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NMR OBSERVATION OF GRAMICIDIN A′ IN PHOSPHATIDYLCHOLINE VESICLES

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Summary

Dimyristoyl phosphatidylcholine was prepared with perdeuterated hydrocarbon chains and sonicated into bilayer vesicles together with gramicidin A′. The ^1H NMR resonance from the tryptophan residues in the gramicidin has a linewidth of approximately 80 Hz, indicating significant local mobility for these residues. Paramagnetic lanthanides added to the aqueous medium cause a chemical shift of this signal indicating that some of the tryptophans may be located in the interfacial region of the bilayer.

Peptide-phospholipid interactions have been studied with proton nuclear magnetic resonance (^1H NMR) spectroscopy using such systems as valinomycin [1] or alamethicin [2] incorporated into aqueous phospholipid dispersions. The perturbations of the phospholipid resonances induced by the peptides provided information about the properties of the system. Clearly, useful information is contained in the resonances of the peptide. We wish to report the first observation of the ^1H NMR signals from a peptide antibiotic, gramicidin A′, incorporated into phospholipid vesicles.

Gramicidin A′ is a mixture of linear pentadecapeptides with antibiotic activity [3]. The most abundant component is valine-gramicidin A:

HCO-Val-Gly-Ala-Leu-Ala-Val-Val-Val-Trp-Leu-Trp-Leu-Trp-Leu-Trp $NHCH_2CH_2OH$.
L L D L D L D L D L D L D L

This peptide serves as a functional model for ion transport through a membrane, the active species being a dimer [4]. The structure of this dimer ion

Abbreviation. [^2H]dimyristoyl phosphatidylcholine, dimyristoyl phosphatidylcholine with perdeuterated hydrocarbon chains

channel is under investigation in several laboratories [5, 6, 7].

^{1}H NMR studies of gramicidin A′ in phospholipid bilayers suffer from the intense background signals from the phospholipids. A significant simplification of the ^{1}H NMR spectrum can be achieved by perdeuterating the phospholipid hydrocarbon chains, as was shown by Kroon et al. [8] for cholesterol in dipalmitoyl phosphatidylcholine vesicles. The ^{1}H NMR spectra of gramicidin A′ in dimethylsulfoxide [9] and of phosphatidylcholine in sonicated bilayer vesicles [10] have previously been assigned.

The ^{1}H NMR spectrum of 4 mol % gramicidin A′ in [^{2}H] dimyristoyl phosphatidylcholine vesicles at 70°C is shown in Fig. 1. Higher sample

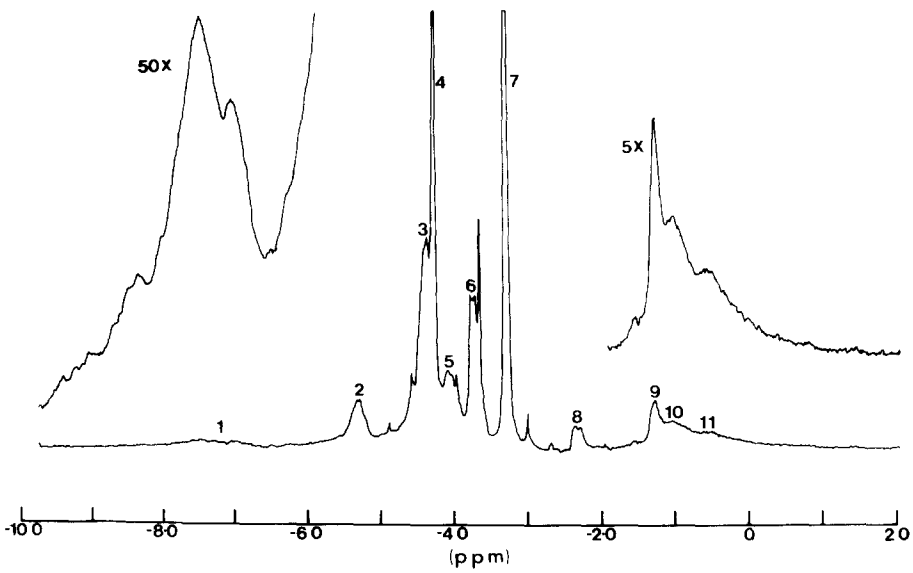

Fig. 1. 80 MHz ^{1}H NMR spectrum of bilayer vesicles of gramicidin A′ (0.5 mM) at 70°C. Peaks are (1) Trp CH, (2) glycerol CH, (3) glycerol CH_2OCO + choline CH_2OP, (4) HO^2H, (5) glycerol CH_2OP, (6) choline CH_2N, (7) choline $N(CH_3)_3$, (8) fatty acyl α-CH^2H, (9) $(CH^2H)_n$, (10) Val CH_3 + Leu CH_3 + CH_2 ^{2}H, (11) Val CH_3 + Leu CH_3. 5000 transients were collected. The chemical shift scale is with respect to internal DSS (2,2-dimethyl-2-silapentane-5-sulfonic acid). 99% perdeuterated myristic acid was prepared by the method of Dinh-Nguyen et al. [14]. [^{2}H] Dimyristoyl phosphatidylcholine was prepared according to Robles and Van den Berg [15].

temperatures did not cause significant further line narrowing. Gramicidin resonances between −2 and −5 ppm are not resolved because of overlap in this spectral region with phospholipid peaks. The gramicidin peaks which are resolved were identified by comparison of the chemical shifts with published values for gramicidin A′ in dimethylsulfoxide [9] and for amino acids in ^2H_2O [11]. The valine and leucine methyl peak at −1.0 ppm can be seen as well as the upfield ringcurrent shifted valine and leucine methyl peak at −0.55 ppm. Considerable overlap from residual hydrocarbon protons in the [^{2}H] dimyristoyl phosphatidylcholine occurs in this region. The other gramicidin resonance which is evident in this spectrum is the downfield aromatic resonance centered at −7.2 ppm. This resonance is a composite of chemically shifted protons from the four aromatic residues in each gramicidin molecule, predominantly (approximately 93%) from tryptophan

residues, with small contributions from phenylalanine and from tyrosine. Since the tryptophan CH resonances range from −7.04 to −7.54 ppm for the amino acid in solution [11], a linewidth component comparable to this frequency range might be expected to contribute to the observed 80 Hz resonance. There may also be a linewidth component resulting from the different local magnetic environments of the individual tryptophans. Because of the difficulty of evaluating the chemical shift contributions to the observed linewidth, detailed information about the motional state of the aromatic residues is not readily obtained from the linewidth. However, an upper limit of 80 Hz can be assigned to the homogeneously broadened component of the linewidth, indicating significant mobility for the aromatic residues of gramicidin A′ within the lipid bilayer. Integration of this peak and comparison with the integral of the phosphatidylcholine glycerol CH peak reveals that 100 ± 10% of the expected tryptophan signal is accounted for in the 80 Hz peak.

Samples prepared with (protonated) dimyristoyl phosphatidylcholine exhibited a detectable gramicidin aromatic resonance. However, the low signal-to-noise ratio and the intense baseline curvature made chemical shift and peak intensity measurements unreliable.

The observation of the tryptophan resonance in the gramicidin-containing [^{2}H]dimyristoyl phosphatidylcholine vesicles permitted experiments to be performed which were aimed at determining the location of these residues within the bilayer. A number of different paramagnetic lanthanides were used to shift the tryptophan peak, as well as to shift the phosphatidylcholine peaks. The lanthanide-induced shift was measured with respect to the $(CH^2H)_n$ peak, which did not shift in these experiments [12]. The lanthanide ion concentrations on both sides of the bilayer were equilibrated, as monitored by the collapse of the choline methyl splitting. At 70°C the half time for this equilibration is minutes, whereas it is hours at 30°C. Lanthanide-induced shifts using Tm^{3+} are shown in Fig. 2. Eu^{3+} caused weak upfield shifts, Pr^{3+} weak downfield shifts. At the high concentrations of Eu^{3+} or Pr^{3+} (>5 mM) required to observe significant tryptophan shifts, these lanthanides caused pronounced sample aggregation. Dy^{3+} gave rise to strong downfield shifts of tryptophan and phosphatidylcholine peaks but was not found to be ideal for these studies because of its noticeable line broadening effects, and also because it shifted an intense resonance, probably the choline CH_2OP signal [12], directly onto the tryptophan peak. The most useful reagent was Tm^{3+}, a strong upfield shifter which caused only slight line broadening. The observed chemical shifts are paramagnetic in origin since the diamagnetic ion Y^{3+} caused only a small shift, and also the Dy^{3+}-induced shifts were downfield while the Tm^{3+} shifts were upfield, in accordance with the established properties of these ions in phospholipid vesicle preparations [13].

A difficulty arises in the interpretation of the tryptophan chemical shifts because the aromatic region peak assignments cannot be made at the relatively low magnetic field strength used for these experiments. It appears that the peak centered at −7.4 ppm shifts upfield while the peak centered at −7.0 ppm does not shift. This differential shifting might suggest that the peaks at −7.4 and −7.0 ppm originate from different tryptophan popula-

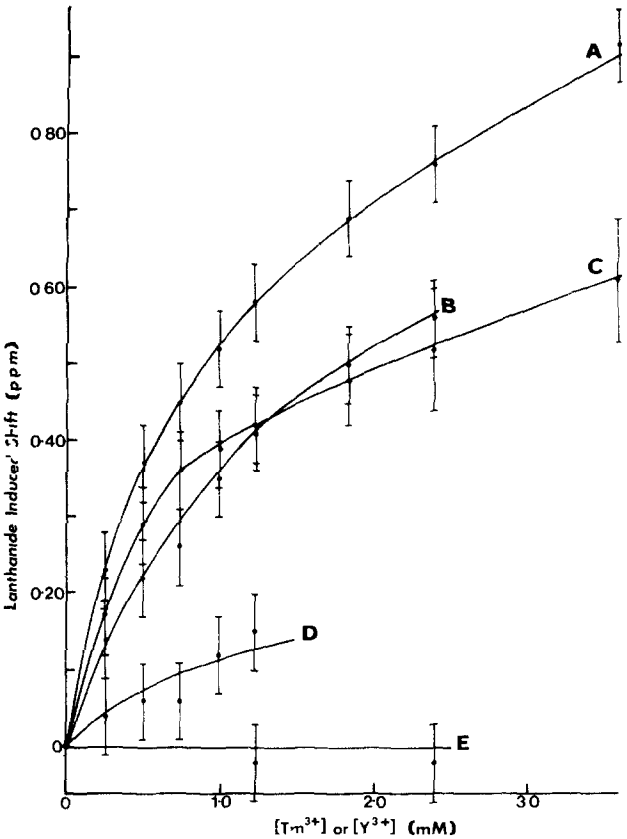

Fig. 2. Lanthanide-induced chemical shifts of phosphatidylcholine and gramicidin A′ protons at 70°C Curves A-D, Tm^{3+} induced shifts. Curve E, Y^{3+} induced shift. (A) choline $N(CH_3)_3$, (B) glycerol CH, (C) Trp CH, (D) fatty acyl α-CH^2H, (E) Trp CH.

tions, implying a peak assignment which has not yet been verified.

The magnitude of the observed lanthanide-induced shift for a part of the tryptophan resonance strongly implies, but does not prove, that some tryptophan residues are located in the interfacial region of the bilayer if it is assumed that the lanthanide binding site is in the phosphate region of the phosphatidylcholine [12]. More precise localization will require the establishment of the binding site for the lanthanides in the gramicidin-containing vesicles.

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