Selective Binding of Fluoresceinated-Avidin to A-Cells of Sectioned Pancreas

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SUMMARY

Fluoresceinated-avidin (FITC-avidin) was observed to bind specifically to a subset of pancreatic islet cells in sections of both human and rat pancreas. FITC-avidin binding was inhibited by excess unlabeled avidin, and by biotin, but not by glucagon, somatostatin, or insulin. Labeling of islets with anti-insulin, anti-glucagon, anti-somatostatin, and anti-human pancreatic polypeptide antibodies showed the avidin binding subset to correspond to islet cells identified by anti-glucagon antibody. Conversely, avidin reacted with no insulin, somatostatin, or cells containing HPP. We conclude that avidin localizes specifically to A-islet cells. Binding may be to a biotin-containing enzyme within the A-cells, but the precise molecular site of binding is currently unidentified. DIABETES *31*:107–109, February 1982.

Avidin is a tetrameric glycoprotein (70,000 MW) that was originally isolated from egg white in 1941.[1] The salient feature of this glycoprotein is its extraordinarily high affinity for biotin (Kd = 10^{-15}), which is in turn an essential enzymatic cofactor involved in CO_2 transfer reactions. It is postulated that egg white avidin serves the function of an antimicrobial factor by tightly binding free biotin and thus depriving bacteria of this essential growth factor.[2–4]

Recently, the avidin-biotin complex has found application in cytochemistry. Biotin can often be covalently attached to molecules such as antibodies or lectins. These biotinylated conjugates can then be incubated with appropriate cells or sections, and visualized with avidin covalently linked to a probe such as fluorescein or ferritin.[5–7] In our laboratory we were investigating a system of biotin-labeled anti-islet antibodies with FITC-avidin when it became apparent that FITC-avidin bound specifically to a subset of cells in the pancreatic islets, independent of biotinylated antibody. This report describes our studies that identify the subset of pancreatic islet cells which react with avidin.

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MATERIALS AND METHODS

Preparation of pancreatic sections. Normal human adult pancreas and Lewis or Sprague-Dawley rat pancreas (age 10 days) were fixed by two different methods. In one method, the pancreatic tissue was immersed in Bouin's solution for 2 h, followed by immersion in 70% ethanol and paraffin embedding. In the alternative method, the tissue was immersed in 3.7% buffered formalin solution for 30 min, followed by washing with Dulbecco's phosphate-buffered saline (PBS) and storage in liquid nitrogen. Following fixation, 5-μm sections were cut from the pancreatic blocks. Immediately before use, the paraffin-embedded sections were treated with xylene and ethanol washes. The frozen sections required no further treatment.[8]

FITC-avidin labeling. Pancreatic sections were incubated for 15 min with a PBS solution containing 1% bovine albumin (Sigma, St. Louis, Missouri). Following this incubation 1 mg/ml FITC-avidin in PBS albumin was applied to the section for 30 min at room temperature. The sections were subsequently washed with PBS, coverslip applied, and viewed with a Leitz fluorescent microscope equipped for epifluorescence.

Double labeling. Rabbit anti-glucagon antibody and rabbit anti-somatostatin antibody (provided by M. Appel, Worcester, Massachusetts), rabbit anti-HPP antibody (Eli Lilly Laboratories, Indianapolis, Indiana), and guinea pig anti-insulin antibody (Cappel Laboratories, Cochranville, Pennsylvania) in 1:50 dilutions were each applied to pancreatic sections. Following a 30-min incubation at room temperature the sections were washed with PBS, and incubated an additional 30 min with a 1:50 dilution of appropriate anti-rabbit or anti-guinea pig rhodamine-labeled anti-sera (Cappel Laboratories). These sections were then washed with PBS and labeled with FITC-avidin as described above.

Blocking experiments. Various potential inhibitors of avi-

din's specific binding were added in combination with FITC-avidin. These included glucagon (Lilly; 1 mg/ml), insulin (Lilly; 2 mg/ml), somatostatin (Calbiochem, LaJolla, California; 50 μg/ml), avidin (Sigma; 10 mg/ml) and biotin (Sigma; 0.1 mg/ml). These solutions were then added to pancreatic sections for labeling in the same manner as FITC-avidin. The sections were subsequently evaluated microscopically for specific FITC-avidin binding and the effect of the various potential inhibitors.

RESULTS

FITC-avidin labeling. After incubation of either human or rat pancreas with FITC-avidin, 15–30% of pancreatic islet cells were brightly fluorescent. This finding was reproduced with both Bouin's and formalin-fixed sections. The fluorescent cells were distributed primarily on the periphery of the islets in the rat pancreas (Figure 1D). In human sections (not shown) positive cells were more widely distributed. The pattern of individual cell fluorescence was usually homogenous, though some cells did demonstrate assymetric labeling with the fluorescence concentrated to one side of the cell. The acinar and ductal portions of the pancreas did not bind FITC-avidin.

Double labeling. Labeling with anti-glucagon, anti-insulin, anti-somatostatin, and anti-HPP antibodies detected with rhodamine fluorescence demonstrated the characteristic topographic pattern of alpha-, beta-, delta-, and PP-cells. Concurrent labeling with FITC-avidin showed a 1:1 cellular correspondence of FITC-avidin binding with alpha-cells (Figure 1A,B). In contrast, the beta-cells (Figure 1C,D), delta-cells, and PP-cells (not shown) did not bind avidin. Double exposure photography verified the shared identity of the alpha- and avidin-labeled cellular subsets showing a full color overlap of the rhodaminated anti-glucagon with fluoresceinated-avidin (not shown).

Labeling inhibition. To explore the mechanism by which avidin bound to alpha-cells, a number of compounds were tested for their ability to inhibit binding. Excess cold avidin was observed to inhibit binding by FITC-avidin with negligible fluorescence in any pancreatic sections. In addition, free biotin was found to markedly reduce FITC-avidin binding. In contrast, high concentrations of glucagon, somatostatin, and insulin had no effect on FITC-avidin binding.

DISCUSSION

Our experiments demonstrate a 1:1 correspondence between avidin binding to pancreatic islet cells and glucagon-containing islet cells. This specific binding of avidin to

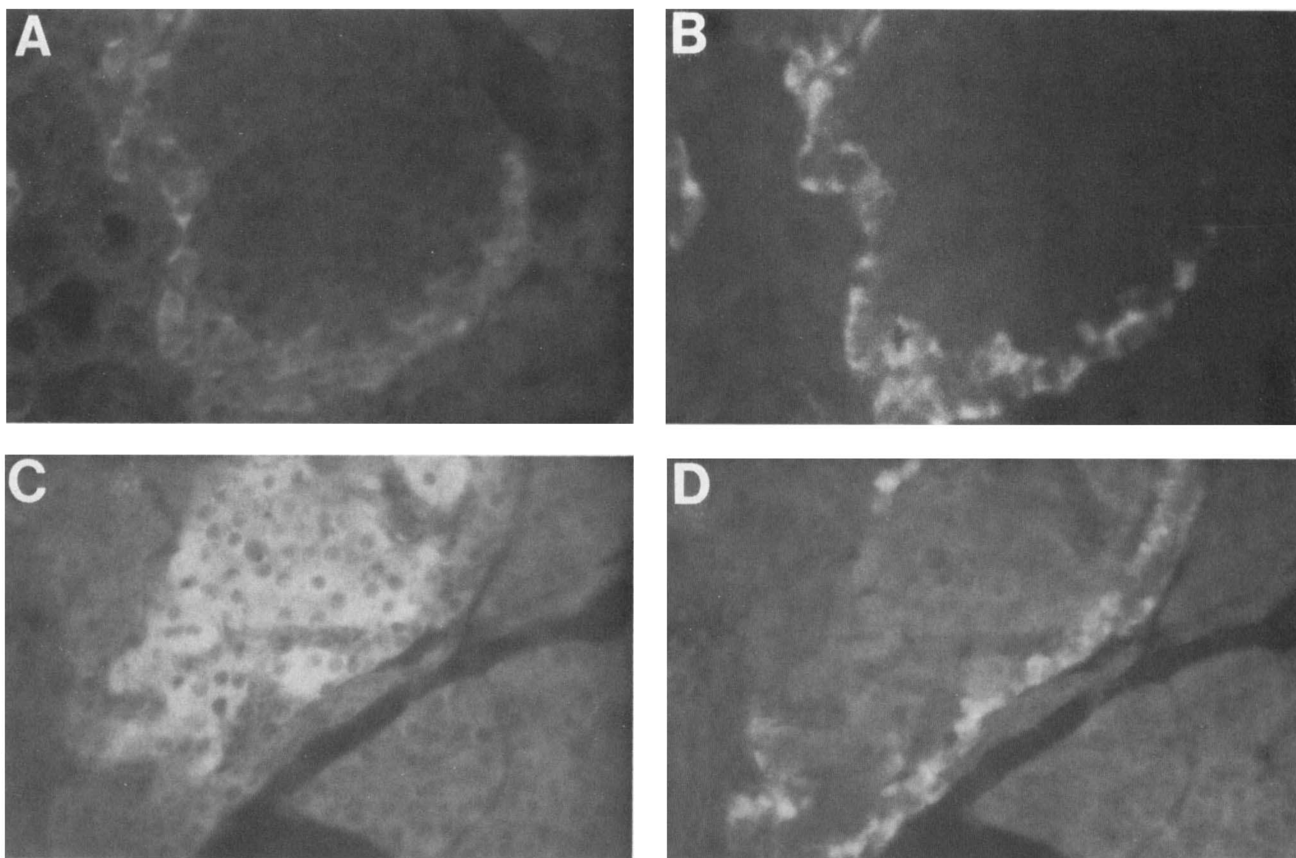

FIGURE 1. (A) Fluorescent photomicrograph of rat pancreatic alpha-cells identified by rabbit anti-glucagon antibody detected with rhodamine conjugated goat anti-rabbit antisera. (B) Fluoresceinated-avidin labeling of the same section of rat pancreas seen in photomicrograph A. (C) Fluorescent photomicrograph of rat pancreatic beta-cells identified by guinea pig anti-insulin antibody detected with rhodamine conjugated rabbit anti-guinea pig antisera. (D) Fluoresceinated-avidin labeling of the same section of rat pancreas seen in photomicrograph C.

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alpha-cells is present in both rat and human pancreatic sections, using both Bouin's and formaldehyde fixation. In contrast, insulin-, somatostatin-, and pancreatic polypeptide-containing islet cells and acinar pancreas evidenced no significant avidin binding.

The biochemical nature of the avidin binding site of alpha islet cells is not known. Our experiments have demonstrated that free biotin eliminated specific FITC-avidin binding to the pancreas as did excess unlabeled avidin. These results are consistent with the site of avidin binding being a biotin-containing molecular species within the alpha-cells, perhaps an enzyme involved in CO_2 transfer reactions. Such a biotin enzyme, specifically found in alpha-cells in high concentration, remains to be identified. Very little is known concerning the enzyme histochemistry of alpha-cells. Previous studies have shown histochemical differences distinguishing exocrine from endocrine pancreatic tissue, and differences when distinguishing the islets of various mammalian species.[9,10] However, no definitive enzymatic histochemical marker differentiating alpha-, beta-, delta-, and PP-cells has been found.[11,12]

Our results also cannot rule out the possibility that a molecular species without biotin could be the site of avidin binding in alpha-cells. Glucagon as a binding site seems unlikely because of the inability of glucagon to block binding. Other molecules, however, that are structurally related to biotin could be the site of avidin binding. Avidin has been shown to bind oxazolidone and imidazolidone derivatives, as well as analogues corresponding to small fragments of biotin including urea, tetrahydrofuran, glycol, and caproic acid.[3] However, an elevated concentration of any of these derivates specific to alpha-cells remains to be shown. Furthermore, it is debatable whether low-affinity conjugates would indeed produce the distinctive binding seen.

In conclusion, the experiments conducted with both human and rat pancreas indicate that avidin binds to alpha pancreatic islet cells. In addition, it is likely that the biotin binding site of avidin is involved in the specific binding. The particular chemical basis for this phenomenon remains to be elucidated.

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