THE APPEARANCE OF CARBOHYDRATE-RICH MATERIAL IN THE DEVELOPING GOLGI APPARATUS OF AMOEBAE

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SUMMARY

The silver proteinate reaction was used to stain carbohydrate-rich substances in normal Amoeba proteus and in the developing Golgi apparatus of renucleated amoebae. Normal cells contained stained material, which probably is glycoprotein, in the cell surface, cisternae at the concave pole of the Golgi apparatus, and cytoplasmic vesicles and vacuoles. Previous radioautographic studies had shown that glycosylation occurs in the Golgi apparatus, and that material in the Golgi apparatus is precursor to the cell surface. Amoebae were enucleated for 5 d, which results in a decline of the Golgi apparatus, the disappearance of the glycoprotein-containing cisternae preceding that of the rest of the organelle. A new nucleus was then transplanted into the enucleate amoebae, bringing about the regeneration of the Golgi apparatus. Small curved cisternae that appeared 30 min after renucleation lacked staining with silver proteinate. By 1 h after renucleation, however, the content of cisternae toward the concave poles of Golgi bodies stained with silver proteinate. The Golgi apparatus in cells fixed 6 h and 1 d after operation resembled that of normal amoebae in both morphology and staining pattern. The results suggest that the developing Golgi apparatus acquired the capacity to participate in assembly of cell-surface material within 1 h after renucleation. This occurred before development of the normal enzymic activity of the Golgi apparatus was completed.

INTRODUCTION

The Golgi apparatus of amoebae depends upon the nucleus for maintenance of its normal form and chemical composition (Flickinger, 1968, 1969, 1978; Wise & Elickinger, 1971). This permits study of the development of the organelle by microsurgical manipulation, because Golgi bodies decline in size and number in the absence of the nucleus (Flickinger, 1968) and are regenerated by transplanting a new nucleus into enucleate cytoplasm (Flickinger, 1969). During development small curved cisternae are succeeded by small stacks and then by normal-sized stacks of saccules (Flickinger, 1969). Cytochemically demonstrable enzymic activities in the forming Golgi apparatus differ from normal, and the sequence of changes during development suggests that Golgi membranes may arise from the endoplasmic reticulum (Flickinger, 1978).

One of the functions of the Golgi apparatus in amoebae is the glycosylation of components of the cell surface, as demonstrated radioautographically following the administration of radioactive sugars (Flickinger, 1974a, 1975). This role is reflected by the presence in cisternae at the concave pole of a filamentous material that resembles the amoeba surface coat, both morphologically (Stockem, 1969) and in staining for a
carbohydrate-rich substance (Revel & Ito, 1967; Stockem, 1969; Wise & Flickinger, 1970a, b) that is probably glycoprotein (Wise & Flickinger, 1970a). The glycoprotein-containing parts of the Golgi apparatus are sensitive to the removal of the nucleus since they disappear within about 1 d after enucleation while other cisternae persist longer (Wise & Flickinger, 1971).

In the present investigation, the appearance of carbohydrate-rich material in the forming Golgi apparatus of renucleated amoebae was studied by cytochemical staining. Since glycosylation normally occurs in the Golgi apparatus (Flickinger, 1975), the appearance of this material serves as an index of the functional maturation of the organelle.

MATERIALS AND METHODS

Cultures of *Amoeba proteus* were maintained in Prescott’s amoeba medium (Prescott & Carrier, 1974) with daily feedings of washed *Tetrahymena*. Amoebae were cut in 2 with the tip of a braking pipette (Prescott & Carrier, 1964; Flickinger, 1968). The enucleated halves were separated from the nucleated parts on the basis of the enucleates’ round shape, failure to attach to the substrate, and immotility. The enucleates were maintained at 21 °C for 5 d and then renucleated by transplantation of nuclei from normal donor amoebae. Pairs of amoebae were placed on the surface of an agar-coated slide (Jeon, 1970). Under a compound microscope at a magnification of 180 ×, the nucleus was transferred from the donor to the recipient by a glass probe controlled by a de Fonbrune micromanipulator. After the operation the renucleates were placed in amoeba medium at 21 °C for intervals of 30 min, 1, 6 and 24 h.

Samples were fixed by immersion for 1 h in Karnovsky’s fixative (Karnovsky, 1965). The cells were rinsed overnight in 0.05 M cacodylate buffer and embedded in a small cube of agar to prevent their subsequent dispersion (Flickinger, 1969). The samples were postfixed in 1 % OsO₄ in 0.1 M cacodylate buffer pH 7.3, dehydrated in a graded series of ethanols followed by propylene oxide, and embedded in Araldite. Silver to pale-gold sections were cut with a diamond knife on a Porter-Blum MT-1 ultramicrotome.

Sections were stained with the silver proteinate method for carbohydrate-rich substances (Thiery, 1967). There were 2 main reasons for choosing this staining procedure. First, the reaction is performed on thin sections, so available blocks containing renucleated amoebae could be used, eliminating the need for much additional microsurgery and specimen preparation. Second, preliminary observations of normal amoebae revealed fine, well localized staining and little or no background over control specimens. Sections were mounted on 200-mesh gold grids and submerged in 1 % periodic acid for 30 min, 3 changes of distilled water for 10 min each, and 1 % thiosemicarbazide or thiocarbohydrazide in 10 % acetic acid for 1–20 h. This was followed by successive rinses: 10 % acetic acid, 10 min; 5 % acetic acid, 5 min; 1 % acetic acid, 5 min, and 3 changes of distilled water, 5 min each. In subdued light, the grids were immersed in a 1 % solution of silver proteinate (EM Laboratories, Elmsford, NY) for 30 min, and then rinsed 3 times in distilled water, 10 min each. The preparations were viewed either without additional staining or following light staining for ~1 min with lead citrate. Controls consisted of immersing sections in distilled water instead of periodic acid. Micrographs were obtained with a Philips EM-300 electron microscope operated at 60 kV.

RESULTS

*Normal amoebae*

The cell surface of normal *Amoeba proteus* consisted of the plasma membrane, which was ~10 nm thick, and a cell coat (Fig. 1). The latter comprised an amorphous
layer next to the membrane, and a layer of filaments which extended outwards for a distance of 150–200 nm from the plasma membrane and amorphous layer. A similar coat lined the inner surface of the membrane of 'fringed' vacuoles in the cytoplasm. The Golgi apparatus comprised multiple Golgi bodies (Figs. 2, 3) that were distributed through the cytoplasm. Each was composed in turn of a stack of approximately 6–8 curved, smooth-surfaced cisternae with expanded ends. Vesicles lay near the margins of the stacks. Vesicles and cisternae toward the concave pole had a content of filamentous material. Other features of amoeba ultrastructure have been described in detail elsewhere (Daniels, 1973; Flickinger, 1973, 1974a).

In sections of normal amoebae prepared with the silver proteinate reaction, staining was observed in the cell surface, parts of the Golgi apparatus, and certain cytoplasmic vesicles and vacuoles. In the cell surface, an electron-dense precipitate stained the filaments of the external layer of the cell coat (Fig. 1). Frequently a granule or thickening along the course of a filament was highlighted. Little staining of the amorphous layer was observed. Similar staining occurred in the lining of the fringed vacuoles (Fig. 3), which are believed to be formed from the cell surface by endocytosis (Flickinger, 1975). In the Golgi apparatus (Figs. 2, 3), a fine electron-dense precipitate was observed over the expanded ends of cisternae and in small vesicles near the concave pole. In some Golgi bodies an expanded cisterna at the concave pole contained stained filaments. Cisternae at the convex pole and the central parts of most of those toward the concave pole usually did not stain. A fine filamentous material in the interiors of members of groups of small vesicles and tubules in the cytoplasm also was stained. No staining was observed in the rough endoplasmic reticulum.

Irregularly shaped, ~30-nm cytoplasmic granules, which varied greatly in abundance from one amoeba to another, stained intensely with silver proteinate (Fig. 3). Their identification is uncertain, but this staining suggests that they may represent a storage form of carbohydrate such as glycogen.

Control sections, from which the periodic acid oxidation was omitted, usually were devoid of precipitate (Fig. 4). In a few cases a very fine, light stippling overlay the entire cell, but selective staining of Golgi apparatus or surface was never observed in controls.

*Renucleated amoebae*

When amoebae were enucleated, Golgi bodies declined in size and number so that they were absent or so few in number that they were no longer detectable morphologically more than 3 days after removal of the nucleus (Flickinger, 1968, 1969). In cells fixed 30 min after introduction of a new nucleus, the first step in regeneration of the Golgi apparatus was the appearance in the cytoplasm of scattered groups of small curved cisternae (Fig. 5). Some of the small cisternae had expanded ends, and in places 2 or 3 were aligned in parallel to form stacks resembling small Golgi bodies. These small cisternae completely lacked staining with silver proteinate. This was not due to failure of the staining reaction on the 30-min sample, because other structures such as the surface and fringed vacuoles were prominently stained in the same sections (Fig. 5).
By 1 h after renucleation, Golgi bodies consisting of stacks of 4–6 curved cisternae were present, and staining with silver proteinate was detected (Figs. 6, 7), even in the smallest examples. A fine precipitate was observed in the expanded ends of cisternae (Fig. 7), particularly those near the concave pole, and when a distended cisterna was visible at the concave face its content also stained (Fig. 6). The precipitate often had a reticulate pattern and appeared to be deposited on fine filaments (Fig. 6). Thus the distribution of the staining in the Golgi apparatus of the 1-h sample was similar to that of the normal organelle.

The pattern of silver proteinate staining of the Golgi apparatus in 6-h and 1-d samples (Fig. 8) also resembled that of normal amoebae, with a fine precipitate highlighting filamentous material in the ends of cisternae and in distended sacculi at the concave pole. The cell surface, fringed vacuoles, and some small vesicles and tubules (Fig. 8) were stained as well in renucleated amoebae at all the intervals.

Some general points about the silver proteinate preparations may be noted. The delicate, very finely granular nature of the precipitate necessitated study of sections that were not stained with lead salts (or were only very lightly stained in this way) so as not to obscure the reaction product. Nevertheless, the localization of the silver proteinate staining was evident because the control sections were very clean, usually lacking precipitate altogether. The overall contrast of the sections was less than usual, however, because staining with lead was omitted.

**DISCUSSION**

The silver proteinate method for staining carbohydrate-rich material for electron microscopy is based on the periodic-acid–Schiff (PAS) reaction of light microscopy. It depends on the detection of aldehyde groups that are produced by periodic acid oxidation of hydroxyl groups on adjacent carbon atoms of sugars. Thiosemicarbazide and thiocarbohydrazide react with the aldehyde groups and subsequently also reduce silver proteinate to metallic silver, which is visualized in the electron microscope (Hayat, 1975). Silver proteinate produces a finer precipitate, localizable at higher resolution, than other procedures based on periodic acid oxidation (Knight & Lewis, 1977). Stainable substances include polysaccharides, glycoproteins, mucoproteins,

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**Fig. 1.** Normal amoeba stained with silver proteinate. A fine granular electron-dense stain is present on the filamentous layer of the cell surface (s). Part of a Golgi apparatus (g) is visible. a, amorphous layer of the cell surface; p, plasma membrane. × 57 000.

The value of the bar in this and the following micrographs is 0.5 μm.

**Fig. 2.** Normal amoeba Golgi apparatus. Silver proteinate staining is present in cisternae at the concave pole (arrows). A mitochondrion (m) is not stained. × 66 000.

**Fig. 3.** Normal amoeba. In addition to staining of cisternae at the concave pole of the Golgi apparatus (arrows), a fringed vacuole (f), and irregularly shaped cytoplasmic granules (b) are stained. × 68 000.

**Fig. 4.** Control for silver proteinate staining, normal amoeba. There is no electron-dense staining of the cell surface (s), Golgi apparatus (g), fringed vacuoles (f), or other structures. × 43 000.
Fig. 5. Renucleated amoeba 30 min after operation. Small curved cisternae (c) are present and some are aligned to form small stacks (arrow), but none are stained with silver proteinate. A fringed vacuole (f) in the field has been stained. × 41000.

Figs. 6, 7. Golgi apparatus 1 h after renucleation. Silver proteinate staining is present at the concave pole, especially in the expanded ends of the cisternae (arrows). The stain appears to be deposited on fine filaments in a distended cisterna (arrowhead). Fig. 6, × 65000; Fig. 7, × 70000.

and some lipids, although the latter are usually extracted in preparation. The amoeba cell surface contains a variety of sugars (Allen, Ault, Winzler & Danielli, 1974) that are capable of participating in this reaction, and previous study has indicated that the carbohydrate-rich material in the cell surface and Golgi apparatus is most likely glycoprotein (Wise & Flickinger, 1970a).
Radioautographic studies have shown that a function of the Golgi apparatus in amoebae is glycosylation of cell-surface components (Flickinger, 1975; Read & Flickinger, 1980). This kinetic evidence, coupled with morphological and cytochemical similarities (Revel & Ito, 1967; Stockem, 1969; Wise & Flickinger, 1970a), suggests that much of the material in the Golgi apparatus stainable with silver proteinate represents cell-surface material either in the process of assembly or completed and awaiting transport to the surface.

Fig. 8. Renucleated amoeba 6 h following operation. The distribution of staining resembles that of normal amoebae, being present in the cell surface (s), cisternae at the concave pole of the Golgi apparatus (g, arrow) and small tubules and vesicles (v). x 69,000.

The capacity of the Golgi apparatus to participate in assembly of carbohydrate-rich material appeared to be acquired early in its development. Only the small cisternae that represented the incipient Golgi apparatus 30 min after renucleation lacked a content of stainable material. Subsequently, carbohydrate-containing material was present in Golgi bodies 1 h after renucleation. This substance already had a normal distribution, although it is uncertain if it was as concentrated as in normal amoebae. Taking into account the likelihood that some time would be required for the material to accumulate in a sufficient amount to be microscopically visible, this observation implies that the developing Golgi apparatus was functionally active in glycosylation.
less than 1 h after introduction of the nucleus. However, despite the resemblance, it is not certain from cytochemical studies whether the material that stains in the developing Golgi apparatus is identical chemically to that in the normal amoeba Golgi apparatus.

The functional development of the Golgi apparatus indicated by staining with silver proteinate preceded completion of its chemical maturation. When the forming Golgi apparatus of renucleated amoebae was tested cytochemically for enzymic activities present in normal amoeba Golgi apparatus, nucleoside diphosphatase and esterase were present in Golgi cisternae from when they were first visible, but thiamine pyrophosphatase was not detected until between 12 and 24 h after renucleation (Flickinger, 1978). Thus the appearance of stainable glycoprotein and presumably the functional capacity to produce and accumulate cell coat material occurred before the Golgi apparatus acquired a normal enzymic complement.

The carbohydrate-rich parts of the amoeba Golgi apparatus are very sensitive to the presence of the nucleus. When amoebae were enucleated, the glycoprotein-containing cisternae of the concave pole were the first to disappear, being detectable for only about a day in the absence of the nucleus (Wise & Flickinger, 1971). In contrast, phosphatase-containing cisternae at the convex pole were present for 1 or 2 more days and retained enzymic activity as long as Golgi bodies were detectable. Why some parts of the Golgi apparatus are more responsive to removal of the nucleus than others is unknown, as is the relation between the phosphatase and glycoprotein-containing cisternae. One possibility is that the glycoprotein-containing cisternae are continually depleted by transport to the cell surface and are dependent for maintenance upon input from the endoplasmic reticulum of the polypeptide parts of glycoproteins, and perhaps membranes as well. Synthesis of the polypeptides could require relatively short-lived messages and thus be sensitive to the presence of the nucleus, or transport from the endoplasmic reticulum to the Golgi apparatus may require the presence of the nucleus, as do other forms of motility in amoebae (Jeon, 1968).

This research was supported by grants from the American Cancer Society (VC-169C, CD-58D). The author is indebted for technical assistance to Ms Kathleen Glasheen and Ms Ellen Kabana.

REFERENCES


Golgi apparatus of amoebae


(Received 1 July 1980)