The occurrence of the intracisternal granules in the acinar cells of the bovine pancreas

By

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The presence of the opaque granules in the rough-surfaced cisterna was primarily described by Palade (1956) in the acinar cells of the pancreas of the guinea pig. Thereafter, several authors have reported on the origin and the fate of this granule particularly in relation to the secretory cycle of the acinar cell, using exclusively guinea pigs as an experimental animal under the condition of re-feeding after a certain period of the starvation (Siekevitz and Palade, 1958, Watanabe and Arakawa, 1963, and Caro and Palade, 1964), or on condition of the stimulation by employing the chemicals which accelerate the secretory function of pancreas (Suzuki, 1958, Watanabe and Arakawa, 1963, and Kurosumi and Kobayashi, 1963). The intracisternal occurrence of the granular structure in pancreas happens barely in rats only after the chemical stimulation or in the abnormal circumstances (Ichikawa, 1958). While, Kurosumi and Kobayashi (1963) could not recognize any intracisternal granules in the pancreas of the rat, mouse, golden hamster and the rabbit, which belong to the rodent order together with the guinea pig. In addition to the acinar cells of pancreas, similar granules have hitherto been found in the vesicular zone of the vitelline body of the mature oocyte of the spider (André and Rouiller, 1957), in the plasma cell of the lymph node of the rat (Wellensieck, 1957 and Thiéry, 1958) and of the mouse (Tranzer, 1962), and in the cancer cells of human mammary gland (Hollmann, 1959).

The present study reports the presence of the intracisternal granules in the acinar cell of the bovine pancreas, and deals with the distribution pattern of some pancreatic enzymes in the competent granule.
Materials and methods

The tissue used in the investigations to be reported was composed exclusively of bovine pancreas, removed within 30 minutes after the sacrifice of the animals in the slaughter house. The tissue block of pancreas was cut further in tiny pieces in the following fixatives: 1% phosphate buffered osmium tetroxide and 5% formalin buffered at pH 7.2 containing both 0.89% sodium chloride and 0.001 M ethylenediaminetetraacetic acid. The tissue fixed in the osmium tetroxide solution for 1 hour at 0°C was then washed briefly with chilled buffered saline, dehydrated with graded dilution of acetone and finally embedded in epoxy resin. The formalin-fixed tissue was allowed to remain in the cold for 1 hour, blotted on the filter paper, immersed in the soybean trypsin-inhibitor solution at the concentration of 20 mg per ml for 1 hour, and successively transferred into the ferritin-conjugated antibody solution prepared against bovine trypsin, chymotrypsin and ribonuclease, respectively. The incubation of the tissue in the tagged antibody solution was carried out at room temperature for 30 minutes, followed by an additional incubation for 90 minutes at 4°C. Some of the tissue blocks received the freezing-thawing procedure before being applied to the conjugated globulin solution, which renders the cell permeable for ferritin-globulin complex to enter into it. After rinsing with several changes of buffered saline, the specimens were post-fixed in 1% phosphate buffered osmium tetroxide for 30 minutes, dehydrated with acetone and embedded in epoxy resin. The ultrathin sections were obtained with the aid of a Porter-Blum microtome, stained with lead hydroxide (Karnovsky, 1961) or with a double staining procedure utilizing both uranyl acetate (Watson, 1959) and lead hydroxide, and examined mostly in a Siemens Elmiskop I. Since the ferritin-conjugated antibody was prepared in the same manner as outlined elsewhere (Yasuda and Isliker, 1965), the detailed description for that was excluded here.

Observations

As demonstrated in Figs. 1 and 6, several opaque granules occur in the rough-surfaced cisternae in the bovine pancreas, being enclosed in the amorphous electron-lucent material. This granular structure belongs to the identical category with that was described by Palade (1956) as "intracisternal granule", in the pancreas of
the guinea pig. The granule is composed of homogeneous material which displays medium electron density but far lighter contrast compared with the opacity of the zymogen granule. The size of the granule varies from one to another in one cutting plane, ranging from 100 m\( \mu \) to 200 m\( \mu \) in diameter, but not exceeding 300 m\( \mu \). The granule is not bounded by the definite limiting membrane, and often, the boarder line between the granule and the surrounding cisternal material is not clear. There is no sharp difference of the density between the central core and the periphery of the granule. The intracisternal granules are found in the delated cisternae of the rough-surfaced reticulum, but not in the narrow alley surrounded by the regularly arranged reticulum. Similar to the pancreas of guinea pigs, these granules occur generally in the rough-surfaced cisternae in the basal portion of the cell, with some exceptional feature in the cisternae around the Golgi area. Furthermore, it should be mentioned that every acinar cells is not always provided with the cluster of the intracisternal granules. Each cell shows the marked difference in the number of the intracisternal granules, ranging from nothing to the big concentration.

Using ferritin-conjugated antibody method, the intracisternal granules are proved to contain trypsin (Fig. 2), chymotrypsin (Fig. 3) and ribonuclease (Fig. 4). The ferritin-globulin complex is localized evenly in the granule, as well as on the surface of the granule. The electron-density tends to turn light in the granules which lodge the ferritin-tagged globulin, in comparison with the opacity of the granule which is treated with heterologous ferritin-antibody solution (Fig. 6). Occasionally, the cisterna is occupied by the amorphous and slightly opaque material which reacts with the ferritin-antibody complex, enclosing no granular structure in it. The deposit of the ferritin granules in Fig. 5 demonstrates the site of ribonuclease in the homogeneous distribution pattern.

**Discussion**

The granular structure occurred in the rough-surfaced cisternae of the acinar cells of the bovine pancreas displays similar character to that originally described by Pala de (1956) in the pancreas of the guinea pig, with respect to its distribution pattern, electron opacity and to the lack of the definite limiting membrane around it. Concerning the condition in which are the granules observed in the pancreas, following treatments have been reported: re-feeding the guinea pig.
after 48 hours starvation (Palade, 1956 and Watanabe and Arakawa, 1963), injection of secretin in the guinea pig (Suzuki, 1958 and 1959), administration of pilocarpine to the rat (Ichikawa, 1959) and to the guinea pig (Watanabe and Arakawa, 1963). While, the granule is recognizable also in the normally fed animals (Watanabe and Arakawa, 1963), and even decreased in number after the injection of carnitine (Kurosumi and Kobayashi, 1963). Considering the experimental condition of the present study in comparison with those mentioned above, bovine pancreas used are taken from the animals which are, in principle, starved for two days before the sacrifice. Since the specimen was randomly collected in the slaughter house, it is not expected to have a definite proof for the strict alimentary condition. Furthermore, the observation on the pancreas of non-starved cattle was not taken place as a control experiment. Thus, the effect of the alimentation on the appearance and disappearance of the granule in the bovine pancreas is obscure in this report. Only assumption is that the possible irregular feeding during the starvation may facilitate the formation of the intracisternal granules. The role which the intracisternal granule may play in the secretory function of the pancreas has long been discussed by several authors. Palade (1956) assumed this granule to be the precursor of the pancreatic enzyme, and later, together with Siekevitz (1958), proved that the submicrosomal fraction which is presumed to contain this granule carries the trypsin-activatable protease. On the other hand, Watanabe and Arakawa (1963) and Kurosumi and Kobayashi (1963) pointed out that the formation of the intracisternal granule is not the indispensable factor in the protein synthesis in the cell. More recently, Palade, Siekevitz and Caro (1962) and Caro and Palade (1964) conjectured that the intracisternal granule is not an obligatory step in the formation of the zymogen granule, and it possibly be a sort of the intermediate product resulted from the abnormal blocking of the synthetic course, in which the secretory protein produced in the rough-surfaced reticulum is being transferred toward the Golgi region. It is not clear from the present experiment whether or not the intracisternal granule is the necessary step in the formation of the zymogen granule. But, the fact, that the granule contains the material which reacts specifically with anti-trypsin, -chymotrypsin and anti-ribonuclease antibodies, coincides with the result of Siekevitz and Palade (1958) reporting that the heavy microsomal fraction from DOC-treated micro-
some consists mostly of the aggregation of the intracisternal granules and shows as high activity of trypsin-activatable protease and ribonuclease as purified zymogen granules do.

It is noteworthy that the ferritin-tagged antibody against ribonuclease lodged on the non-granular component in the rough-surfaced cisternae (Fig. 5). The ferritin-tagged antibodies against other two enzymes examined also combined with the amorphous material in the rough-surfaced cisternae. Provided that, at least some steps of the protein synthesis were taken place in the endoplasmic reticulum, the newly synthesized material thus produced in the cisternae may display the liquid state more probably than the granular feature. In general, attachment of the ferritin-conjugated antibody to the amorphous substance is less frequently observed than to the intracisternal granules. This tendency might be partly because of the high solubility of the newly synthesized enzymes into the fixative employed. As the immunological difference between enzyme and its precursor is not distinguishable as far as trypsin and chymotrypsin are concerned, the deposited sites of the ferritin-conjugated antibodies against two mature enzymes may also represent the sites of each precursor in the cell constituents. Thus, the intracisternal granules may contain either the precursor of the proteolytic enzymes as already presumed by Palade (1956), or the matured enzymes as well. Since ribonuclease has no definite precursor, the positive sites of the immunological reaction between this enzyme and its homologous antibody may represent the sites of the enzyme distribution itself, but not of the precursor. Similar difficulties in the judgement of the immunological reaction are noticed in searching for the difference of the protein composition between the intracisternal granules in the basal cytoplasm and those around the Golgi area. Namely, it is not obvious from the immunocytochemical view-point that the intracisternal granules around the Golgi area are more mature than those in the basal cytoplasm.

**Conclusion**

The intracisternal granule was encountered in the bovine pancreas. Trypsin, chymotrypsin and ribonuclease were localized in the granules by using the immuno-electron microscopic procedure.
Acknowledgment

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Explanation of figures

Fig. 1. Basal cytoplasm of an acinar cell of the bovine pancreas. The intracisternal granules (ig) appear in the dilated cisternae of the rough-surfaced endoplasmic reticulum. The granules are embedded in the material which exhibits fine granular and mesh-like appearance with less electron density than the granules. b : basement membrane, c : collagenous fibers. Fixed with osmium tetroxide, embedded in epon resin and stained with both uranyl acetate and lead hydroxide. Magnification : 69,000×.

Fig. 2. Cytoplasm in the neighbourhood of the Golgi area. The intracisternal granules (ig) combine with the ferritin-conjugated globulin prepared against bovine trypsin. The margin of occasional granules come in contact with the inner surface of the reticulum. Non-granular component is hardly seen in the cisternae. The tissue is fairly distorted after the treatment in the antibody solution. Prefixed in formalin, treated with ferritin-glubulin complex and post-fixed with osmium tetroxide. Stained with uranyl acetate and lead hydroxide. Magnification : 75,000×.

Fig. 3. The feature of the cytoplasm around the Golgi area. Chymotrypsin is localized in the intracisternal granules (ig), as visualized by the deposit of the ferritin granules. A zymogen granule and an immature zymogen granule (iz) are seen in the middle of the picture. v : vacuole. Fixed primarily in formalin, then with osmium tetroxide after the treatment in the ferritin-conjugated globulin solution. Double staining with uranyl acetate and lead hydroxide. Magnification : 75,000×.

Fig. 4. Part of the cytoplasm around the Golgi area. The ferritin-tagged antibody against bovine ribonuclease lodges on the intracisternal granules (ig). The periphery of the granule (left) is slightly denser than the central core. The junctional complex and the diversing bundles of the fine fibrils are seen at the upper part of the picture. The transitional portion between rough- and smooth-surfaced reticulum is observed at the lower middle of the photograph. Fixed with formalin, applied in the ferritin-conjugated antibody solution and post-fixed in osmium tetroxide. Double staining procedure. Magnification : 75,000×.

Fig. 5. Basal portion of the cytoplasm. The ferritin-globulin conjugate prepared against bovine ribonuclease has tagged the non-granular intracisternal substance (is). Some parts of the amorphous material which might have filled the cisterna may diffused out during the fixation and the treatment in the antibody solution. The intracisternal substance (is) which remained even after the several treatments tends to be recognizable along the inner surface of the rough-surfaced reticulum, with the exception of the narrow cisterna where the substance remained still filling up the space. The density of the intracisternal substance is, after being treated with antibody solution, far denser than that in the non-
treated sections shown in Fig. 1. Fixed with formalin, and with osmium tetroxide after the treatment in the antibody solution. Double staining procedure. Magnification: 75,000x.

Fig. 6. The control specimen treated in the ferritin-conjugated anti-vaccinea virus antibody solution. The intracisternal granules are devoid of ferritin granules. The cisternae contain almost no amorphous substance. Prefixed with formalin followed by the treatment in the ferritin antibody solution, and successively post-fixed in osmium tetroxide. Double staining method. Magnification: 60,000x.