Synthesis of the Mating Factor of Saccharomyces cerevisiae and its truncated peptides : The Structure-Activity Relationship

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SUMMARY : The mating factor of Saccharomyces cerevisiae, Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr, has been synthesized to confirm the structure of the natural mating factor. The tridecapeptide has the same biological activity as the natural mating factor. From the studies on the biological activity of its truncated peptide synthesized the minimum sequence of the peptide require for the mating factor was deduced to be as His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln.

INTRODUCTION : In 1956, Levi (1) recognized first the presence of a chemical factor in a culture fluid of the α-mating type cells of Saccharomyces cerevisiae, which is involved in the mating process between α- and a-mating type cells of Saccharomyces cerevisiae. Recently, Stötzler et al. (2) indicated that four different peptides were responsible to the same biological function, and they proposed the structure of two major components among them as below :

Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr (I)

His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr (II)

Independently, Tanaka et al. (3) isolated a mating factor from the culture fluid of α-mating type cells which had the same sequence as the peptide I. They could not recognize the presence of the peptide II in their culture fluid. Instead, they isolated two inactive fragments of the peptide I which must have arisen by cleavage of the peptide bond between Leu-6 and Lys-7, and a longer peptide which was considered to be a precursor peptide of the mating factor.

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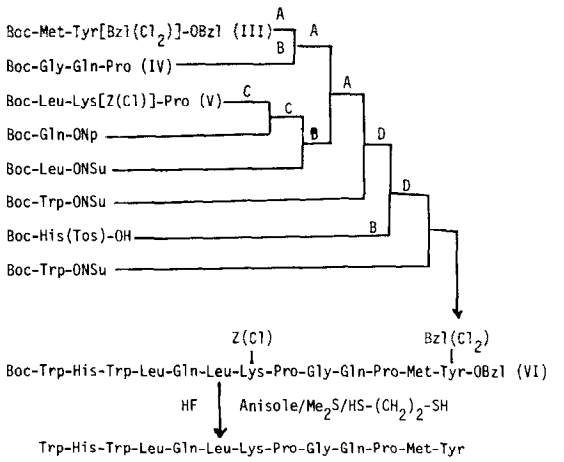

Fig. 1. Synthesis of a Mating Factor of Saccharomyces cerevisiae

A : Trifluoroacetic acid treatment in a presence of Me_2S

B : 1-Ethyl-3-(3-Dimethylaminoprophyl)-Carbodiimide treatment in a presence of 1-Hydroxybenzotriazole

C : Trifluoroacetic acid treatment

D : Trifluoroacetic acid treatment in a presence of Me_2S and $HS-(CH_2)_2-SH$

The synthesis of the tridecapeptide I was undertaken in order to confirm the structure of the natural mating factor by comparing the biological activities and the chromatographic behavior of the two. Furthermore, several truncated peptides were synthesized and the minimum sequence of the peptide required for the mating factor activity was elucidated.

MATERIALS and METHODS : Biological activity of the mating factor synthesized chemically and its truncated peptides was determined by incubating Saccharomyces cerevisiae X-2180 1A, a-mating type cell, at 27°C for 5 hr in the presence of different amounts of each peptide. The activity was expressed as the minimum amount of the peptide necessary for the induction of cellular deformation on the test organisms.

RESULTS and DISCUSSION

Synthesis of Peptides : Synthesis of the tridecapeptide has been carried out by the conventional solution procedure as shown in Fig. 1. Boc-amino

Abbreviations
Boc, t-butyloxycarbonyl ; OBzl, benzyl ester ; $Bzl(Cl_2)$, 2,6-dichlorobenzyl ; Z(Cl), 2-chlorobenzyloxycarbonyl; ONp, p-nitrophenyl ester ; ONSu, succinimido ester ; Tos, tosyl ; All amino acids are L-configuration.

acids which are commonly used for solid phase synthesis were utilized for the synthesis, in which the side-chain functional groups were protected by the stabilized protective groups such as 2,6-dichlorobenzyl for Tyr (4), 2-chlorobenzyloxycarbonyl for Lys (5), and tosyl for His (6). Fragments IV and V were synthesized by the stepwise addition of each amino acid starting from proline benzyl ester. The benzyl ester group in the precursor of compound IV was removed by catalytic hydrogenolysis and that in the precursor of compound V was removed by saponification.

In each removal reaction of the Boc-group of the intermediates by trifluoroacetic acid, the following scavengers were added : Dimethylsulfide (10 eq.) for Met-containing peptide and ethanedithiol (10 eq.) together with dimethyl sulfide (20 eq.) for Trp-containing peptide. The final protected tridecapeptide (VI), which showed mp 175°C (decomp.) and $[\alpha]_D^{25}$-27.3° (c 0.5, DMF), was treated by anhydrous hydrogen fluoride (7) in a HF-reaction apparatus (8) at 0°C for one hour in the presence of anisole, dimethly sulfide, and ethanedithiol as the scavengers. After removal of the excess HF by vaccum distillation, below 0°C, the residue was washed well with ether. The residue was redissolved in 2 N acetic acid, and insoluble material was removed by filtration. The filtrate was passed through a column of Dowex 1 x 2 (AcO^- Form) to remove the bound HF, and the effluent was lyophilized. The crude lyophilizate was purified by gel filtration on Sephadex LH-20 using 2 N acetic acid as a solvent. The main fraction was purified further by partition chromatography on Sephadex G-25 using a mixture of n-butanol-acetic acid-water (4 : 1 : 5 v/v/v). Fractions covering the main peak were combined and concentrated under reduced pressure, followed by lyophilization from 1 N acetic acid. The analytical and biological data of the final product are shown in Table 1. The synthetic tridecapeptide had the same mating factor activity as the natural product. Homogeneity of the peptides synthesized was confirmed by thin layer chromatography, and the Rf value of the tridecapeptide was found to be identical with that of the natural mating factor as shown in Table 1.

Table 1. Analytical Data and Biological Activities of the Synthetic Peptides

Peptides	Optical Rotation $[\alpha]_D^{25}$ (c 0.5,AcOH)	Thin Layer Chromatogr.** Rf^1	Rf^2	Biological Activity
Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr*	-35.6°	0.59	0.74	6×10^{-6} µg/ml
His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr	-38.4°	0.57	0.72	6×10^{-6}
Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr	-43.3°	0.68	0.77	500
Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr	-42.3°	0.55	0.69	500
Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met	-40.0°	0.51	0.63	7.8×10^{-3}
His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met	-33.5°	0.54	0.61	100×10^{-3}
Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro	-29.8°	0.49	0.58	4
Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln	-20.5°	0.49	0.54	6
His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln	-39.0°	0.41	0.47	6
Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln	-30.8°	0.54	0.69	500
Leu-Gln-Leu-Lys-Pro-Gly-Gln	-38.6°	0.42	0.54	500
Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly	-29.8°	0.53	0.57	500
His-Trp-Leu-Gln-Leu-Lys-Pro-Gly	-48.5°	0.50	0.54	500

*) Elemental Anal. Found : C,54.18 ; H,6.94 ; N,14.91%. Calcd for $C_{82}H_{114}O_{17}N_{20}S \cdot 2AcOH \cdot 6H_2O$; C, 54.02 ; H,7.06 ; N,14.65%
The molar ratio of amino acid was determined after hydrolysis of this material at 108°C for 24 hr, using 6 N hydrochloric acid in the presence of 2% thioglycolic acid ; Lys,1.07 ; His,0.95 ; Trp,0.95x2 ; Glu,1.00x2 ; Pro,0.95x2 ; Gly,1.00 ; Met,0.93 ; Leu,1.03x2 ; Tyr,0.98.

**) Developed on Cellulose plate (Merck Art 5552)
Rf^1 : 1-BuOH : AcOH : H_2O (4 : 1 : 5 v/v/v) Upper Phase
Rf^2 : 1-BuOH : AcOH : Pyridine : H_2O (15 : 3 : 10 : 12 v/v/v/v)

Mating Factor Activity of Truncated Peptides : Several truncated peptides were synthesized similary, and their biological activities were assayed in order to know the minimum sequence of the peptide which expresses the mating factor activity. Structure of those peptides synthesized, their Rf values on thin layer chromatography, their optical rotation values and their biological activities were shown in Table 1. As is obvious from Table 1, the dodecapeptide devoid of the N-terminal Trp residue is equally active as the natural tridecapeptide though the undecapeptide lacking the N-terminal Trp-His residues of the natural mating factor was virtually inactive.

When the C-terminal Tyr residue was removed, the biological activity of the dodecapeptide decreased dramatically. Further removal of the amino acid residues from the C-terminus up to Pro-11 produced no decrease in activity. However, the loss of Gln-10 gave an inactive peptide as a mating factor. From these results the minimum sequence of the peptide required for the mating factor activity can be deduced as His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln.

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