Vibrational Circular Dichroism Is an Incisive Structural Probe: Ion-Induced Structural Changes in Gramicidin D

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Vibrational circular dichroism (VCD) measures[1] the differential absorption of left versus right circularly polarized infrared light originating from vibrational transitions of chiral molecules. This differential absorption is typically 5 orders of magnitude smaller than the vibrational absorption. Despite this weak nature of the signal, all $(3N - 6)$ vibrational modes of a chiral molecule (N being the number of atoms in the molecule) can give rise to circular dichroism; thus, VCD can lead to a much more detailed stereochemical information on the molecule of interest. This may be compared to electronic circular dichroism (ECD),[2] where only a limited number of electronic transitions are usually accessible. This becomes all the more important for peptides with aromatic residues, where the peptide electronic transitions occur in the far-ultraviolet region (around 220 nm) and these transitions are obscured by those from the aromatic residues.

Both VCD and vibrational Raman optical activity (VROA),[3] which is the Raman counterpart of VCD, have been shown to be useful for studying proteins. The complicated nature of vibrational modes and overlapping vibrational bands generally pose problems in the interpretation of vibrational optical activity spectra. Nevertheless, the amide I region represents an isolated region without such interpretational problems and provides useful information on the secondary structure of biological molecules.[4]

Gramicidin, a hydrophobic linear polypeptide consisting of 15 amino acids with alternating L- and D-configurations, is an antibiotic produced by *Bacillus brevis*.[5] The amino acid sequence[6] of gramicidin is: HCO-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-XXX-D-Leu-L-Trp-D-Leu-L-Trp-$NHCH_2$-CH_2OH. It is designated as gramicidin A when XXX = Trp, as gramicidin B when XXX = Phe, and as gramicidin C when XXX = Tyr. The mixture of gramicidin A, B, and C in the ratio of 80:5:15 is designated as gramicidin D. Gramicidin has attracted a lot of interest because of its complex conformational behavior, making it a good model for the study of polypeptide folding. When incorporated into phospholipid membranes gramicidin serves as an ion channel,[7] and this attribute makes it an ideal candidate for modeling of ion transport through biomembranes.

Veatch et al.[8] proposed an interconvertible four-species family of double helices, referred to as Species 1, 2, 3, and 4. All four species, and some monomeric forms, are thought to be present for gramicidin in methanol.[9] NMR studies indicated that the structure of gramicidin in solution changes in the presence of cations.[10−12] In the presence of lithium cations in methanol, the structure was proposed to change from multiple conformations in ion-free methanol solution to a random coil structure.[10] In the presence of cesium cations in methanol, the structure was proposed to change to a right-handed *antiparallel* double helix.[11] The structure of gramicidin in methanol in the presence of Ca^{2+} ions was suggested to be a *parallel* left-handed double helix.[12]

The influence of solvents on the structure of ion-free gramicidin has been investigated[9] using VCD, but there are no prior VCD studies on ion-induced structural changes in gramicidin. In this report we present the first VCD study on the structural changes in gramicidin D induced by ions and demonstrate that VCD is an incisive probe of the structure of gramicidin.

The infrared and VCD spectra were recorded at 8 cm^{-1} resolution on a commercial Fourier transform VCD spectrometer, Chiralir (Bomem-BioTools, Canada), with a ZnSe beam splitter, BaF_2 polarizer, optical filter (transmitting below 2000 cm^{-1}) and a 2 × 2 mmHg CdTe detector. Gramicidin D sample was purchased from ICN Biochemicals; CsCl was purchased from Aldrich; $CaCl_2$, LiCl were purchased from Sigma chemical Co; methanol-d_4 was purchased from Cambridge Isotope Labs. All gramicidin concentrations were 4 mg/mL and the temperature was 20 °C. The spectra were measured as functions of increasing ion concentration until no further major changes were observed. For the sake of brevity, only the higher ion-concentration spectra are presented. All spectra were recorded, with 1 h data collection time, in a variable path length cell with BaF_2 windows. The presented spectra are limited to the 1800−1300 cm^{-1} region, which contains the amide I and amide II bands. For all of the spectra presented here, solvent absorption and VCD have been subtracted out; the absorption and VCD spectra were scaled to give a maximum absorbance of 1.0 in the region shown.

The absorption and VCD spectra of ion-free gramicidin in methanol-d_4 are shown in Figure 1a. The amide I absorption band is present at 1635 cm^{-1}, with a strong shoulder in the 1640−1660 cm^{-1} region, and the amide II band is present at 1454 cm^{-1}. The VCD spectrum shows a positive couplet in the amide I region (with negative component at 1651 cm^{-1} and positive component at 1628 cm^{-1}) and negative couplet in the amide II region (with positive component at 1452 cm^{-1} and negative component at 1431 cm^{-1}). These observed features were associated[9] with four species of double helix structures and some amount of a monomeric structure. The absorption and VCD spectra of gramicidin in the presence of Li^+ ions are shown in Figure 1b. The amide I absorption band shifts to 1663 cm^{-1}, and no significant change is seen in the amide II absorption band. VCD in the amide I and II regions decreases gradually as the Li^+ concentration is increased and disappears completely at 2.4 M or higher. The absorption and VCD spectra of gramicidin in the presence of higher

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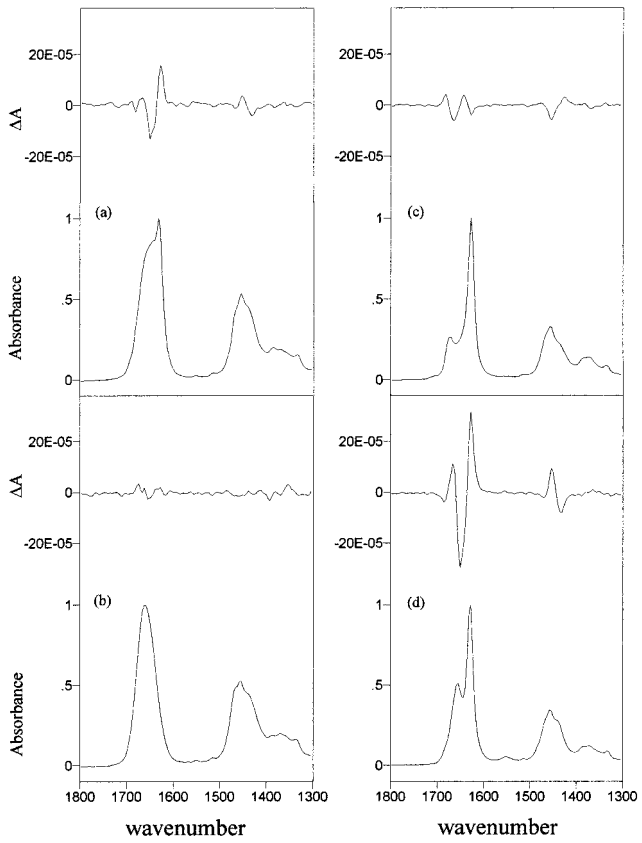

Figure 1. Vibrational absorption (bottom) and VCD (top) spectra of gramicidin D in methanol-d_4 solution: (a) ion-free; (b) in the presence of 2.4 M Li^+ ions; (c) in the presence of 40 mM Cs^+ ions; (d) in the presence of 4.8 mM Ca^{2+} ions. The absorption and VCD spectra were scaled to give maximum absorbance of 1.0 in the region shown. The actual peak absorbances used in the measurements were: 0.33 for (a); 0.26 for (b); 0.58 for (c); 0.55 for (d). Solvent absorption and VCD were subtracted out. The data collection time was 1 h in each case.

concentrations of Li^+ resemble that of ion-free gramicidin[9] in DMSO-d_6, where a random coil monomeric structure was proposed. This observation is consistent with NMR studies[10,11] which also concluded a random coil structure for gramicidin in the presence of Li^+ ions. The absorption and VCD spectra of gramicidin in the presence of Cs^+ ions are shown in Figure 1c. The amide I region shows two well-resolved absorption bands at 1674 and 1628 cm^{-1} and a positive−negative−positive−negative VCD pattern. VCD in the amide II region changes from a negative VCD couplet for ion-free gramicidin to a positive VCD couplet for Cs^+-bound gramicidin. The solution structure for gramicidin in the presence of Cs^+ ions has been shown[11] to be a right-handed *antiparallel* double helix, so the observed VCD features in the presence of Cs^+ ions are attributed to this structure. The absorption and VCD spectra of gramicidin in the presence of Ca^{2+} ions are shown in Figure 1d. The amide I region shows two well-resolved absorption bands at 1655 and 1628 cm^{-1} and a negative(weak)−positive−negative−positive VCD pattern. It is important to note that the VCD sign pattern seen for Cs^+-bound gramicidin is opposite to that seen for Ca^{2+}-bound gramicidin. This can be interpreted as a reversal of the handedness of helical structure which means that the structure of Ca^{2+}-bound gramicidin is a left-handed double helix. However, there must be some other change, besides handedness, between the structures of Cs^+-bound gramicidin and Ca^{2+}-bound gramicidin because the resolved amide I absorption bands in these two cases are separated by 46 and 27 cm^{-1}, respectively; and the VCD intensities for Ca^{2+}-bound gramicidin are larger than those for Cs^+-bound gramicidin. This in fact is the case, since the structure of Ca^{2+}-bound gramicidin is shown[12] to be a left-handed *parallel* double helix while that for Cs^+-bound gramicidin is right-handed *antiparallel* double helix. Thus, the structural information deduced from the present VCD studies is consistent with that deduced from the solution-phase NMR studies.

The far-ultraviolet ECD spectra of ion-free gramicidin[13] shows two negative peaks at ∼213 and 229 nm, which are assigned as $\pi-\pi^*$ and $n-\pi^*$ transitions. (a) These two negative peaks change[13] to one positive peak at 228 nm for both Li^+-bound gramicidin and Cs^+-bound gramicidin. Thus the ECD spectra do not indicate any major structural difference between Li^+-bound gramicidin and Cs^+-bound gramicidin. In contrast, VCD spectra reveal remarkable changes (compare Figure 1b and 1c) for these two, as in NMR studies.[10] (b) ECD spectra of Ca^{2+}-bound gramicidin[14] show the same two negative features as for ion-free gramicidin but with increased intensities, so that the conclusions about the structural changes between ion-free gramicidin and Ca^{2+}-bound gramicidin have to depend only on the intensities. But VCD spectra very clearly show marked differences both in intensities and sign patterns (compare Figure 1a and 1d) for these two cases. (c) The ECD spectrum of Cs^+-bound gramicidin shows[13] a single positive peak at 228 nm, while that of Ca^{2+}-bound gramicidin shows[14] two negative peaks at ∼213 and 229 nm. Thus, the reversal of handedness between these two cases is not that obvious from the ECD data. However, the oppositely signed VCD pattern (Figure 1c and 1d) in going from Cs^+-bound gramicidin to Ca^{2+}-bound gramicidin clearly indicates the reversal of handedness in these two cases. The structural differences among Li^+-bound, Cs^+-bound, and Ca^{2+}-bound gramicidin deducible from ECD spectra are limited probably because aromatic residues comprise ∼25% of the amino acid residues of gramicidin and the electronic transitions from aromatic residues obscure the peptide transitions. Such interference is not present for the vibrational transitions in the amide I region. Thus, VCD serves as a better probe for the structures of peptides that contain aromatic residues.

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