Conformational change of mastoparan from wasp venom on binding with phospholipid membrane

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The conformational change upon binding with phospholipid membrane has been studied of mastoparan from wasp venom, a tetradecapeptide causing the degranulation of mast cells. The 270-MHz ^{1}H-NMR spectra and CD spectra indicate that the mastoparan molecule takes the α-helical conformation in methanol solution, but a much less ordered form in aqueous solution. On binding with phospholipid membrane, the α-helical conformation is formed even in aqueous medium. Such a conformational change is primarily due to the interaction between the aliphatic side chains of mastoparan and the hydrophobic interior of phospholipid membrane, in contrast to the case of melittin from bee venom.

Circular dichroism *Conformational change* *^{1}H-NMR* *Mastoparan* *Peptide-phospholipid interaction* *Venom peptide*

1. INTRODUCTION

Mastoparan is one of mast cell degranulating peptides in the venom of wasp (*Vespula lewisii*) and the primary structure is Ile-Asn-Leu-Lys-Ala-Leu-Ala-Ala-Leu-Ala-Lys-Lys-Ile-Leu-NH_2 [1,2]. A similar peptide, namely mastoparan-X, has been isolated from the venom of *Vespa xanthoptera* [3], and the primary structure is Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-NH_2. It has been suggested that mastoparan enhances the transport of Ca^{2+}, Na^+ and K^+ ions through black membrane [4]. As a first step in the elucidation of the structure–toxicity relationship, we have measured the CD and ^{1}H-NMR spectra and analyzed the solution conformations of mastoparan and the interactions between mastoparan (and mastoparan-X) and phospholipids. The results will be discussed, in comparison with the case of melittin from the bee venom [5–9].

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2. MATERIALS AND METHODS

Mastoparan, N-terminal heptapeptide and C-terminal heptapeptide of mastoparan were synthesized by solution method (to be reported separately). Mastoparan-X was synthesized as in [10]. Egg yolk phosphatidylcholine was isolated by a combination of the method in [11] and silica gel column chromatography. Unilamellar vesicles (diameter 25 nm) of phosphatidylcholine were prepared by the sonication (20 W) for 60 min at 23°C under nitrogen atmosphere and subsequent centrifugation (100000 × *g*) for 60 min. Lysophosphatidylcholine was obtained by the hydrolysis of phosphatidylcholine with phospholipase A_2, and was purified with a silica gel column. The micelle of lysophosphatidylcholine was prepared with a vortex mixer (5 min). The critical micelle concentration (cmc) was determined from the absorbance enhancement of rhodamine 6G at 542 nm on micelle formation [12]. The 270-MHz ^{1}H-NMR spectra were recorded on a Bruker WH-270 spectrometer. CD

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measurements were carried out with a Jasco J-40S spectrometer.

3. RESULTS

3.1. *Circular dichroism*

The CD spectrum of mastoparan in methanol solution is quite different from that in aqueous solution (fig.1). The spectrum in aqueous solution changes drastically on addition of micelles of lysophosphatidylcholine (fig.1). A similar spectral change is also observed on addition of small unilamellar vesicles of phosphatidylcholine. The dependence of $[\theta]_{222}$-value of mastoparan upon the concentration of lysophosphatidylcholine is shown in fig.2. The $[\theta]_{222}$-value is sharply increased above the cmc. However, the CD spectrum of mastoparan is not affected by the presence of 0.1 M phosphate buffer, in clear contrast to the case of melittin [9].

3.2. *Nuclear magnetic resonance*

The amide NH proton resonances of mastoparan in C^2H_3OH solution and in H_2O solution are shown in fig.3. These resonances have not been assigned completely to individual residues yet because of the multiple presence of same residues (4 Ala, 4 Leu, 3 Lys, and 2 Ile). However, the peaks 2 (in C^2H_3OH) and 1′ (H_2O) may be assigned to the main-chain NH proton of Asn^2, because this proton exchanges with labile solvent proton much faster than any other main-chain NH protons [13]. In addition, the amide NH_2 proton resonance peaks 8 and 14′ are assigned to Asn^2 (trans), 15 and 17′ to Asn^2 (cis), 16 and 15′ to C-terminus (trans), and 17 and 16′ to C-terminus (cis), from the spectral comparison with those of the N-terminal and C-terminal heptapeptides of mastoparan. For the 13 main-chain NH proton resonances of mastoparan, the vicinal spin coupling constants $^3J_{NH-C\alpha H}$ have been measured. In C^2H_3OH solution, $^3J_{NH-C\alpha H}$-values are smaller than 5.0 Hz (except for the peaks 2, 6, 10 and 11) whereas in aqueous solution, $^3J_{NH-C\alpha H}$-values are larger than 5 Hz (except for the peak 7′).

The temperature coefficients of NH proton chemical shifts are useful for distinguishing hydrogen-bonded NH protons from exposed ones. For the peaks 4, 5, 7, 12–14, and 16 of mastoparan in C^2H_3OH solution, the negative temperature coefficients are smaller than -4.3×10^{-3} ppm/°C. The coefficients for the peaks 1, 3, 6, 9–11 are in the range $(-4.5 \sim -5.5) \times 10^{-3}$ while those for the peaks 2, 8, 15 and 17 are $> -6 \times 10^{-3}$ ppm/°C.

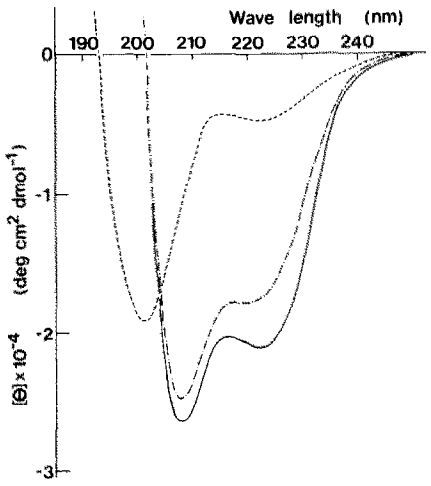

Fig.1. The CD spectra of mastoparan (50 μM, 23°C) in aqueous solution at pH 6 (– – –), in methanol solution (·——) and in aqueous solution in the presence of lysophosphatidylcholine (10 mM) (——). The value of $[\theta]$ is the molar ellipticity as divided by 14, the number of residues.

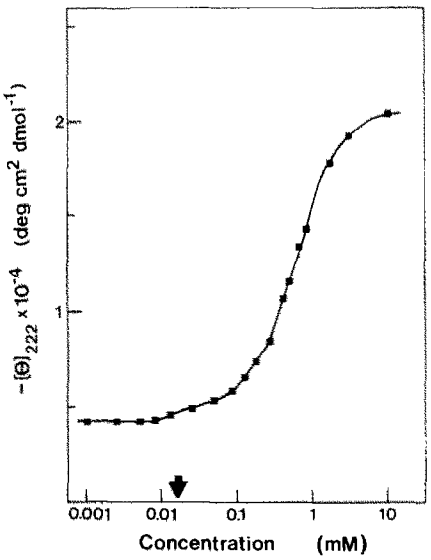

Fig.2. The CD at 222 nm of mastoparan (50 μM, 23°C) in aqueous solution as the function of the concentration of lysophosphatidylcholine (the critical micelle concentration is shown with an arrow).

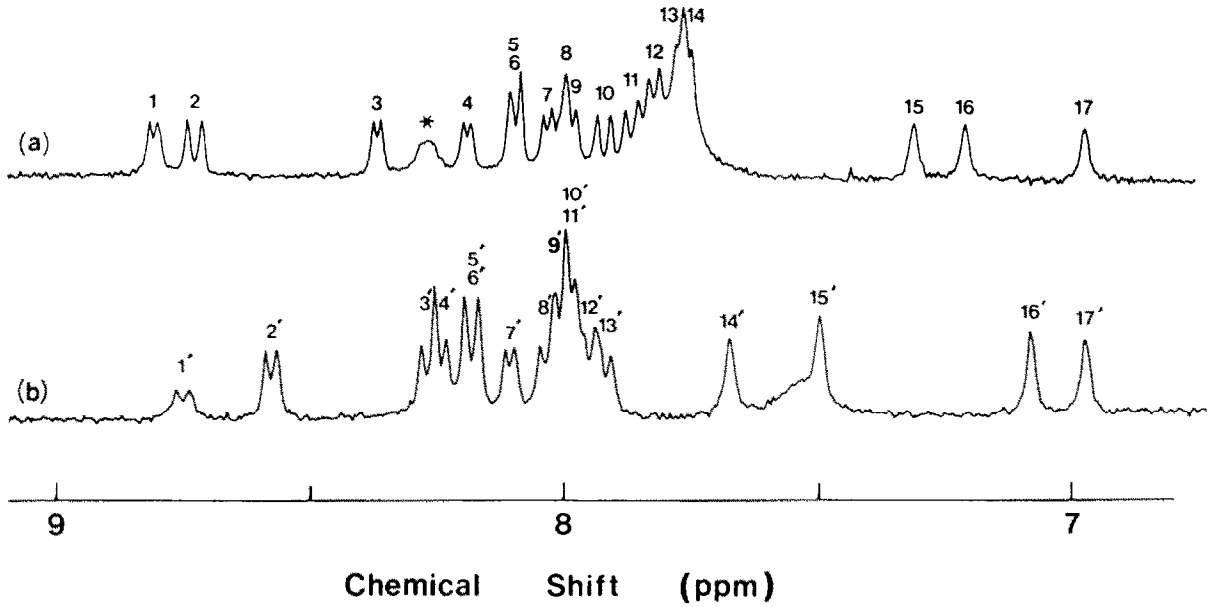

Fig.3. The 270-MHz ^{1}H-NMR spectra (in the NH proton region) of mastoparan (2 mM, 23°C) in C^2H_3OH solution (a) and in H_2O solution at pH 4 (b). The chemical shifts (ppm) are measured downfield from the internal standard of 2,2-dimethyl-2-silapentane-5-sulfonate. The strong solvent resonances are pre-saturated by irradiation for 0.5 s prior to the observation pulse. The peak * in (a) is due to the α-NH_3^+ group of mastoparan which has been prepared by lyophilization of the aqueous solution at pH 3.5.

On the other hand, in aqueous solution, the temperature coefficients for the peaks 7′, 9′, 11′, 13′ and 17′ are in the range $(-4.5 \sim -5.5) \times 10^{-3}$ ppm/°C and those for all other peaks are even larger. These results indicate that many more amide NH protons participate in intramolecular hydrogen bond formation in C^2H_3OH solution than in aqueous solution.

4. DISCUSSION

The CD spectrum of mastoparan in methanol solution is typical of the α-helical form, with negative bands at 208 nm and 222 nm (fig.1). In such an α-helical form, the dihedral angles H-N-C_α-H are nearly 120° and vicinal coupling constants will be about 4 Hz. In fact, in methanol solution, most of the $^3J_{NH-C\alpha H}$-values are as small as 4 Hz. Furthermore, the C-terminal amide NH proton (trans) shows the temperature coefficient of chemical shift as small as -2.7×10^{-3} ppm/°C, indicating that this proton is involved in strong intramolecular hydrogen bond of the C-terminal part in the α-helical form. On the other hand, for the peaks 10 and 11, temperature coefficients are about -5×10^{-3} ppm/°C and the vicinal coupling constants are as large as 7.8 and 7.0 Hz, respectively, which are certainly larger than the value (4 Hz) expected for the α-helical form. These indicate that some amino acid residues (N-terminal or middle part) are not tightly involved in the α-helical form. In fact, from the $[\theta]_{222}$-value, the α-helical content is estimated as about 60% for mastoparan in methanol solution.

In aqueous solution, however, the CD spectrum of mastoparan is typical of nonhelical form, with a negative band around 200 nm. This is consistent with the large $^3J_{NH-C\alpha H}$-values and large temperature coefficients of chemical shifts as obtained for most amide NH protons. These observations indicate that the mastoparan molecule largely takes unordered forms in aqueous solution. However, in the presence of unilamellar vesicles of phosphatidylcholine or micelles of lysophosphatidylcholine, the CD spectrum of mastoparan becomes nearly the same as that in methanol solution (fig.1). Such a spectral change is not induced by the interaction with monomeric lyso-

phosphatidylcholine molecule below cmc (fig.2) or with the phosphate ion. These observations indicate that the conformational change of mastoparan to the α-helical form is primarily due to the interaction of the hydrophobic side-chain groups with hydrophobic interior of phospholipid membrane. In fact, the fluorescence peak of Trp^3 of mastoparan-X in aqueous solution is blue-shifted by 20 nm (with intensity enhancement) on addition of lysophosphatidylcholine micelles or of phosphatidylcholine vesicles, indicating that the indole ring of this tryptophan residue becomes involved in the hydrophobic environment (not shown).

The conformational changes of mastoparan are clearly different from those of melittin. In aqueous solution, the nonhelical conformation of melittin is converted to the α-helical form on addition of phosphate ion [6,9], in sharp contrast to the present case of mastoparan. Accordingly, the conformational change of melittin on binding with membrane [5–9] is probably due to the electrostatic interaction between the positively charged groups of melittin and the negatively charged phosphate groups of phospholipid membrane, rather than hydrophobic interactions. In fact, melittin molecules do not take the α-helical form in ethanol solution [6]. In the hope of elucidating the conformation–toxicity relationship of mastoparan, in comparison with melittin, the NMR analyses of the mastoparan–membrane interactions are now in progress in our laboratory by the use of deuterium-labelled species.

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