Mating Pheromone-Induced Alteration of Cell Surface Proteins in the Heterobasidiomycetous Yeast *Tremella mesenterica*

TOKICHI MIYAKAWA,* TAKASHI KADOTA, YUICHI OKUBO, TAKUSHI HATANO, EIKO TSUCHIYA, AND SAKUZO FUKUI

Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Saijo, Higashi-Hiroshima 724, Japan

Received 19 December 1983/Accepted 12 March 1984

Mating pheromone-induced alteration of the cell surface proteins of haploid cells, presumed to play crucial roles in the specific cell-cell interactions during sexual conjugation of *Tremella mesenterica*, was investigated. Exposed surface proteins were revealed by lactoperoxidase-catalyzed iodination in combination with polyclarlamide gel electrophoresis and autoradiography. From comparison of the molecular species of 125I-labeled surface proteins of the vegetative and the gamete (mating pheromone-treated) cells of the two compatible mating types (ab and AB), it was suggested that a striking change in cell surface structure occurs during the differentiation; although labeled protein species of the vegetative cells of the two mating types were indistinguishable, several new species, both mating type specific and nonspecific, appeared in the gamete cells. Turnover of the labeled proteins of the vegetative cells was negligible, whereas that of the gamete cells was rapid with release of low-molecular-weight labeled proteins in the medium. A role for the labeled surface proteins of the gamete cells in the cell-cell interactions during sexual conjugation was suggested by the following: (i) the surface changes were induced by mating pheromone; (ii) the labeled proteins were preferentially localized on the surface of the mating tube; (iii) the labeled species appeared sequentially during the differentiation; and (iv) mating type-specific species were present in both mating types.

The alternation from asexual to sexual propagation in the life cycle of the heterobasidiomycetous yeast *Tremella mesenterica* is achieved by conjugation of yeast-like haploid cells of two compatible mating types (ab and AB); the resulting conjugants grow as dicaryotic cells with hyphal morphology. Preceding conjugation, the cells are mutually induced to differentiate to gamete cells by the reciprocal exchange of diffusible mating pheromone between the cells of two mating types. Tremerogen A-10 and tremerogen a-13, mating pheromones produced by AB and ab cells, respectively, have been purified to homogeneity and shown to be isoprenyl peptides (6, 7). When induced by the mating pheromones, the G1-arrested yeast-like cell forms an elongated "mating tube" (3). Morphologically, the conjugation on solid medium is accomplished by mutual and directional extension of the mating tube toward the apex of the tube of the partner cell, resulting in cell fusion at the apexes of the two tubes (the process is similar to the one described by Abe et al. [1] with other species of the heterobasidiomycetous yeast *Rhodotorula toruloides*). The specific cell-cell interaction for the conjugation appears to be facilitated by an altered cell surface structure during sexual differentiation (M. Ikeda, unpublished data). Thus, the identification and characterization of exposed cell surface proteins specific to the gamete cell seem to be particularly important for understanding the mechanism of cell-cell interactions. In this report, we describe mating pheromone-induced cell surface changes probed by lactoperoxidase-catalyzed iodination. The results show that striking changes in surface structure occur during the differentiation and suggest that the labeled proteins of gamete cells may be important for the conjugation process.

---

* Corresponding author.

**MATERIALS AND METHODS**

**Microorganisms and growth conditions.** Haploid strains of *T. mesenterica* UBC 6106-1 (mating type AB) and -2 (mating type ab), obtained from R. J. Bandoni, University of British Columbia, Canada, were used throughout the experiments. Cells were grown aerobically at 28°C in liquid minimal medium which contained 20 g of glucose, 3 g of ammonium sulfate, 1 g of KH2PO4, 0.5 g of MgSO4 · 7H2O, and 0.1 g of NaCl per liter of deionized water.

**Assay of mating pheromone.** Determination of mating pheromone activity was performed as follows. To a twofold serial dilution of mating pheromone diluted in 0.5 ml of minimal medium was added 0.5 ml of the assay cells in the mid-logarithmic phase of growth (5 × 107 cells per ml). After a 14-h incubation at 28°C, the percentage of cells with mating tube(s) was scored under a microscope. One unit of pheromone is defined as the amount which induces the characteristic morphological change (mating tube formation) in 30% of the cells tested. One unit of either tremerogen A-10 or tremerogen a-13 is equivalent to 1 ng of pure pheromone (6, 7).

**Preparation of gamete cells.** In all experiments, cell-free culture filtrates of AB and ab cells were used as sources of tremerogen A-10 and tremerogen a-13, respectively. The culture media, which usually contained 32 U of the pheromone per ml, were heated at 100°C for 3 min to inactivate secreted enzymes and were filter sterilized. To prepare the gamete cells, sexual differentiation was induced by incubating the vegetative cells at a concentration of 2 × 107 cells per ml in 5 ml of medium which consisted of equal volumes (2.5 ml) of fresh minimal medium and culture filtrate. Unless otherwise noted, the cell suspension was incubated at 28°C for 12 h with shaking. Vegetative growth stopped in the induced culture within 5 to 6 h of incubation, and mating tubes were formed in ca. 90% of the treated cells by the end
of the 12-h incubation (see Fig. 3). Control (vegetative) cells treated with mating pheromone from the same mating type showed no changes in their growth and morphology.

**Iodination and sample preparation for electrophoretic analysis.** The gamete and the vegetative cells were harvested and washed twice with 20 mM sodium phosphate buffer (pH 7.0) by centrifugation at 2,500 \( \times g \) for 10 min. The washed cells were iodinated by incubation with 20 \( \mu \)Ci of carrier-free Na\(^{125}\)I (Amersham Corp.), 1 \( \mu \)g of glucose oxidase (Boehringer Mannheim Biochemicals), and 1 mg of glucose in 1 ml of phosphate buffer for 30 min at 28°C with gentle shaking. The reaction was terminated by the addition of 0.8 mg of 2-mercaptoethanol to the reaction mixture. The cells were washed five times by centrifugation with 3 ml each of the buffer containing 3 mM NaI. The iodinated cells were suspended in 0.3 ml of the buffer containing 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical Co.) and were disrupted by blending in a Vortex mixer with glass beads (0.3 mm) in a test tube (13 by 100 mm). The suspension of disrupted cells was then transferred to a microfuge tube and centrifuged in a microfuge for 10 min at 10,000 \( \times g \). The sediment (envelope fraction) and the clear supernatant (cytoplasmic fraction) were isolated. Samples for polyacrylamide gel electrophoresis were prepared by the addition of sodium dodecyl sulfate, glycerol, 2-mercaptoethanol, and bromophenol blue to 0.5 ml of water to achieve final concentrations of 2, 10, 5, and 0.008%, respectively. In an alternative method, the labeled cells were disrupted directly in the gel sample buffer of the composition described above. To the pellet of labeled cells in a microfuge tube were added 0.5 ml of the gel sample buffer and glass beads, and the cells were disrupted by vigorous agitation with a Vortex mixer. Proteins were solubilized by heating the resulting suspension at 100°C for 3 min, and the clear supernatant after centrifugation was subjected to gel electrophoresis. Disruption of cells before solubilization was necessary for reproducible results.

**Polyacrylamide gel electrophoresis.** The procedure used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis was a modification of the discontinuous buffer system described by Laemmli (5). The 1-mm-thick slab gel consisted of 16 cm of either 12.5% acrylamide resolving gel or 7.5 to 17.5% linear gradient acrylamide resolving gel (pH 8.8) and 1 cm of 3% acrylamide stacking gel (pH 6.8). After completion of the electrophoresis, the gel was stained with 0.05% (wt/vol) Coomassie blue R-250 in isopropanol-acetic acid-water (5:2:13 [vol/vol/vol]) and destained in methanol-acetic acid-water (1:1:1, [vol/vol/vol]). The gel was then dried under vacuum onto filter paper over a boiling water bath, and an autoradiograph was prepared with Kodak XAR-5 film by using an intensifying screen (Lightning Plus; Du Pont Co.). Gels were calibrated with human erythrocyte membrane proteins (2) and bovine serum albumin as molecular weight markers. Intensity of labeling of the bands was quantitated by densitometric tracing of the autoradiograph by a spectrophotometer (model DMU-33C; Toyo Scientific Industry, Osaka, Japan).

**Electron microscopic autoradiography.** Cells were iodinated as described above, except 100 \( \mu \)Ci of Na\(^{125}\)I was used. The radioiodinated cells were fixed successively with glutaraldehyde and osmium tetroxide as reported previously (3). Ultrathin sections were cut on an ultramicrotome (Sorvall MT-1) and prepared for electronmicroscopic autoradiography. Sakura NR-H3 emulsion was applied, and the coated grids were stored at 4°C for 1 month. The grids were developed with Kodak D-19, stained with lead citrate, and examined in a JEM-7A electronmicroscope at 80 kV.

**RESULTS**

Identification of cell surface proteins of vegetative and gamete cells. The use of lactoperoxidase-catalyzed iodination (4) for the surface-specific labeling of *T. mesenterica* cells was assessed. The vegetative and the gamete cells of mating type *ab* were radioiodinated by the lactoperoxidase-catalyzed reaction, using the \( \text{H}_2\text{O}_2\)-generating system of glucose and glucose oxidase. The \( ^{125}\text{I} \)-labeled proteins of the cytoplasmic and the envelope fractions were analyzed by polyacrylamide gel electrophoresis. Total and \( ^{125}\text{I} \)-labeled proteins were visualized by Coomassie blue staining and autoradiography, respectively. From the profiles of the Coomassie blue staining and the autoradiograph in Fig. 1, it was obvious that only a small number of protein species of the envelope fraction were labeled in both the vegetative and the gamete cells. No radioactivity was apparent in the cytoplasmic fraction (Fig. 1). The autoradiographic electronmicroscopy of the labeled cells (Fig. 2) also supported the idea that the labeling is highly specific to cell surface proteins.

The autoradiographs in Fig. 1 also show a striking difference in the profiles of labeled proteins of the vegetative and the gamete cells. In the vegetative cells, the protein with a molecular weight of 66,000 was the major band resolved by the gel electrophoresis; other bands of very high molecular weights (>250,000) could not be resolved. In the gamete cells, however, the 66,000 protein seen in the vegetative cells diminished, and new species with molecular weights of

![FIG. 1. Comparison of labeled proteins of vegetative and gamete cells of mating type *ab* radioiodinated by lactoperoxidase-catalyzed iodination. Vegetative (V) and gamete (G) cells were radioiodinated, disrupted, and then separated to the envelope and the cytoplasmic fractions by high-speed centrifugation. Proteins of each fraction were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 12.5% acrylamide concentration. The autoradiographs of the envelope and cytoplasmic fractions and the Coomassie blue-stained pattern of the envelope fraction are shown. Molecular weight is shown in thousands (K).](http://jb.asm.org/Downloaded-from HTTP://J.B.ASM.ORG/ On October 19, 2017 by guest)
73,000, 60,000, 43,000, and 20,000 appeared. Such differences seen in the autoradiograph were not visible at the corresponding positions in the Coomassie blue-stained pattern (Fig. 1).

**Localization of ¹²⁵I-labeled components.** The distribution of radioactivity in the labeled cells was examined by electron microscopic autoradiography. Typical micrographs of the vegetative and the gamete cells are shown in Fig. 2. From an examination of ca. 100 cells, ca. 90% of the silver grains were found on the cell periphery in both the vegetative and the gamete cells. The autoradiograph of the gamete cells also showed that the grains were preferentially localized on the mating tube (Fig. 2). From an examination of 100 gamete cells, the density of grains along the sections of the mating tube was estimated to be 3.5-fold that of the nontube counterpart (yeast-form part). In the vegetative cells, there were no significant differences in the densities of grains on the bud and the mother cell.

**Times of the change in the intensity of labeled proteins during sexual differentiation.** The times of change in the intensity of labeling of surface proteins during sexual differentiation from the vegetative to the gamete cells was investigated. Sexual differentiation was induced in ab cells for various periods of time (0 to 15 h) by incubation with mating pheromone, and the treated cells were radioiodinated. The incorporation of radioactivity into each labeled species was quantitated by densitometric tracing of the autoradiograph (Fig. 3). The average length of the mating tubes and the percent incidence of cells with mating tube(s) are also shown as parameters of the extent of sexual differentiation (Fig. 3). The intensity of labeling of the 60,000-dalton protein, one of the differentiation-specific proteins, started to increase after 4 h of mating pheromone treatment concomitant with the appearance of mating tubes, and the increase continued during the differentiation. In contrast, the appearance of other major proteins, the 73,000- and 43,000-dalton species, started after 8 h of pheromone-treatment and after the cells had already formed mating tubes. The 66,000-dalton protein, which was labeled intensely only in the vegetative cell, started to decrease immediately after the pheromone treatment. By the time mating tubes started to appear (4-h pheromone treatment), the level of this protein became ca. 15% of the initial level.

**Turnover of surface proteins.** The turnover rate and the fate of surface proteins during further incubation of the labeled cells were studied. Vegetative and gamete cells of mating type ab were radioiodinated, and the labeled cells were transferred to fresh medium, which allowed for the same mode of growth as before labeling (vegetative growth for the vegetative cell, and sexual differentiation for the gamete cell), as described in the legend to Fig. 4. Viability of the labeled cells determined by colony formation was not affected by the iodination in either cell type. The intensities of major labeled protein bands after 5 and 10 h of incubation was quantitated. The intensity of the 66,000-dalton protein of the vegetative cells decreased only slightly even after 10 h of the incubation (Fig. 4). In contrast, the labeled proteins of the gamete cells decreased rapidly; after a growth period corresponding to one cell doubling time (7.5 h) for vegetative cells, the intensities of bands in the 60,000- to 73,000-dalton region and the 43,000-dalton protein decreased to ca. 60 and 25% of the initial values, respectively. Since the 60,000- and 73,000-dalton bands were not resolved well, the combined intensity of the bands in this region was determined. Proteins released into the medium were analyzed by gel electrophoresis. The result showed that released proteins were mainly

---

**FIG. 2.** Electron microscopic autoradiographs of iodinated vegetative (V) and gamete (G) cells. Arrows indicate silver grains which represent ¹²⁵I atoms covalently linked to proteins. Bar = 2 µm.
digested to small-molecular-weight species unresolvable by the gel system used (data not shown).

The slow turnover rate of the 66,000-dalton protein of the vegetative cells described above was in marked contrast to the rapid decrease of this protein on exposure of the vegetative cells to mating pheromone (Fig. 3). The decrease of this protein during the differentiation may be due to release of this protein from the cell surface as an immediate response of the vegetative cells to the pheromone. To test this possibility, $^{125}$I-labeled vegetative cells were further incubated in fresh minimal medium in the presence of mating pheromone, and the cell-associated proteins were analyzed as described above. The 66,000-dalton protein diminished rapidly during the pheromone treatment (Fig. 4, V→G), in good agreement with the rate of decrease of this protein seen in Fig. 3.

Mating type specificity of the exposed surface proteins. Data presented so far referred to the surface proteins of mating type ab cells only. If the alteration of surface structure during sexual differentiation is involved in cell-cell interaction of mating, the appearance of mating type-specific proteins would be expected on the surface of the gamete cells. Radiiodinated proteins of vegetative and gamete cells of both mating types were compared. To increase the resolution of the high-molecular-weight region of the electrophoretic pattern, a continuous linear gradient of acrylamide concentration (7.5 to 17.5%) was used in this experiment. There were no significant differences in the patterns of labeled proteins of the vegetative cells of the two mating types (Fig. 5). However, significant differences were seen in the gamete cells of the two mating types. The proteins with molecular weights of 73,000 and 60,000 seen in ab cells were not prominent in AB cells. Proteins with molecular weights of 110,000, 77,000, 40,000, and 22,000 were present in gamete cells of this mating type. The differentiation-dependent decrease of the 66,000-dalton protein seen in ab cells was not notable in AB cells, being present in the gamete cells at an intensity similar to that of the vegetative cells. However, it is possible that the 66,000 protein of the vegetative ab cells also decreased by the pheromone treatment with the appearance of new protein species with a similar molecular weight. The appearance of bands with molecular weights of 140,000 and 95,000 were seen in both mating types. These and other results described earlier are summarized in Table 1.

DISCUSSION

Mating pheromone-induced alteration of exposed surface proteins of haploid cells of T. mesenterica was probed by lactoperoxidase-catalyzed iodination. Sexual differentiation was accompanied by a dramatic change in the $^{125}$I-labeled protein species in both mating types. The 66,000 and very-high-molecular-weight proteins (>250,000) were the only major labeled species (occasionally, the 95,000 protein was

FIG. 3. Time course of the changes in the labeling intensity of exposed surface proteins during sexual differentiation. Sexual differentiation was induced in mating type ab cells for various periods of time. The treated cells were radiiodinated and disrupted in the gel sample buffer, and labeled proteins were separated by electrophoresis in 12.5% acrylamide gel. The intensity of labeling of major labeled bands was quantitated by densitometric tracing of the autoradiograph. The percentage of cells with mating tube(s) and average length of mating tubes are shown in the upper panel as parameters of the extent of sexual differentiation. Molecular weight is shown in thousands (K).

FIG. 4. Turnover of labeled surface proteins. Vegetative and gamete cells of mating type ab were radiiodinated and washed extensively to remove enzyme catalysts and unreacted isotope. The labeled cells were allowed to continue sexual differentiation (G) for the gamete cells, and vegetative growth (V) or sexual differentiation (V→G) for the vegetative cells. At 0, 5, and 10 h of incubation, the cells were harvested by centrifugation and disrupted in the gel sample buffer. After gel electrophoresis, the amount of radioactive label in the major labeled bands were quantitated by densitometric tracing of the autoradiograph. The arrow indicates one cell doubling time of the vegetative cell. Molecular weight is shown in thousands (K). OD, Optical density.
weakly labeled) in vegetative cells of mating type ab. Proteins with molecular weights of 73,000, 60,000, and 43,000 (and occasionally 20,000) appeared in gamete cells. The 66,000 protein of ab cells, which was labeled intensely in the vegetative cells, diminished in the gamete cells. It is not yet clear whether these changes are due to de novo protein synthesis or to modification by rearrangement of cell surface structure which resulted in change in accessibility of surface proteins to lactoperoxidase. However, considering that no differences were visible at the corresponding positions in the Coomassie blue-stained patterns of the vegetative and the gamete cells (Fig. 1), the alteration of the labeled pattern may not be due to de novo synthesis. The occurrence of rearrangement of the cell surface is suggested by the observation that the 66,000-dalton protein, which is normally very stably associated with the vegetative cells (Fig. 4), is rapidly released into the medium when the vegetative cells were treated with mating pheromone (Fig. 3 and 4).

Exposed surface proteins of the gamete cells are good candidates for molecules that are involved in the specific cell-cell interactions in mating. Supporting this inference, our unpublished data indicated that sexual interactions be-

![Diagram](image)

**FIG. 5.** Mating type specificity of labeled surface proteins. Vegetative (V) and gamete (G) cells of mating type ab and AB were radioiodinated. The labeled proteins were analyzed as described in the legend to Fig. 4. To increase the resolution of the electrophoretic pattern, a continuous linear gradient of acrylamide concentration (7.5 to 17.5%) was used in this experiment. To avoid confusion arising from the anomalous electrophoretic mobility of glycoproteins in different acrylamide concentrations (8), molecular weights of labeled species of ab cells in the present gel system are those determined in the 12.5% gel, and those of AB cells are estimated from the molecular weights of labeled proteins of ab cells. Molecular weight is shown in thousands (K).

<table>
<thead>
<tr>
<th>Mol wt (10^3)</th>
<th>Mating type</th>
<th>Change in the labeling intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>ab, AB</td>
<td>Increase</td>
</tr>
<tr>
<td>110</td>
<td>AB</td>
<td>Middle</td>
</tr>
<tr>
<td>95</td>
<td>ab, AB</td>
<td>Middle</td>
</tr>
<tr>
<td>73</td>
<td>ab</td>
<td>Appearance</td>
</tr>
<tr>
<td>66</td>
<td>ab</td>
<td>Decrease</td>
</tr>
<tr>
<td>60</td>
<td>ab</td>
<td>Appearance</td>
</tr>
<tr>
<td>43</td>
<td>ab</td>
<td>Appearance</td>
</tr>
<tr>
<td>40</td>
<td>AB</td>
<td>Increase</td>
</tr>
<tr>
<td>22</td>
<td>AB</td>
<td>Appearance</td>
</tr>
</tbody>
</table>

* The terms early, middle, and late indicate that the change in the intensity of the labeled band initiates before, with, or after the apparent progress of sexual differentiation, respectively.

Table 1. Summary of the changes in the intensity of the 125I-labeled surface proteins of haploid cells of T. mesenterica during sexual differentiation

ACKNOWLEDGMENT

This work was supported in part by a grant-in-aid for scientific research (no. 57560106) from the Ministry of Education, Science and Culture of Japan.

LITERATURE CITED

