Preparation of Golgi Membrane Fractions Containing Lectin-binding Sites from Suspension-cultured Rice Cells

Shinn Kimura, Kenichi Matsuda, Toshiaki Mitsui, and Ikuo Igaue

Department of Biosystem Science, Graduate School of Science and Technology, and *Department of Agricultural Chemistry, Faculty of Agriculture, Niigata University, 8050 2-Ikarashi, Niigata 950-21, Japan

Received July 2, 1991

The Golgi complex is one of the major components of the endomembrane system of eukaryotic cells. It participates in various processes of newly synthesized proteins and in the allocation of macromolecules to their correct intracellular or cell-surface destinations. The isolation and characterization of the Golgi complex is essential for biochemical and cell biological studies of this organelle. Recently we reported the isolation of Golgi membranes from cultured rice cells by a linear glycerol density gradient centrifugation. In this work, we attempted to prepare the Golgi membrane-rich fractions using a discontinuous glycerol density gradient centrifugation.

The "burst" microsomal membranes (100,000 × g pellet) were prepared from 5 g of suspension-cultured rice cells (Oryza sativa L. cv. Nipponkai) as previously described. These membranes were suspended in 2.5 ml of 20% (v/v) glycerol in 50 mM maleate-NaOH (pH 6.0). The suspension (1 ml) was layered onto a discontinuous glycerol gradient containing 2 ml each of 30, 35, 45, and 89% (v/v) glycerol in 50 mM glycylglycine-NaOH (pH 7.5) and 1 mM EDTA, and centrifuged at 100,000 × g for 30 min in a Hitachi RPS5-VF vertical rotor and then fractionated into 0.6-ml fractions. A sample of each fraction of the glycerol gradient was used for SDS-PAGE. After electrophoresis, the gel was processed by Western blotting and lectin-peroxidase staining.

Figure 1 shows the activity profiles of various organelle-specific marker enzymes, IDPase for Golgi membrane, NADPH-cyt c reductase for ER membrane, cyt c oxidase for mitochondrial inner membrane, and ATPases for tonoplast and plasma membrane, after the discontinuous glycerol gradient centrifugation. Two peaks of IDPase activity, 1 and 2, were observed (Fig. 1A). The IDPase peak 1 was well separated from NADPH-cyt c reductase, cyt c oxidase, and ATPases (Fig. 1B), but the IDPase peak 2 was not separated from the peaks of other organelle marker enzymes. Figure 2A shows PNA-peroxidase staining of glycopeptides in each fraction of the glycerol gradient. PNA is a galactose-specific lectin, which binds to glycoproteins in the Golgi membrane-rich fractions as previously described. In this experiment, PNA was found to bind to glycoproteins in the fractions of IDPase peak 1 but scarcely to those of IDPase peak 2. The fractions of IDPase peak 1 (fractions no. 2–6) were collected and recentrifuged on a 10 to 50% (w/w) linear sucrose gradient (Fig. 2B). UDPase and GDPase activities, which are associated with the Golgi membranes as previously described, were found in the peak fraction of IDPase activity. The activities of other organelle marker enzymes were not detectable. UEA-I, which is a fucose-specific lectin, was found to bind to glycoproteins in the peak 1 fraction of IDPase. Based on these data, the peak 1 fraction of IDPase is considered as a part of the Golgi membrane fractions, which were obtained by the linear glycerol gradient centrifugation in our previous work. So it seems likely that the peak 1 fraction could not be separated by the linear glycerol density gradient centrifugation although it was separated by the discontinuous glycerol gradient centrifugation.

These results indicated that the peak 1 fraction of IDPase contained a part of the Golgi membranes which had lectin (PNA, UEA-I) binding sites, and this fraction was further purified by re-centrifugation on the sucrose gradient. However, the following possibilities cannot be excluded: (1) the endomembranes that could not be detected by the marker enzymes might have been included in IDPase peak 1 and (2) IDPase peak 1 might have been contaminated by some aggregation of glycoproteins of different organelles (e.g. vacuolar inclusion). Cytotoxicological approaches to detect the lectin binding sites of Golgi complex is in progress in our laboratory.

Abbreviations: IDPase, Inosine diphosphatase; UDPase, Uridine diphosphatase; GDPase, Guanosine diphosphatase; ER, endoplasmic reticulum; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PNA, peanut lectin; UEA-I, Ulex europaeus lectin-I.

References
Fig. 2. Lectin-binding Analysis of Glycoproteins in Each Fraction of Glycerol Density Gradient Centrifugation and Sedimentation of Membranes Obtained from the IDPase Peak 1 Fraction in a Linear Sucrose Gradient.

(A) PNA-peroxidase staining of glycoproteins in each fraction prepared in the experiment shown in Fig. 1. Arrowheads indicate the IDPase peak 1 fraction and the peak 2 fraction.

(B) The fractions of IDPase peak 1 were collected and centrifuged at 100,000 × g for 1 hr. The pellet was resuspended in 0.5 ml of 5% (w/w) sucrose in 50 mM maleate-NaOH (pH 6.0) and layered onto 4 ml of a linear density gradient of sucrose (10—50%, w/w). After centrifugation at 100,000 × g for 3 hr, the gradient was fractionated into 0.3-ml fractions. (Upper panel) IDPase (——), UDPase (——), GDPase (——), and sucrose (○). (Lower panel) UEA-I-peroxidase staining of glycoproteins in each fraction obtained as described above.