Immunoreactive Avidin in the Hen Oviduct Mucosa

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Summary: The detection of avidin in the hen oviduct was studied by immunohistochemistry. Antigenic avidin was demonstrated in secretory granules of both gland cells and nonciliated epithelial cells in the magnum. These immunospecific granules were electron dense and nonhomogeneous especially in acinar cells, and the size varied from small to large (0.7 to 2.2 μm in diameter) in the gland and small (200 to 700 nm) in the epithelium. Epithelial cells containing secretory granules had a strong resemblance to those of the protodifferentiated gland cells appearing in the magnum of chicks pretreated with hormones. No avidin was observed in any epithelial goblet cells or ciliated cells. The findings paralleled those obtained by biotinylated-enzymes affinity cytochemical methods as previously described (Kami and Yasuda, 1982).

Therefore avidin in the hen's oviduct is one of the proteins produced and stored in the secretory granules of the gland cells and protodifferentiated acinar cells located in the epithelial layer, although initiation of the synthesis may be triggered by progesterone. However, it is still not clear whether different hormone-dependent proteins are located in the same granules or not.

Avidin is one of the avian egg-whites that is composed of several kinds of proteins. The major proteins in the magnum gland of the hen's oviduct have been a matter of intense study (Kami et al., 1976, 1978, 1979; Suzuki et al., 1970; Suzuki and Nagato, 1980). These studies have revealed their presence in secretory granules of the gland cells of the laying hen's oviductus proprius.

Nevertheless, in regard to the location of avidin which is one of the minor proteins, it has long been believed (Schrader et al., 1981) that avidin was produced in goblet cells of chicken oviducts pretreated with diethylstilbestrol (DES) and then with progesterone as demonstrated by both immunofluorescent and biotin affinity autoradiographic techniques (Kohler et al., 1968). Although the target-cell specificity of DES and progesterone in regulating the synthesis of cell-specific proteins was shown, the cell types which are capable of avidin synthesis may not be entirely known. Another immunofluorescent antibody study showed that almost all of the epithelial cells were avidin positive 24 hr after the administration of progesterone (Tuohimaa, 1975). However, goblet cells are not present in the immature chicken oviducts (Kellokumpu-
Lehtinen et al., 1976). On the other hand, there has been no report of the presence of avidin in the laying hen's oviduct with the exception of a preliminary report of the present authors who used biotin-labelled enzymes (biotinyl-peroxidase and -alkaline phosphatase) affinity cytochemistry (Kami and Yasuda, 1982). They showed that avidin might be present in the condensing vacuoles and the secretory granules of the gland cells and some epithelial cells of the magnum, but not in goblet cells and ciliated cells.

The aim of the present study was to determine immunohisto- and cytochemically, whether the avidin is located in mucous cells of the hen's oviduct.

Materials and Methods

ANTI-AVIDIN ANTISERUM: The antigen used for immunization was avidin (purchased from E. Y. Laboratories, Inc.), and immune sera were produced in rabbits by injecting a mixture of avidin dissolved in saline and Freund's complete adjuvant. Its immunological specificity was verified by Ouchterlony's immunoprecipitation; it did not react at all with any other egg-white proteins.

CYTOCHEMICAL PROCEDURES: Laying white-Leghorn hens obtained commercially were caged and kept on a natural lighting regimen. Food and water were available ad libitum.

At the level of light microscopy, tissue blocks (5 x 5 x 3 mm) were fixed with 0.5% acetic acid-ethanol, and then embedded in paraffin. Pieces of magnum tissues (2 x 2 x 2 mm) allotted for the electron microscopic histochemistry were excised and fixed in a mixture of 4% paraformaldehyde and 0.25% glutaraldehyde dissolved in 0.1 M phosphate buffer (pH 7.4) for 3 hr at 4°C. After fixation, they were rinsed overnight with the same buffer containing 0.2 M glycine and 7% sucrose.

The pre-embedding indirect (labelled) antibody method was used for the immunoperoxidase cytochemistry. Cryostat and/or Sorvall tissue chopper sections were exposed for 60 min to a 1:100 dilution of the primary antiserum in phosphate-buffered saline (PBS, 0.01 M, pH 7.4) containing 1% bovine serum albumin. This was followed by a 60 min treatment with diluted (1:400) horse-radish peroxidase-labelled goat anti-rabbit immunoglobulin purified according to the methods of Yamashita et al. (1976), and the tissue was allowed to react with substrate medium [containing 2.5 mg of 3,3'-diaminobenzidine 4-HCl per 10 ml of 0.05 M Tris buffer (pH 7.4), to which 0.005% hydrogen peroxide was added]. All incubation steps were separated by exhaustive washing in PBS for 30–60 min. Control sections were prepared by substituting normal rabbit serum for the primary antiserum.

All specimens were postfixed in 1% osmium tetroxide, dehydrated, and embedded in epoxy resin. Ultrathin sections were counterstained with uranyl acetate and viewed in a JEOL 200 CX electron microscope.

Results and Discussion

The luminal surface of the hen oviduct is completely covered with a single layer of epithelial cells composed of both ciliated and nonciliated columnar cells. The latter cells, however, vary widely in appearance and distribution in five segments (Fertuck and Newstead, 1970; Makita and Nishida, 1966; Makita et al., 1973). On the other hand, the tubular glands of the oviduct are also observed in the lamina propria mucosae except in the infundibulum and vagina. The present preliminary study was confined to
immunohistochemical staining of the location of avidin in the middle and lower magnum based on macroscopic boundaries.

The antibody used was generated in rabbits with avidin, and was tested by immunodiffusion that showed a single precipitation line.

At the level of light microscopy, endogenous avidin was detected in gland cells of the magnum (Fig. 1). In the epithelial cells, small patches indicating a positive immunoreaction were also seen in the lower magnum. They were never observed in any epithelial goblet cells nor ciliated cells. Some materials observed in the lumen showed the presence of antigenic avidin. Negative stains in the control experiments confirmed the immunohistochemical specificity.

These findings obtained by immunoperoxidase techniques in the hen oviducts circumvented the restrictions of the early studies of Kohler et al. (1968). They showed that avidin was produced and localized in almost all of the epithelial goblet cells of the immature chicken oviduct stimulated by administration of progesterone and/or progesterone after pretreatment with DES, by using immunofluorescent histochemistry and biotin affinity autoradiography. Tuohimaa (1975), however, showed that some of the gland cells were also avidin-fluorescence positive after a dose of progesterone by which the chicks were estrogen-primed. This is in agreement with our data from the laying hen obtained by the present immunohistochemistry and the biotinylated-enzymes affinity histochemical observations (Kami and Yasuda, 1982). However, this major discrepancy between the results with hens and with immature chicks stimulated with hormones cannot be explained by differences not only in the hormonal environment of the animals but also in the techniques and chemicals.

On the other hand, we have shown by electron microscopical cytochemistry that the immunoperoxidase reaction products indicating avidin by light microscopic histochemistry are consistent findings. As can be seen in Fig. 3, avidin-dependent reaction products in the epithelium of the lower magnum were seen as fine deposits confined to secretory granules in the supranuclear and apical cytoplasm. Although their electron density was as high as that of granules of the magnum gland, the size of the granules (200 to 700 nm in diameter) was very close to that of the protodifferentiated tubular gland cells appearing in the magnum of the chicks pretreated with estrogen or estrogen plus progesterone (Kami and Yasuda, 1983; Palmiter and Wrenne, 1971). Therefore, the authors of this study conclude that non-ciliated epithelial cells containing avidin are so-called progenitor cells of the gland cells. Secretion granules in ciliated cells per se were electron dense and filled with a speckled intragranular network (Kami and Yasuda, 1982; Makita et al., 1973) even in immunocontrol experiments.

Furthermore, the location of antigens in the acinar cells is shown in Figs. 2 and 3, in which both secretory granules in the gland and amorphous materials in the tubular lumen show a specific localization. Various-sized nonhomogeneous granules with a patched core and a dense peripheral region reacted with the antibody. Fully homogeneous condensing vacuoles showing immunoreaction-negative and nonhomogeneous vacuoles with crescent-shaped cap indicating avidin and electron lucent region were also present nearby the nucleus or Golgi areas. The predominant secretion in superficially located regions of the upper and middle magnum was seen as many light granules with a fibrillar matrix. They were easily distinguished from immunoreaction-positive dense granules.

These findings mentioned above paral-
leled the data obtained by biotinylated-enzymes affinity cytochemical methods (Kami and Yasuda, 1982). Nevertheless, it is desirable to inspect the location of both avidin and the other egg-white proteins in the same section because in the present results there remained the big question as to whether different hormone (estrogen and progesterone)-dependent proteins are located in the same granules.

References

Explanation of Figures

Plate I

Fig. 1. Paraffin-embedded section taken from the lower magnum of laying hen's oviduct. Specific immunopositive reaction products for avidin exhibit a intense staining pattern in the magnum gland (MG) and a patchy pattern in the epithelial cells (EP). L: lumen of the oviduct. Not counterstained. x 170.

Fig. 2. Electron micrograph demonstrating an immunoreactive avidin in the tubular gland cells of the magnum. While reaction products are concentrated in almost all of the secretory granules and amorphous materials of the lumen (*, white arrow) of the excretory duct (ED), immature condensing vacuoles (arrowheads) around Golgi apparatus (G) and light vacuole (LV) are free of lavel. Counterstained with uranyl acetate. x 6,900.

Fig. 3. Electron micrograph of part of epithelial cell layer (EP) and magnum gland (MG) in the lower magnum showing positive immunoreaction for avidin over various-sized granules (black and white arrows). CT: connective tissue, L: lumen of the oviduct, PAC: protodifferentiated acinar cell. Counterstained with uranyl acetate. x 6,900.