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Highly Resolved Spectra of Gas-Phase Gramicidin S: A Benchmark for Peptide Structure Calculations

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Theoretical predictions of molecular structure have become ubiquitous in biochemical and biophysical research and are at the heart of in silico drug design. While IR spectroscopy can in principle provide a test of predicted structures, the degree of constraint imposed depends on the spectral resolution. For small peptides in the gas phase, high-resolution IR−UV double-resonance spectra in both the light-atom stretch region[1−8] and the amide I and II regions[6,9,10] can convincingly distinguish between calculated structures. Structural identification becomes less convincing for lower-resolution spectra resulting from the use of broader IR laser sources[3,4,11,12] and/or from thermal broadening.[13,14] Here we report high-resolution electronic spectra and conformer-selective vibrational spectra of the doubly protonated natural antibiotic gramicidin S (cyclo-VOLFPVOLFP)[15] in the gas phase. Our method employs a table-top optical parametric oscillator (OPO) with a line width of ∼1.5 cm^{-1} and records linear IR spectra in the 6 μm region, which was demonstrated previously only on small peptides.[6,10] The narrow line width, together with cooling in an ion trap and the conformational selectivity afforded by IR−UV double resonance, allows us to measure highly resolved vibrational spectra on larger peptides that can serve as a critical benchmark for testing structure calculations.

Much of our experimental apparatus has been described in detail elsewhere.[7,8] Briefly, we generate protonated peptides in the gas phase using a nanospray ion source. The ions of interest are preselected by a quadrupole mass filter and stored in a 22-pole ion trap kept at 6 K, where they are cooled by collisions with helium. The trapped ions are then interrogated by UV photofragmentation or by depletion of this fragmentation signal in an IR−UV double-resonance scheme. For the latter, we use 1.5−2 mJ of 6 μm light delivered by an IR OPO (LaserVision) together with difference-frequency mixing in a $AgGaSe_2$ crystal.

The electronic spectrum of gramicidin S (GS) already provides some indication of its structural features. Figure 1 shows UV photofragment spectra of doubly protonated GS obtained under the coldest (Figure 1a) and slightly warmer (Figure 1b) conditions. We assign the transitions in these spectra to at least three different conformers, which we have designated as **A**, **B**, and **C**. A vibrational progression of strong transitions (A1−A4) with a spacing of 16 cm^{-1} appears in the region above 37 700 cm^{-1}, and we assign these peaks to conformer **A**. Transitions A2−A4 are each clearly split by 3.9 cm^{-1}. We attribute this splitting to vibronic (exciton) coupling between the two Phe chromophores, and we consider below its implication for the structure of GS. Comparison of spectra (a) and (b) in Figure 1 reveals that the relative intensity of only the first peak of the progression (A1) increases with increasing temperature. This suggests that A1 is a hot band and that the next peak of the progression, A2, is the band origin of conformer **A**. The intensities of the peaks labeled C and D change differently than those of conformer **A** with increasing temperature, implying

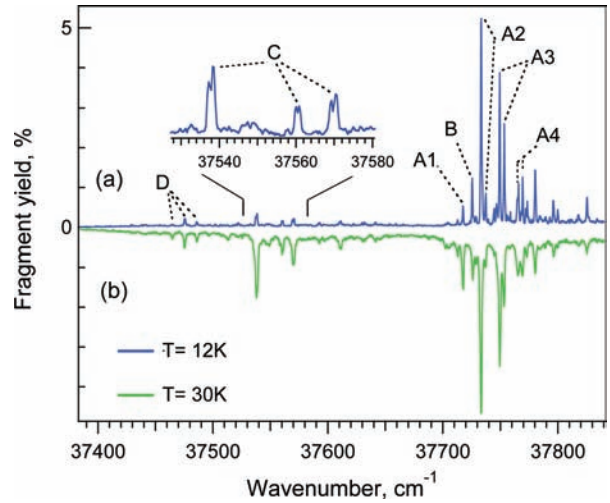

Figure 1. Electronic spectra of doubly protonated GS cooled to (a) ∼12 and (b) ∼30 K. The inset in (a) shows details of the three peaks labeled C. The vibrational temperature of the ions was estimated from the relative intensity of the hot band, A1. Spectrum (b) was detected using the IRLAPS technique.[16]

that they correspond to two higher-energy conformers. A closer look (Figure 1a inset) reveals that the peaks labeled C are split by ∼1 cm^{-1}, and we also attribute this splitting to exciton coupling of the two Phe chromophores.

Splitting due to coupling of electronic transition dipole moments (TDM) of two phenyl groups scales with their separation R as $1/R^3$. It is also governed by the relative orientation of the TDMs.[17] The diphenylmethane (DPM) molecule[17,18] can be used to estimate the magnitude of exciton splitting in GS. The two phenyl groups are separated by 0.4 nm in DPM[18] and 1.6 nm in the structure of GS calculated at the BP86/SVP level (Figure S1 in the Supporting Information). Scaling the splitting of 123 cm^{-1} in DPM[18] to the calculated spacing in GS would result in a ∼2 cm^{-1} splitting, which is on the same order as that observed in conformers **A** and **C**. This supports our suggestion that the splittings are excitonic in nature and implies that the difference between the splittings in **A** and **C** arises from a larger spacing between chromophores in conformer **C** and/or a difference in their relative orientations.

The position of the band origin in the electronic spectrum also contains some structural information. The band origin of conformer **A** (37 733.5 cm^{-1}) is significantly shifted to higher energy with respect to those of gas-phase neutral Phe (37 535 cm^{-1})[2] and several protonated helices where the charge is remote from the Phe chromophore.[7] Such an unusually large blue shift suggests that the phenyl groups are in close proximity to the charges, which are located on the side chains of the ornithine residues. This is confirmed by experiments in which solvation of the charges with

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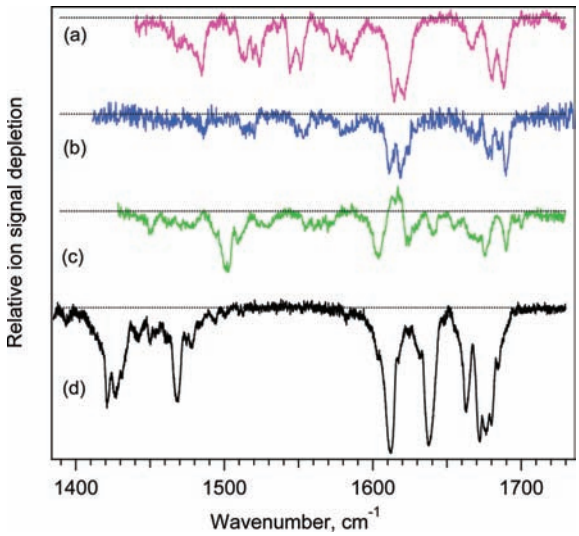

Figure 2. IR−UV depletion spectra of doubly protonated GS: (a) conformer **A**; (b) conformer **B**; (c) conformer **C**; (d) conformer **A** of the deuterated peptide. The strongest peak in (d) corresponds to a depletion of 82%. The substantial signal above the baseline in (c) around 1617 cm^{-1} is an artifact (IR−UV "gain") due to absorption at this wavenumber by the more abundant conformer **A**.

crown ether molecules (18-crown-6) removes this shift (see Figure S2). Moreover, the fact that conformer **A** exhibits only a single split electronic band shifted to higher frequency indicates that both phenyl groups interact with the charged ornithine side chain in a symmetrical structure. The lack of a significant shift in the band origin of conformer **C** may reflect the remoteness of charges from both chromophores.

While the electronic spectrum provides indirect evidence for the presence of at least three conformers, IR−UV double-resonance spectroscopy can unambiguously verify this. For example, fixing the wavenumber of the UV excitation laser on transitions A2 or A3 in Figure 1 produces identical IR depletion spectra (Figure 2a). This supports our assignment that the UV transitions in this progression belong to the same conformer (**A**). IR depletion of the small peak at 37 538 cm^{-1} produces a different IR spectrum (Figure 2c), supporting our assignment that this transition belongs to a conformer different from **A**, which we designated above as **C**. IR depletion of the transition labeled B produces an IR spectrum (Figure 2b) that is similar to, but reproducibly different from, the spectrum of conformer **A**, and we assign this to a third conformer, **B**. We thus have clear evidence of three conformers of doubly protonated GS in the gas phase and a hint of at least one additional conformer (**D**).

In addition to verifying the presence of several conformers, the IR spectra in Figure 2 provide further information on the conformer structures. In Figure 2a, the five peaks lying above ∼1600 cm^{-1} belong to the 10 bands that have primarily C═O stretch character (amide I), and the peaks at lower wavenumber arise from the NH bending vibrations (amide II), although there is some mixing between the stretches and bends in both regions. This assignment is confirmed by comparison of this spectrum with that of deuterated (NH → ND) GS (Figure 2d), where the ND bends are strongly shifted to lower frequency, as expected. The appearance of less than 10 CO bands in the undeuterated molecule could come from either cancellation of transition intensity for carbonyls that are almost exactly antiparallel[9] or overlapping bands that are simply symmetrically equivalent, even in a noncoplanar arrangement. The shifts of these bands upon deuteration allow us to partially resolve some of them, supporting the latter explanation. This suggestion is further supported by calculations of vibrational spectra for the lowest-energy conformers of GS (Figure S3), which exhibit characteristic doublets for C═O stretches in identical residues. The backbones of these calculated structures are highly symmetric (C_2) (Figure S1), similar to the β-sheet structure of GS in the condensed phase.[19,20] However, none of our calculated spectra, nor those reported recently by Kupser et al.,[14] exhibit an acceptable match with our measured spectra, indicating that a more sophisticated computational approach is necessary in order to determine the structure of a molecule as large as GS.

In summary, highly resolved electronic spectra of cold, gas-phase gramicidin S suggest a symmetrical structure in which the Phe chromophores are only weakly coupled to each other but interact strongly with charged Orn side chains. Highly resolved, conformer-specific IR spectra in the amide I and II regions reinforce the suggestion of a symmetrical structure, as the C═O stretch bands seem to occur in unresolved doublets. These spectra, which are the highest-resolution ones available for a decapeptide, together with the qualitative constraints that they imply, stand as a challenge and a benchmark for computations of peptide structure.

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Supporting Information Available: Calculated geometry and computational method, UV spectra of GS and the complex of GS with two 18-crown-6 molecules, and sample calculated spectra for the low-energy structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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