Spectroscopic Evidence for Peptide-bond Selective UV Photodissociation

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ABSTRACT

We study the photodissociation induced by ultraviolet excitation of amide bonds in gas-phase protonated peptides. Jointly, mass spectrometry and cold ion spectroscopy provide evidence for a selective non-statistical dissociation of specific peptide bonds in the spectral region of the formally forbidden $n \rightarrow \pi^*$ transition of amide groups. Structural analysis reveals that the activation of this transition, peaked at 226 nm, originates from the non-planar geometry of the bond. In contrast, the statistical dissociation in the electronic ground state appears to be the main outcome of the $\pi \rightarrow \pi^*$ excitation of the peptide bonds at 193 nm. We propose a tentative model that explains the difference in the fragmentation mechanisms by the difference in localization of the electronic transitions and the higher amount of vibrational energy released in the electronic excited state upon absorption at 193 nm.

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TOC GRAPHICS

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Amide is a chemical group common to all peptides and proteins, in which it links together amino acids by peptide bonds. Each amide group is an inherent chromophore that can be used for energizing these molecules *via* electronic excitation. In solution phase the electronic absorption by peptide bonds is broad and typically lies at wavelengths shorter than ~ 220 nm.¹⁻² The weak absorption band around 210 nm and the much stronger one peaked at 190 nm were previously assigned to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively.²⁻⁶ Photodissociation by ultraviolet light (UVPD) is one of the recently emerging techniques in mass spectrometry (MS) that takes advantage of high abundance and positional regularity of peptide bonds for efficient sequencing of proteins *via* excitation of $\pi \rightarrow \pi^*$ transitions.⁷⁻¹¹ Compared with dissociation due to absorption by a few aromatic residues present in a protein (statistically only 11.5%), the presence of almost the same number of amide chromophores as the number of residues drastically increases fragmentation efficiency upon electronic excitation of peptide bonds. Despite numerous studies¹²⁻¹⁷ and an extensive use of UVPD in proteomics, the detailed mechanism of this process remains obscure: structural variety and large size of studied molecules make a comparative analysis of UVPD mass spectra difficult; the validated geometries of studied ions are usually unavailable which reduces confidence in quantum chemistry calculations of electronic transitions; internal energy is not well determined in room-temperature experiments; etc. Elimination/suppression of these factors should sharpen and, perhaps, decouple different processes and steps involved in UVPD. Herein we report on a spectroscopic–mass spectrometric (2D UV-MS) study of model protonated peptides that are cooled to cryogenic temperature and whose accurate gas-phase structures are known. The experiment is supported by quantum chemistry computations of electronic transitions in one of the peptides (see SI for details). We address two questions related to the photophysics of peptide bonds: (i) what is the prevailing mechanism of UVPD in the studied

peptides; (ii) can UVPD be peptide bond-selective and tell us something about the geometry of individual amide groups in peptides?

Our experimental approach for 2D UV-MS fingerprinting of cold biomolecules was described in detail elsewhere (see SI for details).¹⁸⁻²⁰ The cooling removes, essentially, all thermal vibrational energy from the studied ions, such that their internal energy after UV excitation by a single photon is determined solely by the photon energy. The experimental method allows for monitoring photodissociation mass spectra continuously as a function of UV wavelength within a wide spectral range that covers both $n \rightarrow \pi^*$ and (partially) $\pi \rightarrow \pi^*$ transitions.

Figure 1. Photodissociation (a) mass spectra of [Ac-PheAla₅Lys+H]¹⁺ peptide recorded at 226 and 193 nm and normalized to 1 mJ of UV OPO pulse energy, and (b) UV spectra of the peptide obtained from 2D UV-MS fingerprints for different fragments and for the sum of all of them. For graphical clarity the UV spectra are shown scaled to about the same maximum relative yield. (c) Fragmentation yields of *b*-type fragments in collision-induced dissociation and the depletion of the parent ion (P) as a function of activation potential. (d) Optimized geometry of the conformer A of the peptide; hydrogen bonds are shown by dashed lines; the insert shows a close view of the 5th amide group with the H atom sticking out of the peptide bond plane.

Figure 1a shows the UVPD mass spectra of the N-acetylated α -helical heptapeptide [Ac-PheAla₅Lys+H]¹⁺ recorded at 226 nm and 193 nm, which correspond to the excitation of $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions, respectively. Accurate intrinsic geometries of this peptide were validated by IR cold ion spectroscopy.²¹ Nearly 80% of the ions were attributed to the pair of conformers that differ only by the orientation of the Phe side chain.²¹⁻²² We therefore consider in our analysis below only one of these conformers, originally named A. The observed UVPD mass spectra contain a variety of fragments, of which the b_n/y_{7-n} ($n=1-6$) are, by far, the most abundant; a few low abundant fragments of *a*, *x*, and *z*-type were detected only at 193 nm. Figure 1b shows the photodissociation spectra for the most abundant fragments of the heptapeptide derived from its 2D UV-MS fingerprint. Overall, the spectra of the highly abundant fragments b_2 , y_3 , b_3 , and b_4 are almost identical. They all contain a highly intense rising edge below 200 nm that corresponds to the $\pi \rightarrow \pi^*$ transition similar to the UV absorption by a single peptide bond in [Ala₂+H]¹⁺ dipeptide,²³ a long tail appearing at longer wavelengths due to the overlap of this and the weaker $n \rightarrow \pi^*$ transition and, on top of the tail, a band around 214 nm which corresponds to the absorption by the Phe residue.²⁴

In order to unravel the mechanism of the UVPD process we measured collision-induced dissociation mass spectra of the peptide in the HCD cell of the Exactive MS at several activation

potentials (AP). The spectra (Fig. S1a) are dominated by *b/y*-type fragments which are typical for thermal dissociation. The overall similarity of the UVPD and HCD mass spectra suggests statistical nature of the UVPD mechanism at 193 nm. This is further supported by our quantum chemistry calculations showing that the $\pi \rightarrow \pi^*$ transitions of the peptide are strongly delocalized over 3-5 amide groups (Tables S1 and S2). This implies that the fragmentation observed at 193 nm cannot result from bond-selective excitation. Although the energy of a 193 nm photon is sufficient to break a peptide bond in the excited state, the excitation does not yet ensure a fragmentation: the rich network of hydrogen bonds holds the two parts of a vibrationally cold ion together. This confinement slows down the rate of prompt dissociation and releases some vibrational energy in the electronic excited state of the ion. Statistically, this vibrational energy and the delocalization of the electronic excitation should accelerate internal conversion to the electronic ground state S_0 ²⁵⁻²⁶ making this process competitive to the hindered prompt dissociation. The subsequent dissociation of the ions in S_0 thus suppresses the characteristic for “prompt” dissociation *a/x* and *c/z* fragments. It is worth mentioning that the described dynamical picture can (potentially) be observed only if the UV excitation is performed by an ultrashort coherent laser pulse that is shorter than the lifetime of the absorbing electronic state. Only such excitation prepares a non-stationary quantum state that evolves in time.²⁷ A typically employed 10 ns UV laser pulses excite subsets of molecular “quasi” eigenstates which amplitude may evolve on a timescale longer than the pulse duration.

Figures 1a and 1b suggest that the mechanism of UVPD at 226 nm for the b_5 fragment might be different from the one described above. This fragment has a very low relative abundance at 193 nm while it is the second most abundant at 226 nm (Table S3). In contrast to the spectra of all other fragments, the spectrum of the b_5 fragment exhibits a prominent band centered at ~226 nm.

A similar effect has been observed also for [Ac-Ala₆Lys+H]¹⁺ helical peptide (Fig. S3), whose accurate structure, however, is not known. What is the origin of this band? Does it stem from a specific UV absorption of the 5th peptide bond or simply reflect the secondary statistical dissociation in the electronic ground state of the peptide?

Although UV and high-energy collisional activation processes are different, one can expect certain consistency between their energy dependences, if UVPD is statistical. Figure 1c (and S1b) shows the yields of HCD fragments as a function of AP. At low AP (below ~33 V), where the secondary fragmentation is negligible, *b*₅ fragment is the most abundant followed by *b*₄ and *b*₃ ones. The high abundance of these fragments reflects the low energy barriers for the cleavage of these bonds, most likely due to the hydrogen bonds that link the 3rd to 5th amide groups directly to the protonated side chain of Lys. At high AP the secondary fragmentation becomes important, subsequently, for the largest, *b*₅, to the smaller (*b*₄ to *b*₂) fragments. This observation prompts us to explain the low relative abundance of *b*₅ photofragment at 193 nm by the secondary fragmentation. Taking the ratio of the yields for *b*₅ (Fig. 1a) at 193 nm and 226 nm (1:3) nm together with the fragmentation at these wavelengths of single peptide bond in Ala₂H⁺ (90:1), we derived that as much as 99.7% of *b*₅ undergoes secondary fragmentation at 193 nm. If the *b*₅ band around 226 nm indeed reflects the *b*₅ band centered at 38 V in the HCD spectrum, the 226-193 nm wavelength range should correspond to AP range that spans entirely across this HCD band. A band similar to that for *b*₅ at 226 nm therefore should be observed also for *b*₄ and, perhaps, *b*₃ fragments. We do not detect such bands in UVPD spectra of *b*₄, *b*₃, which implies a substantial secondary fragmentation of *b*₅ already below 226 nm. In the corresponding AP region (>40 V) the yield of HCD to *b*₅ decreases monotonically however, while the UVP yield of this fragment increases between 240 nm and 226 nm. An additional UVPD source of *b*₅ is to be invoked to remove the

contradiction. We thus assign UVPD band of b_5 around 226 nm to an additional UV absorption in this wavelength region by the 5th peptide bond followed by its non-statistical cleavage that yields b_5 . If so, what is the origin of this additional absorption and of the site selective dissociation?

Being free from any strong perturbations, amide groups exhibit planar geometry. This makes the $n \rightarrow \pi^*$ transitions symmetry-forbidden due to the orthogonality of the n and π^* orbitals.³ Strong hydrogen bonding may alter the geometry and break this orthogonality, thus activating the transition. The known geometry of the tested helical peptide allows us to relate the non-planarity of the peptide bonds to their UVPD spectra. It turns out that the 5th peptide bond is the only one with a substantial non-planar geometry in the molecule (Figure 1c, Table S4). The distortion is caused by the strong non-covalent bonding of the oxygen of the 5th amide to NH_3^+ of Lys and of the hydrogen of this amide to the oxygen of the first amide. Quantum chemistry calculations show that, due to this distortion the absorption by the 5th peptide bond at 226 nm is twice stronger than by the others. Calculations also show that, different from the $\pi \rightarrow \pi^*$ transitions, the $n \rightarrow \pi^*$ excitation appears to be localized (Tables S1, S2, and Fig. S4). The elevated abundance of b_5 fragments at 226 nm is exactly the spectroscopic manifestation of the geometry distortion and the localized character of the electronic excitation. The vibrational energy released upon this excitation in the upper electronic state is lower by $7.6 \cdot 10^3 \text{ cm}^{-1}$ compared with the excitation by a 193 nm photon. We speculate that the deficit of vibrational excitation and the localization of electronic excitation reduce the rate of internal conversion leaving enough time for a proton transfer with a subsequent cleavage of the bond yet in the electronically excited state.

The spectroscopic signature of a non-planar geometry of a peptide bond was also observed in the peptide that contains a proline residue (Figure 2a). Among natural amino acids, Pro is the only one with cyclic structure of its backbone. Because of this, the nitrogen atom of the side chain is

involved in a peptide bond making it structurally distinct from the others. Figure 2b shows the fragment-specific UVPD spectra of the $[\text{GlyProGlyGly}+\text{H}]^{1+}$ peptide whose structure was reported previously.²⁸ With the exception for the y_3 fragment, the UV spectra of all abundant fragments appear nearly identical and similar to that of the planar peptide bond in the $[\text{Ala}_2+\text{H}]^{1+}$ dipeptide.²³ Apart from the still substantial relative fragmentation intensity at short wavelengths, the spectrum of y_3 in figure 2b resembles the one for the b_5 fragment of the helical peptide (Fig. 1b), where the respective amide group has a non-planar geometry. In the gas phase $[\text{GlyProGlyGly}+\text{H}]^{1+}$ resides in *cis*- and *trans*- states, which are almost equally populated under mild conditions of an electrospray ion source.²⁸ A close analysis of the geometries of these conformers reveals a substantial (13.8°) tilt of the CO moiety with respect to the CNC_α plane of the Pro residue in the *cis*- conformer. This distortion activates the $n \rightarrow \pi^*$ transition, while the planar geometry of all peptide bonds in *trans*- $[\text{GlyProGlyGly}+\text{H}]^{1+}$ allows for the $\pi \rightarrow \pi^*$ transitions only. This explains the relatively high intensity of the y_3 spectrum at 193 nm, which is not conformer-selective and reflects the sum of the fragmentations of both conformers of the peptide.

Figure 2. (a) Geometry of cis-conformer of [GlyProGlyGly+H]¹⁺ peptide reconstructed from the data in ref.²⁸; (b) UVPD spectra of the peptide obtained from its 2D UV-MS fingerprint for the most intense photofragments (Fig. S5).

In conclusion, our study of model peptides provides evidence of selective fragmentation of peptide bonds upon excitation of $n\text{-}\pi^*$ transitions in non-planar amide groups. Such distorted geometry of peptide bonds activates the transition, while local nature of this electronic excitation and the low amount of vibrational energy released in a molecule after the excitation enables selective dissociation of the bonds. Overall, the study demonstrates the high potential of the 2D UV-MS approach in disentangling the complex process of UVPD.

ASSOCIATED CONTENT

Supporting Information. Computational and experimental details; HCD and UVPD spectra; computed electronic transitions; geometry of molecules.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interests.

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Supporting Information

Spectroscopic Evidence of Peptide-bond Selective UV Photodissociation.

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1. Experimental

Protonated gas-phase ions are produced from 1:1 water-methanol solution with 1% of acetic acid using electrospray ionization source (Proxeon), and enter into a two-stage electrodynamic ion funnel²⁹ (Spectroglyph) through 0.7 mm ID, 10 cm long metal capillary, orthogonally to the funnel axis. The funnel guides the ions into a home-made octupole RF trap, where they are accumulated and thermalized for approximately 100 ms. The ions are then released from the trap and mass-selected by a quadrupole mass filter (Extrel), bent 90° by an electrostatic quadrupole bender, decelerated by a stack of electrostatic lenses and, finally, guided by an octupole ion guide into a cold octupole ion trap. The trap is mounted on a cold head (T=6 K) of a two-stage closed cycle refrigerator (RDS-407, Sumitomo). The ions are trapped and cooled in the octupole to T=10 K by collisions with He gas, which we pulse into the trap right before the arrival of the ion packet.³⁰ After fragmentation of parent ions by a 5 ns pulse of VUV/UV optical parametric oscillator (OPO), both parent and fragment ions are released from the cold trap. The released ion package of ions is 90° bent by the second electrostatic quadrupole bender and analyzed in an Orbitrap-based broadband mass spectrometer (Exactive-II, Thermo Scientific, Bremen). We fill the cold trap in 10 Hz cycles and run the UV OPO at the same repetition rate. We typically take an average of 20 laser shots per data point in spectral measurements.

The energy of the OPO pulses was continuously recorded and kept within 1-1.2 mJ and to 0.15-0.2 mJ intervals for the 280-209.5 and 210-193 nm spectral regions, respectively, to compensate for very different absorption cross sections in the two regions and to avoid two-photon absorption (checked by linear power dependence); the fragment ion signals were normalized on the power. A 2D UV-MS fingerprint of an ion is measured by scanning the wavelength of OPO while recording mass spectra of all appearing charged photofragments at each wavelength. We applied this approach to two model protonated peptides, for which the exact 3D intrinsic geometries were determined earlier: an α -helical (N-acetylated) peptide [Ac-PheAla₅Lys+H]¹⁺ and a proline-containing peptide [GlyProGlyGly+H]¹⁺.

The peptides of >95% purity and the solvents of HPLC grade were purchased from Bachem Inc. (Switzerland) and used without any further purification.

2. Computational details

The structure of conformer **A** of [Ac-PheAla₅Lys+H]¹⁺ from Ref.²¹ was re-optimized using density functional theory with the CAM-B3LYP³¹ functional and the 6-311G* basis set.³² Vertical excitation energies (VEE) were calculated for the first 20 singlet excited states using time-dependent density functional theory (Table S2). The long-range corrected hybrid functional CAM-B3LYP has a good performance on benchmark datasets for VEEs and oscillator strengths and it is also efficient for larger-sized molecules with average deviations of about 0.3 eV from the experimental absorption maxima³³. We also performed VEE calculations with the LC- ω PBE³⁴ functional for the CAM-B3LYP/6-311G* optimized geometries to verify that the excited states remain similar using different methods (Table S3). Structure optimization and excited state calculations were all performed with the Gaussian 09 program suite,³⁵ while the TheoDORÉ program³⁶ was used to analyze the excitations.

To analyze the electronic transitions, the peptide is divided into 9 segments (Figure S3). The electron and hole populations are calculated using Löwdin population analysis for these segments. Highly localized transitions have high hole and electron populations on only one segment (states 1-10 in Tables S3 and S4), while delocalized transitions have multiple segments with significant hole and electron populations (e.g., states 11, 13-19 of Table S3). Natural transition orbitals of both localized and delocalized states are illustrated in Fig. S4.

The calculated excitation wavelengths of 223 (and 221) nm with CAM-B3LYP (and LC- ω PBE) agree very well with the UVPD experiment (226 nm) for the n - π^* type transition of the 5th peptide bond (state 2 of Tables S2 and S3). The calculated excitation energies (160-180 nm) of the π - π^* transitions agree well with the rising edge observed in the UVPD experiments around 193 nm.

Figure S1. a) HCD mass spectra of helical heptapeptide $[\text{Ac-PheAla}_5\text{Lys+H}]^{1+}$ measured at 34 and 43 activation potentials. **b)** Fragmentation yields of different fragments obtained from collision-induced dissociation and the depletion of the parent ion (P) as a function of activating potential.

Figure S2. Separation of $[\text{Ac-PheAla}_5\text{Lys+H}]^{1+}$ into segments for the computational analysis of electronic transitions presented in Tables S2 and S3.

Table S1. Excitation wavelengths (λ), oscillator strengths, hole (h) and electron (e) populations calculated at the CAM-B3LYP/6-311G* level. The definition of the peptide segments is given in Figure S2. The b and y fragments corresponding to the breakage of the respective peptide bonds are also given.

state	λ / nm	osc. str.	Seg. 1		Seg. 2		Seg. 3		Seg. 4		Seg. 5		Seg. 6		Seg. 7		Seg. 8		Seg. 9	
							b_1, y_6		b_2, y_5		b_3, y_4		b_4, y_3		b_5, y_2		b_6, y_1			
			h	e	h	e	h	e	h	e	h	e	h	e	h	e	h	e	h	e
1	225	0.001	1.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	223	0.006	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.9	0.9	0.0	0.0	0.0	0.0
3	217	0.004	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0
4	215	0.003	0.0	0.0	0.1	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	214	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	213	0.000	0.0	0.0	1.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	211	0.003	0.0	0.0	0.0	0.0	0.1	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	209	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0
9	202	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
10	201	0.014	1.0	0.9	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	181	0.085	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.2	0.1	0.3	0.3	0.3	0.3	0.1	0.0	0.0	0.0
12	179	0.197	0.9	0.5	0.0	0.1	0.1	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	178	0.482	0.5	0.4	0.0	0.1	0.2	0.3	0.1	0.1	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
14	177	0.229	0.3	0.2	0.1	0.1	0.0	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.2	0.2	0.1	0.0	0.0	0.0
15	175	0.095	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.2	0.2	0.2	0.2	0.2	0.1	0.0	0.0	0.0
16	174	0.213	0.2	0.1	0.0	0.0	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.0
17	172	0.119	0.2	0.1	0.0	0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.2	0.1	0.1	0.0	0.0	0.0	0.0
18	171	0.203	0.6	0.2	0.1	0.2	0.2	0.5	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19	169	0.145	0.2	0.1	0.4	0.4	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.1	0.0	0.1

Table S2. Excitation wavelengths (λ), oscillator strengths, hole (h) and electron (e) populations calculated at the LC- ω PBE/6-311G* level. The definition of the peptide segments is given in Figure S3. The b and y fragments corresponding to the breakage of the respective peptide bonds are also given.

state	λ / nm	osc. str.	Seg. 1		Seg. 2		Seg. 3		Seg. 4		Seg. 5		Seg. 6		Seg. 7		Seg. 8		Seg. 9	
							b_1, y_6		b_2, y_5		b_3, y_4		b_4, y_3		b_5, y_2		b_6, y_1			
			h	e	h	e	h	e	h	e	h	e	h	e	h	e	h	e	h	e
1	223	0.000	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	221	0.005	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.9	0.9	0.0	0.0	0.0	0.0
3	215	0.003	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0
4	214	0.002	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	213	0.000	0.0	0.0	1.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6	212	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
7	209	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	208	0.002	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.9	0.0	0.0
9	201	0.001	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0
10	197	0.014	1.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	173	0.281	0.1	0.1	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.3	0.2	0.3	0.3	0.0	0.0	0.0	0.0
12	173	0.482	0.8	0.8	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13	171	0.622	0.9	0.9	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14	170	0.085	0.0	0.0	0.0	0.0	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.0	0.3	0.3	0.1	0.0	0.0	0.0
15	168	0.059	0.0	0.0	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.4	0.4	0.2	0.2	0.0	0.0	0.0	0.0
16	167	0.134	0.0	0.0	0.1	0.1	0.1	0.1	0.2	0.3	0.4	0.4	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0
17	165	0.286	0.0	0.0	0.0	0.0	0.4	0.4	0.2	0.2	0.1	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
18	163	0.304	0.0	0.0	0.3	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.1	0.1
19	162	0.071	0.0	0.0	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.3	0.3	0.1	0.1
20	153	0.000	0.9	0.9	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table S3. Relative intensities of fragments in UVPD of [Ac-PheAla₅Lys+H]¹⁺.

Amide	<i>b_n</i>	<i>y_{6-n}</i>	<i>b_n</i> + <i>y_{6-n}</i>	<i>b_n</i> / <i>y_{6-n}</i>
n	193 nm			
1	0.12	0.045	0.17	2.76
2	0.30	0.038	0.34	7.95
3	0.64	0.116	0.76	5.54
4	0.72	0.186	0.90	3.85
5	0.04	0.172	0.21	0.22
6	0	0.126	0.13	0
	226 nm			
1	0.018	0.013	0.312	1.42
2	0.046	0.012	0.582	3.93
3	0.056	0.019	0.742	2.97
4	0.132	0.04	1.720	3.30
5	0.120	0.021	1.413	5.63
6	0	0.014	0.140	0

Figure S3. Screen shot of photofragment UV and mass spectra of [Ac-Ala₆Lys+H]¹⁺ heptapeptide. The spectra are labeled by the fragment identities and UV wavelengths.

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Figure S4. Natural transition orbitals showing the location of the electron before and after excitation for (a) the $n-\pi^*$ transition of the 5th amide group (state 2 of Table S2) and (b) one of the $\pi-\pi^*$ transitions (state 11 of Table S2).

Table S4. Dihedral angles of the OCNH peptide bonds in the two most abundant conformers of $[\text{Ac-PheAla5Lys+H}]^{1+}$.

Peptide bond	Dihedral angle, °		
	Conformer A Present work	Conformer B Ref 21	Conformer A Ref 21
1	177.4	-177.4	178.6
2	-176.2	-174.4	-175.8
3	177.3	176.4	176.9
4	-172.3	-172.5	-172.4
5	-163.3	163.2	-162.9
6	-174.0	-173.0	-172.9

Figure S5. Photodissociation mass spectrum of $[\text{GlyProGlyGly}+\text{H}]^{1+}$ peptide, measured with UV OPO wavelength at 212.8 nm.

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