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INDUCTION OF SEXUAL CELL AGGLUTINABILITY OF a MATING TYPE CELLS BY α-FACTOR IN SACCHAROMYCES CEREVISIAE

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Summary

Mating hormone, α-factor, which inhibits DNA synthesis and causes characteristic changes in cell morphology in a mating type cells, was also responsible for induction of sexual cell agglutinability of a mating type cells.

Mating reaction in haploid strains of Saccharomyces cerevisiae is achieved through an orderly progression of three sequential events; sexual cell agglutination during which cells recognize opposite mating type cells, cell fusion (plasmogamy) to form zygote, and nuclear fusion (karyogamy) to form diploid (1).

When cells grown at 25° C were mixed with heat-killed or living tester cells of opposite mating type , sexual agglutination was observed (constitutive agglutinability). When cells grown at 36° C were mixed with heat-killed tester cells, no sexual agglutination was observed. When the cells were mixed with living tester cells, however, sexual agglutination was observed after a lag period (inducible agglutinability) (2). Since this phenomenon suggests that sexual agglutinability of cells grown at 36° C is induced by living cells of opposite mating type, the possibility was tested that the α-factor in the culture filtrate may induce sexual agglutinability.

Materials and Methods

Yeast strains, media and culture conditions: Prototrophic haploid strains of Saccharomyces cerevisiae, K27-3B (a) and T22 (X2180-1B,

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α) were used. Modified YPD (mY, 5% glucose, 1% peptone, 0.5% yeast extract, 0.5% KH_2PO_4 and 0.2% $MgSO_4.7H_2O$), MV (3) and PG (1% glucose and 0.1% peptone) were used for yeast culture, preparation of culture filtrate and induction of agglutinability, respectively. Yeast culture was carried out at 25°C or 36°C on a reciprocal shaker.

Induction of sexual agglutinability: Cells from late logarithmic phase culture of K27-3B grown in mY medium at 36°C were harvested, washed and suspended in water. Absorbance at 530 nm (A_{530}) of the suspension was adjusted to 4. For induction of agglutinability, a half ml of the cell suspension was mixed with 9 ml of phosphate buffer (10 mM, pH 5.5) containing purified α-factor and 0.5 ml of 10 times concentrated PG and then the mixture was incubated at 36°C.

Purification of α-factor: 100 ml of 2 day-old culture of T22 were inoculated into 20 liter of MV medium in jar fermenter (Ishiyama Kagaku Kikai, Tokyo) and cultured for 48 hours at 25°C. After removal of cells by centrifugation, cell-free culture filtrate was obtained by filtering culture fluid through glass fiber filter (GF/C, Whatman, England). Purification of α-factor from the filtrate was carried out essentially according to Duntze et al.(4). α-Factor activity was determined in mY medium at 25°C 3 hours after incubation by testing two-fold dilution series. The most diluted test culture which showed characteristic change in cell morphology called "shmoo" was determined as containing one unit of α-factor. Although direct comparison is impossible since different assay system was used, our preparation of α-factor had activity similar to that reported previously (4).

Enzyme treatment: Purified α-factor (1 μg/ml) was treated with proteolytic enzymes, Pronase E (Kaken Kagaku, Tokyo), pepsin (Worthington Biochem. Co., Freehold, N.J.), trypsin (P-L Biochem., Milwaukee) and α-chymotrypsin (ICN Pharmaceuticals, Cleveland) for 60 min. at 36°C. The enzymes except pepsin were dissolved in 1 mM calcium acetate before use. Tris-HCl buffer (30 mM, pH 7.0) was used for pronase, trypsin and α-chymotrypsin, and sodium acetate buffer (10 mM, pH 3.5) for pepsin. Activity of α-factor to cause a-shmoos or to induce agglutinability was determined after inactivating enzyme by boiling for 5 min. at 100°C.

Synthetic α-factor: Synthetic α-factor (5) was provided by the courtesy of Dr. Hiroshi Kita, Central Res. Inst., Suntory Ltd., Osaka.

Agglutination assay: Agglutination assay was carried out as described previously (2) except the use of living cells instead of heat-killed cells as a tester.

Results

Induction of sexual agglutinability by purified α-factor

As shown in Table 1, purified α-factor could intensely induce sexual agglutinability of a mating type cells in the phosphate buffer enriched with both glucose and peptone (PG). It could not

Table 1. Induction of sexual agglutinability by α-factor

Medium[1)]	Agglutination index
PB	1.03
PB + glucose	1.04
PB + peptone	1.01
PB + glucose + peptone[2)]	1.03
PB + glucose + peptone	1.34
PB + glucose + peptone[3)]	1.38

Purified α-factor (1 μg/ml) was added to each medium.
1) Concentration of glucose and peptone was 1% and 0.1%, respectively.
2) No α-factor was added.
3) Synthetic α-factor instead of purified one was added at the concentration of 2 μg/ml, although this value was not accurate because of small amount of it.

induce agglutinability in the phosphate buffer alone, or in the same buffer supplemented with either glucose or peptone. Agglutinability-inducing activity of purified α-factor was inactivated with pronase and pepsin which are also known to destroy a-shmoo-forming activity. Trypsin and α-chymotrypsin had virtually no effect on both activities. These results suggest that sexual agglutinability is induced by α-factor. Furthermore, synthetic α-factor could also induce agglutinability. Unfortunately, shortage of synthetic material could not allow us to perform further experiments.

Intensity of agglutinability as a function of concentration of α-factor

As shown in Fig. 1, the intensity of sexual agglutinability increased with increasing amount of added α-factor up to the concentration of 1 μg/ml. In this system, a-shmoo were not formed even at the highest concentration tested, i.e. 10 μg/ml.

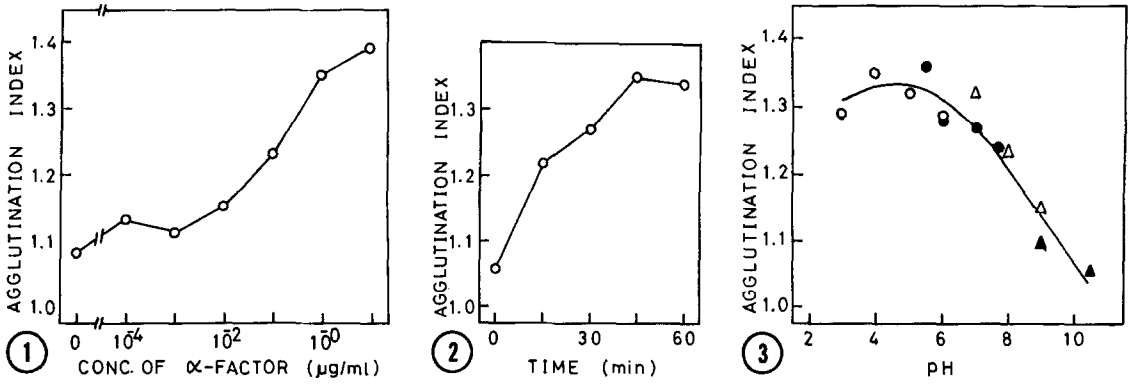

Fig. 1. Relationship between intensity of sexual agglutinability and concentration of purified α-factor. Cells were incubated at 36°C in PG medium containing various ammount of purified α-factor. After an hour, cells were harvested, washed and subjected to agglutination assay.

Fig. 2. Time course of the induction of sexual agglutinability. Cells were incubated at 36°C in PG medium which contained 1 μg/ml of purified α-factor. At time intervals, cells were withdrawn, washed and subjected to agglutination assay.

Fig. 3. pH dependency of induction of the agglutinability. Cells were incubated at 36°C in various buffer solution (10 mM) containing 1 μg/ml of purified α-factor, 1% of glucose and 0.1% of peptone. After an hour, cells were harvested, washed and assayed for agglutination. ○ ; citrate buffer, ● ; phosphate buffer, △ ; Tris-HCl buffer and ▲ ; carbonate buffer.

Time course of the induction of agglutinability

As shown in Fig. 2, sexual agglutinablity began to be observed 15 min. after incubation, increased with time, and reached the maximum level after 45 minutes.

pH dependency of the induction of agglutinability

Fig. 3 shows that the degree of sexual agglutinability was maximal at pH 3 to 7, and decreased with increasing in pH value. This pH dependency is similar to that of a-shmoo-forming activity of α-factor (4).

Discussion

It was showed that mating hormone, α-factor from Saccharomyces cerevisiae inhibits DNA synthesis and causes characteristic change in cell morphology of opposite mating type cells (4,6).

The present study suggests another characteristic of this mating hormone, i.e. the induction of sexual agglutinability. The possibility, however, can not be excluded that the sexual agglutinability-inducing substance is not identical with α-factor, since the α-factor preparation used in present study may not be completely pure.

Further studies are necessary to clarify mechanism underlying different phenomena caused by α-factor, inhibition of DNA synthesis, shmoo formation and induction of sexual agglutinability.

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