IODINATION OF ZETA PROTEIN BY LACTOPEROXIDASE, CHLOROPEROXIDASE AND CHLORAMINE T

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Summary: Normal and Rous sarcoma virus transformed chicken embryo fibroblasts cultured on petri dishes were labeled with ^{125}I by two enzymic methods and one chemical method. The enzymic labeling systems employed chloroperoxidase and lactoperoxidase. Hydrogen peroxide was generated by glucose oxidase and glucose. The chemical method used chloramine T as the oxidant for iodide ion. After solubilization of cells, SDS disc gel electrophoresis and gamma counting, it was found that only one cell protein was predominantly labeled in all three reactions. This protein, a major cell surface protein, has been previously identified and termed Zeta protein. Zeta protein disappears from transformed cells and is susceptible to trypsin digestion.

Lactoperoxidase has been used widely to specifically label cell surface sturctures with iodine atoms because under certain conditions the active iodinating species remains enzyme bound and hence cannot penetrate into the cell. Using this enzymic labeling reaction, a cell surface protein, termed Zeta protein, was found in abundance in normal cells but was found in greatly diminished quantity in virus transformed cells (1-3). Zeta protein is also susceptible to trypsin digestion and can be stripped from normal cells by brief treatment with trypsin.

It has been suggested that the specific labeling of Zeta protein by lactoperoxidase might be the result of a special interaction between lactoperoxidase and Zeta protein (4). Thus, it has been argued that other surface glycoproteins might also disappear or appear as the result of transformation but would not be detected by the lactoperoxidase label. In this paper, we report that no special interaction is required for the iodination of Zeta protein by lactoperoxidase. Enzymatic iodination of chicken embryo fibro-

blasts by chloroperoxidase as well as the chemical iodination of normal cells serves to specifically label Zeta protein.

Materials and Methods: Chloroperoxidase was isolated from Caldariomyces fumago. The method of purification has been previously reported (5). Rous sarcoma virus was obtained from Dr. Michael J. Weber. Lactoperoxidase and trypsin were obtained from Sigma, glucose oxidase was purchased from Worthington, chloramine T was from Metheson, Coleman and Bell, and ^{125}I was obtained from New England Nuclear.

Cell Cultures - Primary chicken embryo fibroblasts were prepared from ten-day old chicken embryos. All the internal organs of the embryos were removed and the body walls were dispersed in 0.25% trypsin. Cells were plated in 100 mm petri dishes at a density of 1 x 10^7 cells per plate. The growth medium was Dulbecco's modified Eagle's medium supplemented with 10% tryptose phosphate broth, 4% calf serum and 1% heat inactivated chicken serum. Each petri dish contained 12 ml of medium. Transformed cells were prepared by infecting cells with wild type Rous sarcoma virus 6 hours after plating using one infectious particle per cell. Secondary cell cultures were prepared after 4 days by trypsinizing primary cultures with 0.05% crude trypsin. All secondary cultures were plated in 60 mm petri dishes. Normal cells were plated at a density of 6 x 10^5 cells per plate and virus-infected cells were plated at a density of 8 x 10^5 cells per plate. Cells were iodinated after a 48 hour growth period.

Iodination - All iodinations were carried out using 15 µC of ^{125}I-iodide for each petri dish. The total amount of sodium iodide added to each plate, including both ^{125}I and ^{126}I, was 0.0067 µg.

Lactoperoxidase iodination was essentially carried out according to the procedure previously reported (1). The method was altered slightly by using 15 µC of carrier iodide and 25 µg of glucose oxidase in 1 ml of phosphate buffered saline (0.01 M phosphate), pH 7.2. After iodination, the cells were washed twice in phosphate buffered saline. Washing was carried out by adding the phosphate buffered saline directly to the dishes with swirling.

The chloroperoxidase iodination of the cells was carried out at pH 6.9. Otherwise, the conditions for chloroperoxidase labeling were identical to those used for lactoperoxidase labeling.

Chloramine T iodination was carried out according to the procedure described by Sonda (6). The reaction was carried out at room temperature for 10 minutes at pH 6.9 in phosphate buffered saline.

Trypsinization to Remove Zeta Protein - After iodination, the labeled cells were incubated in 1 ml phosphate buffered saline containing 10 µg of trypsin (2X crystallized) for 10 minutes at 37°.

SDS Disc Gel Electrophoresis and Counting - Upon completion of the iodination reaction or after trypsinization, 0.3 ml of 2% SDS and 1% mercaptoethanol in 0.01M sodium phosphate buffer, pH 7.2, was added directly to each dish. Cells were scraped from the plate and boiled until they were solubilized.

The disc gel electrophoresis system was adopted from the Weber and Osborn procedure (7) with minor modifications. The gels contained 7.5% acrylamide. The final concentration of phosphate in all the buffers was 0.05M. Gels were 1 cm in diameter. Electrophoresis was performed at a constant current of 20 mA per gel. After electrophoresis, gels were cut into 1 mm slices and counted in a gamma counter.

Results and Discussion: Normal chicken embryo fibroblasts grown in monolayer were iodinated, solubilized in SDS, and the total cell proteins separated by gel electrophoresis. Fig. 1 depicts the radioactivity profiles

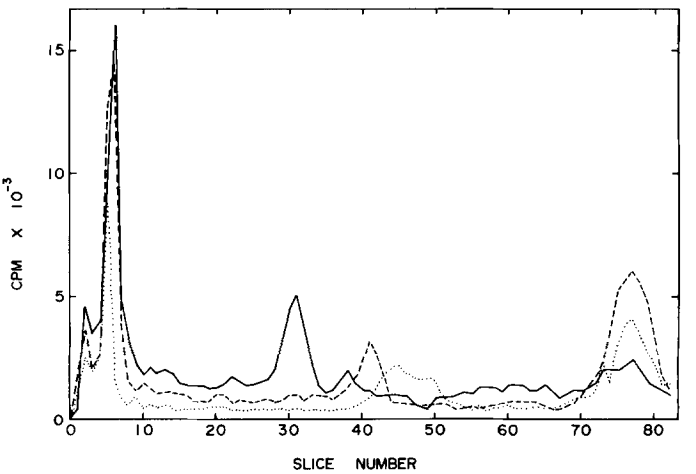

Figure 1. Iodination of normal chicken embryo fibroblasts. The iodination reactions (lactoperoxidase ——, chloroperoxidase ·····, and chloramine T -----) were carried out on cells in monolayer as described in Methods and Materials. The cells were solubilized and the proteins separated by SDS-polyacrylamide gel electrophoresis (see Methods). The curves show the radioactivity profiles obtained by counting sliced gels.

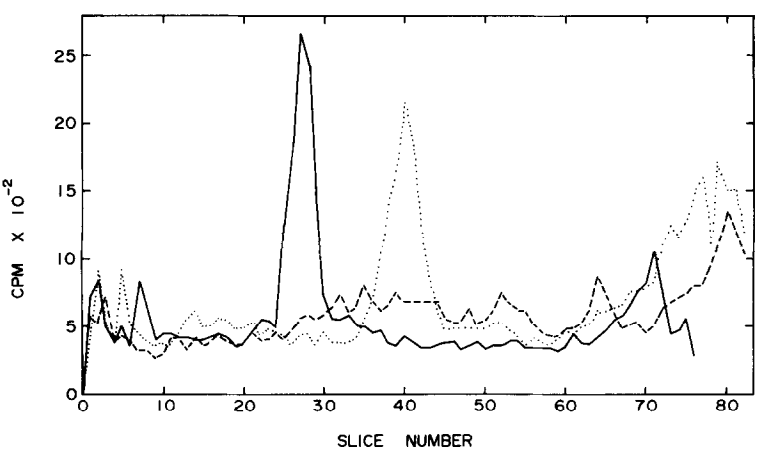

Figure 2. Iodination of Rous sarcoma virus transformed chicken embryo fibroblasts. The transformed cells were treated the same as the normal cells in Figure 1. The curves (lactoperoxidase —— , chloroperoxidase ·····, and chloramine T -----) show the SDS gel radioactivity profiles for each type of iodination.

obtained by counting the sliced gels. Profiles for lactoperoxidase, chloroperoxidase and chloramine T labeling show a major peak near the top part of each gel and some minor peaks. After transformation by Rous sarcoma virus, the major high molecular weight iodinated peak disappears. The same labeling

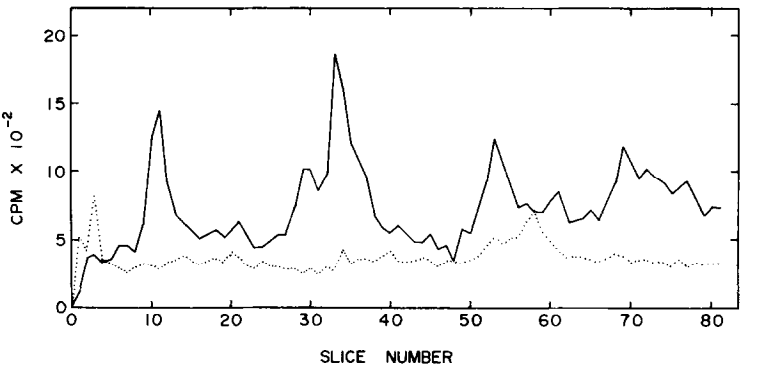

Figure 3. Trypsinization of normal chicken embryo fibroblasts after iodination. Cells which had been iodinated using lactoperoxidase (———), or chloroperoxidase (·····) were exposed to trypsin for 10 min (see Methods), solubilized and the proteins separated on SDS polyacrylamide electrophoresis.

pattern holds for lactoperoxidase, chloroperoxidase and chloramine T iodination (Fig. 2).

The high molecular weight iodinated protein is destroyed by exposing whole cells to trypsin digestion as shown in Fig. 3. Little of the material remains after a 10 minute incubation of normal cells with trypsin.

The radioactive material labeled in these experiments corresponds to the major cell surface protein previously labeled with lactoperoxidase and termed Zeta protein (1-3). We therefore conclude that both chloroperoxidase and chloramine T label Zeta protein in a fashion analogous to lactoperoxidase. These experiments rule out the possibility that the lactoperoxidase labeling of Zeta protein depends on some special interaction between lactoperoxidase and Zeta protein. In addition these results rule out the need for an enzyme-bound iodinating intermediate in order to confer specificity for Zeta protein labeling. The results with chloramine T indicate that a freely diffusable electrophilic chemical iodinating species is able to specifically label Zeta protein.

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