GOLGI APPARATUS IS ONE CONTINUOUS ORGANELLE IN PANCREATIC EXOCRINE CELL OF MOUSE\textsuperscript{1,2}

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The three-dimensional structure of the Golgi apparatus (GA) in mouse pancreatic exocrine cells was studied under an ordinary transmission electron microscope and also a high voltage electron microscope. From observations of thick sections of ZIO (Zinc-Iodide-Osmium solution)-reacted pancreatic tissues, GA was found to be a complex three-dimensional structure with its basic configuration summarized as follows: 1. Golgi cisterna is a long elongated ribbon-like structure and Golgi stack is composed of several ribbon-like cisternae organized from cis to trans. 2. The ribbon-like Golgi stack has wide expanded areas in some portions where it branches into 2 or 3 ribbon-like stacks. 3. The Golgi stack often shows a huge circular arrangement, about 10 \( \mu \text{m} \) in diameter, maintaining the polarity from cis to trans. 4. In some portions the direction of polarity of the stack changes: the Golgi stack in some parts rotates in the long expanding direction (X axis) maintaining each cisternal continuity. 5. According to the observations of 1 \( \mu \text{m} \) thick serial sections, the continuity and polarity of the Golgi stack are maintained three-dimensionally (X, Y and Z axes) and GA, as a whole, was found to be one huge continuous reticular organelle in the cell.

The three-dimensional structure of GA had already been studied under the light microscope before the introduction of the electron microscope to cell biology (8, 10, 11), but because of the limited resolution of light microscopy, the results were lacking in precision and clarity. Recent developments in the high voltage electron microscope (HVEM) and scanning electron microscopy (SEM) added other aspects to the study of the three-dimensional structure of GA with their higher resolution (12–14, 17). Concerning studies under HVEM, the methods for staining GA have practically been limited to Friend’s osmium impregnation method (3), but the ultrastructures of cell organelle stained by this method were often found to be disintegrated and swollen. In addition, precise understanding of the three-

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dimensional ultrastructures of GA was often hampered by the coarse precipitates of reduced osmium. Another method using SEM with high resolution was developed by Tanaka and Fukudome (17) but their descriptions were limited to the parts of whole GA.

Recently we applied the Zinc-Iodide-Osmium method for demonstrating GA and found it to be suitable for the study of the three-dimensional structure of GA, especially for observation under HVEM. Here we report the three-dimensional structure of GA of pancreatic exocrine cell with clearer and better preserved morphology than ever reported, and attempt to describe the whole three-dimensional organization of GA in the cell with serial thick sections under HVEM.

MATERIALS AND METHODS

Male ddY-strain mice fed ad libitum were anesthetized with pentobarbital (Somnopentyl) and perfused at first with heparinized physiological saline for a few min and then 2% glutaraldehyde in 0.1 M phosphate buffer containing 5% sucrose, pH 7.4 at room temperature for 5 min from the left ventricle. After removal of the pancreas, the tissue was trimmed into 1–2 mm³ blocks which were immersed in the same fixative for 2.5 hr at 4°C. Next, the tissue blocks were rinsed at first with 0.1 M phosphate buffer, pH 7.4, with 8% sucrose at 4°C for 1 hr, and then with Tris-HCl buffer, pH 7.4, at 4°C for 10 min. The composition of Tris-HCl buffer was 0.05 M Tris, 0.57 N NaCl, 5 mM CaCl₂, and 15 mM MgCl₂. pH was adjusted to pH 7.4 with HCl, and this solution was used as Tris-HCl buffer. This solution is a twice diluted solution of that used by Vrensen and de Groot (18). The tissue blocks were soaked in ZIO solution for 16–20 hr at 4°C and kept in the dark with gentle agitation. ZIO solution was prepared shortly before each incubation: 3 g Zn powder was dissolved ultrasonically in 20 ml distilled water and 1 g iodine crystal was slowly added to Zn solution with stirring. After cooling, this mixture was filtered and 4 ml of this solution was mixed again with 2 ml of Tris-HCl buffer, pH 7.4, and 2 ml of 2% osmium tetroxide aqueous solution.

After incubation, tissue blocks were stained en bloc with 0.5–1% uranyl acetate aqueous solution for 30 min at 50°C in the dark. The tissues were then dehydrated in graded ethanol and embedded in Spurr's resin. Ultrathin sections or 1–5 μm thick sections were cut with an LKB Ultrotome III 8800 and ultrathin sections were observed under a JEOL 100 CX at 80 kV as an accelerating voltage and thick sections under a Hitachi H-1250 M (National Institute for Physiological Sciences, Okazaki, Japan) at 1,000 kV as an accelerating voltage. 1 μm thick serial sections were picked up separately on the grid-free small areas of formvar coated 50 meshes (508 μm). Before observation with HVEM, the same cells to be observed and the faces of each section were checked with the light microscope. Most micrographs obtained from 1 μm thick sections were taken at tilting angles of ±8–10° and then stereo-paired micrographs were viewed with stereoscope.
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RESULT

Observations of ultrathin section of ZIO reacted tissues

Ultrastructures of the cell organelle incubated in ZIO solution were finely preserved and there were no ultrastructural differences between the specimen treated for routine morphology and ZIO-stained specimen. By the ZIO method, the Golgi stack of pancreatic exocrine cell of mouse was stained especially in the cis side (Fig. 1). In the pure morphological study there were some cases where we could not identify the polarity, presumably existing in the Golgi stack, but in the case of ZIO reacted pancreatic tissue the differentiation between the trans and cis sides of GA were relatively easy because of the intensely stained cis side and the weakly stained condensing vacuoles locating in the trans side of GA (CV in Fig. 2). Interestingly enough, the intensity of ZIO staining tended to be strong in the cis cisterna of GA and rather weak in the trans side and condensing vacuole. However, the cisternal density was homogenous and staining patterns of Golgi stacks were very similar in each cell though many Golgi stacks appeared to be separated from each other within the cell. This staining pattern of Golgi stacks strongly suggests the morphological relationship among these Golgi stacks. It was confirmed that these densely stained materials actually represent the structural expansions of Golgi stacks, by the fact that, in high magnification, the dense materials were clearly demarcated by the cisternal membrane (Fig. 1 inset). One of the most characteristic features of GA manifested by ZIO staining was the specific fenestration existing in the cis-most cisterna (Fig. 1, 1-inset, 2, 3). These pores were observed on the transverse cut-face of the Golgi stack as the intermittent faded spaces sandwiched by dense stained cisterna, and on the tangential cut-face of cis cisterna as relatively uniform pores of 50–100 nm in diameter (Fig. 2).

Observations of thick sections of ZIO reacted tissues

The appropriate thickness of the specimen for observation with HVEM depends on the transmission power (i.e. related to the accelerating voltage) and the depth of focus of the HVEM. As far as we have tried, the images obtained from 1 μm thick sections were found to be best for the morphological analysis.

First of all, it was remarkable that the Golgi stacks were observed as large and long elongated structures (Figs. 3–5). The staining patterns observed in ultrathin sections appeared to be similar in thick sections, but increased transmission power ascribed to HVEM tended to diminish the images of the weakly stained or unreacted structure that could be clearly observed in thin sections. As the thick sections of ZIO reacted material could be visualized by HVEM, it became possible to observe

Fig. 1. Ultrathin section of ZIO-reacted pancreatic exocrine cell. Many Golgi stacks are easily identified by densely stained cisternae. Note the difference in density between the cis and trans side and between mature zymogen granules and immature zymogen granules. The structural relations among these GA are indicated by the similar staining pattern by ZIO. Inset. Ultrathin section of ZIO reacted pancreatic exocrine cell. Higher magnification of the Golgi stack. It is clear that these electron dense materials are surrounded by the cisternal membrane (arrows), indicating that electron dense figures represent the structural images of Golgi stacks.

Fig. 2. Heavily stained cis-most Golgi cisterna is observed in the right side as an intermittent wavy line, but in the left side as fenestrated expansion. This fact indicates that the cis-most Golgi cisterna has specific fenestration, and therefore, in its transverse cut-face of ultrathin section, it appears as intermittent cisterna, not as continuous cisterna. CV: condensing vacuole
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Fig. 3. 1 μm thick section of ZIO-reacted pancreatic exocrine cell. The specific curving of Golgi stack is clearly demonstrated. Golgi stack appears to rotate its cisterna in an elongated direction at the portions indicated by arrowheads. Fenestrated cis-most cisterna is obvious. White arrows: trans side. Black arrows: cis side.

Fig. 4. 1 μm thick section of ZIO-reacted pancreatic exocrine cell. Stereo-paired micrographs taken at tilting angles of ±10°. Golgi cisternae are observed to expand their cisternae as ribbon-like structures, having cisternal branches in several portions. N: nucleus
Fig. 5. 1 μm thick section of ZIO-reacted pancreatic exocrine cell. Circular arrangement of the Golgi stack, about 12 μm in diameter. Black arrows indicate the cis side of Golgi stack and white ones the trans side. Note the portion marked by white arrowheads where the trans side and the cis side of Golgi stack interchange their spatial arrangement.

Fig. 6. 1 μm thick section of ZIO-reacted pancreatic exocrine cell. Stereo-paired micrographs taken at tilting angles of ±8°. The torsion of the Golgi stack is clearly revealed by the three-dimensional vision of these stereo-paired micrographs. The densely stained cisternae (cis side) show the cisternal rotation around their developing direction. The weakly stained cisternae (trans side) are found to be accompanying these cis cisternae. Note that the spatial relationship between the cis and trans sides is exchanged between upper and lower arrows. White arrows: trans side, Black arrows: cis side.
more continuous and larger three-dimensional organization of GA than with the ultrathin sections. The features of the Golgi stacks obtained from 1 μm thick sections were variable but there several characteristic structures existed among them:

a. Every Golgi cisterna was a basically long, well-developed ribbon-like structure with some width rather than large extended square, and therefore the Golgi stack was recognized as the overlapping of several ribbon-like cisternae one after another.

b. The spatial arrangement of the Golgi stack often tended to be circular and the trans side of the Golgi stack faced mostly to the inside of the circle. (Fig. 4, 5)

c. Golgi stack shows bifurcation or trifurcation in the wide portion into 2 or 3 stack branches. These branches also had Golgi cisternae, and they were ultimately connected with other Golgi stacks to form circular loops. (Fig. 4, 7)

d. General spatial arrangement of Golgi stacks were such as we described above, but in some portions the trans and cis sides of the Golgi stack interchanged their spatial positions by the cisternal torsion around their elongating axis. We could obtain two-dimensional images indicating this feature with ultrathin sections, but as this kind of cisternal movement was indeed three-dimensional, the spatial relationships between the trans and cis sides of the Golgi stack were more clearly understood by stereoview based on the stereo-paired micrographs of thick sections (Fig. 5, 6).

The electron micrographs obtained from thicker sections more than 2 μm were not suitable for precise morphological analysis of GA, but stereo-paired micrographs of 5 μm thick sections provided better information concerning the three-dimensional organization that could not be obtained from 1 μm thick sections (Fig. 7). Though these images lost their accuracy, Golgi stacks were observed like threads composing a huge reticular complex. Many branched Golgi stacks appeared to have interconnections among them and to form many loops.

![Fig. 7](image-url) 5 μm thick section of ZIO-reacted pancreatic exocrine cell. Stereo-paired micrographs taken at tilting angles of ±4°. Heavily stained Golgi stack appears to be three-dimensionally interconnected and, as a whole, results in the "glomerular" structure located in the supranuclear region.
**Observations of thick serial sections of Z10 reacted tissues**

In order to examine the intracellular extension of GA, we prepared 1 μm thick serial sections which covered the whole cell. The number of serial sections that we used for this purpose were about 15–20 sections for one whole cell, but the intracellular space where occupied by GA was almost included within 7–10 serial sections among them. In other words, GA was found to localize within the confined intracellular space whose diameter was 7–10 μm. As we could see in the Fig. 8, the plasma membranes of each cell sometimes appeared to be too obscure to trace the whole cell, because they do not appear as clear lines but as obscure areas as far as we observed them in thick sections. However, we could identify the border of neighboring cells by carefully following the contour of the densely stained cell.
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The relatively dark cells that are shown in Figs. 8a–n are slices of the same cell but in Fig. 8n the intercellular spaces (arrowheads) and the fragment of the next neighboring cell could be observed. Figs. 8e–l show the serial images of GA in this dark cell. Three-dimensional structures of these GA showed the similar basic features that are described above (a–d). The intracellular arrangement of Golgi stacks appeared to be very flexible and free, so that we could follow the cisternal connections among several Golgi stacks that seemed to be separated from each other.

The three-dimensional organization of GA appearing in these sections are shown in Figs. 9a–h with stereo-paired micrographs. These figures were arranged ac-
Fig. 10a, b. Superimposed micrographs obtained from two micrographs of 8i and 8j (corresponding to 9e and 9f) for 10a, and 8j and 8k (corresponding to 9f and 9g) for 10b. By superimposing 2 micrographs obtained from successive two 1 μm thick sections of ZIO reacted material, clearer structural information can be obtained than that from the direct observation of 2 μm thick section. Golgi stacks indicated by circles are identical portions where the highest (white arrows) and lowest points (black arrows) in 9e-g are fused respectively.
cording to the three-dimensional order: three-dimensionally the lowest portion of Fig. 9a are the lowest of all Figs. 9a–h and the highest portions of Fig. 9h are the highest of all Figs. 9a–h and there are structural connections among this series of sections. For example, the two points observed as considerably higher portions of Golgi stacks indicated by white arrows in Fig. 9e appeared to be the identical portions with the three-dimensionally lower two points indicated by black arrows in Fig. 9f. The same with Fig. 9f and Fig. 9g. Superimposed images from two successive sections show cisternal connections of Golgi stacks between those two sections (Fig. 10a, b) and therefore the reticular images of higher organization of GA could be observed more clearly.

DISCUSSION

Three-dimensional, morphological analysis of cell organelles is a fundamental requirement for the study of cell biology. Up to now, most of the morphological studies of cell organelles were made with the transmission electron microscope (TEM). So it is inevitable that the structural images obtained from an ordinary TEM are from two-dimensional cross-sections of the organelles. However, it should be kept in mind that most of the cellular components are three-dimensional and it would be very difficult to construct the three-dimensional images from only two-dimensional electron micrographs. In the case of GA, because of its peculiar structure and various shapes depending upon the kind of tissue, many generalized schemes about the three-dimensional structure of GA have been presented, but as a matter of fact, morphological studies of the three-dimensional organization of GA are very few.

In general, there are two ways of TEM analysis for an elucidation of the three-dimensional structure of GA. The first is the three-dimensional reconstruction of TEM images of GA obtained from serial thin sections, and the other is the stereoscopical observations of stereo-paired electron micrographs taken under HVEM using thick sections. The former approach is a useful method for relatively finer structure but not for larger ones because it is not so easy to make several hundred serial thin sections and to reconstruct images from them. As compared to the former, it is easy to prepare the thick sections, even serial thick sections, containing all the information covering the whole cell for HVEM. However, in the case of HVEM, it is a problem to obtain the sharp images of GA with high resolution and an appropriate density of cytoplasm for understanding cellular orientations.

These problems can be solved by Zinc-Iodide-Osmium (ZIO) staining. The ZIO method, more widely Sodium or Potassium-Iodide-Osmium method, is a classical method and there are numbers of modifications of these methods by many investigators (1, 4, 6, 7, 9, 15, 18). Most of these methods seemed to lack consistency in their result, i.e. the reproducibility of the method and the staining mechanism is not well understood, either. But under certain conditions, certain whole cell or certain cell organelle could be visualized clearly to an extent more than any other method has ever achieved. As a preliminary experiment we have tried to observe the thick sections stained by Friend’s osmium impregnation method or other cytochemical methods such as the demonstration of NADPase activity according to the
procedure of Smith (16) as modified by Clermont et al. (2) under HVEM. But the reaction product of Friend's method was coarse and unsatisfactory to demarcate the Golgi cisternae. In addition, the morphology of cell organelles often showed structural distortion which may have been due to a prolonged osmium impregnation without aldehyde-prefixation. One of the best cytochemical staining methods for Golgi cisternae is the detection of the localization of NADPase activity. However, even though Golgi cisternae appeared to be stained very clearly by this method in ultrathin sections, the density of the reaction product in thick sections was not high enough to visualize their structure under HVEM.

The details of the reaction mechanism between ZIO solution and aldehyde-fixed pancreatic tissue is not clear at present. The chemical nature of dense material observed in GA was found to be almost only osmium and no other significant elements were detected by electron probe X-ray microanalysis (Noda and Ogawa, unpublished data). This result is not consistent with the result by Gilloteaux and Naud (5), who showed zinc osmate associated to Ca$^{2+}$ affinity sites. However, it might be possible that the pancreatic tissue, after aldehyde-fixation, loses a considerable amount of divalent cations before the chemical reaction with ZIO solution, and that all densely reacted areas do not represent Ca$^{2+}$ affinity sites. In addition to that, it would be difficult to regard all the densely stained cells which are

![Schematic representation of the axes of the Golgi stack](image)

**Fig. 11.** Schematic representation of the axes of the Golgi stack. Basically Golgi stack can be regarded as the overlapping of ribbon-like cisternae. Distinguishable three sides of Golgi stack are expressed in this scheme as three axes of three-dimensional Golgi stack. Note that these coordinates can rotate around the X axis and that branches of Golgi stack occur along the Y axis with all cisternae.
observed in some cases in tissue blocks as being the site of Ca\(^{2+}\) affinity. Thus, it seems to be reasonable to consider the densely stained material observed in the present work to be different from the dense material reported by Gilloteaux and Naud (5) from the point of not only the morphological appearance but also the chemical nature.

As an aid to an understanding of the three-dimensional feature of ribbon-like Golgi stacks, it may be convenient to introduce the three-dimensional and topographical coordinates (X, Y and Z) on the Golgi stack (Fig. 11):

1) let X axis be the elongating direction of ribbon-like Golgi cisternae,
2) let Y axis be the rectangular line to X axis on the plane formed by the cis-most cisterna of the Golgi stack,
3) let Z axis be the line perforating the Golgi stack from cis to trans vertically to both X and Y axes.

After defining topographical coordinates as above, the several basic features of Golgi stack can be described as follows: The Golgi stack is known to have the polarity of trans and cis side (along the Z axis), but it has two distinctive additional sides; one is the elongating side of Golgi cisterna (X axis) and the other non-elongated lateral side (Y axis). In an ultrathin section, we cannot distinguish the latter two sides, because both cut-faces on YZ plane and XZ plane of Golgi stack look like the "transverse" section. The width of the Golgi stack as the cisternal distance along the Y axis is varied from 0.5 \(\mu\)m to 1 \(\mu\)m, but there are often relatively wide portions. The bifurcations or trifurcations of Golgi stacks appeared to occur in these areas and each cisterna developed from the lateral side of the identical cisterna along Y direction. However, it should be noted that these coordinates are defined topographically on the Golgi stack. The torsion of the Golgi stack that we have described in the result can be understood as the rotation of the coordinates around X axis (Fig. 3, 5, 6).

Thus, the true three-dimensional configuration of GA can be obtained from 1 \(\mu\)m thick, serial sections of a whole cell, an observation of 5 \(\mu\)m thick sections and superimposed micrographs, and these observations indicate that findings obtained from a 1 \(\mu\)m thick section represent a part of the huge continuous reticular Golgi "complex".

Judging from an extreme flexibility of the structure of GA, it is not unlikely that the characteristic three-dimensional structure of GA is dynamic and easily changeable depending on the physiological state of the cell. Questions, however, such as whether these connected structures of GA reflect a synchronization of the cellular secretory activity toward different intracellular sites, e.g. intercellular canaliculi, or whether there might exist regional heterogeneities in the whole GA per se, await further clarification.

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