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

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Abstract

Himac, now part of the Eppendorf Group, has a profound record of over 60 years of experience developing high-speed floorstanding-ultracentrifuges tailored to customer needs. In this Application Note, the use of the ultracentrifuge of the CPNX-series (here CP80NX) in combination with swing-bucket rotor P32ST and rotor P40ST from Himac for the isolation of Golgi from rice seedlings will be demonstrated. Obtaining high-quality Golgi isolates is critical for further analysis and understanding of this organelle. It is shown how this can be achieved by the sequential application of differential pelleting and discontinuous sucrose density gradient centrifugation.

Figure 1: Ultracentrifuge of the CP-series in combination with swing-bucket rotors P32ST and P40ST

Introduction

The Golgi apparatus of eukaryotic cells first was 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 [2].
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 (Figure 2) [1].

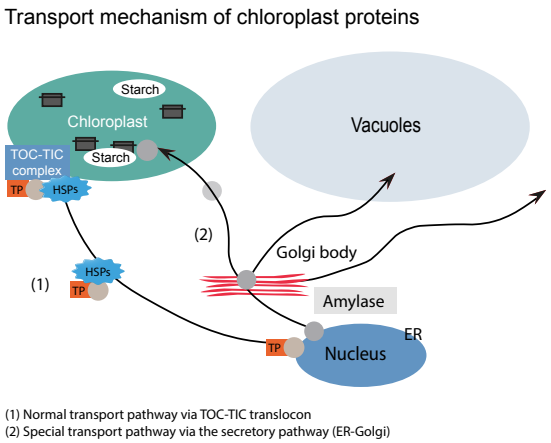

Figure 2: Protein transport in plant cells via (1) the Toc-Tic translocation and (2) via the Golgi apparatus and the secretory pathway.

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 formes a complex structure with other membrane systems like the endoplasmic reticulum (ER) [2], 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 [2]. Density gradient centrifugation is one of the most established techniques used for the enrichment of specific membranes [2]. 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. The isolation of high-quality Golgi fractions using Centrifuge CP80-NX with the combination of the rotors P32ST and P40ST will be described in the following section.

Materials and Methods

Materials used

Centrifuge CP80NX with the following swing-bucket rotors:

1. Rotor P32ST for 40 mL PET tubes
2. Rotor P40ST for 13 mL PET tubes

First step:

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

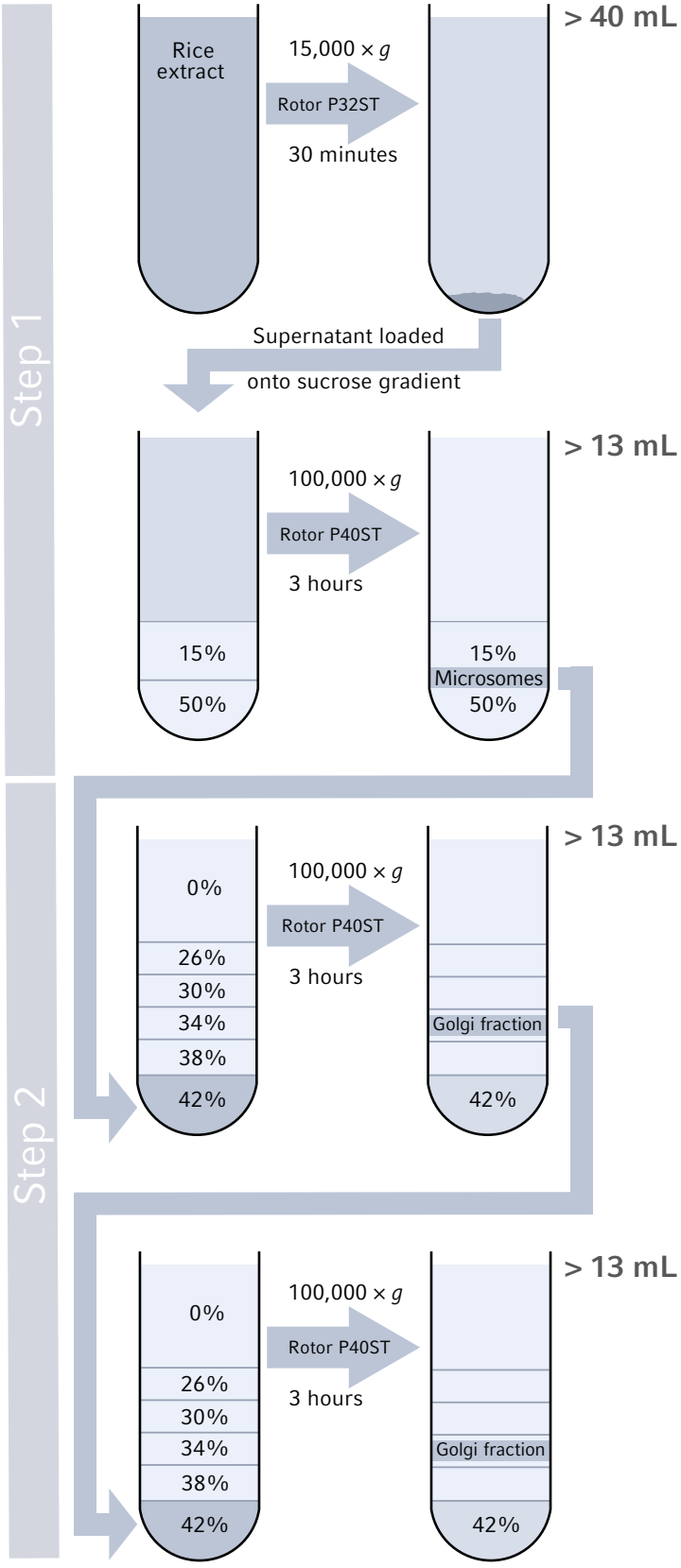

Figure 3: Isolation of Golgi bodies by a sequence of discontinuous sucrose gradient centrifugation steps. High purity is achieved by two separate floating steps.

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.

By floating centrifugation twice using long narrow 13 mL tubes, highly purified Golgi can be isolated from the microsome. The Golgi fraction (2) is recovered and subjected to assays and blotting analyses. 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 [2].

Hence, highly purified Golgi membranes are essential for the analysis and investigation of, e.g. the Golgi proteome [1] 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 (see Figure 3). 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 (Figure 3). It was shown by Asakura et al. [4] that the purity of the Golgi body fraction was improved significantly after the second floating step.
The quality of the Golgi fraction can be checked by the presence of marker enzymes like UGPase (Uridindiphosphate-Glucose-Pyrophosphorylase: Cytosol), RbcL (Ribulose bisphosphate carboxylase large chain: plastid), COXII (Cytochrome c oxidase subunit 2: mitochondria), and ARF (Adenosyl-Ribosylation-Factor: Golgi) by immunoblot analysis [1].

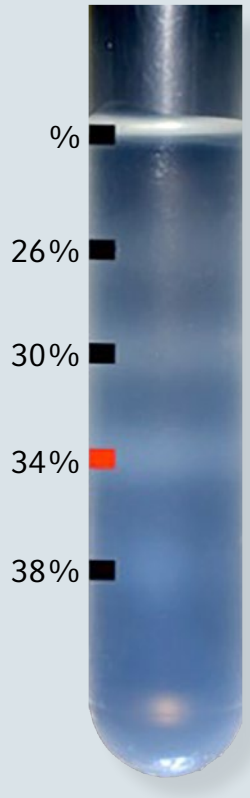

Figure 4: Sub fraction of the rice Golgi apparatus after multiple steps of discontinuous sucrose density gradient centrifugation. Golgi fraction between 34% and 38% sucrose solution indicated in red.

Conclusion

The use of the combination of the two rotors P32ST and P40ST is ideal for the isolation of Golgi. It allows the shift from higher (40 mL) to lower volumes (13 mL) with high performance. The special long and narrow shape of the Himac 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.

Literature

[1] Oikawa K. et al. (2018): Proteomic Analysis of Rice Golgi Membranes Isolated by Floating Trough Discontinuous Sucrose Density Gradient, Methods in Molecular Biology, Chapter 6, 91-105.
[2] Parsons H.T. et al. (2012): The Current State of the Golgi Proteomes, Proteomic Applications in Biology, Dr. Joshua Heazlewood (Ed.).
[3] Kitajima A. et al. (2009): The Rice α-Amylase Glycoprotein Is Targeted from the Golgi Apparatus through the Secretory Pathway to the Plastids; The Plant Cell, Vol. 21: 2844-2858.
[4] Asakura T. et al. (2006): Isolation and proteomic analysis of rice Golgi membranes: Cis-Golgi membranes labeled with GFP-SYP31. Plant Biotechnology 23, 475–485.

Ordering information

Description	Order number
Centrifuge CP80NX EU Region / India (230V)	5720 108 012
Centrifuge CP80NX North America (208V)	5720 108 013
Centrifuge CP80NX Asia-Pacific /without Japan (220V)	5720 108 011
Rotor P32ST, swing-bucket, 6 × 40 mL	5720 214 003
Rotor P40ST, swing-bucket, 6 × 13 mL	5720 214 002
PET Tubes, 13 mL	5720 411 107
PET Tubes, 40 mL	5720 411 148

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