No.110

himac APPLICATION

January 2003

Separation of cell organelle using easily prepared density gradient of Nycodenz®

CP-MX series preparative ultracentrifuge, P28S swinging bucket rotor

Experiment: Separation of rat liver cell organelle using the continuous density gradient without use of density gradient former

Cell organelle can be separated from a homogenate of liver etc. by the density gradient centrifugation using the ultracentrifuge. However, there was a tendency to avoid density gradient centrifugation because the conventional methods required the use of a density gradient former and the operation was complicated.
This time, cell organelle were separated from a rat liver homogenate according to the procedure described in himac APPLICATION No. 108.

Continuous density gradient can be easily made according to the procedure below using Nycodenz® as the density gradient solution.

(1) Dilute Nycodenz® to the specified concentration.
(2) Freeze it at -20℃ or -80℃.
(3) Leave it at the ambient temperature to melt.

That is to say, you can obtain a continuous density gradient of Nycodenz® in the tube just by pouring the specified-concentration Nycodenz® in the tube, freezing it for preservation, taking it out of the freezer, and leaving it in the ambient temperature for melting. Separation of organelles in such a way is utilized as a part of proteome research and it seems to be important more and more in the future.

1. Equipment used
   Centrifuge: CP-MX series preparative ultracentrifuge
   Rotor: P28S swinging bucket rotor
   Centrifugal tubes: 40PA tubes

2. Result of separation

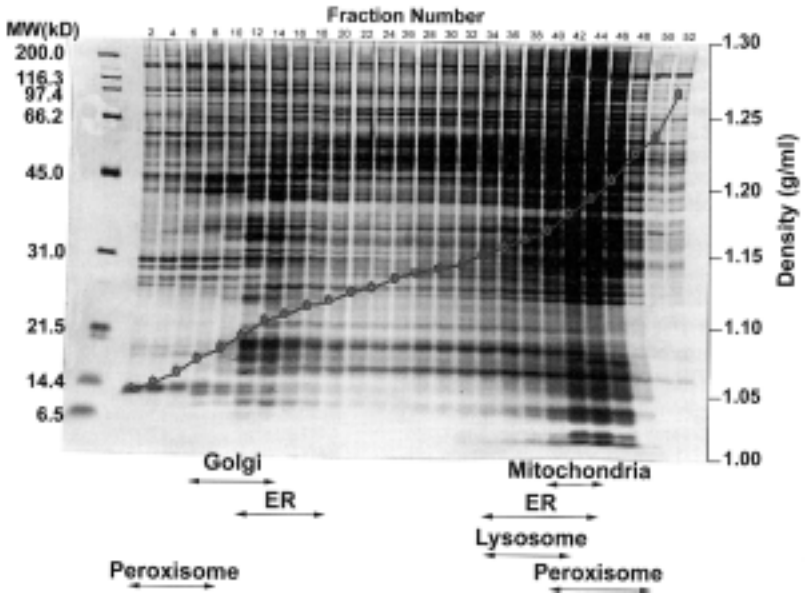

Fig. 1 Result of electrophoresis (1-D SDS-PAGE) for each fraction after centrifugation
(Amount of sample: 3 μl)

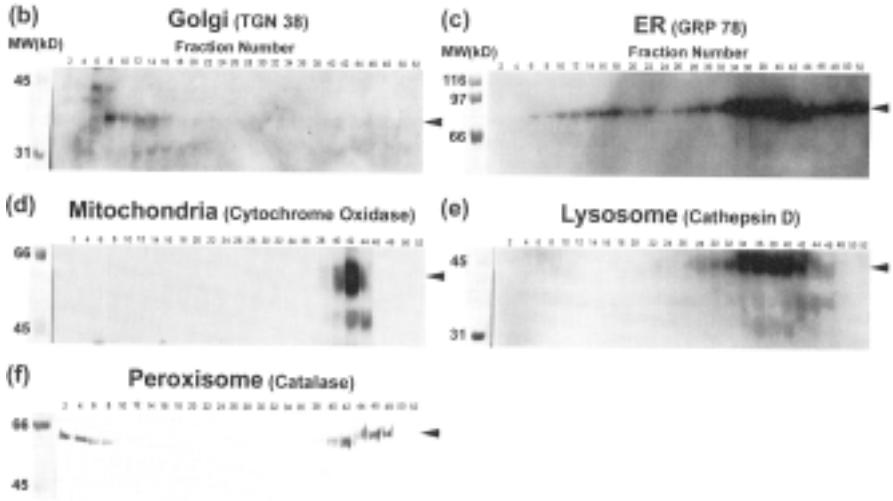

Fig. 2. Western blotting result between fractionated samples and index proteins of each cell organelle

3. Separating conditions
Speed: 25,000 rpm (Average RCF: 82,200xg)
Time: 180 minutes
Temperature: 4℃
Sample: Rat liver homogenate
Amount of sample: 3.5 ml (Concentration: About 14mg/ml)
Density gradient solution: Nycodenz® (made by Nycomed Pharma AS (Oslo, Norway))
Initial concentration of Nycodenz®: 20%(w/v)（1.105g/ml）
Amount of Nycodenz®: 32 ml
Amount of fraction: 0.75 ml/Fraction

A fractionator is required for fraction after centrifugation. ALC-20 fractionator (made by Advantec Toyo Co., Ltd.) is reasonable and usable for fraction after centrifugation as well as Hitachi's DGF-U fractionator. Both fractionators are equipped with a level sensor and perform fractionation from the upper surface of the band in the centrifugal tube after centrifugation.

The above result was provided by Professor Kimie Murayama, Division of Biochemical Analysis, Central Laboratory of Medical Sciences, Juntendo University School of Medicine.

(References)
Kimie Murayama, Tsutomu Fujimura, Masataka Morita and Noriko Shindo, Electrophoresis, 2001, ***22***, 2872-2880.

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