Spectroscopy

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Spectroscopy of Isolated Gramicidin Peptides**

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Many details of intrinsic properties of biomolecules are masked by elements of the biological environment, such as the solvent and intermolecular interactions. Separation of the inter- and intramolecular aspects of biomolecular function requires the study of isolated molecules under solvent-free conditions or with a controlled and limited number of solvent molecules. This can be achieved by gas-phase spectroscopy; however, this approach has been limited to small molecules with masses below a few hundred amu. In 1985 Rizzo et al. reported the first molecular beam experiments with tryptophan[1,2] followed by work on di- and tri-amino acids.[3–5] Ever since, the hope to extend this technique to larger molecules has met with very little success. Although electrospray and

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MALDI have allowed mass spectrometry of very large ionic biomolecules,[6,7] the optical spectroscopy of neutral molecules in the gas phase has been largely restricted to systems with up-to-three residues.[8–12] An exception is a recent report by Bakker et al. on the nonapeptide DSIP (delta-sleep-inducing peptide)[11] with a molecular weight of 849 amu. Herein we report on the first double-resonance IR–UV spectroscopy of isolated gramicidin, a family of 15-residue peptides with molecular weights of 1845–1898 amu. The results are consistent with a secondary structure similar to that in the bulk.

This result shows not only that it is possible to bring these peptides into the gas phase intact but also that they adopt unique structures that reveal details of the intramolecular stabilizing forces. Furthermore, the results show that high-resolution spectroscopy can be performed on these peptides in spite of the large number of internal degrees of freedom. This capability opens the way to molecular-level studies of intermediate-size isolated peptides and of their interactions with individual molecules, such as water, in mass-selected clusters.

This approach employs laser-desorption jet-cooling, followed by double-resonance IR–UV spectroscopy.[13–16] Briefly, we placed biomolecules in the gas phase by pulsed laser desorption, followed by entrainment in a supersonic expansion of argon.[17] We performed electronic spectroscopy in the ultraviolet range of the spectrum by resonance-enhanced two-photon ionization (R2PI). We detected the ions in a TOF-mass spectrometer. We recorded IR spectra by employing an IR "burn" laser pulse, followed after 100 nanoseconds by a UV "probe" laser pulse, which two-photon ionizes through the S1 state. When the IR laser is tuned to a resonance it depletes the ground state, which causes a decrease in the ion signal. In this way, we can record ground-state IR spectra of mass-selected, as well as optically selected molecules and clusters. Furthermore, even when the UV absorption is broad, it is possible to obtain sharp IR spectra with this technique. IR–UV double-resonance spectroscopy is a highly sensitive structural probe because it allows us to measure OH and NH stretch frequencies that shift significantly upon hydrogen bonding.

Gramicidin is a biosynthetic product from *Bacillus brevis* that derives its functionality from forming a monovalent cation-selective channel in the lipid bilayer of targeted cells.[18,19] The channel is built from two peptides back to back, each in a single-stranded right-handed β-helical motif with 6.5 residues per turn. Each peptide is arranged with the carboxylic terminus at an interface of the bilayer and the protected amino terminus buried inside (see Figure 2). As the structure does not rely on a polar solvent, it is possible that the helix persists in the gas phase. Gramicidin consists of a mixture of components that each differ in one residue; Table 1 lists their sequences and formula weights. Figure 1 shows the two-photon ionization mass spectrum of a commercial mixture that is characterized by the parent masses and the absence of fragmentation. By monitoring each of the parent mass peaks as a function of ionization wavelength or hole-burning wavelength, we obtained the spectra of the separate components. The R2PI spectra are broad (not shown) but appear to correlate to one conformer, or a family of closely related conformers, because the double-resonance spectra were identical for all UV probe wavelengths tested. Remarkably, in spite of the large scope of the conformational landscape available to a peptide with up to 15 residues, all peptides in this study appear to adopt a single type of conformation without the aid of a solvent or other element of biology.

Table 1: Sequence of components of gramicidin: HCO–Val–X–Ala–D-Leu–Ala–D-Val–Val–D-Val–Trp–D-Leu–Y–D-Leu–Trp–D-Leu–Trp–$NHCH_2CH_2OH$.

Component	X	Y	M_r [amu]	Average composition
A	Gly	Trp	1880	80–85%
B	Gly	Phe	1845	6–7%
C	Gly	Tyr	1861	5–14%
D	Ala	Trp	1898	1%

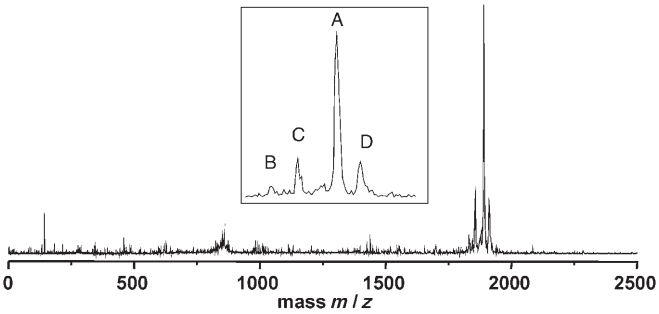

Figure 1. TOF mass spectrum of a gramicidin mixture with its relative abundances listed in Table 1.

Figure 2 shows the IR–UV spectra for each of the four components. Ab initio computations are currently under way to aid in the detailed analysis of these spectra. The structure optimizations, required before vibrational frequencies can be calculated, will take some time for a molecule this size. However, in the absence of such computations, we can already make some observations based on the spectra. The data are all consistent with a helical conformation: free indole NH frequencies are consistent with the tryptophan residing on the outside of the helix as is the free tyrosine OH frequency. Along the backbone, all peptide NH groups are hydrogen bonded leading to an intense broadened and red-shifted peak. The large-amplitude overtone of the NH bending mode at about 3100 cm^{-1} is also indicative of strong NH group hydrogen bonding. Comparison of the spectra of gramicidin A and C demonstrates sequence specificity. The difference between the two sequences is in a single residue, which is a tryptophan (W) in one case and a tyrosine (Y) in the other. The trace for the C form clearly shows the tyrosine OH stretch frequency that is completely absent in trace A.

To gauge the sensitivity of this approach to local structure, Figure 3 compares spectra for three different folding motifs. Figure 4 shows the spectrum of one of these, gramicidin S, in more detail. Its symmetrical β-sheet-type structure leads to pairs of equivalent functions: one pair of free NH_2 (rectangle), two pairs of free NH (solid gray), and two pairs of

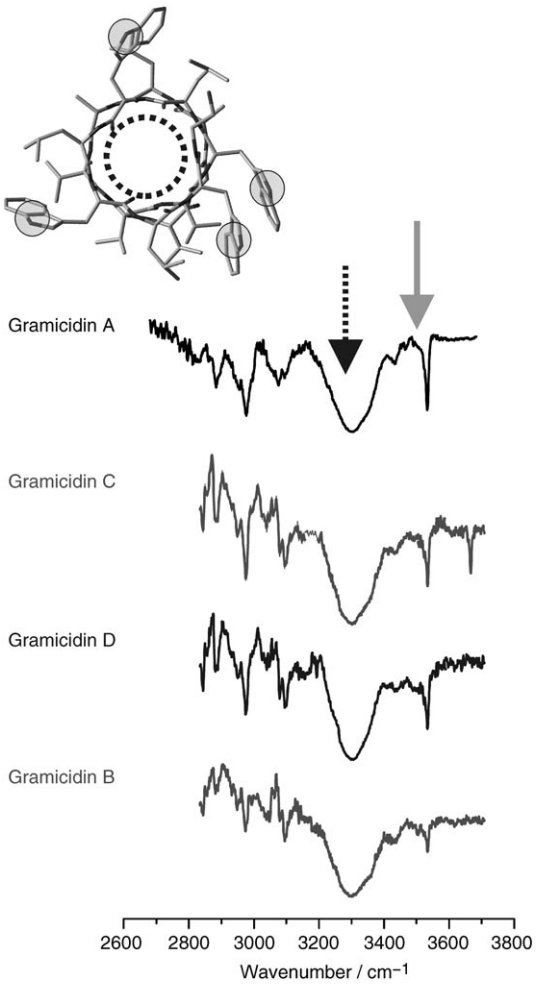

Figure 2. IR–UV double-resonance spectra of gramicidin A–D recorded on the masses of the four components shown in Figure 1. The dotted arrow indicates the wavelength region corresponding to hydrogen bonded NH from the backbone, outlined with a dotted circle in the structure at the top. The gray arrow indicates the wavelength region for free indole NH, outlined with gray circles.

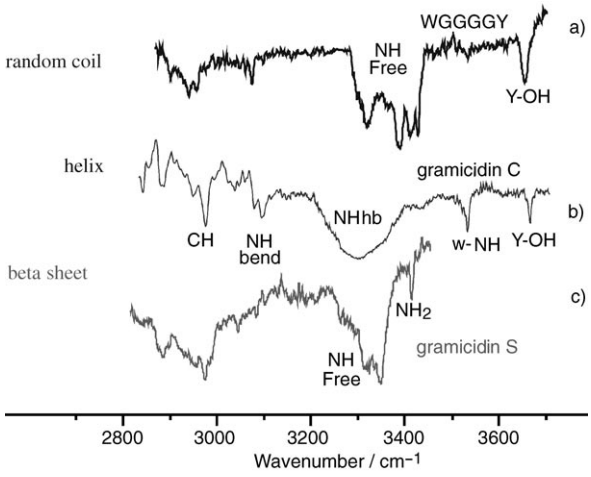

Figure 3. IR–UV double-resonance spectra of three different peptide sequences. The spectra show the distinct response for three different folding motifs. hb = hydrogen bonding.

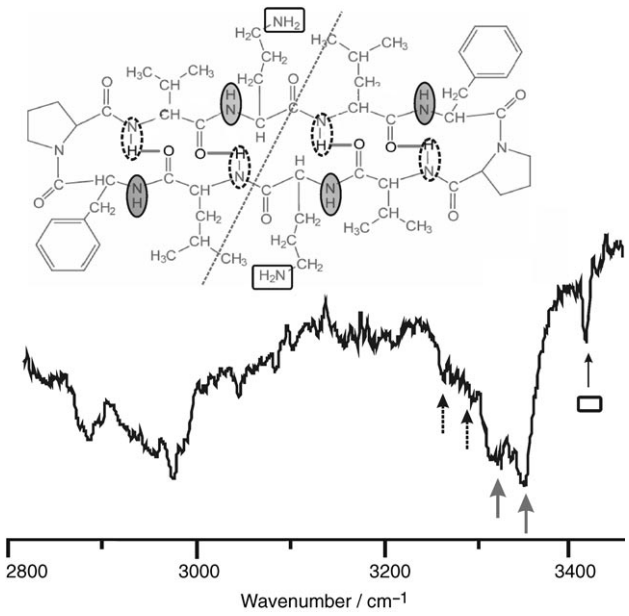

Figure 4. IR–UV double-resonance spectra of gramicidin S recorded on its parent mass. The solid gray arrows indicate the wavelength region corresponding to free or weakly hydrogen bonded NH, dotted black arrows indicate wavelengths for hydrogen bonded NH and the rectangle marks the region for free NH_2. Corresponding moieties in the molecule are marked with the same codes.

hydrogen bonded NH (dotted black). This is consistent with the appearance of the spectrum, which contains the free NH_2 peak as well as structure in the NH peak, which can be interpreted as two shifted and two unshifted peaks. Once again, the secondary structure appears to be maintained in the gas phase in this case. This may be contrasted with the spectrum of WGGGGY in the top trace of Figure 3. Glycine is a helix breaker and thus we would expect this to be a model for a random coil-like structure. Indeed, the NH stretch region is not strongly broadened and shifted as is the case for gramicidin, and the isolated NH frequencies are evident. Furthermore, this spectrum does not show a free indole NH, indicating that the tryptophan is now interacting with other parts of the molecule, rather than being on the outside of a helix. We also obtained the spectra for dimers and trimers of WGGGGY, which show a transition to the bulk spectrum of this compound.

In the analysis of IR spectra of peptides, it is customary to use the amide I and II regions in the mid-IR range, at around 1500 cm^{-1}, for structure analysis. The near-IR region presented herein is usually ignored because, in bulk data, it is insufficiently diagnostic. As can be seen, however, with the increased resolution afforded by the gas phase under jet-cooled conditions, even the near-IR region can be used for structure elucidation. Part of the mid-IR region can also be studied here through the overtones, particularly for the NH bending modes. Preparations are currently under way to perform these same experiments at a free-electron laser facility to access the mid-IR range.[11,20] Traditional analysis relies heavily on deconvolution of overlapping peaks and is based on model peak shapes. The ability to study that same

region in the gas phase with higher resolution and the ability to study isolated peptides should make this approach significantly more powerful.

The main objective of gas phase spectroscopy is not necessarily structure determination but rather the study of local interactions in isolated molecules. The current results demonstrate that different folding motifs lead to different IR signatures that are much more distinct than what is normally used for structure elucidation by infrared absorption in the condensed phase. The great increase in IR resolution in isolated, cooled peptides opens the way to sensitive probing of local interactions that stabilize folding structures. The mass selectivity in the technique makes it possible to obtain size-dependent cluster spectra. One possible application is the systematic study of mass-selected clusters with water. By following the IR signatures as function of the number of water molecules attached to a peptide, it should be possible to study the role of the solvent at the molecular level.

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