Experiment: Effectiveness of pelleting by means of a fixed angle rotor (13.5-ml tubes and 35° of cavity angle) designed for micro ultracentrifuges

Separation of cell organelles from a homogenate of liver etc. by means of a centrifuge is called “cell fractionation”. In most cases, initial rough separation for the cell fractionation is done by differential pelleting without the use of density gradient solutions. A fixed angle rotor is used for the initial separation because it is suitable for pelleting. The new S55A fixed angle rotor has the same capacity as the commonest fixed angle rotors (popularly called “rotors intended for 12-ml tubes”) designed for preparative ultracentrifuges, and its cavity angle is 35°. It is larger than the conventional rotors’ cavity angle (24° – 26°).

This time, difference in pellet forming angles and effectiveness between the new S55A fixed angle rotor and the conventional P90AT fixed angle rotor (cavity angle: 26°) were examined as described below.

1. Equipment used
   - Centrifuge: CS150GXL micro ultracentrifuge
   - Rotor: S55A fixed angle rotor
   - Centrifugal tubes: 10PC thick-walled tubes

2. Centrifugal conditions
   - Speed: 12,000 rpm
   - Time: 20 minutes

3. Specifications of S55A fixed angle rotor
   - Maximum speed: 55,000 rpm
   - Maximum RCF: 260,000xg
   - Rotor’s cavity angle: 35°
   - Rotor capacity: 13.5 ml tube × 8 pcs. = 108 ml
   - Actual capacity when using 10PC thick-walled tubes: 7.3 ml tube × 8 pcs. = 58.4 ml
   - K-factor when using 10PC thick-walled tubes at 55,000 rpm: 56
   - Rotor material: Aluminum alloy
   - Applicable centrifuges: CS-GXL series and CS-GX series micro ultracentrifuges
4. Comparison of pellet forming state and effectiveness between S55A and P90AT angle rotors

Fig. 1 Pellet forming state when using the new S55A fixed angle rotor (Rotor’s cavity angle: 35°)

Fig. 2 Pellet forming state when using the conventional P90AT fixed angle rotor (Rotor’s cavity angle: 26°)

After centrifugation, while the lower edge of the pellet formed by means of the new S55A fixed angle rotor is near the bottom of the centrifugal tube, the lower end of the pellet formed by means of the conventional P90AT rotor is stopped at the inside wall of the centrifugal tube. Even if the difference in the pellet forming angles is slight, it gives an effect on removal of the supernatant. The higher the top edge of the pellet, the lower amount of supernatant may be removed. This is because the liquid interface comes closer to the pellet surface as removal of the supernatant proceeds, and fine particles are released from the pellet surface and mixed into the supernatant upon contact of the liquid interface with the pellet surface. Such a difficulty is frequently found in separation of cell organelle. Therefore, S55A fixed angle rotor having 35° of rotor’s cavity angle is more effective.