Isolation and Enrichment of Golgi Bodies from Rice Seedlings Using Density Gradient Ultracentrifugation

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Introduction

The Golgi apparatus of eukaryotic cells was first described more than 120 years ago by Camillo Golgi. Advances in (electron) microscopy revealed the complex structure while further biochemical analysis enlighted various functions of this organelle within the cell [1].

In cells of higher organisms, the Golgi apparatus is responsible for the synthesis of complex polysaccharides and the processing and distribution of proteins to other organelles as part of the secretory pathway [2].

One example of such a protein is α-amylase, a glycosidase responsible for the hydrolysis of starch molecules within plants. It was shown that α-amylase is synthesized at the endoplasmic reticulum (ER) ribosomes, glycosylated within the ER-lumen, and then transported into the Golgi apparatus for oligosaccharide modification [3].

However, as the Golgi apparatus forms a complex structure with other membrane systems like the endoplasmic reticulum (ER) [1], it is particularly difficult to isolate distinct parts of this organelle. Indeed, fractions of Golgi membranes are often contaminated with parts of other connected membrane systems like the vacuole [1]. Density gradient centrifugation is one of the most established techniques used for the enrichment of specific membranes [1].

In this Application Note, we describe a technique using a sequence of differential pelleting and density gradient centrifugation to obtain fractions of Golgi apparatus membranes from rice seedlings. The applied technique allows to gain extracts of high purity and quality for further downstream analysis like mass spectrometry.

Materials and methods

Materials used

Centrifuge CP80NX (Eppendorf) with the swing-bucket rotors P32ST for 40 mL PET tubes and P40ST for 13 mL PET tubes.

First step

Microsome purification process by using rotors P32ST (40 mL PET tube) and P40ST (13 mL PET tube).

1. Centrifuge the purified rice extract at 15,000 × *g* for 30 min at 4 °C in 40 mL PET tube in a swing-bucket rotor and discard the pellet.
2. Load the 11 mL supernatant on the top of 1 mL of 15% sucrose solution over the 1 mL of the 50% sucrose cushion in the 13 mL PET tube.
3. Centrifuge at 100,000 × *g* for 3 h at 4 °C and subsequently collect the microsome fraction trapped on the cushion of 50% sucrose solution.

Second step

Golgi purification process from microsome fraction by using rotor P40ST (13 mL PET tube).

1. Adjust the collected fraction to 42% sucrose density with 60% sucrose buffer using a refractometer. On top of this solution load 1–2 mL of another discontinuous sucrose density gradient consisting of 1 mL 26%, 30%, 34%, and 38% sucrose layer each. Fill carefully up with water to 13 mL.
2. Centrifuge at 100,000 × *g* for 3 h at 4 °C and subsequently collect the Golgi fraction (1) floating as boundary phase between 34% and 38% sucrose layer briefly.
3. Adjust the collected Golgi fraction to 42% sucrose density again, and then apply 1–2 mL to the second discontinuous sucrose gradient consisting of 1 mL 26%, 30%, 34%, and 38% sucrose layer each. Fill carefully up to 13 mL.
4. Centrifuge at 100,000 × *g* for 3 h at 4 °C and collect the Golgi fraction (2) floating as boundary phase between 34% and 38% sucrose layer.

All sucrose concentrations based on w/w.

Results and discussion

The use of discontinuous density gradient centrifugation is a standard method for isolating or enriching subcellular components. In most cases, differences in the sedimentation coefficient or specific densities are used to obtain a separation. The characteristics regarding these parameters in organelle isolation applications are mainly defined by the composition of the respective membranes. A clear separation is often a challenge, especially for Golgi bodies closely connected with other membrane systems [1].

Hence, highly purified Golgi membranes are essential for the analysis and investigation of e.g. the Golgi proteome [2] or specific proteins within the organelle. Here, we describe an effective method using two different swing-bucket rotors in combination with successive discontinuous sucrose density gradient centrifugation steps to obtain high-quality isolates of Golgi bodies.

After removing the cell debris in the first centrifugation step, the supernatant is loaded onto a first discontinuous sucrose gradient (Fig. 1). Between the 15% and 50% sucrose phases, a fraction of microsomes is accumulated. This fraction is used for further purification by two steps of floating discontinuous sucrose gradients where the Golgi bodies accumulate between the 34% and 38% sucrose phase of the gradient (Fig. 2).

It was shown by Asakura *et al.* [4] that the purity of the Golgi body fraction was improved significantly after the second floating step.

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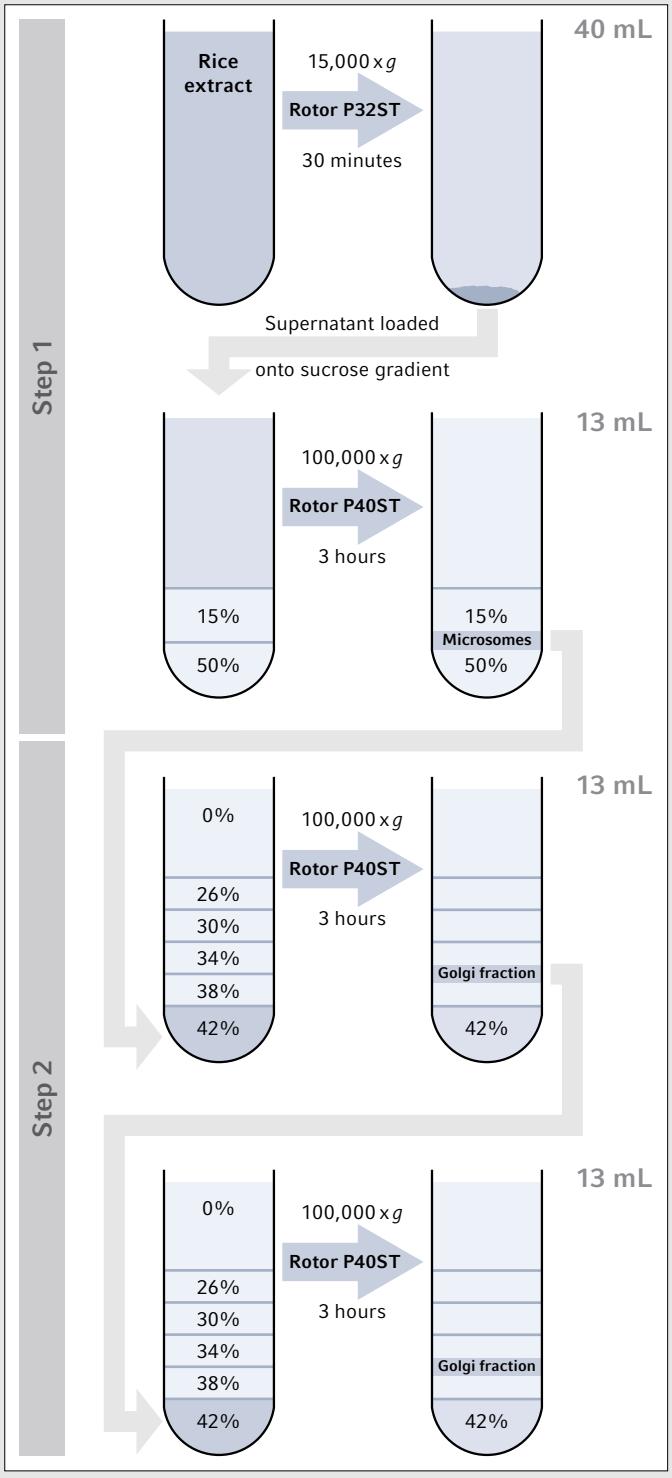

Fig. 1: Isolation of Golgi bodies by a sequence of discontinuous sucrose gradient centrifugation steps

The quality of the Golgi fraction can be checked by the presence of several marker enzymes (e.g. UGPase [Uridindiphosphate-Glucose-Pyrophosphorylase: Cytosol], RbcL [Ribulosebisphosphate carboxylase large chain: plastid] by immunoblot analysis [2].

Conclusion

The combination of the two rotors P32ST and P40ST is ideal for the isolation of Golgi bodies. It allows the shift from higher (40 mL) to lower volumes (13 mL) with high performance. The special long and narrow shape of the 13 mL PET tubes allows a longer floating distance, which increases the purity of the Golgi fraction. Besides, the top-loading of the rotor inserts eases the delicate handling of sucrose gradients and minimizes the risk of unintended mixing.

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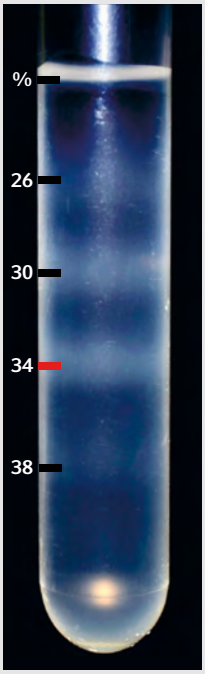

Fig. 2: Sub-fraction of the rice Golgi apparatus after multiple steps of discontinuous sucrose density gradient centrifugation. Golgi fraction between 34% and 38% sucrose solution indicated in red

Literature

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[4] Asakura T. *et al.* (2006): *Plant Biotechnology* 23, 475–485.