Transferred NOE analyses of conformations of peptides as bound to membrane bilayer of phospholipid; mastoparan-X

Kaori Wakamatsu, Tsutomu Higashijima, Masahiko Fujino[+], Terumi Nakajima[†] and Tatsuo Miyazawa*

Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo, Bunkyo-ku, Tokyo 113, [+]Chemical Laboratories, Central Research Division, Takeda Chemical Industries, Yodogawa-ku, Osaka 532 and *[†]Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101, Japan*

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The 270-MHz ^{1}H-NMR spectra of mastoparan-X, a tetradecapeptide toxin from hornet venom, have been observed in the presence of vesicles of perdeuterated dilauroylphosphatidylcholine ([$^2H_{64}$]DLPC). By the analysis of transferred nuclear Overhauser effects (TRNOE), the mastoparan-X molecule as bound to [$^2H_{64}$]DLPC vesicles is found to take an α-helical form in the C-terminal (and central) part. The TRNOE analysis with perdeuterated phospholipid is a unique method for the elucidation of conformation of peptide (and drug) molecules as bound to phospholipid membranes.

^{1}H-NMR *Mastoparan-X* *Membrane-bound conformation* *Perdeuterated phosphatidylcholine* *Physiologically active peptide* *Transferred NOE*

1. INTRODUCTION

A number of biologically active peptides, such as melittin [1], glucagon [2], adrenocorticotropic hormone [3], and mastoparans [4], have been found to interact with phospholipid membranes. In particular, for α-mating factor from *Saccharomyces cerevisiae* and analog peptides, the biological activities have been found to correlate with the conformations of membrane-bound molecules rather than the molecular conformations in aqueous solution [5]. This indicates the importance of detailed analyses of the conformations of peptide molecules in phospholipid membranes.

Here, we have analyzed the proton NMR spectra of mastoparan-X as bound to perdeuterated phospholipid bilayer. Mastoparan-X is one of mast cell degranulating peptides in the venom of *Vespa xanthoptera* [6] and the primary structure is:

Ile–Asn–Trp–Lys–Gly–Ile–Ala–Ala–Met–
Ala–Lys–Lys–Leu–Leu-NH_2.

* To whom correspondence should be addressed

By the analyses of transferred nuclear Overhauser effects (TRNOE) [7], we have succeeded in elucidating the conformation of mastoparan-X molecules as bound to phospholipid bilayer. Such an analysis of TRNOE of biologically active peptides will become a unique method for detailed conformation studies of membrane-bound peptides in relation with biological activities.

2. MATERIALS AND METHODS

Mastoparan-X was synthesized by solution method [8]. Perdeuterated dilauroylphosphatidylcholine ([$^2H_{64}$]DLPC) was synthesized as in [9]. Unilamellar vesicles were prepared by the sonication (20 W) of [$^2H_{64}$]DLPC suspension in ^2H_2O for 30 min at 23°C under nitrogen atmosphere. The 270-MHz ^{1}H-NMR spectra were recorded on a Bruker WH-270 spectrometer. TRNOE experiments were performed with the gated irradiation of the protons of free peptide molecules in an exchange equilibrium with bilayer-bound molecules, and negative NOE enhancements of other proton

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resonances (of free molecules) were extracted by the difference method.

3. RESULTS

The 270-MHz ^{1}H-NMR spectrum of mastoparan-X in ^2H_2O solution is shown in fig.1a, where the assignments of proton resonances are also listed in part (detailed assignments will be reported separately). The ^{1}H-NMR spectrum of mastoparan-X in the presence of [$^2H_{64}$]DLPC is shown in fig.1b. The resonances of residual protons of [$^2H_{64}$]DLPC are indicated with arrows in fig.1b; the degree of deuteration is as high as 95% for the α-methylene groups of fatty acid chains and is higher than 98% for other methylene and methyl groups. The proton resonances of bilayer-bound peptide molecules are not observed because of significant broadening. The proton resonances of free peptide molecules are broader in the presence of vesicles (fig.1b) than in the absence of vesicles (fig.1a), because of the chemical exchange with bilayer-bound molecules.

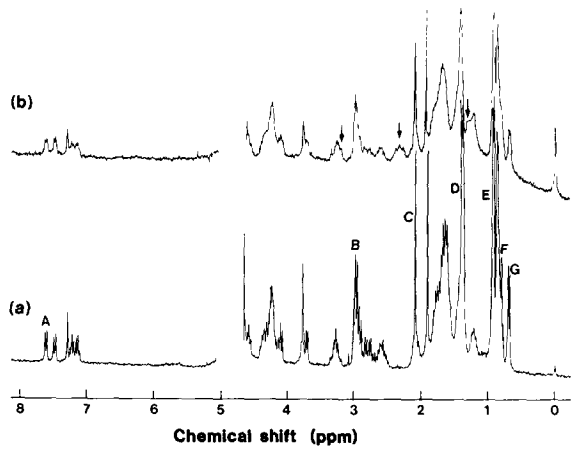

Fig.1. The 270-MHz ^{1}H-NMR spectra of mastoparan-X (1 mM, pH 6, 25°C), (a) in ^2H_2O solution, and (b) in the presence of [$^2H_{64}$]DLPC (the molar ratio phospholipid/peptide being 6) in ^2H_2O solution. The assignments of proton resonances of mastoparan-X are: A (7.60 ppm), Trp^3 indole C4H; B (2.95 ppm), Lys ϵ-CH_2; C (2.08 ppm), Met^9 S-CH_3; D (1.40 ppm), Ala CH_3; E (0.95–0.85 ppm), Ile and Leu CH_3; F (0.82 ppm), Ile^1 δ-CH_3; G (0.68 ppm), Ile^1 γ-CH_3. The resonances marked with arrows are due to residual protons in [$^2H_{64}$]DLPC.

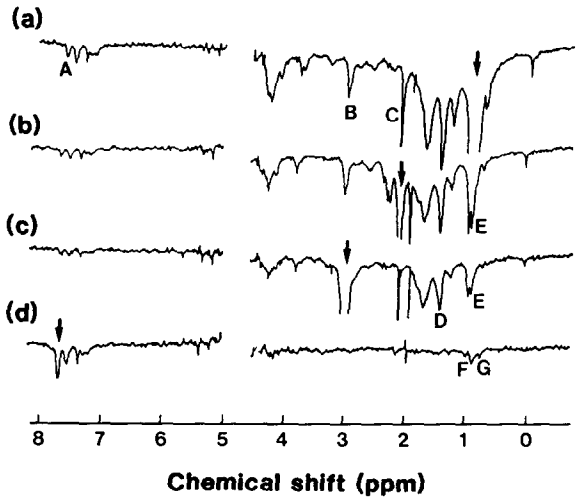

Fig.2. The NOE difference spectra of mastoparan-X in the presence of [$^2H_{64}$]DLPC (the condition is the same as for fig.1b); the irradiated protons are (a) Ile and Leu CH_3; (b) Met^9 S-CH_3; (c) Lys ϵ-CH_2; and (d) Trp^3 indole C4H.

The NOE-difference spectra of mastoparan-X in the presence of [$^2H_{64}$]DLPC vesicles in ^2H_2O solution are shown in fig.2. The observed negative NOE peaks are due to the predominant effects for proton pairs in bilayer-bound peptide molecules in the exchange equilibrium with free molecules, since NOE enhancements are not detected for the free tetradecapeptide molecule in the absence of [$^2H_{64}$]DLPC vesicles in ^2H_2O solution at 25°C. It may be remarked that the observed negative NOE patterns do depend on the irradiation frequencies (fig.2a–d) but not on the duration of gated irradiation over 0.05–0.36 s. These results indicate that the observed negative enhancements are due to transferred NOE but not to spin diffusion [10].

4. DISCUSSION

^{1}H-NMR spectra of peptides in the presence of perdeuterated phosphatidylcholine vesicles have been observed for valinomycin [11]. On addition of phosphatidylcholine vesicles in ^2H_2O solution, the proton resonances of valinomycin are significantly broadened, because of the restriction of molecular motion in membrane bilayer and/or the fast chemical exchange between the bilayer-bound state and the free state. The chemical shifts of such broadened resonances are still measurable and

have been used for the discussion of the conformation of the bilayer-bound molecules [11]. However, for detailed conformation analyses of such molecules, it is required to measure NOE enhancements for a number of proton pairs in the bilayer-bound molecules.

In the course of the ^{1}H-NMR analyses on peptide-phospholipid interactions, we have observed significant negative NOE enhancements for mastoparan-X in the presence of [$^2H_{64}$]DLPC vesicles (fig.2). At the molar ratio (phospholipid/peptide) of 6 in ^2H_2O medium at 25°C, the proton resonances of the bilayer-bound peptide molecules are not observed whereas the proton resonances of the free peptide molecules are clearly observed at practically the same chemical shifts as those of the free molecules in the absence of phospholipid vesicles (fig.1a,b). These observations indicate that the exchange rate between the free state and the bound state at 25°C is slow on the NMR chemical shift scale but is still fast enough for transferred NOE enhancements. Such an exchange rate will become 'intermediate' at higher temperatures. In fact, the proton resonances of the free peptide molecules in the presence of [$^2H_{64}$]DLPC broaden at >25°C.

The conformation of mastoparan molecules as bound to the vesicles of phosphatidylcholine (egg yolk) has been found to be largely α-helical by the analysis of circular dichroism [4]. The conformation of such bilayer-bound molecules of mastoparan-X may be discussed on the basis of the transferred NOE difference peaks of free molecules (fig.2a–d). For example, on irradiation of the CH_3 protons of Ile and Leu residues, negative NOE enhancements are clearly observed for the S-CH_3 proton resonance (C) of Met^9 and the ϵ-CH_2 proton resonances (B) of Lys residues (fig.2a). Conversely, on irradiation of the S-CH_3 protons of Met^9 (fig.2b) or on irradiation of the ϵ-CH_2 protons of Lys residues (fig.2c), a negative NOE enhancement is observed for the CH_3 proton resonances of Ile and Leu residues. These observations are consistent with a largely α-helical conformation of mastoparan-X as bound to phospholipid vesicles. However, on irradiation of the indole C4H proton of Trp^3, negative NOE is observed only for the CH_3 proton resonances of Ile^1 (fig.2d), but not for the CH_3 proton resonance (D) of Ala residues. This indicates that the Trp^3 residue in the N-terminal part of mastoparan-X is not involved in the α-helical form, and accordingly the C-terminal (and central) part of this peptide molecule takes the α-helical form. A two-dimensional transferred NOE analysis of mastoparan-X as bound to [$^2H_{64}$]DLPC is now in progress (in preparation).

Previously, detailed ^{1}H-NMR (and NOE) analyses have been made of the conformations of peptide molecules as bound to micelles of perdeuterated dodecylphosphocholine [12,13]. However, the conformation of mastoparan molecules as bound to micelles of lysophosphatidylcholine is not exactly the same as that of mastoparan molecules as bound to the vesicles of phosphatidylcholine [4]. Since the biological activities of peptides are correlated with the conformations of peptide molecules as bound to the biological membrane [5], it is important to analyse the conformations of peptides as bound to phospholipid bilayer rather than to micelles. As shown here, on mastoparan-X, the analysis of transferred NOE with perdeuterated phospholipid is a unique method for the elucidation of molecular conformations of peptides (and drugs) as bound to phospholipid membranes.

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