gradient solution : Hitachi density gradient fractionator DGF-U, is useful for preparing continuous density gradient solution)

4. Operation

   Put 4 mL of the sample on the bottom of the 13PA tube first, and then layer 6 mL of continuous sucrose density gradient solution (5 to 30% (w/v)) containing TNE buffer on the sample. Set the tube on the P40ST swinging bucket rotor at the CP-WX series ultracentrifuge, and centrifuge at 40,000 rpm (200,000 xg) at 4℃ for 17 hours. Following the centrifugal separation, fractionate 1 mL fraction was harvested from the top to the bottom of the gradient solution and allocate fraction numbers Fr.No.1 to No.10 to the fractions in that order. The lipid raft fractions DRM (Detergent-Resistant Membrane) containing the G protein are in Fr.No.3 to No.5 and the non-raft fractions are in Fr.No.7 to No.10. [*1,*2]



5. Reagents

TNE buffer (25mM Tris-HCl, 150mM NaCl, 1mM EGTA, pH7.5)
TNE buffer + 1% (w/v) Triton X-100
80% (w/v) sucrose in TNE buffer
30% (w/v) sucrose in TNE buffer
5% (w/v) sucrose in TNE buffer

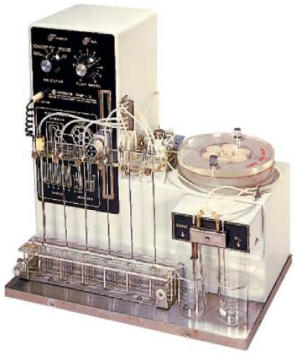
6. References

1) Yuyama K., Sekino-Suzuki N., Sanai Y., Kasahara K.: J Biol Chem. *282*., (2007), 26392-26400.
2) Kasahara K., Watanabe Y., Yamamoto T., Sanai Y.: J Biol Chem. *272*., (1997), 29947-29953

Devices

CP-WX series ultracentrifuge

P40ST swinging bucket rotor



DGF-U Hitachi density gradient fractionator

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