LOCALIZATION OF SORBITOL DEHYDROGENASE IN THE MOUSE ACCESSORY REPRODUCTIVE ORGANS

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It is well known that mammalian spermatozoa utilize fructose as an energy source. In the mouse, the fructose is produced mainly in the seminal vesicle and in the coagulating gland. In this paper, the precise localization of sorbitol dehydrogenase in these organs was studied using an electron microscope. The results indicate that the enzyme is evenly distributed in the cytoplasmic matrix of epithelial cells of the seminal vesicles and the coagulating glands. The light microscopic observations which were performed by early investigators indicated that the distribution of sorbitol dehydrogenase in the epithelial cells of the seminal vesicle was very uniform, while that in the coagulating gland was restricted to the apical region of the epithelial cells. This apparent difference is related not to the local difference of the cytoplasm but to the amount of the cytoplasmic matrix. Since the morphoplasm (nucleus, secretory granules, and the dilated cisternae) showed no reaction, the reaction in these regions might be relatively obscure under light microscopic observation.

Many mammalian spermatozoa utilize fructose instead of glucose as an initial source of energy. The fructose is known to be produced mainly in the accessory organs of reproduction by the reactions of aldose reductase (EC 1.1.1.21) and sorbitol dehydrogenase (EC 1.1.1.14) (2, 5, 6). According to histochemical studies of sorbitol dehydrogenase, it has been shown that the localization patterns were very different in these organs (3, 4). In the coagulating glands, the localization of the enzyme activity was restricted to the apical region of the epithelial cells, while, in the seminal vesicles, it was detectable in all regions of the cytoplasm of the epithelial cells. However, previous works were limited to light microscopic investigations, and ultrastructural localization of the enzyme has not been determined. In this paper, an attempt was made to determine the localization of sorbitol dehydrogenase by the histochemical technique using the electron microscope, and the results were compared with those obtained by light microscopic histochemistry.

MATERIALS AND METHODS

The mouse seminal vesicles and coagulating glands were removed, rinsed quickly in 0.01 M phosphate buffer, pH 7.2, containing 0.8% NaCl (PBS) and followed by fixation in a solution containing 0.25% glutaraldehyde, 50% ethanol, and 0.1 M

cacodylate buffer, pH 7.4, (final concentration) for 2 hr at 4°C. For the electron microscopy, ethanol was omitted from the above fixative. After fixation, these tissues were washed overnight in PBS and 7 μ m thick sections were cut on a cryostat. The sections were placed on slide glasses coated with egg albumin and histochemical staining was performed as described in the previous paper (7). Namely, the following medium was used for the histochemical staining: 5 mg sorbitol, 2 mg nicotine-amide adenine dinucleotide, 0.5 mg tetranitroblue tetrazolium, 0.1 mg phenazine methosulfate and 2 mg bovine serum albumin in 1 ml of Tris-buffer, pH 8.4, at final concentration of 100 mM. Moreover, the solution, in which sorbitol was omitted from the above incubating medium, was used as the control medium. The sections were incubated in the reaction medium for 30 min in the dark at room temperature. After the reaction, the sections were washed in PBS and mounted in glycerin.

For the electron microscopic study, the sections treated for histochemical staining as described above were washed thoroughly in PBS and fixed in 1% cold osmium tetroxide dissolved in 0.1 M phosphate buffer, pH 7.4, for 1 hr. Then, the sections were dehydrated in a graded series of ethanol and embedded in epoxy resin mixture. Ultra-thin sections cut on a Porter-Blum MT-1 ultramicrotome were stained with uranyl acetate and examined under a Hitachi HU-12 electron microscope. Some ultra-thin sections were observed without counter staining.

Part of the tissues from the seminal vesicles and coagulating glands were fixed with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 3 hr in a cold room,

FIGS. 1a and 1b. The distribution of sorbitol dehydrogenase in the seminal vesicle. The enzyme reaction can be seen in all regions of the cytoplasm of the epithelial cells. a) $\times 300$ b) $\times 960$

FIG. 1c. The control reaction, omitting sorbitol from the incubating medium, in the seminal vesicle. No reaction is seen in the epithelium. $\times 280$

FIGS. 1d and 1e. The distribution of sorbitol dehydrogenase in the coagulating gland. The intensive reaction is restricted to the apical region of the epithelial cells, and the reaction at the basal area is weak. a) $\times 300$, b) $\times 960$

FIG. 1f. The control reaction, in which sorbitol was omitted from the incubating medium, in the coagulating gland. No specific reaction can be seen in the epithelium. $\times 280$

FIG. 2a. Epithelial cell of the seminal vesicle of the mouse. The rough-surfaced endoplasmic reticulum is very well developed in all regions of the cytoplasm and Golgi apparatus and many secretory granules are scattered in the supranuclear region. Stained with uranyl acetate and lead citrate. $\times 10,000$ Each bar represents 1 μ m.

FIG. 2b. Epithelial cell of the coagulating gland of the mouse. At the apical cytoplasm, the cellular matrix containing organelles is relatively abundant, while, at the perinuclear and the basal cytoplasm, most of the cytoplasmic area is occupied by enormously dilated cisternae of the rough-surfaced endoplasmic reticulum, and the amount of cell matrix containing organelles is very small. Stained doubly with uranyl acetate and lead citrate. $\times 6,600$

FIGS. 3a and 3b. The localization of sorbitol dehydrogenase in the epithelial cells of the seminal vesicle. a) apical region. b) perinuclear region. The reaction deposits (indicated by arrows) are localized in the cytoplasmic matrix. But neither the secretory granules nor nucleus contain reaction products. A few reaction deposits are seen in the cristae of mitochondria. a) Not stained. $\times 15,000$ b) Stained with uranyl acetate. $\times 15,000$

FIGS. 3c and 3d. The localization of sorbitol dehydrogenase in the epithelial cells of the coagulating gland. a) apical region. b) perinuclear region. Reaction products appear sparsely in the scanty cell matrix of the cytoplasm, but are not present inside the widely dilated cisternae of the rough endoplasmic reticulum. As in the case of the seminal vesicle, a small amount of reaction deposits is localized in mitochondria. Uranyl acetate staining. a) $\times 9,000$. b) $\times 12,500$

followed by post-fixation in 1% osmium tetroxide for 1 hr, and tissues were dehydrated in graded series of ethanol and embedded in epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined in the electron microscope.

RESULTS

1. Light microscopic observation.

In the seminal vesicles, reaction products for sorbitol dehydrogenase were recognized in all regions of the cytoplasm of epithelial cells (Figs. 1a, 1b). On the other hand, in the coagulating glands, the intensive reaction was restricted only to the apical region of the epithelial cells and reaction in other regions was weak (Figs. 1d, 1e). The control specimens which were reacted in the substrate-free medium showed no positive reaction (Figs. 1c, 1f).

2. Electron microscopic observation.

The ultrastructure of the epithelium of the seminal vesicle showed that the rough-surfaced endoplasmic reticulum was well-developed in all regions of the cytoplasm, and the Golgi apparatus and many secretory granules were scattered in the luminal areas of the cytoplasm (Fig. 2a). In the case of the coagulating gland, rough-surfaced endoplasmic reticulum were much more abundant. However, its cisternal spaces were very swollen, and the dilation of cisternae was especially prominent in the supranuclear and infranuclear regions. At these regions, most of the cytoplasm was occupied by its dilated cisternae, and as a result the cytoplasmic matrix was only scarcely visible (Fig. 2b).

The reaction products for sorbitol dehydrogenase in the seminal vesicles were scattered in the cytoplasmic matrix but were recognized neither in the nucleus, cisterna of endoplasmic reticulum nor secretory granules (Figs. 3a and 3b). In the coagulating glands, reaction products were dispersed in all regions of the cytoplasmic matrix but they were not recognized either in the nucleus nor dilated cisternae. The amount of reaction products in the cytoplasmic matrix at the apical region seemed to be the same as that at the dilated cisternae-rich region (Figs. 3c, 3d). Frequently, a small amount of reaction products was recognized in the cristae of mitochondria in both cells.

DISCUSSION

It is thought that spermatozoa utilize fructose instead of glucose as an energy source (5). According to our previous report (7), in the testis, this change of energy source occurs at a late stage of spermatogenesis and the spermatids utilize the fructose produced in Sertoli cells. Afterwards, spermatozoa utilize the fructose produced at the seminal vesicle and the coagulating gland (5, 6). As shown in the previous paper, the site of fructose synthesis in the Sertoli cells was restricted to the cytoplasm adjacent to the late stage spermatids. However, in the epithelial cells of the seminal vesicle and the coagulating gland, the reaction of sorbitol dehydrogenase was recognized sparsely in all regions of the cytoplasmic matrix. No specialized portion of fructose synthesis was observed in mouse seminal vesicle and the coagulating gland.

Light microscopic observation showed that a positive reaction for sorbitol dehydrogenase in the seminal vesicle was recognized in all regions of the cytoplasm but that in the coagulating gland was restricted to the apical region. These results seem to be contradictory. However, the fine structural feature of the coagulating gland was very different from that of the seminal vesicle. The cytoplasm of the epithelial cell of the coagulating gland was occupied by widely dilated endoplasmic reticulum except at the apical region. Since no positive reaction was seen in the dilated endoplasmic reticulum, a positive reaction might be apparent only at the apical region at the light microscopic level. Germino *et al.* (1) claimed that the positive reaction of sorbitol dehydrogenase in the seminal vesicle of the bull could be divided into three reactive portions: apical, supranuclear and infranuclear regions. However, since secretory granules and the nucleus never showed a positive reaction, that in the secretory granules-rich region and nuclear region appeared to be relatively weak at the light microscopic level.

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