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Amino Acid Substitution of Mating Factor of Saccharomyces cerevisiae: Structure-Activity Relationship

Yoshihiro Masui*, Takaharu Tanaka**, Naoyoshi Chino*
Hiroshi Kita** and Shumpei Sakakibara*

*The Peptide Institute, Protein Research Foundation
Minoh, Osaka 562, Japan

**Central Research Institute, Suntory Ltd.
Shimamotocho, Mishimagun, Osaka 618, Japan

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Summary : Analogs of the mating factor of Saccharomyces cerevisiae, Trp[1]-His[2]-Trp[3]-Leu-Gln-Leu[6]-Lys[7]-Pro[8]-Gly-Gln-Pro[11]-Met[12]-Tyr[13], from which amino acids were eliminated or substituted for other amino acid, were synthesized. These analogs showed lower biological activity than the natural mating factor if assayed after 6 hours incubation with a-mating type cells of S. cerevisiae. However, if assayed after 24 or 48 hours incubation, the situation changed, i.e. the analogs in which Leu[6] or Lys[7] were replaced by the corresponding D-isomer, showed higher mating factor activity than the unsubstituted mating factor. The same result was obtained with the analogs in which Met was replaced by norleucine.

INTRODUCTION

The mating factor of Saccharomyces cerevisiae is a peptide produced by α-mating type cells, which is indispensable to the mating process between α- and a-mating type cells (1). Its amino acid sequence was determined to be Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr (2, 3, 4) and was confirmed by the chemical synthesis of the peptide (3, 6).

As was reported in our previous paper (5), the mating factor was degraded by both α- and a-mating type cells of S. cerevisiae into a hexa- and a hepta-peptides by cleavage of a peptide bond between Leu[6] and Lys[7], both of which were inactive as mating factor. Besides, during the course of our studies to determine the minimum sequence of the peptide required for the mating factor activity (6), the changes of biological activity due to the sequential elimination of amino acid residues from either amino- or carboxyl-

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terminal ends of the mating factor peptide differed dramatically. The observations stimulated our interest to explore the effects of amino acid substitution on the mating factor activity.

The present paper describes the properties of chemically synthesized analogs of the mating factor of S. cerevisiae along with their biological activities.

MATERIALS and METHODS

Biological activity of the mating factor analogs was assayed by incubating S. cerevisiae X2180 1A, a-mating type cell, at 27°C for 6, 24 and 48 hours in the presence of the peptide to be examined (4). The activity was expressed as the minimum amount of a peptide necessary to induce the morphological changes in the test organisms.

Syntheses of peptides were carried out according to the conventional solution procedure under the conditions described in the previous paper (6).

RESULTS and DISCUSSION

Analytical Properties of Analogs of Mating Factor ——— A large number of mating factor analogs of S. cerevisiae were synthesized chemically to examine the effect of substituting amino acid residues in the mating factor by other amino acids on the biological activity. Homogeneity of each peptide synthesized was checked by thin layer chromatography, amino acid analysis and element analysis. The values of optical rotation and Rf on cellulose plates were summarized in Table I.

Substitution of Trp^1 of Mating Factor ——— As the dodecapeptide devoid of N-terminal Trp residue was as active as the natural tridecapeptide (6), the substitution of Trp^1 by other amino acids was anticipated to have little effect on its biological activity. Substitution by phenylalanine and sarcosine gave slightly less active peptides, but replacement by D-tryptophan, tyrosine or leucine reduced its activity to 1/1,000 – 1/10,000 of that of the natural mating factor. Replacement with lysine led to a complete loss of the activity (Table IA).

Substitution of His^2 of Mating Factor ——— Substitution of His^2 by any other amino acid reduced its activity to 1/100,000 – 1/1,000,000 of its original activity (Table IB). Furthermore, the replacement of His^2 by D-histidine

Table I. Properties and Biological Activity of Mating Factor Analogs

Amino Acid Replacement*	Optical Rotation $[\alpha]_D^{25}$ (c=0.5, AcOH)	Rf** A	B	C	Biological Activity*** pg/ml
Natural Mating Factor	-35.6°	0.59	0.74		6
A. Trp^1 was substituted by					
[Phe^1]	-36.1°	0.56	0.73		7 x 10
[Sar^1]	-47.0°	0.46	0.65		1.3 x 10^2
[Leu^1]	-38.5°	0.62	0.76		3 x 10^3
[Tyr^1]	-36.1°	0.59	0.73		1.5 x 10^4
[Trp^1]	-48.0°	0.59	0.75		8 x 10^4
Bz des Trp^1	-52.5°	0.64	0.82		4 x 10^5
[Lys^1]	-35.5°	0.46	0.61		3 x 10^8
B. His^2 was substituted by					
[Lys^2]	-36.5°			0.42	2.5 x 10^5
[Phe^2]	-35.9°			0.61	4 x 10^5
[Leu^2]	-43.0°			0.67	9 x 10^6
[D-His^2]	-39.8	0.69	0.81		3 x 10^8
C. Trp^3 was substituted by					
[Ala^3]	-41.6°	0.55	0.69		1 x 10^2
[Phe^3]	-42.7°	0.65	0.74		1.4 x 10^2
[Leu^3]	-45.1°	0.59	0.74		2.3 x 10^2
[Tyr^3]	-34.6°	0.53	0.73		8 x 10^2
[Lys^3]	-38.2°	0.49	0.64		2.1 x 10^4
[Gly^3]	-37.2°	0.53	0.64		2.9 x 10^5
[Asp^3]	-40.9°	0.51	0.62		5 x 10^5
des Trp^3	-35.4°	0.57	0.73		5 x 10^8
D. Pro^8, Pro^{11}, Met^{12} and Tyr^{13} were substituted by					
[$Nleu^{12}$]	-38.1°	0.64	0.78		2 x 10^2
[D-Tyr^{13}]	-37.3°	0.60	0.74		1.5 x 10^4
[Gly^{11}]	-18.5°	0.57	0.74		1.5 x 10^4
[Gly^8]	-25.7°	0.55	0.72		2 x 10^4
[Phe^{13}]	-36.2°	0.64	0.69		3 x 10^4
[Tyr-NH_2]	-43.0°	0.59	0.74		6 x 10^4
[Val^{12}]	-41.8°	0.63	0.77		1 x 10^5
E. Leu^6 and Lys^7 were substituted by					
[D-Leu^6]	-27.0°	0.60	0.76		1.5 x 10^4
des Trp^1 [D-Leu^6]	-37.0°	0.59	0.74		4 x 10^8
[D-Lys^7]	-26.9°	0.60	0.75		1.1 x 10^5
des Trp^1 [D-Lys^7]	-36.1°	0.57	0.68		3 x 10^8
[D-Leu^6, D-Lys^7]	-21.4°	0.59	0.72		2.4 x 10^6

* Trp^1-His^2-Trp^3-Leu-Gln-Leu^6-Lys^7-Pro^8-Gly-Gln-Pro^{11}-Met^{12}-Tyr^{13}

**A 1-BuOH:AcOH:H_2O (4:1:5, upper phase)
B 1-BuOH:AcOH:Pyridine:H_2O (15:3:10:12)
C 1-BuOH:AcOH:H_2O:n-Hexane (4:1:5:0.5, upper phase)

*** Minimum amount needed to induce the morphological changes in a-mating type cells.

gave an inactive peptide. These results suggest strongly that His^2 of the mating factor constitutes an essential part of the peptide needed for mating factor activity. This conclusion is further supported by the evidence that, though the des Trp dodecapeptide was fully active as mating factor, the removal of His^2 of the peptide resulted a complete loss of the activity (6).

Substitution of Trp^3 of Mating Factor ——— The result of substituting Trp^3 can be divided into two groups according to the effect of replacement of Trp^3 with other amino acids on biological activity. Namely, those carrying hydrophobic side chains and others carrying hydrophilic side chains. The substitution by amino acids of the former group reduced its activity slightly, but replacement by amino acids of the latter group yielded peptides with extremely low activity (Table IC). As the dodecapeptide having an identical amino acid sequence with the mating factor except for the absence of Trp^3 showed no activity, the sequence of His-Trp, not Trp-His, and the hydrophobicity of the tryptophan could be essential for activity.

Substitution of Pro^8, Pro^{11}, Met^{12} and Tyr^{13} of Mating Factor ——— Replacement of Pro^8 or Pro^{11} with glycine gave peptides which showed 1/2,000 of the original activity. Substitution of Met^{12} by valine or norleucine lowered its activity to 1/16,000 and 1/15, respectively. Replacement of Tyr^{13} with D-tyrosine, phenylalanine and tyrosine amide also gave much less active peptides. These results are summarized in Table ID. From these results even the slight changes of conformation induced by the substitution of any one of the amino acid residues in the mating factor by others were reflected in the decrease of its biological activity.

Substitution of Leu^6 and Lys^7 of Mating Factor ——— As shown in Table IE, substitution of Leu^6 by D-leucine yielded a less active peptide. Combined replacement of either Tyr^{13} or Met^{12} of the peptide resulted in complete loss of activity. The same was true for the replacement of Lys^7 by D-lysine.

Though the dodecapeptide devoid of the N-terminal Trp residue was as active as the natural tridecapeptide, complete abolition of its biological

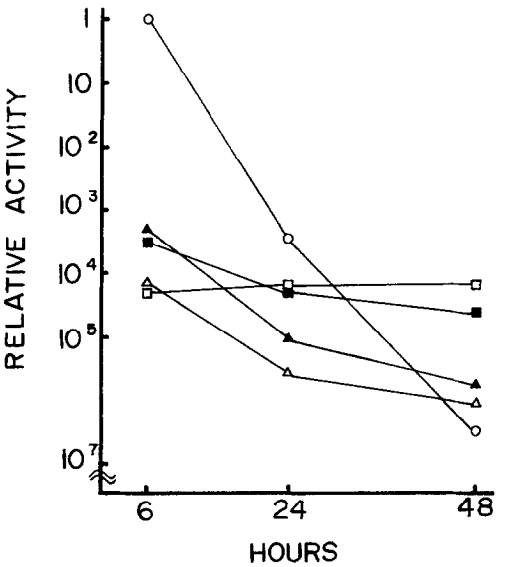

Fig. 1. Effect of Incubation on Mating Factor Activity. Relative activity was expressed as a ratio of minimum amount of each analog at the time of assay to the amount of natural mating factor needed at 6 hours incubation. ○, natural; ■, [D-Leu^6]; □, [D-Lys^7]; ▲, [D-Tyr^{13}]; and △, [D-trp^1] mating factors.

activity was observed when its Leu^6 or Lys^7 was substituted by D-leucine or D-lysine.

Effect of Amino Acid Substitution on the Degradation of Mating Factor —— The substitution of any amino acid residue in natural mating factor always gave a less active peptide when its activity was assayed after 6 hours incubation at 27°C (Table I). But the situation changed when peptides were assayed after 24 and 48 hours incubation. Since mating factor is degraded by the cleavage of the peptide bond between Leu^6 and Lys^7, the replacement of either one with their corresponding D-isomer gave peptides more resistant toward degradation by a-mating type cells. As shown in Fig. 1, 6 pg/ml of the natural mating factor is enough for expression of its biological activity after 6 hours incubation, but fairly rapid loss of its activity followed. In contrast, a peptide having D-lysine in place of Lys^7 lost no activity even

after 72 hours incubation, and, as a result, had more potent biological activity than the original mating factor when assayed after 24 hours incubation with the test organisms. The same conclusion could be drawn about the peptides in which D-leucine replaced Leu^6 or norleucine replaced Met^{12}, although these two peptides were degraded gradually.

The possibility that the low activity expressed by the mating factor analogs having D-amino acid in them is due to the contaminating natural mating factor derived from L-isomers of respective D-amino acid preparations could be excluded by competetion between the original and substituted mating factor (unpublished data). Analysis of the mode of function of these mating factor analogs is now in progress using the conditions described in the preceeding paper (7).

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