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REQUIREMENTS OF CHEMICAL STRUCTURE FOR HORMONAL ACTIVITY OF LIPOPEPTIDYL FACTORS INDUCING SEXUAL DIFFERENTIATION IN VEGETATIVE CELLS OF HETEROBASIDIOMYCETOUS YEASTS

Eiko Tsuchiya and Sakuzo Fukui

Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo

Yuji Kamiya

Institute of Physical and Chemical Research, Wako-shi, Saitama-ken

Yoji Sakagami

Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Tokyo

Masahiko Fujino

Central Research Division, Takeda Chemical Industries, Ltd., Yodogawa-ku, Osaka

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Biological activities of both peptidyl and isoprenyl constituents of yeast sex hormones (lipopeptides), rhodotorucine A and farnesyl tremerogen A-10, were examined. The chemically synthesized simple peptides (peptidyl constituents) and farnesyl cystein (isoprenyl one) did not exhibit any biological activities, such as hormonal and hormone-inhibitory (or -accelerative) activities. The mixture of both constituents also had no activity. Then, it is assumed that the heterobasidiomycetous yeasts (Rhodosporidium toruloides and Tremella mesenterica) recognize the total structure of lipopeptidyl molecule for the biological response, sexual differentiation, and the term "Pattern Recognition" is proposed for the mode of this recognition.

Recently, we isolated two yeast sex-hormones, rhodotorucine A and tremerogen A-10, from culture media of Rhodosporidium toruloides M-919 and Tremella mesenterica IFO-9310, respectively, and determined their chemical structures to be lipopeptides containing S-isoprenyl cystein at the C-terminus (1,2, 3,4,5). These compounds have the hormonal activity inducing sexual differentiation in the haploid cells of the selective strains of heterobasidiomycetous yeasts at a low concentration, 10^{-9} to 10^{-10} M (2,5,6).
In this report, we present some results on relationship between biological activity and chemical structure of the hormones, and the mode of hormone recognition by the cells of hormone-sensitive strain is discussed.

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Materials and Methods

Microorganisms: Rhodosporidium toruloides M-1057 (mating type a) (7) and Tremella mesenterica IFO-9313 (mating type ab) (6) were used as rhodotorucine A-sensitive and tremerogen A-10-sensitive strains, respectively. Yeast sex hormones (mating hormones) and their structure constituents: Rhodotorucine A used here was a purified preparation (2,8). Farnesyl tremerogen A-10, which contains S-farnesyl cystein as the C-terminus amino acid in tremerogen A-10, was chemically synthesized and purified by Fujino et al. (detail will be reported elsewhere), and then we used this synthetic hormone instead of a natural one, tremerogen A-10 (5). Farensyl tremerogen A-10 is functional at a concentration of 1 ng/ml (approximately 0.7 nM), and its Rf value in thin layer chromatography (TLC) on cellulose plate is 0.62 in a solvent system A (n-butanol, acetic acid, pyridine, water; 30:6:20:24, volume ratio). SH-rhodotorucine A and SH-tremerogen A-10, these are isoprenyl-free peptides, were also chemically synthesized and purified by Fujino et al. (detail will be reported elsewhere). Rf values of these simple peptides in TLC (cellulose plate) are as follows: SH-rhodotorucine A, 0.61; SH-tremerogen A-10, 0.35 in a solvent system A and SH-rhodotorucine A, 0.54; SH-tremerogen A-10, 0.37 in a solvent system B (ethylacetate, n-butanol, acetic acid, water; 1:1:1:1, volume ratio). S-farnesyl cystein was chemically synthesized by Kamiya et al. (detail will be reported elsewhere). For chemical S-farnesylation of both cystein and SH-tremerogen A-10, all-trans farnesyl bromide was employed. Chemical structures of mating hormones and their constituent-compounds used here are listed below.

Rhodotorucine A:
H-Tyr-Pro-Glu-Ile-Ser-Trp-Thr-Arg-Asn-Gly-Cys(S-farnesyl)-OH,
SH-rhodotorucine A:
H-Tyr-Pro-Glu-Ile-Ser-Trp-Thr-Arg-Asn-Gly-Cys(SH)-NH_2 and -OH,
tremerogen A-10:
H-Glu-His-Asp-Pro-Ser-Ala-Pro-Gly-Asn-Gly-Tyr-Cys(S-isoprenyl)-OR,
farnesyl tremerogen A-10:
H-Glu-His-Asp-Pro-Ser-Ala-Pro-Gly-Asn-Gly-Tyr-Cys(S-farnesyl)-NH_2,
SH-tremerogen A-10:
H-Glu-His-Asp-Pro-Ser-Ala-Pro-Gly-Asn-Gly-Tyr-Cys(SH)-NH_2,
S-farnesyl cystein:
Cys(S-farnesyl)-OH.
Chemical structures of isoprenyl and R in tremerogen A-10 are not confirmed yet.

Assay of hormonal activity: Hormonal activity was determined as the activity of mating tube formation, a parameter of sexual differentiation. An approximately 10^3 cells of Rh. toruloides M-1057 or Tr. mesenterica IFO-9313 were previously adhered onto a cover glass by the method of Abe et al. (9) and immersed into YS medium (yeast extract 1%, sucrose 1% and NaCl 0.25%)containing the indicated amount of the hormone or/and its constituent(s), followed by incubation at 27°C for 5 hr (assay incubation). The cells adhered on a cover glass were not removed during the incubation. After the incubation, number of mating tube-formed cells was counted under a microscope. Activity was presented as the percentage of mating tube-formed cells per total cells. Sometimes, prior to the assay incubation, the cells adhered were incubated with the constituent compound for 10 min at room temperature (preincubation).

Results and Discussion

It is well-known that polypeptidyl hormones produced by mammalian system are simple peptides, except some peptidyl hormones containing oligo-saccharide as minor constituent. Recently, chemical structure of α factor, which had

been isolated as a mating hormone secreted by Saccharomyces cerevisiae (mating type α), an ascomycetous yeast, was also confirmed to be a simple peptide, H-Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-Gln-Pro-Met-Tyr-OH (10,11). These findings suggest that the essential structure for hormonal function is a peptidyl structure. However, as described above, both rhodotorucine A and tremerogen A-10 are the lipopeptides containing S-isoprenyl cystein, hydrophobic amino acid, at the C-terminus. In order to clarify the requirement of chemical structure in these lipopeptide-molecules for hormonal activity, the mating tube-forming activity of two isoprenyl-free peptides, SH-rhodotorucine A and SH-tremerogen A-10, was determined. As seen in Table I, these peptides did not exhibit any hormonal activity at a concentration range of 1 ng to 10 μg/ml.

If the SH-peptides can bind to the mating hormone-receptor site(s), which locates on cell surface and is specifically required for induction of mating tube formation, the peptides should behave as competitive inhibitor of the hormone. In the assay incubation mixture containing both mating hormone and its SH-peptide even at respective concentrations of 10 ng and 10 μg/ml, any inhibition of mating tube formation was not observed (Table I). Thus, it is concluded that SH-peptide did not bind to the functional receptor site(s) for the mating hormone.

The biological activity of S-farnesyl cystein, a lipophilic part of the hormone molecule, was also examined. However, the lipophilic constituent did not show any activity as mating hormone or as hormone-inhibitor (or -accelerator). This finding indicates that farnesyl cystein also does not compete with the mating hormones to the functional receptor site(s).

In general sense, the lipophilic constituent may have a function to modify the membrane structure to facilitate the transport of extracellular information substance into the cells. Then, the effect of preincubation of assay cell with farnesol (Tokyo Kasei Chemicals, Tokyo) or S-farnesyl cystein on the cell response to SH-peptides was examined. To the farnesol- or S-

Table I Biological Activity of Mating Hormones and Their Constituents

Compounds in Assay Incubation Mixture	Mating Tube Formation,%	
	Rhodosporidium toruloides M-1057	Tremella mesenterica IFO-9313
Rhodotorucine A, 2 units/ml	60	0
SH-rhodotorucine A, 10 ng -10 μg/ml	0	0
Tremerogen A-10, 2 ng/ml	0	80
Farnesyl tremerogen A-10, 2 ng/ml	0	80
SH-tremerogen A-10, 10 ng -10 μg/ml	0	0
Farnesyl cystein, 10 ng -10 μg/ml	0	0
Farnesol, 10 ng/ml	0	0
Rhodotorucine A, 2 units/ml + SH-rhodotorucine A, 10 ng -10 μg/ml	60	0
Rhodotorucine A, 2 units/ml + Farnesyl cystein, 10 ng -10 μg/ml	70	0
SH-rhodotorucine A, 10 μg/ml + Farnesyl cystein, 10 μg/ml	0	0
Farnesyl tremerogen A-10, 2 ng/ml + SH-tremerogen A-10, 10 μg/ml	0	80
Farnesyl tremerogen A-10, 2 ng/ml + farnesyl cystein, 10 μg/ml	0	80
Rhodotorucine A, 2 units/ml	60[a]	
Rhodotorucine A, 2 units/ml + SH-rhodotorucine A, 10 μg/ml	60[a]	
Rhodotorucine A, 2units/ml + farnesyl cystein, 10 μg/ml	70[a]	
SH-rhodotorucine A, 10 μg/ml + farnesyl cystein, 10 μg/ml	0[a]	

a, Preincubation (10 min) was performed with farnesol 10 μg/ml, farnesyl cystein 10 μg/ml or SH-rhodotorucine A 10 μg/ml.
By the assay incubation (5 hr) with farnesol 10 μg/ml, cell lysis was induced.
Farnesol 10 ng/ml did not show any effect on biological activity of mating hormones and their constituents.
Preincubation with farnesol, farnesyl cystein or SH-tremerogen A-10 did not affect on the biological activity of farnesyl tremerogen A-10.
SH-tremerogen A-10, 10 μg/ml + farnesol, 10 ng/ml (or farnesyl cystein, 10 μg/ml) gave no biological activity.

farnesyl cystein-preincubated cells, however, SH-peptides did not exhibit any biological activity.

Furthermore, it was found that Asn-Gly-Cys(S-farnesyl)-OH and Thr-Arg-Asn-Gly-Cys(S-farnesyl)-OH, these were derived from rhodotorucine A by treatment with trypsin and -chymotrypsin, respectively, have no hormonal activity (Kamiya et al., unpublished data).

Consequently, it is assumed that the yeast cells can recognize only the total structure of hormone molecule resulted from intramolecular restriction by peptidyl and S-farnesyl parts. We would like to propose the term "Pattern Recognitiom" for the mode of this typed-recognition. In order to justify this conception for the recognition and to present the strict requirement of chemical structure for hormonal activity, chemical syntheses of many kinds of model peptidyl-compounds are in progress.

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