HITACHI

himac APPLICATION

No. 101 DECEMBER 1999

Subject Separation and fractionation of proteins and complexes by means of an ultracentrifuge using a density-gradient solution prepared by the DGF-U fractionator

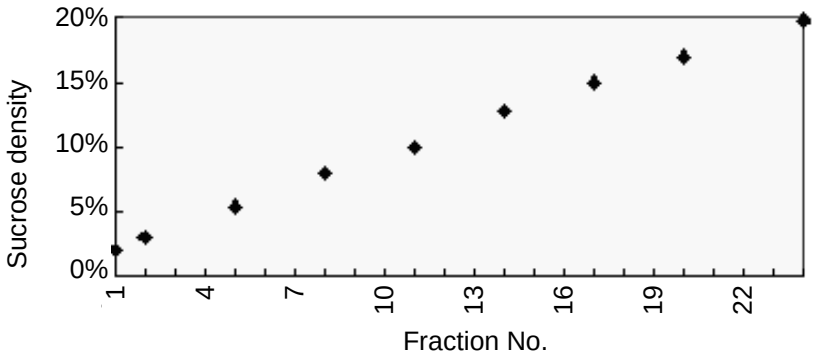
Model DGF-U fractionator and P28S2 swing rotor for ultracentrifuges

Experiment:
Separation of triton extracted from rat-derived culture nervous tissues by means of an ultracentrifuge using a sucrose density gradient solution prepared by the DGF-U fractionator to research whether tissue-derived proteins form complexes or not

The ultra-centrifugation/fractionation method using a density gradient solution is effective for not only the separation of macromolecular proteins comprised of multiple sub units but also for the research whether complexes are formed by protein-protein interactions or not.[1)]
The DGF-U fractionator is usable to prepare a density gradient solution and to extract the separated layers after centrifugation for such an experiment. This time, triton extracted from rat-derived culture nervous tissues[2)] was fractionated by ultra-centrifugation using a sucrose density gradient solution prepared by the DGF-U fractionator.

1. Result of experiment

[A]



[B]

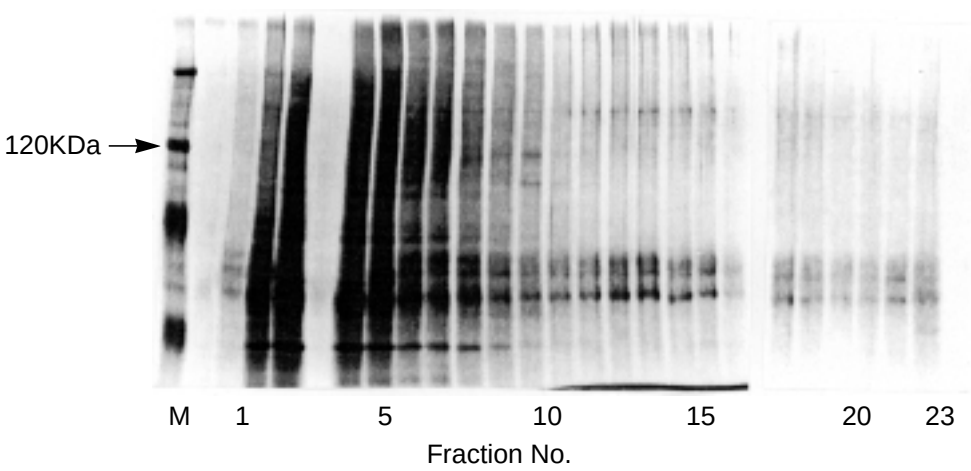


Fig. [A]: Sucrose density gradient prepared
Fig. [B]: Electrophoresis of fractions after ultra-centrifugation

As is evident from the electrophoresis after ultra-centrifugation, protein group compositions vary from fraction to fraction. While most of the proteins are collected at the upper layers of the density gradient (Fr. No. 2 to 5), only the bands whose molecular weight is about 150 kDa and the bands whose molecular weight is from 55 to 65 kDa are collected at the higher density layers (Fr. No. 12 to 16). In this density gradient, thyroglobulin whose molecular weight is about 670 kDa is collected at Fr. No. 12 and 13. Therefore, it is presumed that these proteins are not monomers but form large complexes by thyroglobulin.

2. Procedures of experiment

Prepare 13 ml of 5 - 20% linear sucrose density gradient solution in each tube (16PA tubes) by means of the DGF-U fractionator.

↓

Lay 1.5 ml of sample solution of triton extracted from rat-derived culture nervous tissues [2] on the above sucrose density gradient solution.

↓

Put the 16PA tubes in the P28S2 swing rotor and load the rotor in the Hitachi preparative ultracentrifuge. Perform centrifugation at 25,000 rpm and 4˚C for 20 hours (acceleration mode: 2, deceleration mode: 2).

↓

Perform fractionation for every 0.6 ml (total of 24 samples) then perform electrophoresis 10 µl per fraction.

3. Instruments used

Centrifuge: Hitachi preparative ultracentrifuge
Rotor: P28S2 swing rotor
Tube: 16PA tubes
Instrument to prepare density gradient solution: Hitachi DGF-U fractionator

4. References

1) Piperno G. and Mead K., *Proc.Natl.Acad.Sci.U.S.A.*, 94, 4457-4462 (1997).
2) Tsuda M., Tashiro T. and Komiya T., *J.Neurochem.*, 68, 2558-2565 (1997).

■ Hitachi DGF-U fractionator

- Suitable for preparation of a density gradient solution and for extraction of the fraction layers after centrifugation
- Solution can be injected or extracted from the level.
- Amount of solution can be optionally changed within the range 0 - 5 ml/min.
- Density gradient solution can be prepared in 6 tubes by one operation (it is also possible to prepare in 3 tubes or 1 tube).

The data shown in this himac application was provided by Dr. Tashiro, Associate Professor of Department of Molecular and Cellular Neurobiology, School of Medicine, Gunma University. For further information, please contact Hitachi Koki Scientific Instruments Group.

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