Isolation and amino acid sequence of a mating pheromone produced by mating type α cells of *Saccharomyces exiguus*

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Received 23 April 1986; revised version received 9 June 1986

A peptide, termed α^{se} pheromone, was isolated as a mating pheromone from culture filtrate of mating type α cells of *Saccharomyces exiguus*. The peptide showed both agglutinability-inducing activity to *a* cells of *S. cerevisiae* and shmoo-inducing action to *a* cells of *S. cerevisiae*, *S. kluyveri* and *S. exiguus*. The amino acid sequence of α^{se} pheromone was determined as H-Trp-His-Trp-Leu-Arg-Leu-Ser-Tyr-Gly-Gln-Pro-Ile-Tyr-OH by mass spectrometry, sequence analysis and enzymatic digestion.

(Saccharomyces exiguus) *Mating pheromone* α^{se} *pheromone* *Amino acid sequence*

1. INTRODUCTION

The mating process of heterothallic *Saccharomyces* yeast progresses through interactions between cells of the opposite mating types *a* and α. Mating pheromones produced by each mating type cell act on cells of the opposite mating type to regulate the mating process. Oligopeptides, α^{sc} pheromones (α factors, α substance-I_A) of *S. cerevisiae* [1,2] and α^{sk} pheromones of *S. kluyveri* [3,4] have been characterized as mating pheromones excreted from α cells. These peptides have similar structures in their amino acid sequences and show cross-action between the two species, i.e. both α^{sc} pheromones and α^{sk} pheromones act on each *a* cell of *S. cerevisiae* and *S. kluyveri* to bring about formation of large pear-shaped cells (shmooing).

We isolated the two heterothallic strains, THE1-16C and THE1-16B having opposite mating types from the homothallic diploid strain of *S. exiguus* Yp74L-3 through a mutation at a homothallism-controlling gene by ethylmethanesulfonate (EMS) treatment [5]. The strain Yp74L-3, which had been isolated from partially rotted leaves in Yakushima island (Japan) in 1970, was identified as *S. exiguus* in 1975 and has been deposited in the Institute for Fermentation, Osaka, as IFO accession no.1081. THE1-16B produced a pheromone inducing shmooing and/or sexual agglutinability in *a* cells of *S. cerevisiae* and *S. kluyveri*, and THE1-16C cells, indicating that THE1-16C and THE1-16B are *a* and α strains, respectively [5]. The α-mating pheromone produced by THE1-16B of *S. exiguus* was named α^{se} pheromone. Here, we describe the isolation, biological activity and amino acid sequence of α^{se} pheromone determined as shown in fig.1.

Abbreviations: HPLC, high-performance liquid chromatography; SIMS, secondary ion mass spectrometry

2. MATERIALS AND METHODS

α cells of *S. exiguus* (THE1-16B) were precultured in VHG-BII medium whose glucose content was reduced to 2% [6] by shaking at 27°C for 22.5 h. The pre-culture (400 ml) was transferred to a jar fermentor containing 18 l MMT medium [7].

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00145793/86/$3.50

The cells were cultured at 27°C with aeration (18 l/min) and agitation (200 rpm) for 23 h. The 4 batches of cultures were pooled and filtered through Celite 545, and the culture filtrate (70 l) subjected to purification of the α^{se} pheromone.

Biological activity of the pheromones inducing sexual agglutinability was investigated using strains of *S. cerevisiae* T55s-41 (inducible α strain) and T56 (constitutive α strain) as in [8]. Induction of shmoo by the pheromone was examined with the α strain of *S. exiguus* (THE1-16C), *S. cerevisiae* (T55) [8] and *S. kluyveri* (IFO 1893) [9] in YGH medium [8]. These biological tests were performed at 28°C using a reciprocating shaker.

Analytical HPLCs were undertaken on a column of Nucleosil 5C18 (6 × 150 mm) with the solvent systems described in each case at a flow rate of 1 ml/min, and the peaks detected by UV absorption at 220 nm.

Amino acid compositions were determined by acid hydrolysis of each peptide (2–5 nM) with 5.7 N HCl containing 3% thioglycolic acid in an evacuated tube at 110°C for 20 h, followed by amino acid analysis on a Hitachi 835 amino acid analyzer.

Mass spectrometric analysis by SIMS was performed on a Hitachi M80 spectrometer as in [3].

Sequence analysis was performed on a Beckman 8900 protein/peptide sequencer using 3 nM peptide. For digestion with enzyme, the peptide was incubated with TPCK trypsin (Worthington) in 0.05 M ammonium acetate buffer (pH 6.5) at 27°C for 19 h at an enzyme:substrate ratio of 1:20.

3. RESULTS

3.1. *Isolation*

Culture filtrates of α cells of *S. exiguus* showed pheromone activity to induce sexual agglutinability in inducible *a* cells of *S. cerevisiae*. The active principle in the culture filtrate was purified according to the procedure outlined in fig.1. The active peptide thus obtained showed a peak at t_R 23.0 min by analytical HPLC with a solvent system of acetonitrile-0.1% trifluoroacetic acid (29:71). By the same HPLC analyses, α^{sc1} pheromone (α factor, α_1) showed a peak at t_R 16.4 min and α^{sk2} pheromone at t_R 17.1 min. Therefore, the peptide was found to be different from any of the known α-mating pheromones and was termed α^{se} pheromone, which was obtained in a yield of around 0.1 mg from 1 l culture filtrate.

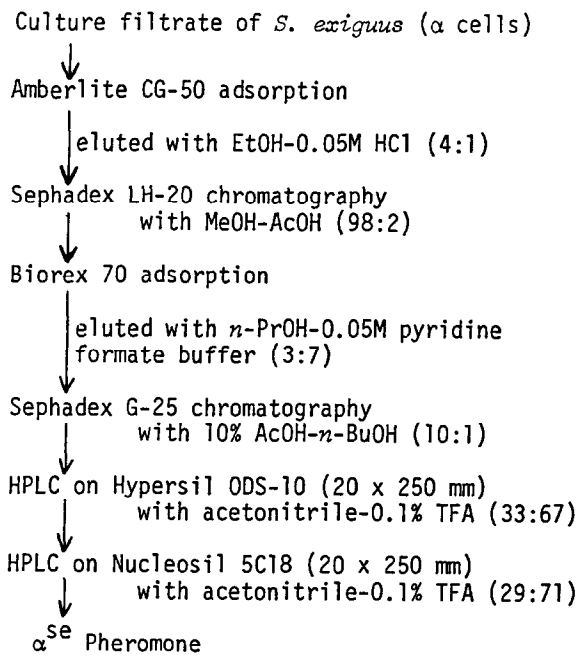

Fig.1. Isolation procedure of α^{se} pheromone.

3.2. *Biological activity*

The purified α^{se} pheromone induced sexual agglutinability of the inducible *a* strain of *S. cerevisiae* (T55s-41). Further, the pheromone caused shmooing of *a* cells not only in *S. exiguus* but also in *S. cerevisiae* and *S. kluyveri* as shown in table 1.

3.3. *Amino acid sequence*

On amino acid analysis of the acid hydrolysate the following amino acids (molar ratio to Leu = 2) were detected in α^{se} pheromone: Ser (0.9), Glu (1.0), Pro (0.9), Gly (1.0), Ile (0.9), Leu (2.0), Tyr (1.9), His (0.9), Trp (1.5) and Arg (0.9). Mass spectrometric analysis by SIMS gave a quasimolecular ion at m/z 1718. This value corresponded to the molecular mass of a peptide composed of 1 Ser, 1 Gln, 1 Pro, 1 Gly, 1 Ile, 2 Leu, 2 Tyr, 1 His, 2 Trp and 1 Arg with free N- and C-termini. Thus, α^{se} pheromone was confirmed as being composed of the above 13 amino acids.

Table 1

Biological activity of α mating pheromone

	Induction of sexual agglutinability in inducible *a* strain of *S. cerevisiae*	Induction of shmoo formation in *a* cells of		
		S. cerevisiae	*S. kluyveri*	*S. exiguus*
α^{se} pheromone	1 ng/ml	5 μg/ml	1 μg/ml	50 μg/ml

Minimum concentrations to show biological activity are indicated

Table 2

Sequence analysis of α^{se} pheromone

	Degradation step												
	1	2	3	4	5	6	7	8	9	10	11	12	13
PTH-amino acid identified	Trp	His	Trp	Leu	Arg	Leu	Ser[a]	Tyr	Gly	Gln	Pro	Ile	Tyr

[a] Dehydro Ser was detected by monitoring UV absorption at 319 nm

PTH amino acid from each cycle was identifed by HPLC on an Ultrasphere ODS column (4.6 × 250 mm) with a solvent of trifluoroacetate-acetate [10] modified as follows. Buffer A: a solution containing 18.3 mM trifluoroacetic acid and 0.4 mM acetic acid was adjusted to pH 6.2 with 1 M NaOH, mixed with acetonitrile (90:10, v/v). Buffer B: a solution of 13.8 mM trifluoroacetic acid was adjusted to pH 3.5 with 1 M NaOH, mixed with acetonitrile (25:75, v/v). Buffers A and B were mixed in a gradient program from 99:1 to 7:93 in 9 min and reversed to 99:1 in 3 min at a flow rate of 1.2 ml/min at 50°C. UV absorption at 269 nm was monitored

Sequence analysis was carried out with a protein sequencer and the resulting PTH-amino acids were unambiguously identified as shown in table 2.

Digestion of the peptide with trypsin gave two peptide fragments, Tr-1 and Tr-2. Each peptide fragment was purified by analytical HPLC with a solvent system of acetonitrile-0.1% trifluoroacetic acid (gradient programmed from 20:80 to 35:65 in 20 min and held at 35:65 for 10 min). The peaks of Tr-2 and Tr-1 appeared at t_R 17.8 and 25.3 min, respectively. The amino acid composition was determined by amino acid analysis of the acid hydrolysate of each peptide. Tr-1 was composed of 1 Leu, 1 His, 2 Trp and 1 Arg, whereas Tr-2 of 1 Ser, 1 Glu, 1 Pro, 1 Gly, 1 Ile, 1 Leu and 2 Tyr. These compositions were well explained by the cleavage at the arginine residue of the sequence deduced from sequence analysis. Therefore, the amino acid sequence of α^{se} pheromone was determined as shown in table 2 and also in fig.2.

4. DISCUSSION

α^{se} pheromone was characterized as the third α-mating pheromones of *Saccharomyces* yeast and confirmed to have pheromone activity to any *a* cells of the three species *S. cerevisiae, S. kluyveri* and *S. exiguus*. Since the other two pheromones, α^{sc} pheromones and α^{sk} pheromones, showed cross-action between *a* cells of *S. cerevisiae* and *S. kluyveri* [3], these three pheromones have the same biological action. α^{se} pheromone obtained in this study appears to be different in amino acid composition, since it contains arginine and isoleucine instead of lysine and methionine which are usually contained in the other two pheromones. However, in the primary structures of these three peptides, close structural similarities exist at the N-terminal four residues and at the C-terminal five residues as shown in fig.2. These two parts of structural similarities in α-mating pheromones of *Sac-*

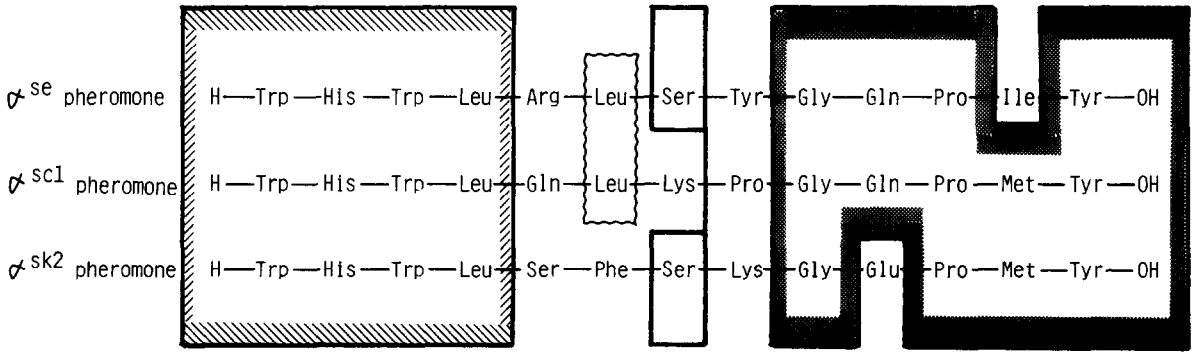

Fig.2. Similarities in primary structures of α-mating pheromones of *Saccharomyces*. α^{se} pheromone of *S. exiguus*, α^{scl} pheromone of *S. cerevisiae*, α^{sk2} pheromone of *S. kluyveri*.

charomyces became distinct through characterization of α^{se} pheromone. This may indicate that the amino acid sequence of four residues at the N-terminal part and five residues of the C-terminal part is important for biological activity to be exhibited.

ACKNOWLEDGEMENTS

We would like to thank Dr Y. Narahashi of this institute for her kind help in amino acid sequence analysis and Mr M. Chijimatsu of this institute for amino acid analyses. Part of this work was supported by Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

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