19. Purification and its Structure-activity Relationship of Mating Factor of Saccharomyces cerevisiae

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Mating factor activity found in the culture fluid of \( \alpha \)-mating type cells of \textit{Saccharomyces cerevisiae},\textsuperscript{1} plays substantial role during the mating between \( \alpha \)- and \( \alpha \)-mating type cells through the inhibition of DNA replication in \( \alpha \)-mating type cells.\textsuperscript{2}

The purification of the mating factor was carried out by Duntze \textit{et al.} and their purified sample was reported as a peptide complexed with cupric ion.\textsuperscript{3} In their subsequent paper, they have claimed that four peptides responsible for mating factor activity could be isolated.\textsuperscript{4} The purification of this mating factor was performed independently in our laboratory. In contrast to the factor prepared by Duntze \textit{et al.},\textsuperscript{3} our purified factor is a colorless peptide, not associated with cupric ion and very stable in aqueous solution between pH 1.0 and 10.0. Besides, the purified factor is very stable against heating in 0.01 N HCl at 105°C over 20 hours.

The present paper describes the purification procedure of mating factor from the culture fluid of \( \alpha \)-mating type cells of \textit{S. cerevisiae} X-2180 1B and its complete amino acid sequence along with the study on the structure and activity relationship using the synthetic peptides. The purification procedure described in this paper is much simpler and milder than that by Duntze \textit{et al.}\textsuperscript{3} Our procedure yields mating factor with a higher specific activity in a better recovery.

For the purification of mating factor, X-2180 1B cells, \( \alpha \)-mating type cell, were cultured in Burkholder's synthetic medium.\textsuperscript{5} The activity of the mating factor in the culture fluid increased rapidly during the exponential phase of cell growth and reached maximum at late log phase, followed by the rapid decline under our culture condition.

\textit{S. cerevisiae} X-2180 1B cells were inoculated and grown in 40 liters of Burkholder's synthetic medium aerobically at 30°C for 40 hours. After removal of cells by centrifugation, 80 ml of acetic acid was added to the supernatant. The acidified supernatant was loaded onto a phosphocellulose P-1 column (16×30 cm) (Whatman Ltd.)

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previously equilibrated with 2% acetic acid. The column was washed thoroughly with 2% acetic acid, followed by the elution of mating factor with 1.25 M pyridine-acetic acid (pH 5.0). The fraction containing the activity was lyophilized. The lyophilized sample was dissolved in 1 M acetic acid and charged to an Amberlite IRC 50 column (2.5 × 15 cm) (Rhom and Haas Ltd.) equilibrated with 1 M acetic acid, followed by washing with 0.4 M pyridine-acetic acid (pH 5.0) and 0.8 M pyridine-acetic acid (pH 5.0). The activity was eluted with 2.0 M pyridine-acetic acid (pH 5.0). The active fraction was lyophilized again. The residue was dissolved in 80% methanol: acetic acid (10:1) and applied to a Sephadex LH 20 column (1.3 × 180 cm) (Pharmacia Fine Chemicals) equilibrated with the same solvent mixture. As shown in Fig. 1, when monitored the effluent by absorbance at 280 nm, one minor (Fraction A) and two major peaks (Fractions B and D) emerged from the column in this order. Most of the activity was found in Fraction B.

Lyophilization of this fraction gave more than 1 mg of colorless powder, which was homogeneous chromatographically on a thin layer cellulose plate (E. Merck, Art. 5716), developed with 1-butanol: pyridine: acetic acid: water (15:10:3:12), and visualized with ninhydrin, Pauly and Ehrlich reagents.

Amino acid analysis of its acid hydrolysate (5.8 N HCl plus 0.5% thioglycolic acid at 105°C for 20 hours) resulted in the following composition: Lys (1.06), His (1.03), Trp (1.85), Glx (1.90), Pro (2.24), Gly (1.0), Met (0.73), Leu (2.04) and Tyr (0.84).
Recovery of amino acids was 96%. Two Glx were proved to be glutamine by the amino acid analysis on both hydrolysates prepared by aminopeptidase M (1% NH₄HCO₃ at 40°C for 4 hours) and carboxypeptidase Y (1% NH₄HCO₃ at 40°C for 4 hours).

A few picogram/ml of the purified factor was sufficient to induce morphological changes on α-mating type cells, X-2180 1A of S. cerevisiae. However, our purified factor did not possess any agglutinating activity as was reported by Sakurai et al.8 even in higher concentrations. The specific activity of purified mating factor is about 2×10⁸ units/mg.

The amino acid sequence of the mating factor was elucidated by Edman degradation on the intact factor and three peptides derived by thermolysine digestion of the factor (1% NH₄HCO₃ at 40°C for 2 hours) as shown in Fig. 2. The carboxyl terminal tyrosine was also confirmed by the use of carboxypeptidase A. The identical structure to the present result for mating factor was recently reported independently by Stötzel et al.10

We could not find any peptide which has a histidine as the N-terminal residue through our entire purification procedure. These findings may suggest that mating factor of dodecapeptide reported by Stötzel et al.3 and by Sakurai et al.⁸ is the artificial product formed during the purification.

When the mating factor was prepared from the culture fluid of late stationary phase, Fraction B in Fig. 1 diminished remarkably with concomitant increase of Fraction D and the appearance of Fraction C as shown in Fig. 3. From the amino acid sequence of the peptides isolated from Fractions C and D (Fig. 2), these two peptides seemed to be the degradation products of mating factor. As to Fraction A in both in Figs. 1 and 3, it might be a peptide of premating factor.

Synthetic tridecapeptide (S. Sakakibara and Y. Masui, Protein Research Foundation) based on the proposed amino acid sequence, possessed a full activity as the mating factor. Synthetic and natural peptides also behaved identically on thin layer chromatography and electrophoresis. Dodecapeptides lacking the N-terminal Trp or C-
terminal Tyr were as active as the natural mating factor.

Synthetic peptides lacking either N-terminal Trp-His or C-terminal Gln-Pro-Met-Tyr, were devoid of mating factor activity.

In order to correlate structure with the activity, over 50 analogues were already synthesized and testing for their activities are in progress.

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