INTRACELLULAR LOCALIZATION OF ACID PHOSPHATASE AND TRYPSINOGEN ON AN IDENTICAL SPECIMEN IN RAT PANCREAS

SHUJI YAMASHITA, NOBORU YAMAMOTO* AND KENJIRO YASUDA

*Department of Anatomy, School of Medicine, Keio University, Shinanomachi,
Shinjuku, Tokyo 160 and Department of Anatomy*, School of
Medicine, Kitasato University, Sagamihara, Kanagawa 228*

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Fine intracellular localization of acid phosphatase (AcPase) and trypsinogen was investigated in an identical specimen of rat exocrine pancreas. The tissue was embedded in acrylamide gel after AcPase enzyme histochemical reaction, and ultrathin sections were prepared successively from the gel block. The trypsinogen was detected on the cut face of each section by use of the immuno-ferritin method.

AcPase activity was recognized in lysosomes, Golgi vacuoles, Golgi vesicles in trans face and in the innermost Golgi lamellae. Trypsinogen occurred in secretory granules and Golgi vacuoles. The Golgi vacuoles were classified into three types on the basis of their contents: i.e., vacuoles containing both AcPase activity and trypsinogen-immunoreactivity, vacuoles containing only trypsinogen-immunoreactivity, and vacuoles showing only AcPase activity. The first type of the Golgi vacuoles was most frequently observed and the third type of Golgi vacuoles, rarely.

On the basis of the result, it is concluded that lysosomes and secretory granules develop simultaneously from the trans face of the Golgi apparatus. It is suggested that one role of lysosomal enzymes may be intracellular modification of secretory proteins in the Golgi mature face of rat exocrine pancreas.

Blobel and his coworkers recently reported that both secretory proteins and lysosomal enzymes crossed the microsomal membrane with the aid of signal peptides (2, 4, 14, 15). Most secretory proteins and lysosomal enzymes consist of glycoproteins, and these proteins may be glycosylated in the Golgi apparatus in which glycosyltransferases are concentrated (1, 5, 20, 25). Many investigators have reported that secretory granules were formed in the Golgi trans area, and AcPase was also recognizable in that area in exocrine and endocrine glands under enzyme histochemical examination (3, 6, 8, 9, 11, 22). These findings suggest that the Golgi apparatus represents a main organella for the translocation of secretory proteins and lysosomal enzymes into secretory granules and lysosomes, respectively. However, it is difficult to morphologically distinguish whether Golgi vacuoles located in the Golgi trans face give rise to secretory granules or to lysosomes.

In the present study, we attempted to demonstrate the intracellular localization of AcPase (a marker enzyme of lysosome) and trypsinogen (a secretory enzyme) in the rat pancreas by use of both enzyme histochemical and immunohistochemical

methods on identical specimens. Attention was particularly focused on the distribution of both enzymes in the Golgi trans area. In order to detect both enzymes in the same specimen, the tissue blocks were incubated in AcPase reaction medium first, then embedded in acrylamide gel (26, 27). Ultrathin sections were prepared successively and the distribution of trypsinogen was examined by the immuno-ferritin method.

MATERIALS AND METHODS

Anti-rat trypsinogen rabbit γ -globulin was a gift from Dr. Tetsuro Ono (Department of Anatomy, Miyazaki Medical School, Miyazaki).

Fixation: The pancreas was removed from a Wistar rat under chloroform anesthesia and fixed for 4.5 hr at 4°C in a fixative consisting of 4% paraformaldehyde, 0.05% glutaraldehyde and 0.025% CaCl_2 dissolved in 0.1 M cacodylate buffer, pH 7.2. The fixed tissues were then washed overnight in 0.1 M cacodylate buffer, pH 7.2, containing 7.5% sucrose.

AcPase reaction: Non-frozen sections of about 70 μm in thickness, were prepared with a Smith-Farquhar tissue sectioner (Ivan Sorvall). The sections were washed with 7.5% sucrose for 2 hr, and then incubated with AcPase reaction medium at 37°C for 20 min. Cytidine-5'-monophosphate was used as substrate (13). Substrate-free medium was employed in a control experiment for the enzyme histochemistry. After the reaction, the sections were washed in 7.5% sucrose for 90 min.

Acrylamide embedding: The washed sections were placed in acrylamide embedding medium (27) and kept in the dark at 4°C overnight. The acrylamide solution containing the sections was polymerized with a day-light type fluorescent lamp. After polymerization, the gel containing the section was cut into small pieces and washed in 0.1 M cacodylate buffer, pH 7.2, containing 7.5% sucrose. The gel blocks were fixed again in 0.5% glutaraldehyde dissolved in 0.1 M cacodylate buffer, pH 7.2, containing 5% sucrose, in order to preserve the fine structure of the tissue and to prevent loss of antigen during the preparation of ultrathin sections. The gel blocks were then washed overnight with 0.1 M cacodylate buffer, pH 7.2, containing 7.5% sucrose, and successively washed with 5% sucrose for 1 hr. The gel blocks were finally dried on silica gel.

Immunohistochemical staining: Ultrathin sections were prepared on the MT2-B ultramicrotome (Sorvall) equipped with a glass knife. Grids holding the sections were floated on 0.1 M cacodylate buffer, pH 7.2, containing 1% bovine serum albumin. The staining procedure used for the immuno-ferritin method was described previously (27). After the immunohistochemical reaction, the grids were washed with 0.1 M phosphate buffer, pH 7.2, containing 0.7 M NaCl, and successively washed with distilled water. The specimens were then post-fixed in OsO_4 vapor for 30 min. For the control staining, normal rabbit γ -globulin was employed in place of the anti-trypsinogen. The specimens were examined under a Hitachi HU-12A electron microscope without any electron staining.

RESULT

In the exocrine cells of the pancreas, the Golgi apparatus generally had four to five lamellae. Golgi vacuoles were found in the trans area of Golgi lamellae, and many small vesicles were recognized in both the cis and trans face of the lamellae.

AcPase: Strong AcPase activity was observed in granular and tubular shaped lysosomes (Figs. 5, 6). AcPase reaction deposits were also localized in the Golgi vacuoles, vesicles of the Golgi trans region and innermost Golgi lamellae. The AcPase activity of the Golgi vacuoles was usually seen along the inside of the limiting membrane of the vacuoles.

Trypsinogen: Ferritin particles demonstrating the localization of trypsinogen, were recognized in secretory granules and Golgi vacuoles (Figs. 1-5). However, very few ferritin particles were present in the Golgi lamellae/or in the vesicles of the Golgi trans face. The other organellae including the lysosomes and endoplasmic reticulum were not stained with anti-trypsinogen.

AcPase and trypsinogen in Golgi vacuoles: The Golgi vacuoles were heterogeneous with respect to both their size and contents. The Golgi vacuoles were classified into three types on the basis of their contents: i.e., vacuoles containing both AcPase and trypsinogen, vacuoles containing only trypsinogen, and vacuoles showing only AcPase activity. The first type of Golgi vacuoles was most frequently observed while the third type of the Golgi vacuoles was rarely recognizable. These different types of Golgi vacuoles were sometimes localized even within an identical Golgi apparatus. It was also found in some cells that the Golgi vacuoles had no reaction deposits of AcPase but contained trypsinogen, in spite of the fact that strong AcPase activity was noticed in the lysosomes in the same specimen. These results are summarized in Table 1.

In some experiments, after having been embedded in acrylamide, the tissue was cut into 70 μ m thick sections and these were incubated with AcPase reaction medium. Ultrathin sections were successively prepared and stained by the immunoferritin method. Similar results were obtained according to the localization of both AcPase and trypsinogen.

Substrate-free medium was employed for control examination of the AcPase reaction. No lead deposits were present in the pancreatic exocrine cells when the incubation time was less than 20 min. No ferritin particles were seen in the cells when normal rabbit γ -globulin was used in place of the specific antibody (Fig. 6).

TABLE 1. *Distribution of AcPase and trypsinogen in pancreatic acinar cells of the rat*

	AcPase	Trypsinogen
Golgi apparatus		
vacuoles	+	+
innermost lamellae	+	±
small vesicles in trans face	+	±
Secretory granules	—	+
Lysosomes	+	—

DISCUSSION

In this study, we investigated the intracellular distribution of AcPase and trypsinogen in identical specimens. The method described under "Materials and Methods" was used for the following reasons. (1) When AcPase reaction was performed on ultrathin sections prepared from the tissue embedded in acrylamide gel, significant loss of the enzyme activity was observed. (2) No diffusion of lead deposits was observed when the tissue was embedded in acrylamide gel after the AcPase reaction. Trypsinogen was selected among secretory enzymes in pancreas in the present study, since it was assumed that each of the secretory enzymes of the exocrine pancreas coexisted in the secretory granules (12, 17, 21, 24).

Our results suggest that lysosomes and secretory granules are formed concurrently from the trans face of the Golgi apparatus in rat pancreatic acinar cells. These results support the views of cytochemists (3, 6, 16, 18, 22), who have reported that immature secretory granules and AcPase active vacuoles develop from the Golgi trans face.

Our results indicated that the Golgi vacuoles were divided into three groups on the basis of their observed contents; vacuoles containing both AcPase activity and trypsinogen, vacuoles containing only trypsinogen and vacuoles showing only AcPase activity. One of the roles of the lysosomes in secretory cells might be to degrade endogenous secretory products when the discharge of the products is depressed under certain physiological conditions (7, 22). Ikehara *et al.* (10) reported that pro-albumin was converted to albumin in secretory vesicles of rat liver and almost the same results have reported by Quinn *et al.* (19). Smith *et al.* (23) suggested that acid protease included in immature secretory vesicles may also participate in the conversion of proinsulin to insulin in rat pancreas. The first type of Golgi vacuoles was

AcPase and trypsinogen were localized by us in identical specimens employing both enzyme histochemical and immuno-ferritin methods in rat exocrine pancreas. After the AcPase reaction, immunohistochemical staining was carried out on ultrathin sections prepared from the tissue embedded in acrylamide gel. For the demonstration of AcPase activity, CMP was used as the substrate. The sections were examined without any electron staining.

FIG. 1. AcPase activity and trypsinogen in identical specimens of a Golgi vacuole. Ferritin particles which demonstrate the localization of trypsinogen are present in the secretory granules, but AcPase activity is not seen in the granules. $\times 42,800$

FIG. 2. Both AcPase and trypsinogen are present in the Golgi vacuole. AcPase activity is recognized along the inner face of limiting membrane of the Golgi vacuole. Trypsinogen is seen in the secretory granules. $\times 49,100$

FIG. 3. Three Golgi vacuoles are present in the Golgi apparatus. Both AcPase and trypsinogen are localized within one of the vacuoles, whereas the other vacuoles contain only trypsinogen. In the Golgi cisternae, ferritin particles (trypsinogen) are barely recognizable. $\times 46,000$

FIG. 4. Innermost Golgi lamella showing AcPase activity. AcPase and trypsinogen are localized simultaneously in the Golgi vacuole. $\times 46,600$

FIG. 5. Lysosomes heavily stained with AcPase activity. Trypsinogen is present in the secretory granules, but is barely recognizable in the lysosomes. $\times 52,300$

FIG. 6. Control experiment. Tissue was incubated with AcPase reaction medium, and then embedded in acrylamide. Ultrathin sections were successively stained with normal rabbit γ -globulin instead of anti-trypsinogen antibody. AcPase is present in the tubular shaped lysosomes. No ferritin particles are seen in the specimen. $\times 50,000$

most frequently observed. This result suggests an additional role of lysosomal enzymes in the intracellular modification of secretory proteins in the Golgi mature face in the secretory gland. However, following possibility is undeniable from this finding. Lysosomal and secretory proteins have different "recognition markers" for the intracellular translocation, and they are transferred to lysosomes and secretory granules by the "recognition markers" in the trans face of the Golgi apparatus. But the "recognition markers" are not so strict that the "recognition markers" are recognized in the different manner and, as a result, some Golgi vacuoles may contain both lysosomal enzyme and secretory protein. In any event, AcPase was not recognized in secretory granules. It is still unknown whether AcPase coexisting with trypsinogen is excluded furthermore from Golgi vacuoles to other cell organelles, such as lysosomes and Golgi vesicles, or inactivated in mature secretory granules. The third type of Golgi vacuoles was rarely recognized. When the ultrathin sections were incubated with ferritin and then embedded in epoxy resin and cross sections were prepared from the epon blocks, the innermost part recognized ferritin particles was 50 nm from the surface of the gel. Therefore, the third type of the Golgi vacuoles may localize in the deep position from the cut face, so that ferritin particles can not interact with them.

Though AcPase was employed as a marker of the lysosome in this study, the localization of another lysosomal enzymes, such as cathepsins and glycosidases, could be defined on a electron microscopic level to elucidate the role of lysosomal enzymes in the secretory cells.

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