Antigenicities of Several Cell Wall Mannan Preparations and Cell Envelope Preparations from Baker's Yeast*

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Antigenicity of mannan preparation obtained by the usual method so far published and that prepared by our method was investigated with the rabbit antiserum against the intact yeast cell. The antigenicity of the former was a little less than the latter, but both mannan preparations were much less in the antigenicity as compared with the intact yeast cell. Thus the preparation of an intact surface antigen was attempted through treating the yeast whole cells successively with acetic acid, pepsin, acetone and ether. The cell envelope obtained by the procedure was almost intact microscopically and maintained the antigenic activity similar to that of the intact cells. The dry weight of the cell envelope was a half of the whole cell and its composition was sugar 60%, crude protein 32% and lipid 5%.

For detecting minute changes in the cell surface more easily, an immunological method using the agglutination reaction with the antiserum against the intact baker's yeast was devised in our laboratory. The effects of various physical or chemical treatments on surface of baker's yeast cells were investigated by this method. From the results of these experiments, it was suggested that protein or peptide is also involved in antigenicity to some extent, and that such antigenic peptide is located more internally than cell wall mannan fraction.

The antigen of yeast cells which display the agglutination reaction has so far been reported to be due to mannan of yeast cell surface and not to be due to glucan located more internally than mannan. Moreover, some authors have suggested that no other components than mannan are involved in precipitation activity. Therefore, in order to solve the contradiction between the results of our laboratory and the papers by the researchers described above, respective antigenicity of the mannan preparation used in the above reports and that prepared by our method was investigated by reaction with the antisemum against the intact yeast cells. In addition to the experiments, the present paper deals with the preparation of the intact cell surface antigen from the whole yeast cells, since the antigenicity of above mannan preparations was much less than that of intact yeast cell.

MATERIALS AND METHODS

Materials. Pressed baker's yeast (Saccharomyces cerevisiae) with no additives was obtained from Oriental Baker's Yeast Co. and stored at 0~5°C. The yeast was used within a month, and is described as fresh yeast. Rabbit antiserum was prepared according to the previous report. Mannan was prepared by Edwards' method and by the mild method from cell wall fraction obtained by the differential centrifugation of disintegrated yeast cells, respectively. The former was a gift from Mr. Okubu of Laboratory of Wood Chemistry of our Faculty, and designated as $M_1$. The latter was prepared according to the procedures shown in Fig. 1, and respective mannans were designated as $M_1$ and $M_2$.

Estimation of antigenicity. Antigenicity of yeast cell surface was estimated from the capacity of yeast cells which could absorb antibodies from the antisemum according to the method of the previous paper. Antigenicity of mannan preparations was investigated by Ouchterlony's double immunodiffusion test and

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* Immunochemical Studies on Surface of Yeast Cell, Part III Part II, see ref. 2.
Fig. 1. Preparation of Cell Wall Mannan by the Mild Method.

and these lines fused completely. This result suggests that either of three preparations has common antigenic determinants. In $M_E$, however, an outer line was very broad and an inner line was obscure (Fig. 2A). Moreover, the precipitation lines against $M_2$ were formed farther from the well of the antiserum than those of $M_1$ (Fig. 2B).

Figure 3 shows the quantitative immunoprecipitation curve of three mannan preparations. Mannan $M_1$ gave almost the same curve as mannan $M_E$, but $M_2$ required more amount than $M_1$ and $M_E$ to give the

RESULTS

Figure 2 shows the double immunodiffusion test of several mannan preparations with the antiserum. To the center well was added the antiserum and to the surrounding well was added each sample (2 mg/ml). Three mannan preparations $M_1$, $M_2$ and $M_E$ formed two precipitation lines against the antiserum estimated by agglutination test of the remaining antibody after absorbing antibodies by precipitation with each mannan.

Analysis of yeast cell envelope. Each sample was dried in a weighing bottle at 100°C to a constant weight to obtain dry weight. Protein content was calculated by multiplying the nitrogen content by 6.25 which was determined by the micro Kjeldahl method. Lipid content was determined according to Folch's method. Each sample was extracted by chloroform-methanol (2:1) and the extract in a weighing bottle was dried under reduced pressure at room temperature until a constant weight was reached. Sugar content was determined by phenol-sulfuric acid method.

Fig. 2. Ouchterlony Double Immunodiffusion Test of Mannan Preparations with the Antiserum.

The concentration of mannan preparations $M_1$, $M_2$ and $M_E$ was 2 mg/ml, and the antiserum (A) was not diluted. The diameter of wells was 5 mm and the distance between wells was 15 mm from center to center. The diffusion was carried out in 1% agar gel of saline at 7°C for 2 days.

Fig. 3. Quantitative Immunoprecipitation of Mannan Preparations.

Each incubation mixture contained 0.5 ml saline solution of mannan preparation and 0.5 ml of the antiserum diluted 5 times. It was incubated at 37°C for 60 min and at 4°C for 2 days. After the incubation, immunoprecipitate was collected by centrifugation, washed with saline 3 times, and the amount of the precipitated protein was determined by the micro Kjeldahl method. ○—○, $M_1$; •—•, $M_2$; ⅹ—ⅹ, $M_E$. 
maximum protein precipitation. It was estimated from these immunoprecipitation curves that 0.4 mg of mannan per 1 ml of five times diluted antiserum is suitable for immunoprecipitation reaction. Hence, antibody was absorbed by each mannan preparation using the above values.

As shown in Table I, absorption of antibody stopped when the procedure of absorption was over 3 times. Antigenicity of $M_1$ was not different qualitatively from that of $M_2$. Contrary to this, antigenicity of $M_E$ might be slightly different from $M_1$ and $M_2$, because the remaining antibody titre in the absorption by $M_E$ was larger by one step than in that by $M_1$ or $M_2$. Even in $M_1$ prepared in the mild procedures, however, antigenicity was much less than that in fresh yeast cells and this result implied that there are some kinds of antigen other than mannan which play the major role on the yeast cell surface. Therefore the preparation of the more complete surface antigen from yeast cells was attempted through the removal of unnecessary components from whole cells.

Figure 4 shows the preparation of cell envelope purified partially as surface antigen. Pressed yeast was liquefied within about half a day when the vapor of acetic acid was added to the surface of pressed yeast at 30°C in a closed vessel such as a desiccator or a vessel for diffusion analysis. From 10 g of pressed yeast, the yields of Pellet A, Pellet P and acetone-ether treated cell envelope (AE cell envelope) were 2.625, 1.625 and 1.575 g as dry weight, and the ratio of each yield to dry weight of fresh yeast was 84, 52 or 50%, respectively.

Table II shows the antigenicity of each preparation. Each preparation equivalent in amount to fresh yeast cell from which the preparation was derived, was used in an absorption by each fraction (12.5 mg of fresh yeast per 1 ml of reaction mixture in one absorption according to the previous report). Antigenic activity of each fraction was almost equal to that of fresh yeast.

Table III shows the chemical composition
TABLE II. ANTIGENICITY OF EACH PREPARATION OF YEAST CELL SURFACE ANTIGEN

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<thead>
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<th>Dilution of antiserum</th>
<th>Blank Agglutination reaction</th>
<th>After absorption by</th>
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<td>Fresh yeast</td>
<td>Pellet A</td>
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DISCUSSION

1) Antigenicity of yeast mannan preparations was significantly less than that of intact yeast cells against the antiserum taken from a rabbit immunized with intact yeast cells (Table II). Although mannan has so far been regarded as the only antigen of the yeast cell surface, the results in the present paper suggest that there are some kinds of antigen other than mannan which play the major role in the cell surface antigen. From the results in the previous paper that formaldehyde or diethyl pyrocarbonate, a reagent reacting with amino groups, decreased antigenicity by one step, and from the result in the present paper that M1, which had been treated with Pronase, retained the maximum antigenic activity of mannan preparations but its antigenicity was less than that of intact yeast cells, peptides may be inferred to contribute significantly to cell surface antigens in the intact yeast.

2) The precipitation lines against M2 were formed farther from the well of the antiserum than those of M1 (Fig. 2B) and M2 required more amount than M1 to give the maximum protein precipitation (Fig. 3). These results mean that antigenic activity of M2 is lower than that of M1, which might be due to the treatment in autoclave. A broad precipitation line of M8 in the immunodiffusion test (Fig. 2A) might mean that M8 contained various sizes of antigen. Moreover, antigenicity was less retained in M8 than in M2 or M1 (Table I). Such properties of M8 might have been caused by the more violent treatment in the preparation of M8 than in that of M2 or M1.

3) As Roelofsen reported that the amount of cell wall of baker's yeast was 20% as dry weight,150% of impure components was still contaminated in the cell envelope fraction prepared in the present study, which retained antigenicity completely. Afterwards, a white powder of the cell envelope fraction was obtained by the performance of exhaustive washing in each process of which the yield became 42% per dry weight of yeast cells, and in the extracts of Pellet A by a lytic enzyme were recognized some thermolabile antigens. The isolation of these thermolabile antigens and their contribution to the antigenicity of the intact yeast cell surface are now being researched.
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REFERENCES

7) Y. Konishi and A. Misaki, Abstracts of Papers, Annual Meeting of the Agricultural Chemical Society of Japan, Kyoto, April, 1976, p. 222.