

Physiological activities of peptides are correlated with the conformations of membrane-bound molecules

α -Mating factor from *Saccharomyces cerevisiae* and analog peptides

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α -Mating factor from *Saccharomyces cerevisiae* and analog peptides are found, by fluorescence analyses, to interact with phospholipid membrane. The circular dichroism analyses indicate that the physiological activities of α -mating factor and analog peptide are correlated with the conformations of the membrane-bound molecules rather than with the molecular conformations in aqueous solution. This suggests that the conformation change of peptide hormone upon binding with phospholipid membrane is an essential step for the recognition by the receptor.

α -Mating factor	<i>Saccharomyces cerevisiae</i>	Fluorescence	Circular dichroism
Membrane-bound conformation		Conformation-activity relationship	

1. INTRODUCTION

α -Mating factor from the α -mating type cell of *Saccharomyces cerevisiae* specifically inhibits the growth of the *a*-mating type cell by suppressing the initiation of DNA synthesis and induces morphological changes [1,2]. The primary structure of α -mating factor is:

Trp-His-Trp-Leu-Gln-Leu-Lys-Pro-Gly-
-Gln-Pro-Met-Tyr [3,4]

synthesized α -mating factor and various analog peptides have the single substitution of L-His² by D-His² accompanied with the complete loss of activity [5,6]. We had analyzed the ¹H and ¹³C NMR spectra of α -mating factor and analog peptides in aqueous solution and found that α -mating factor and active analogs partly take some ordered con-

formation even in aqueous solution [7]. Here, however, we have observed the effects of phospholipid-binding on the fluorescence and CD spectra of α -mating factor and analog peptides, and have found that the physiological activities of these peptides are correlated with the conformations of membrane-bound molecules rather than the conformations in solution.

2. MATERIALS AND METHODS

α -Mating factor and analog peptides were synthesized as in [5,6]. Egg yolk phosphatidylcholine and lysophosphatidylcholine were prepared as in [8]. L- α -Phosphatidyl-L-serine from bovine brain was purchased from Nakarai Chemicals and was purified with CM-52 cellulose column chromatography [9]. Small unilamellar vesicles of phosphatidylcholine-phosphatidylserine (1/1) (to be abbreviated as SUVCS) were prepared by the sonication (20 W) for 30 min at 0°C under nitrogen atmo-

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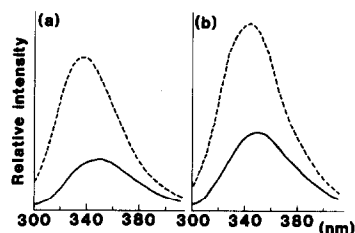


Fig. 1. The fluorescence spectra of (a) α -mating factor and (b) [D-His²]- α -mating factor (50 μ M, 23°C) in aqueous solution (pH 7.2, Tris-HCl buffer, 50 mM NaCl) (—) and in the presence of lysophosphatidylcholine (2.5 mM) (---). The fluorescence is excited at 288 nm.

sphere and subsequent centrifugation ($100\,000 \times g$) for 60 min. All the measurements were performed in 50 mM Tris-HCl buffer (pH 7.2, 50 mM NaCl) at peptide for CD spectra and 50 μ M for fluorescence spectra at 23°C. CD measurements were carried out with a JASCO J-40S spectrometer and fluorescence spectra were recorded on a Hitachi MPF-3 spectrophotometer.

3. RESULTS

3.1. Fluorescence

The fluorescence spectra (with the excitation at 288 nm) have been observed of α -mating factor and [D-His²]- α -mating factor (fig. 1). In the presence of the micelles of lysophosphatidylcholine, the fluorescence peak of tryptophan indole rings is blue-shifted by 13 nm for α -mating

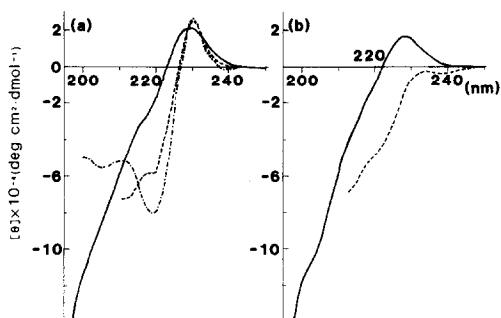


Fig. 2. The CD spectra of (a) α -mating factor and (b) [D-His²]- α -mating factor (100 μ M, 23°C) in aqueous solution (pH 7.2, Tris-HCl buffer, 50 mM NaCl) (—), in the presence of SUVCS (2.5 mM) (---), and in the presence of lysophosphatidylcholine (10 mM) (···).

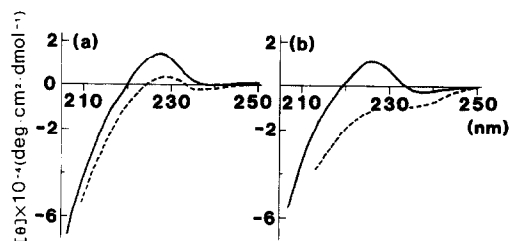


Fig. 3. The CD spectra of (a) [L-Ala³]- α -mating factor and (b) [Gly³]- α -mating factor (100 μ M, 23°C) in aqueous solution (pH 7.2, Tris-HCl buffer, 50 mM NaCl) (—) and in the presence of SUVCS (2.5 mM) (---).

factor and by 6.5 nm for [D-His²]- α -mating factor, with the intensity enhancements of 3.0-times and 2.4-times, respectively. Such blue-shifts and intensity enhancements are observed only above the critical micelles concentration of egg yolk lysophosphatidylcholine ($\sim 20 \mu$ M).

3.2. Circular dichroism

The CD spectrum of α -mating factor in aqueous solution changes drastically on addition of SUVCS (fig. 2a). The CD spectrum of α -mating factor in the presence of SUVCS is similar to that in the presence of the micelles of lysophosphatidylcholine (fig. 2a). The CD spectrum of [D-His²]- α -mating factor in aqueous solution also changes drastically on addition of SUVCS (fig. 2a). The CD spectra of the active [L-Ala³]-analog (with 6% activity relative to α -mating factor [6]) and the inactive [Gly³]-analog (with 0.002% activity [6]) are also shown in fig. 3.

4. DISCUSSION

The analysis of the fluorescence spectra of α -mating factor indicates that this peptide molecule is bound to the micelle, but not to the monomeric molecules, of lysophosphatidylcholine. The fluorescence blue-shift and intensity enhancement of the indole ring upon binding with the micelle indicate that Trp¹ and/or Trp³ residues of α -mating factor become involved in the hydrophobic environment of the micelle. For the inactive [D-His²]-analog peptide, however, the blue-shift and intensity enhancement upon binding with the micelles are significantly smaller than those for the active

α -mating factor (fig. 1a,b). This observation indicates that the indole rings of the membrane-bound molecule of the [D-His²]-analog are in a less hydrophobic environment than the α -mating factor.

The analyses of CD spectra of α -mating factor (fig. 2a) indicate that the conformation of this peptide is significantly affected upon binding with phospholipids. On addition of the micelles of lysophosphatidylcholine, the positive CD peak of α -mating factor is red-shifted by 3 nm and is enhanced in intensity. A similar spectral change is also observed of α -mating factor on binding with SUVCS.

The CD spectra of α -mating factor and the inactive [D-His²]-analog are remarkably similar in aqueous solution, but they are quite different from each other in the presence of SUVCS (figs. 2a,b). Thus, on addition of SUVCS, the positive CD peak of the [D-His²]-analog at 228 nm disappears, leaving a weak negative peak at around 238 nm (fig. 2b). These CD data indicate that the conformation of this inactive analog as bound to SUVCS is quite different from the membrane-bound conformation of α -mating factor.

In fact, this is further supported by the analyses of the CD spectra of the active [L-Ala³]-analog and the inactive [Gly³]-analog (fig. 3a,b). Again, the CD spectra of these analogs in aqueous solution are similar to those of α -mating factor and the inactive [D-His²]-analog. However, on addition of SUVCS, the positive CD peak of the [Gly³]-analog at 225 nm disappears, leaving a weak negative peak at around 235 nm (fig. 3b). Such a spectral change of the inactive [Gly³]-analog is similar to that of the [D-His²]-analog on addition of SUVCS. On the other hand, for the active [L-Ala³]-analog (fig. 3a), the positive CD peak at 237 nm is still observed (red-shifted by 2 nm) in the presence of SUVCS. However, the intensity of this positive peak is significantly low, in good correlation with the low activity (6% relative to α -mating factor) of the [L-Ala³]-analog.

The CD spectral change of α -mating factor on addition of SUVCS is appreciably larger than the spectral change on addition of small unilamellar vesicle of phosphatidylcholine (not shown). This indicates that the electrostatic interaction between the positively charged N-terminal group (with pK_a of 7.5) of α -mating factor and the negatively

charged group of phosphatidylserine is important for the strong peptide-SUVCS binding. This is further ascertained by the observation of the pH dependence of the CD spectra of α -mating factor; the CD spectral changes on addition of SUVCS are enhanced as the pH is lowered from 9.7–5.6 (not shown).

We had analyzed the NMR spectra of α -mating factor and analog peptides in aqueous solution and found that physiologically active peptides take some ordered conformation whereas inactive peptides do not [7]. However, such conformational differences are not observed clearly in the CD spectra of α -mating factor or analog peptides. Nevertheless, the CD spectra of these peptides are significantly affected upon binding with SUVCS, and in the presence of SUVCS, the CD spectra of α -mating factor and active analogs are quite different from those of inactive peptides. These observations indicate that the physiological activities are correlated with the membrane-bound conformations rather than with the molecular conformations in solution. We have also found such correlations between the physiological activities and membrane-bound conformations for the cases of substance P, LHRH, and their analog peptides (submitted). Accordingly, the conformational change of peptide hormone on the interaction with phospholipid membrane is probably an essential step for the recognition of hormone by its receptor.

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REFERENCES

- [1] Bücking-Throm, E. and Duntze, W. (1970) *J. Bacteriol.* 104, 1388–1390.
- [2] Duntze, W., Stötzler, D., Bücking-Throm, E. and Kalbitzer, S. (1973) *Eur. J. Biochem.* 35, 357–365.
- [3] Stötzler, D., Kiltz, H.H. and Duntze, W. (1976) *Eur. J. Biochem.* 69, 397–400.
- [4] Tanaka, T., Kita, H., Murakami, T. and Narita, K. (1977) *J. Biochem. (Tokyo)* 82, 1681–1687.
- [5] Masui, Y., Chino, N., Sakakibara, S., Takana, T., Murakami, T. and Kita, H. (1977) *Biochem. Biophys. Res. Commun.* 78, 534–538.

- [6] Masui, Y., Tanaka, T., Chino, N., Kita, H. and Sakakibara, S. (1979) *Biochem. Biophys. Res. Commun.* 86, 982-987.
- [7] Higashijima, T., Miyazawa, T., Masui, Y., Chino, N., Sakakibara, S. and Kita, H. (1980) in: *Peptide Chemistry 1979* (Yonehara, H. ed) pp. 155-158, Protein Research Foundation, Osaka.
- [8] Higashijima, T., Wakamatsu, K., Takemitsu, M., Fujino, M., Nakajima, T. and Miyazawa, T. (1983) *FEBS Lett.* 152, 227-230.
- [9] Comfurius, P. and Zwaal, R.F.A. (1977) *Biochim. Biophys. Acta* 488, 36-42.