The dipeptide cyclic(glycyltryptophanyl) in the gas phase: A concerted action of density functional calculations, $S_0$–$S_1$ two-photon ionization, spectral UV/UV hole burning and laser photoelectron spectroscopy

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The $S_0$–$S_1$ spectrum of the dipeptide cyclic(glycyltryptophanyl) (cGW) has been recorded by two-photon ionization (R2PI) spectroscopy of thermally evaporated, jet-cooled molecules. The R2PI spectrum contains several vibronic transitions with relatively small spacing. Density functional theory predicts more than 8 conformers. Applying spectral UV/UV hole burning spectroscopy we found that the strongest $S_0$–$S_1$ transitions belong to a single conformer. The transition at 35 058 cm$^{-1}$ is attributed to the $S_1$ origin and the other transitions to inter-ring modes. To obtain further information on the conformer structure, resonant two-colour two-photon ionization photoelectron spectroscopy (R(1 $\nu$ 1')) has been performed via the two most intense $S_0$–$S_1$ transitions. To our knowledge these are the first PE spectra of a dipeptide. The spectra are broad with a smooth onset. The lowest ionization onset lies at 7.709 eV, but is assumed to be not the adiabatic IE. Theoretical calculations with the B3LYP/6-311++G** theory predict that in the most stable neutral conformer an N–H group of the dipeptide ring binds to the indole π system. This structure has the highest IE of all conformers investigated and shows a strong geometry change upon ionization. The fact that the IE of the experimentally observed cGW conformer is higher than that of 3-methylindole is taken as a signature of a repulsion between the two rings in the cation: This could be explained by the N–H group of the peptide ring now interacting with the positively charged indole π system. The conformational assignment by R(1 $\nu$ 1')PI PES is tentative, because all of the lowest-energetic neutral conformers are expected to behave similarly in regard to ionization energies.

I Introduction

The advantage of gas phase measurements is the applicability of spectroscopy methods such as resonant two-photon ionization (R2PI) spectroscopy combined with mass-selective detection, spectral UV/UV or IR/UV hole burning (UV/UV SHB or IR/UV SHB) spectroscopy and resonant two-colour two-photon ionization photoelectron spectroscopy (R(1 $\nu$ 1')PI PES). Further advantages of gas phase investigations are usually sharp spectra which reveal detailed structural information. Most importantly, however, gas phase data can be directly compared to theory. For reviews on biological molecules in the gas phase see ref. 1–4.

Levy and co-workers were the first to perform spectroscopy of di- and tripeptides in the gas phase by using laser desorption with R2PI and mass-selective detection.5,6 For tryptophan-containing peptides they obtained sharp spectra with distinct lines despite the many vibrational degrees of freedom and the many possible conformers. The spectroscopy of laser-desorbed small peptides was later improved by using double resonance methods.7,8 Neutral laser desorption and R2P ionization and laser fragmentation was also introduced as a new species selective inlet system into mass spectrometry.9–11

Thermal evaporation of natural unprotected peptides is impossible.12,13 Up to now only for some modified peptides in which the polar functional groups have been protected by methyl groups a thermal evaporation of intact molecules is reported.14,15 Cyclic dipeptides have no polar N or C terminal and hence, as we show here, can be vaporized intact by heating. To our knowledge up to now no investigation of cyclic peptides in the gas phase has been performed. UV/UV or IR/UV double resonance methods are widely used in spectroscopy of floppy molecules and clusters.7 The strategy to obtain structural information by using zero kinetic energy electron PES (ZEKE7), mass analyzed threshold ionization (MATI7) or high resolution conventional laser PES18 is not new.18–25 In these publications model systems, such as extended molecules with a chromophore and a flexible side chain, have been investigated. Whereas for non-polar side groups sharp PE spectra are observed exclusively18–25 for polar groups sharp spectra are only expected for conformers where the interaction of the charge site and the polar group are minimized, i.e. unfolded, linear structures.18 In folded structures the polar group solvates the charge strongly and structural changes upon ionization become so large that it is impossible to reach the origin of the cation ground state and obtain vibrationally resolved spectra.22 Nevertheless, the IE shift together with theory may provide useful information to allow an identification of the neutral conformation.22 Only in one case of an aromatic molecule with a polar side chain, both folded and unfolded conformers showed sharp vibrationally resolved spectra.24,25

In this paper we report on spectroscopy of the cyclic(glycyltryptophanyl) (cGW) in the gas phase. R2PI spectroscopy with mass selection has been used to record the $S_0$–$S_1$ spectrum. UV/UV SHB was applied to analyze the number of conformers present in the molecular beam. To obtain further...
information on the conformer structure, $R(1 + 1')PI$ PES was performed for the two most intense $S_0 - S_1$ transitions. Density functional theory (DFT) calculations on the B3LYP/6-311+G** level are used to interpret the number and structure of observed conformers, IEs and geometry shifts upon ionization. A tentative conformer identification is given on the basis of a comparison between experiment and theory.

II Experimental

The cyclic peptide cGW was purchased from Bachem. It has been synthesized from pure $l$-tryptophan (W) and hence cGW has also $l$ chirality. The apparatus is described in detail elsewhere.\textsuperscript{18} Briefly it consists (Fig. 1) of a supersonic jet chamber (a), a field-free time-of-flight PE spectrometer (b) and a pulsed linear time-of-flight mass spectrometer (c). A modified pulsed nozzle (General Valve, orifice diameter 300 $\mu$m) was heated to achieve a suitable sample vapor pressure. The indole sample has been added to a sample container above the nozzle and heated to 325 K. The cGW sample, crystalline at room temperature, is placed directly in the nozzle head and heated to 525 K. Ar was used as carrier gas with a backpressure of 5 bar. The jet passed the skimmer (1.5 mm orifice) and crossed the laser beam at right angles in the middle of the PE spectrometer. The time-of-flight PE spectrometer is 900 mm long, $\mu$-metal shielded and covered inside by graphite. Only photoelectrons emitted into the small acceptance angle of the two-stage micro channel plate (MCP) detector were detected and their time measured with high precision in a start-stop logic.

Fig. 1 Experimental setup of the photoelectron spectrometer. Indole has been heated to 325 K and cGW to 525 K to achieve suitable sample vapor pressures. The sample was expanded and cooled in a pulsed rare gas expansion (5 bar nozzle backing pressure) and finally transferred through a skimmer into the field-free PE spectrometer. Electrons emitted in the direction of the micro-channel plate (MCP) detector have been detected and their flight time measured with a start-stop logic. The ions formed in the PE spectrometer drift downwards with their supersonic jet very much depended on the expansion gas and heated to 325 K. The time-of-flight PE spectrometer is 900 mm long, $\mu$-metal shielded and covered inside by graphite. Only photoelectrons emitted into the small acceptance angle of the two-stage micro channel plate (MCP) detector were detected and their time measured with high precision in a start-stop logic.

Note that for photoelectron spectroscopy no ion extraction plates have been used within the PE spectrometer. After ionization, the ions drift downwards with their supersonic jet velocity into a pulsed linear time-of-flight (linTOF) mass spectrometer (mass resolution $m/\Delta m \times 100$). Hence, $R2PI$ mass spectra, mass-analyzed $R2PI$ spectra and $R(1 + 1')PI$ PE spectra with simultaneous mass control can be recorded in the same apparatus. Only for UV/UV SHB experiments three deflection plates were introduced at the ionization site in the PE spectrometer. In this case the ions were formed in an electric field and deflected into the former electron detector. The laser system consisted of two Nd:YAG laser-pumped and frequency-doubled tunable dye lasers (5–7 ns pulse width) and an additional excimer-pumped frequency-doubled dye laser which can be arbitrarily shifted in time with respect to the Nd:YAG-pumped dye lasers.

R2PI spectroscopy

For recording the $R2PI$ spectrum ions were formed in the PE spectrometer and, after drifting down, mass-analyzed in the pulsed linear TOF mass spectrometer. The $S_0 - S_1$ spectrum was recorded by tuning the laser wavelength and recording the parent cations with a gated integrator. For this purpose we used the Nd:YAG-pumped frequency doubled dye laser (resolution in the UV: $< 1 \text{ cm}^{-1}$, 100 $\mu$m pulse$^{-1}$, 1000 mm lens out of focus). We found that the cooling efficiency of the supersonic jet very much depended on the expansion gas and improved with increasing mass of the carrier gas.

UV/UV SHB

For the SHB a three-plate ion optics (repeller plate, intermediate plate, grounded exit plate) was mounted in the PE spectrometer which deflected and accelerated the ions to the electron detector of the PE spectrometer. By changing the MCP voltages suitable for ion detection mass and UV/UV SHB spectra have been measured. The mass resolution $m/\Delta m$ of this lin-TOF is 1500. This setup is needed to deflect the ions formed by the hole burning laser by using a pulsed electric field scheme. For this purpose we used the Nd:YAG-pumped frequency doubled dye laser (resolution in the UV: $< 1 \text{ cm}^{-1}$, 100 $\mu$m pulse$^{-1}$, 1000 mm lens out of focus). We found that the cooling efficiency of the supersonic jet very much depended on the expansion gas and improved with increasing mass of the carrier gas.

For R(1 + 1')PI PE spectroscopy ionization was performed by two frequency-doubled dye lasers pumped by the same Nd:YAG laser. The ionizing laser (laser 1) was time-delayed by 3 ns against laser 1 which performed the $S_0 - S_1$ excitation. The photon energy of laser 2 was not sufficient for a two-photon ionization. The one-photon signal of laser 1 was lower than 5% of the total two-color ion signal. Typical laser pulse energies were 10 $\mu$J for laser 1 and 20 $\mu$J for laser 2 (1000 mm lens, out of focus). To optimize the electron energy resolution, the photon energy of laser 2 was chosen to keep the maximum photon excess energy below 200 meV. For indole we achieved a PE energy resolution $\Delta E = 1 \text{ meV}$ and an absolute accuracy of $\pm 2 \text{ meV}$. The accuracy for cGW was $\pm 3 \text{ meV}$ due to the smooth onset at the ionization onset.

III Theoretical results

Our motivation to perform calculations on cGW was (i) to obtain the relative energies of the neutral and cation
Conformers, (ii) to calculate the vibrational frequencies for the lowest conformers, (iii) to calculate the vertical IEs and (iv) to explore the structural differences between the neutral and cation for the most stable conformers. For conformer assignment we use the comparison of the experimental to calculated data, such as relative neutral conformer energies, vertical IEs and neutral to cation geometry shifts.

So far no ab initio or DFT calculations of a cyclic peptide are reported. We used the density functional B3LYP26 because according to our experience28 it seems to better describe flexible molecules with polar groups than other methods. It is well known that B3LYP systematically underestimates intramolecular electron correlation forces as present in folded molecules. There could be several explanations. One would be an accidental error compensation, another that electrostatic forces dominate the binding.

The greatest advantage of DFT methods is that they are fast enough to allow the use of big basis sets even for large molecules. This is very important for folded molecules because it minimizes the basis set superposition error usually favoring folded molecules energetically. DFT calculations have been performed with the Gaussian 98 program.27 The basis sets 3-21G, 6-31+G* and 6-311++G** were used. The large basis set 6-311++G** was applied for neutral cGW to especially account for diffuse electron distributions. By increasing the basis set from 3-21G to 6-31+G* we observe big changes in the relative conformer energies. A saturation effect occurs when increasing the basis set from 6-31+G* to 6-311++G** (Table 1) which might be due to accidental error compensation for the smaller basis set. We also calculated vibrational frequencies (B3LYP/6-31+G*) for the neutral conformers 1, 2 and 3 which are needed for calculating the Gibbs free energy and also used for a tentative vibrational assignment in section IV.2.

The conformers of neutral cGW

To find the local minima the conformational landscape of cGW has been explored using the program Tinker.28 It forms a general conformational search for the entire potential energy surface by using a hopping algorithm29 which is able to overcome barriers. The program Tinker also minimizes each conformer after its generation. The chosen MM3 force field30 is known to be quite successful in reproducing molecular IR spectra. In order to make sure we do not miss a conformer, in addition also a potential energy surface scan was performed using the OPLS all atom force field.31 The results from the both scans were collected and the conformers processed with B3LYP by stepwise increasing the basis set size.

For discussions of the type of conformers of cGW in Fig. 2a the relevant rotational axes and the resultant structural possibilities are shown. The conformers of cGW can be classified as follows: The peptide ring can be moved into three positions when rotating about the C1–C2 axis (Fig. 2a, right side, see also atomic numbering C0–C2). For each of these conformers the indole ring then can be switched into six positions by rotation around the C0–C3 axis (Fig. 2b, left side). This gives theoretically 18 conformers. The most stable conformers are formed by rotating the C1–C2 axis into the plane which is perpendicular to the indole ring (perpendicular conformer class). The other tilted conformers are sterically unfavoured or unstable. By our calculations 8 stable conformers have been found for neutral cGW. The calculated relative energies with respect to the lowest conformer are presented in Table 1. A full geometry optimization of the neutral conformers was performed by using the B3LYP/6-311++G** method.

The calculations show that the peptide ring (cyclic(glycylglycyl) or 2,5-piperazinedione) is not planar resulting in a double well potential (see Fig. 2b). In the unperturbed cyclic(glycylglycyl) the minima are equal in energy and the barrier is expected to be low because of the high flexibility between the amide groups described in biochemical text books. In cGW this double well is superimposed with a ring-to-ring interaction potential (schematic drawing in Fig. 2b). Depending on the size and steepness of this ring-to-ring interaction potential, for each of the above-mentioned conformers either two sub-conformers a and b (this is the case for conformers 1 and 3), or only one conformer (a or b for conformers 2, 4, 5, 6, 7, 8) exist. The peptide-ring sub-conformers are very similar in energy (energy difference 41 cm⁻¹ for conformers 1a and 1b, and 18 cm⁻¹ for conformers 3a and 3b, see Table 1). The calculated isomerization barrier (B3LYP/6-31+G*) from conformer 1b to conformer 1a is 2 cm⁻¹ and already the zero point vibration would be above the barrier. The same is expected for conformers 3a and 3b for which the energetic difference is even smaller. The accuracy of the reaction barrier calculation is not known. However, even if the isomerization barrier between the a and the b forms would be larger than 2 cm⁻¹ but lower than 300 cm⁻¹, the more unstable form would be converted to the lower conformer in the supersonic expansion.32 In the following we assume that conformers 1b and 3b are experimentally not observable. It is evident, however, that the resulting broad and anharmonic potential strongly reduces the vibrational frequencies in this coordinate, increases the vibrational density and by this favors these conformers on the Gibbs free energy landscape.

In Table 1 the results of the B3LYP density functional calculations of the 8 stable neutral conformers of the dipeptide cGW (see also a and b conformers) using the 3-21G, 6-31+G* and 6-311++G** basis sets are presented. Relative conformer energies are given in cm⁻¹. The four lowest structures are shown in Fig. 3. With the increase of the basis set from 3-21G to 6-31+G* the energetic order of some conformers changes. When increasing the basis set from 6-31+G* to 6-311++G** the conformer order is conserved and the changes in the relative conformer energies become small, which might be due to an error compensation for the smaller basis set. As discussed above, structures 1b and 3b are not stable by zero point vibration. Conformer 2 which is characterized by a C–H···π interaction has a relative energy of 194 cm⁻¹. Conformer 3a which is similar to conformer 1a but with the indole ring switched by 180° lies 355 cm⁻¹ above

<table>
<thead>
<tr>
<th>Conformer</th>
<th>B3LYP/3-21G</th>
<th>B3LYP/6-31+G*</th>
<th>B3LYP/6-311++G**</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>291</td>
<td>0</td>
<td>0</td>
<td>N–H···π'</td>
</tr>
<tr>
<td>1b</td>
<td>291</td>
<td>42</td>
<td>41</td>
<td>N–H···π'</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>219</td>
<td>194</td>
<td>C–H···π'</td>
</tr>
<tr>
<td>3a</td>
<td>431</td>
<td>344</td>
<td>355</td>
<td>As 1a, but indole switched</td>
</tr>
<tr>
<td>3b</td>
<td>431</td>
<td>361</td>
<td>372</td>
<td>As 1b, but indole switched</td>
</tr>
<tr>
<td>4</td>
<td>967</td>
<td>546</td>
<td>531</td>
<td>As 2, but indole switched</td>
</tr>
<tr>
<td>5</td>
<td>1449</td>
<td>673</td>
<td>629</td>
<td>Tilted conformer</td>
</tr>
<tr>
<td>6</td>
<td>335</td>
<td>977</td>
<td>937</td>
<td>C=O···π, repulsive</td>
</tr>
<tr>
<td>7</td>
<td>454</td>
<td>1104</td>
<td>1053</td>
<td>Tilted conformer</td>
</tr>
<tr>
<td>8</td>
<td>2123</td>
<td>1661</td>
<td>1639</td>
<td>As 6, but indole switched</td>
</tr>
</tbody>
</table>

* C1–C2 axis (atomic numbering see Fig. 2a) lies in the plane perpendicular to the indole ring.

Table 1 B3LYP density functional calculations of the most stable neutral conformers of the dipeptide cGW using the basis sets 3-21G, 6-31+G* and 6-311++G**. Relative conformer energies in cm⁻¹. Note that the changes from basis set 6-31+G* to basis set 6-311++G** are small, indicating that the 6-311++G** basis set is large enough.
Fig. 2  (a) Top: The chemical structure and the carbon numbering C₀, C₁ and C₂ relevant for conformer discussion. The rotamer structures of cGW: Six rotations are possible when rotating along the C₀–C₁ axis (left side) and three when rotating along the C₁–C₂ axis (right side). In the four most stable conformers the C₁–C₂ axis lies in a plane nearly perpendicular to the indole plane, therefore termed “perpendicular conformers”. All other rotamers are termed “tilted conformers”. (b) The B3LYP/6-311++G** structure of the peptide ring cyclic(glycylglycyl) is non-planar. As a result the reaction coordinate along the inversion mode has a double well potential. In the peptide cGW the peptide ring-to-indole ring interaction can superimpose a linear potential to the double well. This results in two non-equivalent minima or just one minimum as shown here. Note that the inversion mode is expected to be very anharmonic and should have a considerably lower vibrational energy as calculated by a harmonic approximation.

Fig. 3  (a)–(d) Structures of the four most stable neutral conformers of cGW in their order of energetic stability (see Table 1). Their C₁–C₂ axes lie in a plane perpendicular to the indole ring (see Fig. 2a). Structures 1 and 3 as well as 2 and 4 differ by the orientation of the indole ring. Conformers 1 and 3 are stabilized by a N–H···π interaction and conformers 2 and 4 by a C–H···π interaction. For structures 1 and 3 on a theoretical level second less stable conformers have been found which differ by the peptide ring structure (structures b in Table 1). They have low isomerization barriers to conformers a and are not stable. For further explanation see text.

Conformer 1: \( E_{\text{rel}} = 0 \text{ cm}^{-1} \)  Conformer 2: \( E_{\text{rel}} = 194 \text{ cm}^{-1} \)  Conformer 3: \( E_{\text{rel}} = 355 \text{ cm}^{-1} \)  Conformer 4: \( E_{\text{rel}} = 531 \text{ cm}^{-1} \)
1a. In this conformer the N–H group seems to have better access to the indole π system. The fact that it is less stable than conformer 1a is attributed to a positive partial charge on the carbon in position 4. Only conformers with relative energies well below 365 cm\(^{-1}\) (~\(kT\) at 525 K) are expected to have measurable populations in the beam. This also holds for conformer 2 but not for conformer 3. To calculate the exact thermal population beside the energy difference of the electronic energies also the vibrational zero point energy and the vibrational density of states has to be considered. Assuming harmonic potentials all this would be included in the Gibbs free energy. For a temperature of 300 K it shows an increase of the energetic spacing between conformer 1 and 2 from 355 cm\(^{-1}\) to 675 cm\(^{-1}\). This energy difference is expected to further increase when the strong anharmonicity of the inversion coordinate in conformer 1 would be taken into account. Hence from theory we predict that a great entropy effect is forcing the dipeptide population into conformer 1. The high energy of conformer 3 (355 cm\(^{-1}\)) above conformer 1 should result in a marginal or vanishing population of conformer 3. Comparing conformer 1 and 3 the entropy argument is not helpful because also conformer 3 has a broad double minimum in the peptide ring inversion coordinate. It is well known that barriers lower than 300 cm\(^{-1}\) can be overcome during the supersonic jet expansion.\(^\text{32}\) To compare theoretical data to the experimental data it would be important to calculate the barrier heights between conformers 2 and 1 as well as 3 and 1. Unfortunately, because of the complexity of the coordinates and the size of the system, it is impossible to calculate such isomerization barriers.

The cGW cation

We calculated vertical (6-311++G** basis set) and adiabatic (3-21G and 6-31+G* basis sets) IEs for comparison with the PE results in the hope to be able to assign the structure of the observed conformation. The cation geometry optimization was performed with the 3-21G basis set for all 8 conformers, and with the 6-31+G* basis set for seven conformers. The B3LYP/6-31+G* calculation for conformer 7 did not converge presumably because the potential surface is very flat. We checked the dependence of the cation structural changes on the basis set, but found that the 3-21G structures are very similar to the structures found with the 6-31+G* basis set. The calculated data are collected in Table 2 and the comparison of neutral and cation structures is shown in Fig. 4. We also calculated the vertical IEs of the b conformers of structures 1 and 3, although it is clear that they are not populated due to their low isomerization barriers (see above). The differences between the vertical IEs of conformers a and b nevertheless give hints as to how steep the ion ground state potential at the vertical geometry might be. When optimizing the geometry in the cation ground state the sub-conformers a and b formed by the peptide ring inversion converge for conformers 1a and 1b as well as 3a and 3b to a single minimum. This is explained by a large repulsive peptide ring-to-indole ring interaction in the cation which forces the system into one minimum. A calculation of the geometrical average of the changes of all atomic coordinates between neutral and cation (termed RMS value in Table 2) shows that, except one conformer, all conformers undergo large structural changes upon ionization.

Fig. 4 shows the neutral-to-cation geometry changes of conformers 1, 2 and 6. For this the neutral and the cation structure are overlaid with the indole ring and conformational changes indicated by arrows and lines. In all cases the structural changes are so large that it might be difficult to reach the adiabatic IE. For conformers 1, 2 (Fig. 4a, b) and 3 (not shown) the large geometry change is explained by the difference between the attractive (neutral) versus the repulsive (cation) N–H···π (conformers 1 and 3) or C–H···π (conformer 2) interaction.

In Fig. 4c the neutral and the cationic structures of conformer 6, the most stable cation conformer, are compared. In this conformer the C=O group (note the negative partial charge on O) has access to the indole chromophore. Whereas conformer 6 is rather unstable in the neutral due to the repulsive C=O···π interaction it becomes the most stable conformer when the π system is positively charged.

IV Experimental results and discussion

In the following we present R2PI mass, R2PI S\(0–S1\), UV/UV SHB and R(1′+1′)PI PE spectra of the dipeptide cGW.

IV.1 The R2PI mass spectrum

For recording the R2PI mass spectrum of cGW (Fig. 5) the second harmonic of a Nd:YAG laser-pumped dye laser was

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Table 2: Calculated vertical (B3LYP/6-311++G**) and adiabatic (B3LYP/3-21G and B3LYP/6-31+G*) ionization energies as well as relative cation conformer energies (B3LYP/3-21G and B3LYP/6-31+G*) for the 8 stable neutral conformers of cGW. Note that only one cation structure minimum exists for the neutral conformers 1a and 1b as well as 3a and 3b. We explain this by a superposition of a strong repulsive potential to the inversion double well of the dipeptide ring: In the cation the floppy peptide ring is bent away from the positive indole ring. As a result there is a relatively great vertical IE difference between conformers a and b. Note that the energetic order of cation conformers changes when increasing the basis set from 3-21G to 6-31+G*. The most stable neutral conformer 1 has the highest vertical and adiabatic (B3LYP 6-31+G*) IEs. This agrees well with its structure (Fig. 3a): In the neutral cGW the N–H···π interaction is attractive. Upon ionization this interaction becomes repulsive (N–H···π interaction, see Fig. 4a). The same argument holds for conformers 3 and 4 and a similar argument for the C–H···π interaction of conformer 2. The vertical and adiabatic IEs are given in eV and relative cation energies in cm\(^{-1}\). The RMS value is the square root of the sum of the second power of all atomic displacements upon ionization (given in Å).

<table>
<thead>
<tr>
<th>Conformer</th>
<th>Vertical IE/eV</th>
<th>Adiabatic IE/eV</th>
<th>Rel. energy/cm(^{-1})</th>
<th>RMS/Å</th>
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<tr>
<td>1a</td>
<td>6-311++G**</td>
<td>3-21G</td>
<td>6-31+G*</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>7.754</td>
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<td>7.469</td>
<td>16</td>
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<td></td>
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<td>8</td>
<td>7.476</td>
<td>6.778</td>
<td>7.089</td>
<td></td>
</tr>
</tbody>
</table>

* The two neutral conformers a, b converge to the same minimum. b Calculation did not converge (presumably the potential energy surface is very flat).
tuned to the S0–S1 origin transition. The mass spectrum was recorded by using the PE spectrometer as flight tube for the linTOF. Mass 234 Da corresponds to the parent radical cation. Two fragments with masses 131 Da and 117 Da are observed. These masses correspond to the neutral fragments 3-methylindole (131 Da) and indole (117 Da) which are then ionized by the laser pulse. In the case of a cationic fragmentation of tryptophan instead of mass 117 Da mass 130 Da would be expected as the most intense fragment. Measurements with different laser intensities and wavelengths confirm that both masses are neutral fragments containing the indole chromophore. The ionization of the neutral fragments is a minor contribution in the mass spectrum at low photon energies but increases when coming closer to the indole S0–S1 resonance. Because of the mass selection there should be no influence of the fragment ionization on the R2PI spectrum of cGW. Nevertheless we find a small peak in the R2PI spectrum at exactly the energy of the S0–S1 origin transition of indole which does not show the usual a1 and b1 progressions of cGW transitions. We believe that this peak is due to an intermolecular charge transfer (CT) effect where after ionization the charge hops from indole to the cGW which has the lower IE.

IV.2 Mass selective R2PI spectroscopy: the S0–S1 transition

To record the R2PI spectrum of cGW the second harmonic of a Nd:YAG laser-pumped dye laser was tuned and the intensity of the parent ion monitored as a function of the wavelength. In the R2PI spectrum shown in Fig. 6 an intense first transition is followed by several transitions which have decreasing intensities with increasing energy. The spacing is not regular which may be a hint for the presence of several conformers in the beam. UV/UV SHB spectroscopy proves (see section III.3) that this is not the case: All intense transitions correspond to one single conformer. In the following we make use of this information and assign transitions a1, b1, c1, d1 and presumably Y to vibrations of this conformer. A tentative conformer identification has been made in section IV.4.

The R2PI spectrum of cGW shown in Fig. 6 covers 450 cm⁻¹. No transitions were observed below 35 000 cm⁻¹. Above 35 300 cm⁻¹ the indole vibrations and their many combination bands with vibrations a, b, c and d are expected. The justification to confine our investigation to a range of 450 cm⁻¹ is, that
the four most stable calculated conformers have an attractive peptide ring-to-indole ring interaction and therefore should have similar \( S_0 \rightarrow S_1 \) origin energies. The \( S_0 \rightarrow S_1 \) origin transition of cGW is situated at \( 35.058 \text{ cm}^{-1} \). It lies 180 cm\(^{-1}\) below the \( S_0 \rightarrow S_1 \) transition of indole\(^{44}\) and 178 cm\(^{-1}\) above the \( S_0 \rightarrow S_1 \) transition of 3-methylindole.\(^{35}\) The \( S_1 \) energy in cGW is expected to be influenced by three parameters: (i) The electrostatic effect caused by the increase of the side chain of indole from 3-methylindole to cGW, (ii) the solvation of indole by the peptide ring and (iii) vibrational zero point energy effects.

Concerning (i) we expect in analogy to the alkylbenzene series (relative shift with respect to methylbenzene: \(+110 \text{ cm}^{-1}\) for ethylbenzene, \(+105 \text{ cm}^{-1}\) for propylbenzene, \(+99 \text{ cm}^{-1}\) for butylbenzene\(^{36}\)) a blue shift in comparison to 3-methylindole when increasing the chain size. Concerning (ii): In a structure where the N–H group can interact with the indole \( \pi \) system (see most stable conformer 1 in Fig. 2a), a red shift of the \( S_0 \rightarrow S_1 \) transition is expected when the \( \pi \) system is excited, \textit{i.e.} enlarged,\(^{18}\) because the N–H–\( \pi \) interaction is stabilized further. However, (iii) this shift may be partially compensated by a change in the zero point vibrational energy if some vibrations increase strongly with the excitation to \( S_1 \). Interestingly the origin of \( S_1 \) in cGW lies 353 cm\(^{-1}\) above the \( S_1 \) of the lowest and 163 cm\(^{-1}\) above the second conformer of linear NH\(_2\)G–W–COOH.\(^{5,6,9}\) The fact that our red-shift is not as strong as in linear peptides may be interpreted as follows: The peptide ring interaction forces the functional groups C=O and N–H of the peptide backbone into positions which do not allow ideal solvation of the indole ring. This lengthy discussion shows how difficult it is to interpret the tryptophan \( S_0 \rightarrow S_1 \) frequencies with regard to structural changes of the environment.

The relatively intense transitions to one quantum of the low-frequency modes \( a^1 \) (30 cm\(^{-1}\)) and \( b^1 \) (52 cm\(^{-1}\)) indicate some \( S_0 \rightarrow S_1 \) geometry change in the corresponding coordinates. Interestingly the transition \( a^2 \) is not observed. Only the combination band \( a^2 b^2 \) and tentatively a small transition \( b^5 \) is observed. Because the intense indole vibrations lie above 300 cm\(^{-1}\),\(^{5,6,9}\) the vibrations \( a, b, c (108 \text{ cm}^{-1}) \) and \( d (174 \text{ cm}^{-1}) \) are attributed to vibrations involving a relative motion between the two rings. For conformer 1a the frequency calculation of the neutral ground state gives the following vibrations below 90 cm\(^{-1}\): 21 cm\(^{-1}\) (global ring–ring bending), 39 cm\(^{-1}\) (ring–ring torsion along C\(_1–C_2\)), 51 cm\(^{-1}\) (boat to inverse boat transition A and the probe laser parent ion signal. Note that with this scheme the transition moments of the individual peaks determine the depth of the SHB dip.

In Fig. 7a the UV/UV SHB spectrum of cGW and in (b) for comparison the R2PI spectrum are shown. We confined our investigation on the low-energetic section of the R2PI spectrum of cGW because (i) it contains the most intense transitions and (ii) the lowest-energetic four conformers are expected to lie in this range because of their similar peptide ring-to-indole ring interaction. In Fig. 7a all transitions except E show a strong hole burning effect and hence correspond to one conformer. To investigate the hole burning for transition E in detail, but also to probe accidental overlap of conformer transitions, we carefully checked the degree of hole burning for each transition. Fig. 8 shows the degree of depletion of the probe signal by the hole burning laser for the first six transitions of the R2PI spectrum. The conditions have been the same as for Fig. 7a, except that now the hole burning laser is kept fix at transition A and the probe laser is scanned to the individual transitions A–F. With this scheme the depletion percentage

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**Fig. 7** (a) The spectral UV/UV hole burning spectrum probes the most intense \( S_0 \rightarrow S_1 \) transitions A–F. (b) The R2PI \( S_0 \rightarrow S_1 \) spectrum of cGW for comparison. In (a) the detection laser is set to transition A and the probe laser is scanned through transitions A–F. By this excitation scheme the transition moment of the individual transitions is reflected in the depths of the dips. A–D and F show a strong hole burning effect and hence correspond to one single conformer. The hole burning of transition E stays unclear and is reinvestigated in Fig. 8.

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The ring–ring interaction in the lowest-energetic conformer 1 is attractive. One can expect that this attraction increases in the \( S_1 \) state due to the increase of the size of the \( \pi \) system upon excitation. As a consequence the frequencies of the ring–ring motions would increase. Hence the \( S_1 \) vibrations with the energy 21 cm\(^{-1}\) and 39 cm\(^{-1}\) might correspond to the \( S_1 \) vibrations \( a^1 \) and \( b^1 \). The type of these vibrational motions also agrees with the concept that the N–H–\( \pi \) interaction changes when exciting from \( S_0 \) to \( S_1 \). The \( S_1 \) vibrations observed in the \( S_0 \rightarrow S_1 \) spectra of linear NH\(_2\)G–W–COOH are 24 cm\(^{-1}\) and 38 cm\(^{-1}\) and not too far off our values although the structures are very different.\(^{5,6,9}\) This indicates that the first two or three low-frequency vibrations of all similar-sized chromophore–tail molecules lie in the range of 20–50 cm\(^{-1}\). For conformer 2 for example the first three calculated \( S_0 \rightarrow S_1 \) vibrational frequencies are with 26 cm\(^{-1}\), 41 cm\(^{-1}\) and 56 cm\(^{-1}\) very similar to those of conformer 1. Hence the \( S_0 \) frequencies provide no arguments for conformer assignment.

The \( S_0 \rightarrow S_1 \) R2PI spectrum was recorded with 5 bar Ar nozzle backpressure. Experiments with He and Ne as carrier gas resulted in less perfect vibrationally cooling.

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IV.3 Spectral UV/UV hole burning spectroscopy

(UV/UV SHB)

To clarify the number of conformers in our molecular beam, we used R2PI UV/UV SHB. As mentioned in the experimental section, for the SHB a three-plate ion optics was mounted and the PE spectrometer used as a linTOF mass spectrometer. This setup was needed in order to remove and neutralize the prompt ions formed by the hole burning laser by applying a pulsed electric field. The spectrum was recorded by scanning the hole burning laser while the probe laser was set to the \( S_0 \rightarrow S_1 \) origin transition. Each time when the neutral ground state of the cGW conformer was depopulated by the hole burning laser a dip appeared in the probe laser parent ion signal. Note that with this scheme the transition moments of the individual peaks determine the depth of the SHB dip.
The transition $S_1a_1$ are shown in Fig. 9a and b. Both spectra for ionization of excited $S_1cGW$. The R2PI PE spectra of by the second harmonic of the same Nd:YAG laser was used by the second frequency-doubled dye laser which is pumped by cGW ionized via the vibrationless origin transition of $S_1$ and $a_1$ state is by 6 meV ($\pm$1 meV) lower than when ionized via $S_1a_1$ is attributed to a better Franck-Condon factor to lower vibrational quantum numbers in the cation ground state $D_0$. The hole burning reduces the probe signal by 50% for all transitions. This shows that no origin transition of a minor conformer component is accidentally covered by a vibronic transition of the most stable conformer. Note that theoretically more than 50% of the neutral ground state levels can be depopulated.

IV.4 Photoelectron spectroscopy

Photoelectron spectroscopy is in principle a double-resonance spectroscopy which is able to provide conformer- and state-selective radical cation spectra. The initial hope was that we could sort out and assign the different conformers of cGW by PE spectroscopy alone. To achieve a resolution of 1 meV it is necessary to keep the electron kinetic energy below 200 meV. To fulfill this condition a second frequency-doubled dye laser which is pumped by the second harmonic of the same Nd:YAG laser was used for ionization of excited $S_1cGW$. The R2PI PE spectra of cGW ionized via the vibrationless origin transition of $S_1$ and the transition $S_1a_1$ are shown in Fig. 9a and b. Both spectra show a broad ionization onset with no vibrational structure. According to the calculations of the neutral and cation structures which show a great geometry change upon ionization we believe that we do not observe the origin transition to the cation ground state, but excite to a dense higher excited vibrational manifold. Due to the smooth onset the IE can only be determined with an accuracy of $\pm$3 meV. The fact that the transition A is by 6 meV lower than when ionized via $S_1a_1$ is attributed to a better Franck-Condon factor to lower vibrational quantum numbers in the cation ground state. The PE spectrum of indole (Fig. 9c) taken in the same machine shows that we could resolve vibrational structures if their spacing were larger than 10 cm$^{-1}$. The vibrations of the indole cation ground state have been recently investigated in detail by ZEKE spectroscopy and are not discussed here. The IE value of indole determined by ZEKE spectroscopy allows us to calibrate our indole and cGW PE spectra with an accuracy of $\pm$2 meV.

Unfortunately there is a background in the peptide PE spectra which stems from ionization of neutral fragments, preferentially indole, which is wavelength-dependent. It is small for the wavelength range of the first two $S_1$ transitions of cGW, but increases as the closer the wavelength comes to the indole $S_0-S_1$ origin. Without coincidence techniques it is in principle impossible to distinguish between fragment and peptide photoelectrons. We therefore confined our PE measurements to the first two $S_0-S_1$ transitions.

In Fig. 9 the IE of 3-methylindole (7.514 eV) is also marked. The IEs of substituted indoles should monotonically decrease with chain length (alkylbenzenes$^9$). The fact that with respect to 3-methylindole the IE of cGW is blue-shifted and the smooth onset indicates a relatively strong repulsive interaction between the two rings in the cation, as predicted by theory. Unfortunately the calculation of absolute IEs is relatively inaccurate and the measured IE corresponds neither directly to the calculated adiabatic IE nor the vertical IE. The fact that conformer 1 has the highest IE of all conformers agrees well with the observed blue shift with respect to 3-methylindole.

V Conclusion and tentative conformer assignment

DFT predicts that one conformer (conformer 1) is predominantly stable and further favoured by entropy due to a low-energy peptide ring inversion mode. This is qualitatively in agreement with our R2PI and R2PI SHB spectra in which we attribute the most intense transition in the spectra to one
conformer. In conformer 1 the N–H group of the dipeptide ring interacts with the π system. This interaction is attractive and is expected to increase with S1 excitation. This is qualitatively confirmed by comparison to the calculated low-frequency So vibrations.

The fact that the experimentally observed conformer has an IE which is 195 meV higher than 3-methylindole shows that the solvation of the charge in the indole by the peptide ring is repulsive. The most stable neutral conformer has the highest vertical IE of all 8 conformers and shows a very strong geometry change upon ionization. Hence conformer 1 fulfills all experimental findings. We admit that also conformer 2 and 3 follow the pattern of a attractive ring–ring interaction in the neutral cGW and a repulsive ring–ring interaction in the cationic cGW and that our arguments for the conformer assignment are very weak. For a confident conformer assignment it would be helpful to have several conformers in the molecular beam and be able to compare their IEs. Because the relative energies of calculated IEs are more accurate this would allow a more confident assignment. In conclusion, we propose that conformer 1 is the cGW conformer we observe in our molecular beam.

Our work shows that R2PI and the R(1 + 1)PI PE spectra predominantly provide information about the structural changes in the nearest environment of the chromophore. In cGW, however, the structural changes upon ionization are so great that with conventional high resolution PES a resolution of vibrational structures in the PE spectrum is impossible.

In general a similar neutral-to-cation Franck–Condon problem might exist for peptides or amino acids which have polar groups near the chromophore. The IR/UV double resonance technique may provide additional clues for conformer identification and especially allow us to distinguish between conformers 1 (N–H · · · π interaction) and 2 (C–H · · π interaction).

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