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Purification and Amino Acid Sequence of Mating Factor from *Saccharomyces cerevisiae*

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Mating factor is a peptide excreted into the culture fluid by α-mating type cells of *Saccharomyces cerevisiae* X-2180 1B. The purification of the mating factor was carried out by ion exchange chromatography on phosphocellulose and Amberlite IRC 50 columns, followed by gel filtration on a Sephadex LH 20 column. The factor thus prepared was a peptide composed of Lys_1, His_1, Trp_2, Gln_2, Pro_2, Gly_1, Met_1, Leu_2 and Tyr_1, and was able to induce morphological changes on *a*-mating type cells at a concentration of 5 pg/ml.

The amino acid sequence of the mating factor was determined by the manual Edman degradation method using intact mating factor and its thermolytic peptides. The C-terminal amino acid residue was determined by digesting the factor with carboxypeptidase A. The complete amino acid sequence of the mating factor was established to be as follows:

Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr.

It was suggested by Winge that the mating process between *a*- and α-mating type cells of *Saccharomyces cerevisiae* was controlled by some hormone-like substance(s) (*1*). In 1956, Levi found the activity in the culture fluid of α-mating type cells, and designated the principle responsible for this activity as "mating factor" (*2*).

The most distinctive functions of the mating factor are to inhibit the growth of *a*-mating type cells by suppressing the initiation of DNA synthesis (*3*, *4*) and to induce morphological change of their shapes (*4*, *5*).

The purification of the mating factor was attempted by Duntze *et al.* (*5*) and their success was reported in 1973 (*6*). Their purified factor was a peptide complexed with cupric ion. The purification of the mating factor was also carried out independently in our laboratory, as already reported in a preliminary form (*7*). The mating factor isolated by our procedure, which is simpler and milder than that used by Duntze *et al.*, was a peptide consisted of 13 amino acid residues and was not contaminated with cupric ion.

The present paper describes the purification procedure for the mating factor from the culture medium of α-mating type cells of *S. cerevisiae* X-2180 1B, and some of its properties, together with the amino acid sequence of the mating factor.

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MATERIALS AND METHODS

Yeast Strain and Medium—Haploid strains of *Saccharomyces cerevisiae* X-2180 1B (α-mating type cell) and X-2180 1A (a-mating type cell) (both strains were provided by Dr. R. Mortimer, University of California) were used for the preparation of the mating factor and for assay of its activity.

For the purification of the mating factor, cells of *S. cerevisiae* X-2180 1B were grown aerobically at 30°C for 40 h in Burkholder's synthetic medium, pH 5.0; the composition (*8*) of the medium is shown in Table I.

Assay of the Activity—Activity was determined by incubating *S. cerevisiae* X-2180 1A at 27°C for 5 h in the presence of the mating factor. The titer of the activity was expressed as the reciprocal of the highest dilution producing deformation of the test organisms.

Amino Acid Analysis—Fifty μg of the sample dissolved in 0.2 ml of 5.8 M HCl containing 0.5% thioglycolic acid was hydrolyzed in a sealed, evacuated tube at 105°C for 20 h (*9*). Amino acid analysis was carried out according to Spackman *et al.* (*10*) with a Hitachi KLA 5B amino acid analyzer.

Glutamine was determined with a sample (50 μg) digested with carboxypeptidase Y (*11*) at 40°C for 24 h using an enzyme-to-substrate ratio of 1 : 500 (by weight).

Analytical Procedure—A Gilford 2000-2 spectrophotometer was used for ultraviolet absorbance measurements. The purity of the sample was checked by TLC on a Merck cellulose-glass plate (DC Fertigplatten cellulose) with 1-butanol: pyridine: acetic acid: water (15 : 10 : 3 : 12, by volume) and by paper electrophoresis on Toyo Roshi 51 A filter paper at 50 volts/cm and pH 6.4 (pyridine: acetic acid: water=10 : 0.4 : 180, by volume). The peptides were stained with the ninhydrin, Pauly and Ehrlich reagents (*12*).

Digestion with Thermolysin—After digestion of the mating factor (1 mg) with thermolysin (40 μg) at 40°C for 4 h in 1% ammonium bicarbonate–0.01 M $MgCl_2$ (pH 8.0), the reaction was stopped by lowering the pH to 2.0 with dil. HCl. The resulting peptides were separated by TLC on a cellulose-glass plate with a solvent system of 1-butanol: pyridine: acetic acid: water (15 : 10 : 3 : 12, by volume).

Amino Acid Sequence—Sequential degradation of the purified peptides was performed by the manual Edman degradation method (*13*) with slight modifications. Direct identification of phenyl thiohydantoin derivatives of amino acids was carried out by chromatography on a silica gel-glass plate (Merck, Silica Gel 60F-254 with fluorescent indicator) with Sjöquist solvent V (n-heptane: ethylene dichloride: propionic acid= 45 : 25 : 30, by volume) according to Noda and Narita (*14*).

The phenylthiohydantoin derivative of histidine was identified by TLC with a solvent system of o-xylene: 95% ethanol: acetic acid (50 : 50 : 0.5, by volume) according to the method of Inagami (*15*). Phenylthiohydantoin derivatives on the chromatogram were detected under ultraviolet illumination (Chromatovue, Ultraviolet Products, Inc.).

TABLE I. The composition of culture medium for the yeast. The pH of the culture medium was 5.0.

Glucose	20.0 g	H_3BO_3	0.60 gm
Asparagine	2.0 g	$CuSO_4 \cdot 5H_2O$	0.04 mg
KH_2PO_4	1.5 g	$ZnSO_4 \cdot 7H_2O$	0.31 mg
$(NH_4)_2SO_4$	2.0 g	Thiamine·HCl	0.20 mg
$MgSO_4 \cdot 7H_2O$	0.5 g	Inositol	10.00 mg
$CaCl_2 \cdot 2H_2O$	0.3 g	Ca pantothenate	0.20 mg
KI	0.1 mg	Pyridoxine·HCl	0.20 mg
$FeSO_4 \cdot 7H_2O$	0.2 g	Nicotinic acid	0.20 mg
$MnSO_4 \cdot 4H_2O$	0.04 g	Biotin	2.00 mg
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.02 g	Water	1 liter

For characterization of the newly appearing N-terminal residue at each step of the degradation, the dansylation method was used. Dansyl amino acids were characterized by two-dimensional TLC on polyamide sheets (Cheng Chin Trading Co., Taiwan) (*16*).

The C-terminal sequence of the intact mating factor was determined by the use of carboxypeptidase A. The digestion was carried out at 40°C with an enzyme-to-substrate ratio of 1 : 300 (by weight) for various periods (*17*). The amino acids released in the digests were estimated on an amino acid analyzer.

Chemicals—The culture medium was prepared by using reagent-grade inorganic salts. Phosphocellulose P-1 (Whatman Ltd.), Amberlite IRC 50 (Rohm and Hass Ltd.), and Sephadex LH 20 (Pharmacia Fine Chemicals AB) were obtained from Wako Pure Chemicals Ltd. (Tokyo). Carboxypeptidase Y and thermolysin were gifts from Dr. R. Hayashi (Kyoto University) and Dr. H. Matsubara (Osaka University), respectively.

RESULTS

Purification of Mating Factor—For fractionation of the mating factor, cells of *S. cerevisiae* X-2180 1B were removed by centrifugation from the culture fluid (pH 4.2) after incubation at 30°C for 40 h. Eighty ml of acetic acid was added to 40 liters of the supernatant and the solution was placed on a phosphocellulose column (H^+ form, 16×30 cm) previously equilibrated with 0.35 M acetic acid. The column was washed with 5 liters of the same acetic acid solution and the mating factor was eluted with 2 liters of 1.25 M pyridine-acetic acid (pH 5.0). The fraction containing the activity was concentrated *in vacuo* at 40°C, then diluted 5-fold with water and evaporated to dryness. The evaporated sample (50 mg) was dissolved in 200 ml of 1 M acetic acid and quickly placed on an Amberlite IRC 50 column (H^+ form, 2.5×15 cm) previously equilibrated with the same 1 M acetic acid, because the crude preparation from the phosphocellulose column tended to lose its activity fairly rapidly. The column was washed successively with 500 ml of 0.4 M pyridine-acetic acid (pH 5.0), and 500 ml of 0.8 M pyridine-acetic acid (pH 5.0). The active principle was eluted with 2 M pyridine-acetic acid (pH 5.0). The first eluate (100 ml, light yellow in color) was discarded, and the next portion (200 ml, dark brown in color), containing the active material, was collected. After lyophilization of the effluent containing the activity, it was dissolved in 2 ml of 80% methanol: acetic acid (10 : 1, by volume) and insoluble material was removed by centrifugation. The supernatant (red in color) was applied to a Sephadex LH 20 column (1.3×180 cm) previously equilibrated with the same solvent mixture. Peptides were eluted with the same methanol-acetic acid, and the elution was monitored by measuring the absorbance at 280 nm. One minor and two major peaks emerged from the column, as shown in Fig. 1. Most of the activity was found in Fraction B and minor activity was associated with Fraction C.

Examination of the purity by TLC revealed that Fraction B contained a single entity positive to the ninhydrin, Pauly and Ehrlich reagents (R_f, 0.74). Paper electrophoresis at pH 6.4 also indicated the homogeneity of the peptide in Fraction B (the rate of migration relative to lysine was 0.02).

The purification procedure for the mating factor and the activity recovered at each step are summarized in Table II.

Properties of the Mating Factor—As shown in Fig. 2, the absorption spectrum of the mating

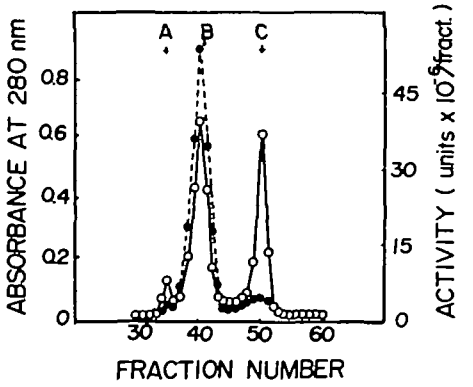

Fig. 1. Chromatography of the mating factor on Sephadex LH 20. A column of LH 20 (1.3×180 cm) was equilibrated with 80% methanol : acetic acid (10 : 1, by volume) then the sample solution (2 ml) was applied and the column was developed with the same solvent at a flow rate of 20 ml/h. Three ml fractions were collected. ○, Absorbance at 280 nm; ●, activity (units/one fraction).

TABLE II. Purification of the mating factor.

Purification step	Volume (ml)	Amount of mating factor (mg)	Total activity ($\times 10^6$ units)	Specific activity ($\times 10^6$ units)	Yield (%)
Culture fluid	40,000	—	280	—	100
Phosphocellulose (H^+ form)	200	50	270	5.4	96
Amberlite IRC-50 (H^+ form)	2	3	250	83	89
Sephadex LH 20	10	1	200	200	71

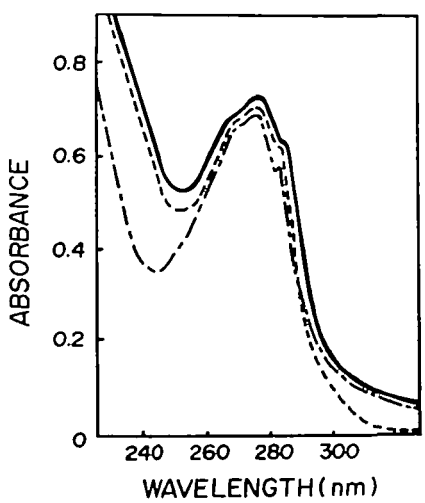

Fig. 2. Absorption spectra of the mating factor and a mixture of tryptophan and tyrosine. ——, Mating factor (120 μg/ml) in 0.05 M sodium carbonate buffer (pH 10); – – –, mating factor (120 μg/ml) in 0.05 M acetate buffer (pH 4.0); - - - -, tryptophan (26 μg/ml) and tyrosine (11.5 μg/ml) in 0.05 M sodium carbonate buffer (pH 10).

factor in acidic solution showed a maximum at 280 nm, shoulders at 275 nm and at 287 nm, and a trough at 245 nm. Changes in its absorption spectrum due to the presence of tyrosine were observed on raising the pH from 5 to 10. The absorption spectrum of the mating factor in 0.05 M sodium carbonate buffer (pH 10.0) coincided well with that of a mixture of tryptophan and tyrosine (2 : 1, mol/mol).

A striking difference in the properties of the mating factor observed between the crude and purified preparations was their stabilities in aqueous solution. The purified mating factor was stable at pH's between 1.0 and 10.0, but crude preparations lost their activity completely after 20 h at pH 8.0 at 4°C.

TABLE III. Amino acid composition of the mating factor. The sample was hydrolyzed with 5.8 M HCl containing 0.5% thioglycolic acid at 105°C for 20 h. All values are calculated assuming that one glycine residue was contained in one molecule of the mating factor.

Amino acid	g residue per 100 g peptide	Molar ratio	Nearest integer
Lysine	8.0	1.06	1
Histidine	8.4	1.03	1
Tryptophan	20.0	1.85	2
Glutamic acid	14.5	1.90	2
Proline	12.8	2.24	2
Glycine	3.4	1.00	1
Methionine	5.7	0.73	1
Leucine	13.6	2.04	2
Tyrosine	8.0	0.84	1
Total	94.4		13

Amino Acid Composition—The amino acid composition of the mating factor is shown in Table III. Recovery of the constituent amino acids on a weight basis was nearly quantitative, suggesting that the factor isolated was not contaminated with non-peptidic material. Amino acid analysis of the carboxypeptidase Y digest of the mating factor suggested that the two residues of glutamic acid existed as glutamine, as shown in Table IV. In our present chromatographic system, the peak position of glutamine on the analysis chart overlapped the threonine peak. We calculated the amount of glutamine from the peak area corresponding to threonine, since no threonine could be detected in the acid hydrolysate (Table III). We

TABLE IV. Amino acid composition of the carboxypeptidase Y digest of the mating factor. The sample was digested with carboxypeptidase Y at 40°C for 24 h at a ratio of the enzyme to substrate of 1 : 500 (by weight).

Amino acid	g residue per 100 g peptide	Molar ratio
Lysine	8.4	1.03
Histidine	4.0	0.46
Tryptophan	9.4	0.79
Glutamine	13.9	1.67
Proline	11.1	1.81
Glycine	3.6	1.00
Methionine	4.0	0.96
Leucine	12.6	1.76
Tyrosine	10.6	1.03
Total	77.6	

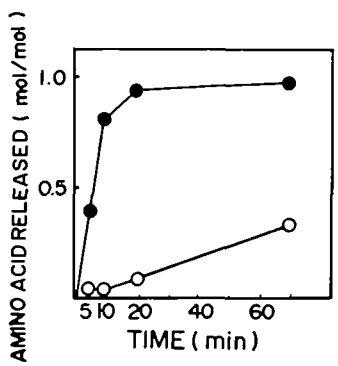

Fig. 3. Rate of release of amino acids from the mating factor with carboxypeptidase A. Digestion was carried out at 40°C at a substrate-to-enzyme ratio of 300. ●, Tyrosine; ○, methionine.

concluded from these results that the mating factor was a tridecapeptide containing Lys_1, His_1, Trp_2, Gln_2, Pro_2, Gly_1, Met_1, Leu_2, and Tyr_1; its molecular weight was calculated to be 1,684.

Amino Acid Sequence of Mating Factor—The amino acid sequence of the mating factor (0.3 μmol) was elucidated from its N-terminus by the manual Edman degradation method up to the 9th residue as Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-. The digestion of the factor with carboxypeptidase A suggested C-terminal -Met-Tyr, as shown in Fig. 3.

To determine the complete amino acid sequence of the mating factor, it was digested with thermolysin. Three peptides (Th-1, Th-2 and Th-3) could be separated from the digest by TLC on a cellulose-glass plate developed with 1-butanol: pyridine: acetic acid: water (see Table V). A part of the cellulose powder containing the peptide was extracted with 10% acetic acid. The yields and amino acid compositions of the three peptide are listed in Table V; together with their N-terminal residues.

TABLE V. Amino acid composition of thermolytic peptides derived from the mating factor. The sample was hydrolyzed with 5.8 M HCl containing 0.5% thioglycolic acid at 105°C for 20 h. Results are expressed in molar ratios.

Amino acid	Th-1	Th-2	Th-3
Lysine		1.03 (1)	
Histidine	1.00 (1)		
Tryptophan	1.87 (2)		
Glutamic acid		1.01 (1)	0.94 (1)
Proline		2.16 (2)	
Glycine		1.02 (1)	
Methionine		0.77 (1)	
Leucine		1.00 (1)	1.00 (1)
Tyrosine		0.79 (1)	
Total number of residues	3	8	3
R_f[a]	0.66	0.62	0.54
Re[b]	0.10	0.27	0.09
Yield (%)	58	63	47
N-terminus[c]	Trp	Leu	Leu

[a] Determined by TLC on a cellulose-glass plate with 1-butanol : pyridine : acetic acid : water (15 : 10 : 3 : 12, by volume). [b] Relative mobility in paper electrophoresis at pH 6.4 (pyridine : acetic acid : water = 10 : 0.4 : 180, by volume) as compared with lysine. [c] Determined by the Edman degradation method.

The following amino acid sequences were deduced by the manual Edman degradation method for the three peptides:

Th-1: Trp-His-Trp
↘ ↘ ↘

Th-2: Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr
↘ ↘ ↘ ↘ ↘ ↘ ↘ ↘

Th-3: Leu-Gln
↘ ↘