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# Membrane-induced peptide structural changes monitored by infrared and circular dichroism spectroscopy

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ABSTRACT

As more peptide secondary structures deduced by infrared spectroscopy (IR) have been reported in the literature, there have been overlaps in assignments of elements of secondary structure to carbonyl vibrational frequencies. We have investigated this phenomenon with regards to the use of IR for monitoring membrane-induced structural changes using conformationally diverse peptides. These IR studies, complemented by circular dichroism (CD) experiments, revealed that peptide–solvent interactions can mask membrane-induced conformational changes monitored by IR. A structural transition from random coil to α-helix upon the binding of mastoparan X to a membrane was clearly observed by CD but obscured in the amide I region of the IR spectrum. In addition, unlike the buried helical peptides gramicidin D and P16 in micelles, the amide II peak for mastoparan X was absent, likely due to H–D exchange. This suggests information on the peptide's membrane-bound solvent accessibility could be obtained from this region of the spectrum.



## 1. Introduction

Infrared spectroscopy (IR) provides an opportunity to quickly obtain structural information on peptides and proteins in a variety of different chemical environments. This is potentially useful for examining the partitioning features of membrane active peptides. Since the biological activity of these peptides can depend directly on the lipid composition of the membrane bilayer, IR can provide an easily accessible and simple method for monitoring the effects of lipid composition on protein conformation. IR for structural analysis of small peptides is particularly advantageous since there is likely only a minimal amount of secondary structure within the molecule, resulting in simpler spectra that should not require high degrees of spectral deconvolution for interpretation. However, previous studies have shown that the analysis of protein IR spectra to deduce contributions from different forms of secondary structure must be approached with caution [1,2]. 2D IR spectroscopy is also being introduced as a new method to circumvent some of the inherent ambiguities in 1D spectra [3,4].

In IR, the amide I band (approximately 1600–1700 $cm^{-1}$) resulting from the CO stretching vibration has been shown to be the most sensitive to backbone conformation and the amide II band (approximately 1510–1580 $cm^{-1}$) resulting from the N–H bending vibration coupled to the C–N stretching vibration has also been suggested to be somewhat sensitive to protein secondary structure [5–12]. This latter band is suggested to be even more sensitive to the protonation state of the amide nitrogen [13]. Although specific amide I, and sometimes amide II, bands have been assigned to distinctive types of secondary structure, these characteristic frequencies sometimes overlap which could result in mis-assignment of the band. In order to provide further insight into the interpretation of IR data for small peptides, we have methodically used IR and circular dichroism (CD) spectroscopy to compare the structures of four structurally diverse membrane-associating peptides in different chemical environments. All peptides in this work have been previously studied in different solvents by various spectroscopic methods including CD, NMR, X-ray crystallography, and to some extent, IR. Of the peptides in this study, leucine enkephalin (Leu-Enk), gramicidin (Gram), and to a lesser extent mastoparan X (MPX) have been characterized by IR in some solvents, while P16 has not.

Leu-Enk (Tyr-Gly-Gly-Phe-Leu), a small peptide hormone with morphine-like activity, has been shown to form β-type structures in a variety of different solvents. However, the precise nature of these structures (β-sheet, β-turn, β-bend) has been controversial over the years and has been suggested to be dependent upon environmental factors such as overall peptide charge and nature of the solvent system [14–18]. GramD (L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp), an antimicrobial peptide from *Bacillus brevis*, is a heterogeneous mixture of pentadecapeptides with conservative variations in the hydrophobic amino acids at positions 1

*Abbreviations:* CD, circular dichroism spectroscopy; DCPC, dicaproylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DTPC, ditetradecylphosphatidylcholine; Gram, gramicidin; GramA, gramicidin A; GramD, gramicidin D; IR, infrared spectroscopy; Leu-Enk, leucine enkephalin; MPX, mastoparan X; MeOH, methanol; MLV, multilamellar vesicle; TFA, trifluoroacetic acid; TFE, trifluoroethanol.

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and 11. Structural studies on GramA, a component of GramD, have suggested that the molecule is dimeric with several different oligomerized hydrogen-bonded forms. These differences in oligomerization are thought to be a function of environment as well as a reflection of different functional states of the peptide [19].

P16 (Ac-Lys-Lys-Gly-$(Leu)_7$-Ala-$(Leu)_7$-Lys-Lys-Ala-$NH_2$) is an engineered lysine-flanked polyleucine peptide that has been shown to form a stable, hydrophobic transmembrane α-helix in a model membrane environment [20]. P16, like GramD, is not soluble in aqueous solution. MPX (Ile-Asn-Trp-Lys-Gly-Ile-Ala-Ala-Met-Ala-Lys-Lys-Leu-Leu-$NH_2$), a channel-forming peptide from wasp venom, has been shown to be random coil in aqueous solution and to assume an amphipathic α-helical conformation (from residues 3–14) in the presence of micelles, bicelles, or other membrane-mimetic solvents like trifluoroethanol (TFE) [21–24]. Although they have the same secondary conformation, unlike P16, MPX has been shown to associate at the membrane interface of zwitterionic bilayers [23]. These two latter peptides will therefore allow for direct comparison of effects of solvent and mechanism of membrane-association on the IR spectrum.

In this work, the secondary structure of these four membrane-binding peptides was studied by IR and CD in the presence and absence of different membrane-mimetics. Solvents examined for each peptide were chosen based on peptide solubility, for comparison to previous work, and to allow for comparison of the effect of aqueous vs membrane-like systems on peptide conformation. The membrane-mimetic solvents (alcohols, micelles, bilayers) have been shown to induce structure in these peptides and can therefore be used to examine solvent effects on the IR spectrum when the same secondary structure is present; this was particularly important in the examination of MPX. Results are compared to previous structural analyses by IR and additional spectroscopic methods. Consequences for the use of IR spectroscopy in adequately probing membrane-induced structural changes will be discussed.

## 2. Experimental

### 2.1. Materials

Leu-Enk, GramD, and spectroscopy solvents were purchased from Sigma-Aldrich (St. Louis, MO) and MPX was obtained from Bachem (King of Prussia, PA). P16 was synthesized using standard FMOC chemistry on a MilliGen 9050 solid-state peptide synthesizer and purified by high performance liquid chromatography following previously described procedures [20]. Dicaproylphosphatidylcholine (DCPC) and dimyristoylphosphatidylcholine (DMPC) were obtained from Avanti Polar Lipids (Alabaster, AL). All purchased chemicals and solvents were used without further purification.

### 2.2. IR

All samples for infrared spectroscopy were prepared by dissolving the peptides in the appropriate solvent ($D_2O$, alcohol, 5 mM DCPC monomers, 20 mM and greater concentration DCPC micelles [25] prepared in $D_2O$ (pH 7.1)). Concentrations were chosen based on instrument sensitivity and a final concentration of 1.5 mg peptide/mL yielded suitable signal/noise ratios for all peptides. This corresponded to: 1 mM MPX, 0.6 mM P16, 2.7 mM Leu-Enk, and 0.8 mM GramD. Additional samples with 15 mg/mL (27 mM) Leu-Enk were also examined. The pH of the peripheral membrane peptides ranged from 4.7 to 4.9 for aqueous Leu-Enk samples and 4.4 to 6.0 for aqueous MPX samples. Additional samples of Leu-Enk in DMPC multilamellar vesicles (MLVs) were prepared using a 15:1 lipid:peptide weight ratio as described previously [15]. Background subtracted spectra were obtained on a Perkin-Elmer Spectrum 1000 FT-IR spectrometer using $CaF_2$ windows. Each spectrum was acquired at room temperature with 300 scans at a resolution of 2 $cm^{-1}$. Absorbance numbers were converted to molar absorptivity for each spectrum and plotted using Igor Pro 5.04B. Spectra were smoothed using a 2nd order 13-point Savitsky–Golay function.

### 2.3. CD

Samples for CD spectroscopy were prepared by dissolving the peptide in each solvent system at concentrations based on instrument sensitivity. Resulting concentrations were: 1 mM MPX in $H_2O$ and 0.4 mM MPX in all other solvents, 0.4 mM P16 in all solvent systems, 1 mM Leu-Enk in $H_2O$ and 484 mM DCPC micelles and 0.07 mM Leu-Enk in 20 mM DCPC micelles, and 84 μM GramD in all solvent systems. The pH of DCPC micelles was 5.2 and the final pH values of the peripheral membrane peptides ranged from 4.4 to 4.9 for aqueous Leu-Enk samples and 6.2 to 6.7 for aqueous MPX samples. Spectra were obtained on an Aviv Model 215 circular dichroism spectrometer equipped with a Peltier-type thermoelectric cell holder using a 1 mm path quartz cuvette. Spectra were acquired in 1.0 nm intervals. Background was subtracted prior to conversion to molar ellipticity.

## 3. Results

### 3.1. IR assignments

Table 1 shows some characteristic frequencies in the amide I and amide II regions of the IR spectrum that have commonly been used as reference points for structural assignments. However, these frequency ranges are sometimes close together or overlap significantly. For example, as shown in Table 1, a band at 1640–1645 $cm^{-1}$ has been assigned to both antiparallel β-sheet and random coil conformation in aqueous solution. It is also not uncommon for a given secondary structure to result in a frequency outside of these ranges. Shifts in characteristic amide I frequencies have been observed due to solvent interaction or membrane-association even though the peptides maintained the same secondary structure under different conditions. For example, studies in Table 1 have shown that a peak at 1650 $cm^{-1}$ is characteristic of a buried α-helix in proteins whereas this peak is shifted to around 1632 $cm^{-1}$ for solvated α-helices. These differences are thought to arise from changes in hydrogen bonding patterns

**Table 1**
Previously determined characteristic IR frequencies as a function of secondary structure.

| Frequency ($cm^{-1}$) | Secondary structure | Source |
|---|---|---|
| Amide I | | |
| 1620–1640 | β-sheet(parallel, antiparallel) | [5,31] |
| 1675 ± 4 | β-sheet(antiparallel) | [5,31] |
| 1615–1637, 1696 | β-sheet | [42] |
| 1640 | β-sheet(antiparallel) | [42] |
| 1630(strong) + 1680(weak) | β-sheet(antiparallel) | [10] |
| 1645 ± 4 | Random coil | [5,31,43] |
| 1650 | α-helix | [9] |
| 1652 | α-helix | [44] |
| 1655 ± 4 | α-helix | [5] |
| 1650–1658 | α-helix | [13,45] |
| 1660–1670, 1680–1696 | β-turn | [5,31] |
| 1680 | β-turn | [13] |
| Amide II | | |
| 1547 | β-sheet | [14] |
| 1548 | α-helix | [44] |
| 1550(strong) + 1530(weak) | β-turn | [37] |
| Membrane-associated Amide I | | |
| 1610–1628 | β-sheet | [1] |
| 1640–1645 | Random coil | [1] |
| 1650–1655 | Buried α-helix | [1] |
| 1632 | Solvated α-helix | [46–48] |
| 1656–1670 | $3^{10}$ helix | [1] |
| 1670–1680 | β-turn | [49] |