Immobilization of *E. coli* Cell as an Antigen by Radiation Polymerization Method

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E. coli NIJ cells were immobilized by radiation polymerization of 2-hydroxyethyl methacrylate at low temperatures. The immobilized *E. coli* cells as an antigen were reacted with peroxidase labeled *anti-E. coli* in competition with the free *E. coli* cells. It was found that *E. coli* cells can be assayed quantitatively with the immobilized *E. coli* cells in disc form. Microorganisms such as gram negative bacteria had no specific affinity to *anti-E. coli*. Cross reactivity of the immobilized *E. coli* cells with the *E. coli* cells from various strains was examined.

Introduction

The studies of immobilization of various biofunctional substances such as enzymes, microbial cells, tissue cells, and physiologically active substances and their applications have been interested and progressed. Some immobilized enzymes have achieved application on a technical scale. Various possibilities for the application of immobilized enzymes have been described and reviews have been recently have published on the different methods of immobilization of enzymes on various carriers [1–4].

We have developed a new immobilization method by radiation polymerization at low temperatures, by which various biofunctional substances can be fixed physically on the surface of the porous polymer matrix [5–7]. We have applied this method to the immobilization of antibodies such as anti-α-fetoprotein for enzyme immunoassay of α-fetoprotein [8]. In this work, *E. coli* NIJ cells as an antigen were immobilized by the radiation polymerization method and the antibody aggregative reaction of the immobilized *E. coli* cells was studied.

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Materials and Methods

Materials

E. coli NIJ cells were obtained from Professor K. Hotta in Institute of Chemistry for Microbiology and other bacteria cells from Professor S. Takao in Strain Preservation Facility of Hokkaido University. 2-Hydroxyethyl methacrylate (HEMA) as a monomer was obtained from Shin Nakamura Chemical Co., Ltd. and used without further purification. Bacteria cells were cultivated in a normal agar cultivation medium at 37 °C with stirring in the case of cultivation under anaerobic condition.

Immobilization

The *E. coli* cells (5×10^7 cells/ml in 100 mM phosphate buffer, pH 7.0) were mixed with the monomer in 2:1 volume ratio and put into a glass ampoule of 8 mm diameter and 200 mm length. The ampoule was frozen at −78 °C and irradiated by γ-ray from ^{60}Co source with dose of 1×10^6 rad. The polymerized products were cut into 20 μm thin membrane form (disc) by a microtome for the use.

Assay

One piece of the disc, 0.5 ml cell suspension solution in the buffer solution, 0.1 ml *anti-E. coli* IgG-peroxidase which was obtained from rabbit serum and labeled with peroxidase and 0.4 ml phosphate buffer saline (PBS, pH 7.2) were mixed in a flask.

The reaction was carried out 25 °C for 3 h. After reaction, the disc was washed with the PBS several times and transferred into another flask containing 2.0 ml saline.

The enzyme reaction was carried out with the disc using a mixture of *o*-phenylene diamine (60 mg) and 0.75% H_2O_2 solution (1.0 ml) in 50 mM sodium citrate solution (9 ml). The enzyme activity was examined by measuring the absorbance of the reaction solution at 492 nm.

Results and Discussion

The activity of the immobilized *E. coli* cells was examined by the aggregation reaction with *anti-E. coli* in the competition with the free *E. coli* cells (Fig. 1). The immobilized *E. coli* cells are bound with the enzyme-labeled antibody by antigen-antibody reaction. The relationship between absorbance and the concentration of added free *E. coli* cells is shown in Fig. 2, which is a typical standard curve of enzyme immunoassay.

The result in Fig. 2 shows that the immobilized *E. coli* cells are effectively bound with the enzyme-labeled antibody as well as the free *E. coli* cells. It is possible to assay the cell concentration of the added free *E. coli* cells by this method. The increase of the absorbance at low cell concentrations as well as at high cell concentrations are rather small, because the enzyme-labeled antibody might be reacted with the free cells easily than the immobilized *E. coli* cells.

The cross reactivity of the immobilized *E. coli* cells was examined by the competition reactions with the free *E. coli* cells cultured under various conditions. The result is shown in Fig. 3. The relation curves between absorbance and cell concentration in the *E. coli* cells cultured under various conditions agreed with each other, indicating that the *E. coli* cells can be specifically reacted with the enzyme-labeled antibody. There was not difference in the specificity of antibody aggregation by cultivation conditions of the *E. coli* cells.

The cross reactivity of the immobilized *E. coli* cells with various gram negative bacteria was examined. Various gram negative bacteria were used instead of the free *E. coli* cells for the competition with the immobilized *E. coli* cells, and the relation curves between absorbance and bacteria concentration is shown in Fig. 4. The absorbance had no dependence on the added cell concentration. This fact means that the antibody reacted predominantly with the immobilized *E. coli* cells and has the specific affinity to the *E. coli* cells, while it had no specific affinity to gram negative bacteria.

The cross reactivity of the immobilized *E. coli* cells with various *E. coli* cells was examined. *E. coli* cells from various strains were used instead of the free *E. coli* NIJ cells and the antibody aggregation reaction was carried out. The result in shown in Fig. 5. According to this result, all *E. coli* cells from different strains gave similar relation curves between absorbance and cell concentration, indicating that the *anti-E. coli* has an affinity for all *E. coli* cells from the various strains.

From these results, the immobilized *E. coli* cells are aggregated by the presence of the antibody of the *E. coli* cells with a specific affinity competing with the free *E. coli* cells which have also a specific affinity to the antibody. It was found that a quantitative assay of th e antibody of the *E. coli* cells is possible using the immobilized *E. coli* cells obtained by radiation polymerization method at low temperatures. The preparation of the immobilized *E. coli* cells in disc form by the present method is based on physical trapping, some of which conatin covalent binding (radiation cross-linking) by irradiation. A large amount of *E. coli* cells can be, therefore, immobilized in the disc having a porous polymer matrix. The porous structure varied mainly with monomer concentration. The formation of the porous structure in the polymer matrix was one of the most characteristic features of the discs obtained by radiation polymerization of hydrophilic monomers such as HEMA at low temperatures. The porous structure in the discs increases the surface

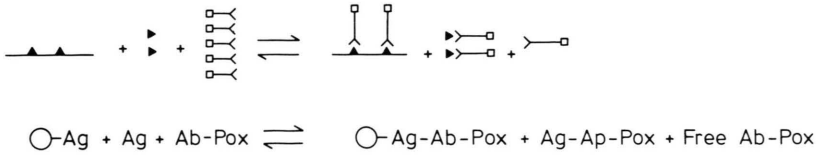

Fig. 1. Scheme for enzyme immunoassay with immobilized *E. coli* cells. ○-ag: immobilized antigens; ag: free antigens; ab-pox: peroxidase-labeled antibody.

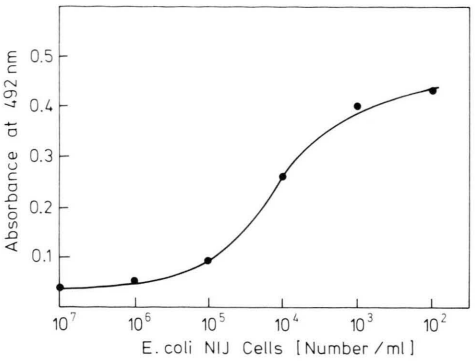

Fig. 2. Relationship between absorbance and cell concentration the free *E. coli* cells.

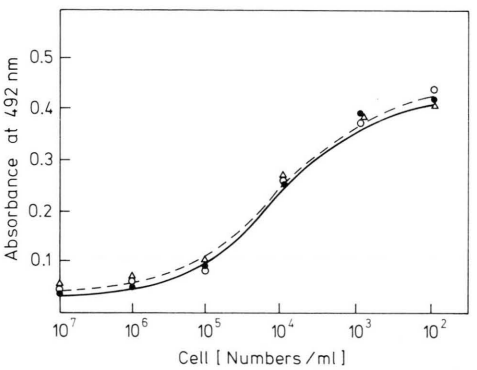

Fig. 3. Cross reactivity of the immobilized *E. coli* cells with the free *E. coli* cels cultured under various conditions. ● The cells cultured under aerobic conditions (in liquid medium); ○ the cells cultured under anaerobic conditions (in liquid medium); △ the cells cultured in solid medium.

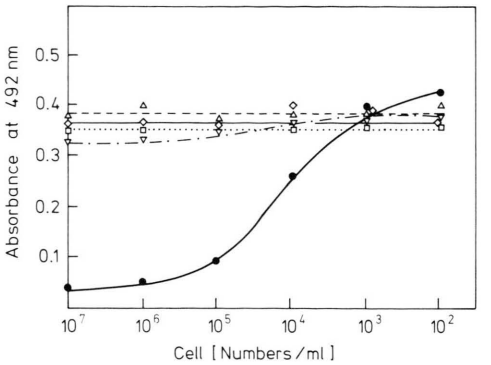

Fig. 4. Cross reactivity of the immobilized *E. coli* cells with various free gram negative bacteria. ● *E. coli* NIJ; △ *Seratia marcescence* AHU 1135; ▽ *Proteus vulgaris* AHU 1144; □ *Aerobacter aerogenes* AHU 1540; ◇ *Cactobacillus arubinosus* AHU 1413.

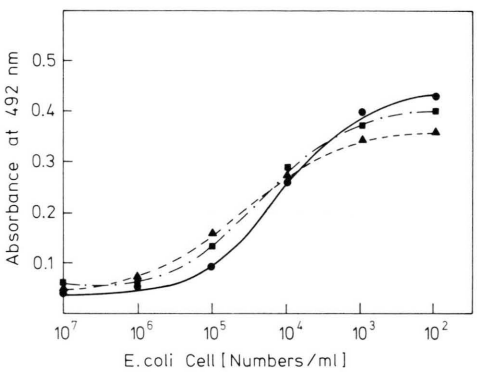

Fig. 5. Cross reactivity of the immobilized *E. coli* cells with various *E. coli* cells. ● *E. coli* NIJ; ▲ *E. coli* AHU 1014; ■ *E. coli* AHU 1040.

area. Furthermore, the discs were flexible and were very smaller than immunoreagents such as glass beads. The preparation of the discs is simple process and many discs can be made from a piece of the polymerized products by slicing, indicating that the production cost is cheap.

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