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Fourier transform infrared spectra of the polypeptide alamethicin and a possible structural similarity with bacteriorhodopsin

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FTIR spectra of alamethicin have been obtained in KBr disk, methanol and in aqueous lipid dispersion (above and below the lipid phase transition). The solution structure of this polypeptide in methanol has been shown by recent studies (Esposito et al. (1987) Biochemistry 26, 1043–1050) using NMR spectroscopy to be predominantly α-helical in content. It may therefore be regarded as a model structure for the interpretation of the spectra of certain biomembrane proteins. A comparison of the spectra with that obtained with bacteriorhodopsin shows spectral similarities, e.g. the presence of a high-frequency amide I maximum at 1661–1663 cm^{-1} and shoulders near 1640 cm^{-1} and 1620 cm^{-1}.

Alamethicin is a natural polypeptide that has antibiotic activity [2] it is extracted from the fungus *Trichoderma viride* as a mixture of related compounds, of which the main component has the following sequence [3]: Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-Phol, where Aib is α-aminoisobutyric acid ($(CH_3)_2C(NH_2)CO_2H$) and Phol is phenylalaninol, the α-amino alcohol derivative of phenylalanine.

Alamethicin forms voltage-gated ion channels in membranes [4–7]. Two main classes of models have been proposed for the way in which this gated channel operates. These are the conformational change models and the helix dipole models. For example, Hall et al. [8] proposed a conformational transition between a bent structure and a linear one as the basic gating event. This model is related to the one proposed by Fox and Richards [9], although the 'open' and 'closed' states are not the same. Models involving the helix dipole moment include those where the helix 'flips' across the membrane and those where the helices in the channel move with respect to each other [10–12]. These proposals for the action of the channel remain speculative since the structure of alamethicin in the lipid bilayer is not known in detail. At present there is some evidence that it adopts a mainly α-helical conformation in the membrane [13].

The X-ray structure of alamethicin, crystallized from methanol [9] is predominantly helical. The structure shows that after the initial α-helix segment, Pro-14 introduces a bend that shifts the axis of the subsequent 3_{10}-helix formed by the C-terminal residues. Structural studies have been also carried out in various solvents. Circular dichroism

Abbreviations: CD, circular dichroism; FTIR, Fourier transform infrared; NMR, nuclear magnetic resonance; DMPC, dimyristoylphosphatidylcholine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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studies of alamethicin [14,15] have earlier been used to indicate that the polypeptide shows a helix content which can range from 20% to 45% in a variety of organic solvents. NMR studies [15,16] have been used to determine the correct primary structure. Later Davis and Gisin [17], used 600-MHz ^{1}H-NMR to assign many of the resonances. From the different isotopic exchange rates observed for the backbone NH's, a rigid α-helical structure was inferred for the N-terminal portion of the molecule, with a more flexible C-terminus.

Alamethicin has been studied in methanol and in water by both one- and two-dimensional (2D) NMR techniques [18–20]. From analysis of coupling constants and relaxation parameters, Banerjee et al. [18] inferred a head to head dimeric association of the peptide via intermolecular hydrogen bonds between groups on residues 15–20, arranged in an extended parallel β-pleated structure. Esposito et al. [1] have recently presented a ^{1}H-2D-NMR study of purified alamethicin in methanol at 500 and 300 MHz. The resonances, including the Aib residues, were assigned. Characteristic NOE patterns for a helix were observed, and it was possible to make detailed comparison of the structure of the molecule in solution with the crystal structure described by Fox and Richards [9]. Contrary to the previous reports of Banerjee and co-workers [18], the conformation of the molecule in solution is considered to be similar to the crystal structure i.e. it is predominantly α-helical.

It has been argued that alamethicin does not constitute a model peptide for membrane proteins because of its high content of Aib amino acids. However, the fact that it is a hydrophobic peptide, which is predominantly α-helical and whose crystal structure and solution structure are known, makes it a useful peptide for comparison at this stage of development of FTIR spectroscopy applied to protein structure. Model polypeptides which can provide basic structural information as reference compounds for interpreting the FTIR spectra of biomembrane proteins, e.g. bacteriorhodopsin, are urgently required. In this paper we examine the FTIR spectra of alamethicin and make comparison with the spectrum of bacteriorhodopsin.

Alamathicin used in this study was obtained from Sigma Chemical Company. L-α-Dimyristoylphosphatidylcholine was also purchased from Sigma and its purity checked by thin-layer chromatography.

Infrared spectrum of alamethicin in a KBr disk was obtained using a protein concentration of 0.5% (w/w). Spectra of alamethicin in methanol were obtained with 1–2 mg of the polypeptide dissolved in 100 μl of the solvent. Spectra were recorded at 15°C and 25°C by signal averaging 64 scans with a resolution of 4 cm^{-1}.

Alamethicin in lipid dispersions were prepared as follows; 1 mg of alamethicin and 5.2 mg of DMPC was dissolved in chloroform, corresponding to a lipid/alamethicin molar ratio of 15:1. After evaporation of the solvent, the samples were dried in a vacuum. To each of the dried samples was added 100 μl of 10 mM phosphate-buffered saline (pH 7.4), and the samples were incubated above the transition temperature of DMPC for about 3–4 h. At the lipid/polypeptide molar ratio of 15:1 used in this study, alamethicin is likely to be incorporated into the lipid membrane in an aggregated form [21]. Infrared spectra were recorded at 20°C and 30°C by signal averaging 400 scans with a resolution of 4 cm^{-1}.

Spectra were obtained using a Perkin-Elmer 1750 FTIR spectrometer equipped with a TGS detector and Perkin-Elmer 7300 computer for data acquisition and analysis. Samples in solvent were placed in a thermostatted Beckman FH-01 CFT microcell fitted with CaF_2 windows and a 6 μm tin spacer. Temperature control was achieved by means of a cell jacket of circulating water. The spectrometer was continuously purged with dry air to eliminate water vapour absorptions from the spectral region of interest. A sample shuttle was used to permit the background to be signal-averaged concurrently with the sample.

Solvent spectra were recorded in the same cell and under the same instrument conditions as sample spectra. Difference spectra were obtained by digitally subtracting solvent spectra from the corresponding sample spectra [22,23]. The infrared spectrum of bacteriorhodopsin presented in this study is similar to that published by Lee et al. [23]. Description of the experimental procedure used to obtain the spectrum can be found there.

Fig. 1 shows the FTIR absorbance spectrum of alamethicin in methanol at 15°C in the 1800–1500

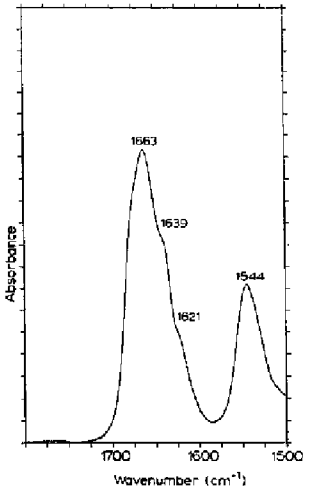

Fig. 1. FTIR spectrum between 1500 and 1800 cm^{-1} of alamethicin in methanol at 15 °C after solvent subtraction.

cm^{-1} region after subtraction of the methanol absorption. Methanol was chosen as the solvent for alamethicin so that comparisons can be made with the NMR studies made on this polypeptide by other workers [1]. The spectrum of alamethicin at 15 °C (Fig. 1) and 25 °C (not shown) were identical.

Fig. 2 shows the spectra of alamethicin in aqueous dispersions of dimyristoylphosphatidylcholine recorded above (Fig. 2b) and below (Fig. 2a) the transition temperature of the lipid. These spectra are similar to the spectrum of alamethicin in methanol with the amide I and amide II frequencies in similar position. The band at 1737 cm^{-1} in Fig. 2 represents absorbance from ester carbonyl groups of the lipid [24]. With the exception of the band at 1640 cm^{-1}, which appears to be more pronounced above the phase transition, the spectra of alamethicin above and below the phase transition of the lipid are similar. Further experiments are in progress where we are using using derivative and deconvolution procedures to monitor any changes in alamethicin structure which may occur as a function of temperature and lipid phase transition.

The spectrum of dry alamethicin in a KBr disk (not shown) is also similar to those recorded in methanol and aqueous lipid dispersions. The amide I band is, however, broader.

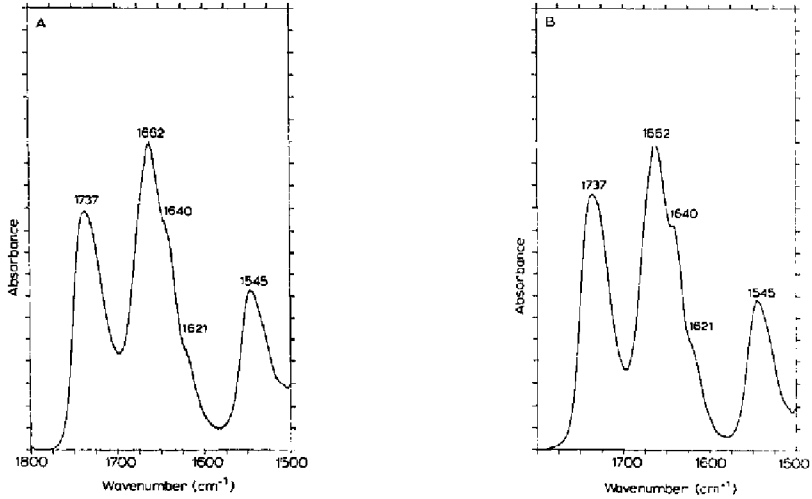

Fig. 2. FTIR spectrum between 1500 and 1800 cm^{-1} of alamethicin in aqueous DMPC dispersion, after water subtraction, at a molar lipid/peptide ratio of 15 : 1. The spectra were recorded at 20 °C (A) and 30 °C (B).

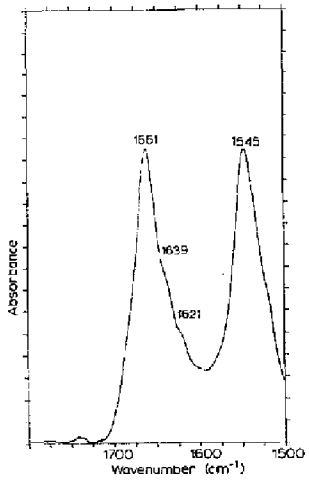

Fig. 3. FTIR spectrum of bacteriorhodopsin between 1500 and 1800 cm^{-1} in Hepes buffer (pH 6.8) at 20 °C, after subtraction of the spectrum of the aqueous buffer. A description of the experimental procedure used to obtain the spectrum has been published previously [23].

The spectrum of bacteriorhodopsin in the 1800–1500 cm^{-1} region is shown in Fig. 3. It can be seen that the spectra of alamethicin and bacteriorhodopsin in the amide I and amide II regions are similar. Both spectra exhibit an amide I maxima near 1661–1663 cm^{-1} with shoulders near 1640 cm^{-1} and 1621 cm^{-1}. The frequency of the main amide I band at 1661–1663 cm^{-1} is higher than normally observed for typical α-helical polypeptides and proteins [24,25]. The amide II/amide I intensity ratio is higher in the spectrum of bacteriorhodopsin compared to that observed with alamethicin (Figs. 2 and 3). Additional high-frequency components occur near 1675 cm^{-1} and 1685 cm^{-1} (seen after second-derivative analysis) for alamethicin and bacteriorhodopsin, respectively.

Previous Raman studies and CD studies of alamethicin had suggested that the conformation of alamethicin in methanol and in lipid membranes was predominantly helical but also contains some β-sheet secondary structure [13]. (The helix content as determined by Raman spectroscopy was deduced to be considerably higher than that derived from the CD measurements.) Further, Vogel [13] suggested that his Rama data was in agreement with the NMR measurements of alamethicin by Bannerjee et al. [18] i.e. where the first nine or ten residues at the C-terminus form a parallel β-sheet. Vogel indicated that the alamethicin structure in fluid lipid membranes and in methanol solution as deduced from his Raman data were similar [13]. He also suggested that when a change of the physical state of the lipid membrane takes place, a conformational transition from helix to β-structure occurs in the C-terminal segment. The conclusions of the recent 2D NMR studies of Esposito et al. [1] of alamethicin in methanol differ from that of Banerjee et al. [18]. They interpreted their data to show that the structure of alamethicin in methanol is predominantly α-helical and has no β-sheet content. The Raman data of Vogel [13] can in fact be reinterpreted to be in good agreement with this more recent NMR data and structural deduction [1].

Band frequency correlations of the amide I and amide II region with secondary structures have been based previously, on infrared spectra of water soluble proteins and polypeptides [24,25]. If we use these correlations and apply them to alamethicin we would deduce that the band at frequency of 1663 cm^{-1} is associated with the presence of an α-helical secondary structure, a band at 1639 cm^{-1} indicates the presence of β-sheet secondary structure and the band at 1621 cm^{-1} could also be associated with the presence of β-sheet [24,25]. It has been reported by Krimm and co-workers that β-turns of type I, II and III can also absorb near 1640 cm^{-1} [26,27]. Type III β-turn corresponds to one turn of a 3_{10}-helix, whereas type I and II are non-helical conformations [26,27]. A further feature of the alamethicin FTIR spectrum is that the main amide I band frequency is higher than occurs with many water soluble proteins containing α-helical structure (Refs. 24, 25 and our own unpublished results). Such a high amide I band frequency in the spectrum of bacteriorhodopsin has been attributed to the presence of α_{II}-helical structures [28]. In an α-helix the plane of the peptide group is essentially parallel to the helix axis, whereas in α_{II} it is

tilted with the N-H bond pointing inward to the axis.

If we accept however that the structural determination of Esposito et al. [1] is correct, i.e. that alamethicin exists as a regular helical structure and has no β-sheet content in methanol, we must reinterpret the FTIR spectra of alamethicin. We need of course to bear in mind the different time scales of the NMR and infrared method. The NMR (10^5 s^{-1}) structure represents an average structure neglecting protein dynamics whilst the infrared method provides a fast snap shot (10^{13} s^{-1}) of the polypeptide structure.

The values of the dihedral angles (ϕ, ψ) and of the interatomic distances as deduced from NMR are not precise enough to exclude α_{II} helices although existance of any β-sheet structure can be ruled out [1]. Furthermore the X-ray diffraction structure of alamethicin does not show the presence of either β-sheet or α_{II}-helical structures [9]. Clearly the classical and previous infrared band frequency correlations are unsatisfactory when applied to this polypeptide.

The high frequency amide I absorption band which we observe must be a reflection of the particular structure of alamethicin, coming from an overlap of absorbance from the 3_{10}-helices and normal α-helices present. In this regard, Krimm and co-workers [27] have calculated that a band near 1665 cm^{-1} in the infrared spectra of poly(α-aminoisobutyric acid) can arise from 3_{10}-helices, however, they used dihedral angles for 3_{10}-helix (ϕ, $\psi = -45°$, $-30°$, see Ref. 27) which differed from the experimentally found values (ϕ, $\psi = -60°$, $-30°$, see Ref. 29). Crystal structures of small peptide fragments of alamethicin and poly(α-aminoisobutyric acid) are in a 3_{10}-helical conformation [30,31]. A larger Aib-containing peptide adopts an α-helical conformation in crystals [32]. The content of α-aminoisobutyric acid in alamethicin is about 40–45%.

It is also of interest to compare the infrared spectrum of alamethicin with that of melittin. Like alamethicin, melittin also forms voltage-gated pores in lipid bilayers. At position 14 both polypeptides possess a proline residue, responsible for the bent helical structure found in the crystals [9,33]. Although both polypeptides are α-helical, the X-ray studies show that melittin does not possess any 3_{10}-helical regions [33]. Whereas alamethicin shows the unusually high amide I maxima near 1662 cm^{-1}, melittin displays a 'normal' amide I frequency of 1656 cm^{-1} (Ref. 34 and our unpublished data).

With regard to the bands at 1639 cm^{-1} and 1621 cm^{-1}, the component at 1639 cm^{-1} may arise from vibrations associated with the 3_{10} α-helices.

An interesting feature of the alamethicin FTIR spectrum is its similarity to that of bacteriorhodopsin (see Figs. 2 and 3). The three-dimensional structure of bacteriorhodopsin has been determined to a resolution of about 6Å using electron diffraction and electron microscopy [35]. According to this study the protein in the membrane exhibit seven transmembrane α-helices with no indication that part of the structure is β-sheet as suggested by Jap et al. [36]. However, unlike alamethicin [1,9] the resolution of this study [35] is not sufficient to resolve structural details for bacteriorhodopsin at an atomic level. Previous infrared spectroscopic studies of bacteriorhodopsin have used the classical correlations of band frequency and secondary structure. The high frequency amide I band has been previously assigned to the presence of an α_{II} helix [28]. The shoulder near 1640 cm^{-1} in the infrared spectrum of bacteriorhodopsin has been interpreted by many workers as providing strong evidence for substantial β-sheet content in this protein [23,36–38].

The spectral similarity between alamethicin and bacteriorhodopsin may, however, indicate a common folding arrangement of their polypeptide chain. If this is the case the transmembrane helices in bacteriorhodopsin could adopt a conformation similar to the helices in alamethicin, i.e. bent α-helices with short 3_{10}-helical regions rather than the α_{II}-helices previously proposed.

Although the 3_{10}-helix conformation is reported to occur rarely in protein structures [29], it is interesting to note that on the basis of its amino acid sequence, a significant amount of this type of structure is proposed to occur in the Na^+ channel which is an integral membrane protein [39,40].

Our studies show the difficulties which occur at the present time of making unequivocal deductions of secondary structure of membrane proteins from FTIR spectroscopy. The similarity of infrared

band frequency of alamethicin and bacteriorhodopsin is provocative. It could provide an alternative interpretation of the structure of this membrane protein to that based on previous interpretations of infrared spectra [23,36–38]. Further studies including polarised infrared spectroscopic data may resolve this situation.

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