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Article

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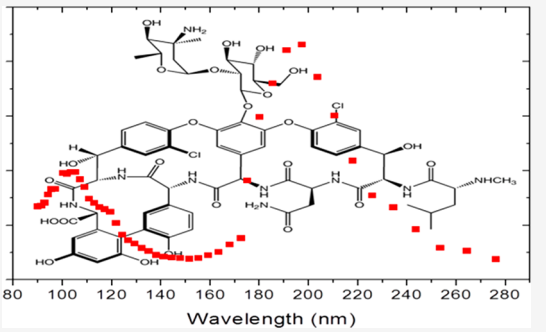
ABSTRACT: The gas phase offers the possibility to analyze organic molecules by ultraviolet−vacuum ultraviolet (UV−VUV) spectroscopy without any solvent effect or limitation in terms of spectral range due to absorption by the solvent. Up to now, the size and chemical composition of neutral molecular systems under study have been limited by the use of vaporization methods based on thermal heating. Soft sources of gas-phase thermolabile molecular systems such as electrospray or matrix-assisted laser desorption ionization are appealing alternatives to heating-based techniques, but they lead to the production of ions. In such cases, UV−VUV action spectroscopy is then the method of choice to study the electronic structure and corresponding photodynamics of these gas-phase molecular ions. However, previous investigations have shown that the UV−VUV action spectrum of a given molecular ion depends on the charge state, which in many cases might be a caveat. Here, by means of synchrotron radiation coupled to mass spectrometry and through the test case of the glycopeptide antibiotic vancomycin noncovalently bound to a deprotonated small peptide, we show that the UV−VUV photofragmentation spectrum of neutral thermally fragile organic molecules can be obtained via charge-tagging action spectroscopy.



INTRODUCTION

Ultraviolet (UV) spectroscopy is one of the most widely used techniques in analytical chemistry, especially for measuring the absorption of molecules in solution. The obtained spectra are crucial for identifying whole molecules or chemical groups within a larger system. UV spectroscopy also allows probing the electronic structure of molecular systems, which is useful in understanding and predicting the consequences of light−molecule interaction, as well as the reactivity of these systems. However, solution-phase spectra suffer from the dependence toward the solvent and the difficulty in controlling the stoichiometry of noncovalent complexes, which are key issues in many fields of chemistry and biology, including host−guest chemistry, molecular recognition, drug screening, sensors, etc. UV spectroscopy of molecular systems in the gas phase is an alternative approach that probes the intrinsic molecular properties, which can directly be modeled by state-of-the-art calculations.[1]

One major advantage of studying isolated molecular systems is the ability to extend the spectral range to the short wavelengths, i.e., in the vacuum ultraviolet (VUV) or even soft X-ray regions, increasing the amount of information obtained, by allowing electrons from inner-valence and even core orbitals to be excited or ejected from the molecule. Such investigations have benefited, in the past decades, from the advent of high-flux and tunable sources of ionizing photons such as synchrotrons, triggering a wealth of studies on small neutral systems.[2−4] However, they all rely on detecting the product ions coming from ionization of the target neutral molecular system, which is not always possible with UV light. Therefore, several experimental techniques have been applied to the measurement of the absorption of neutral molecular systems without ionizing them, notably cavity ring-down spectroscopy.[5] Nevertheless, in all these studies, the size of the molecule is limited because the vaporization techniques used most often involve thermal heating, which induces chemical reactivity for relatively large thermolabile and floppy molecules. Notably, regarding biologically relevant molecules,[6−8] a trade-off between thermal decomposition and signal intensity exists.[9,10] The use of aerosol thermodesorption may allow a slight extension of the size range of neutral studies but with limited target densities.[11−13]

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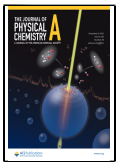

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An alternative consists in taking advantage of the soft ion sources such as electrospray ionization (ESI) or matrix-assisted laser desorption ionization (MALDI); they have allowed action spectra of molecular ions to be measured in the UV, VUV, or soft X-ray ranges, notably of biologically relevant systems,[14] in particular proteins[15−17] but also peptides,[18−22] carbohydrates,[23] and DNA strands.[24,25] In action spectroscopy, the mass spectrum of ionic products from photoabsorption is usually recorded as a function of photon energy. A great advantage of action spectroscopy as compared to direct absorption lies, in certain cases, in its very high sensitivity as a zero-background technique. However, very often, the action spectrum of an isolated molecular system depends on the charge state: for instance, the number of protons in a protonated molecule usually changes its fragmentation yield and channels because proton transfer can lead to bond cleavage, for instance in protonated peptides.[26] Increasing the charge state has also an effect on the geometrical structure of the system: it often induces unfolding of proteins, for instance, and can thus influence reactivity (notably charge transfer).[17,27] These undesired effects can be circumvented by studying the neutral molecule and recording its photofragmentation yield as a function of photon energy. However, then one is facing the issues of bringing intact fragile neutral molecules into the gas phase. In this context, we propose here the following strategy: in order to detect, by mass spectrometry, the fragment ions from photofragmentation of a given neutral molecule, the latter can be noncovalently bound to an ion acting as a charged tag, and such a noncovalent complex can be brought intact into the gas phase by ESI or MALDI, for instance. Several conditions must be fulfilled in order to obtain the photofragmentation spectrum of the neutral molecule:

1. The yield of product ions formed after photoabsorption by the tag ion is negligible compared to that of the neutral molecule in the photon energy range considered.
2. The noncovalent interactions between the tag ion and the neutral molecule do not strongly modify the electronic states of the latter.

Condition 1 might be fulfilled in the UV−VUV range by using singly charged negative ions as tags: indeed, their electron photodetachment energy is very low (on the order of a few electronvolts, for instance, 2.15 ± 0.15 eV for deprotonated phenol and 2.31 ± 0.15 eV for deprotonated indole),[28] so they are expected to mainly create *neutrals* after photoabsorption at higher photon energy, which are not detected by mass spectrometry. Therefore, these events will not pollute the photofragmentation spectrum of the neutral molecule. Note that in the high-energy VUV range, two electrons will possibly be ejected from the tag anion, leading to the formation of a monocation: however, it is not expected to dominate below 14 eV in the photon energy range studied here. Besides, electron detachment is known to be a process occurring before inter- and intramolecular vibrational energy redistribution (IVR); thus, noncovalent binding with a large molecule would not quench it.[15] However, electronic excitation without electron detachment might still happen and must be quantified in the singly charged negative tag ion. Positive tag ions are not expected to be a good choice because their photoabsorption can trigger ionization and/or fragmentation of the complex and thus positive ions that may pollute the spectrum of the neutral molecule. Condition 2 must be checked case-by-case by considering the type of noncovalent interactions existing in the complex and the photon energy range probed.

In this Article, we report the proof-of-principle that the UV−VUV photofragmentation spectrum of an isolated fragile neutral macromolecule can be obtained by action spectroscopy of its noncovalent complex with a small anion. We use synchrotron radiation as a source of photons, irradiate the anionic complexes in an ion trap, and detect photoabsorption thanks to mass spectrometry analysis of the produced anionic fragments. As a test case, we choose the noncovalent complex between vancomycin (denoted V) and the deprotonated $Ac_2{}^{L}K^{D}A^{D}A$ peptide (denoted R). Vancomycin is a last-resort antibiotic against infections caused by Gram-positive bacteria: it specifically recognizes a precursor of the main constituent of the bacterial cell wall, preventing its renewal and leading to cell death. Previous studies have established that the peptidic C-terminal $^{D}A^{D}A$ sequence is mandatory for high affinity between vancomycin and the receptor.[29] The noncovalent interactions responsible for this behavior have been revealed by structural investigation of the crystallized V + R complex by X-ray diffraction,[30] and they are depicted in Figure 1. Five H-bonds

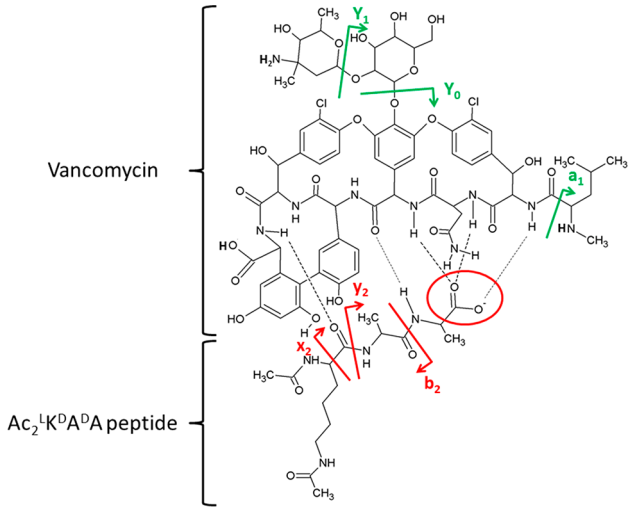

Figure 1. Chemical structure of vancomycin ($C_{66}H_{75}Cl_2N_9O_{24}$, average mass 1449.3 amu) bound to the $Ac_2{}^{L}K^{D}A^{D}A$ peptide that mimics the receptor. The standard nomenclature is used for the peptide backbone as well as for oligosaccharide fragmentation and hence for the main fragments observed after photoabsorption. Arrows indicate on which side of the cleaved bond the charge is located.

link the two peptidic moieties, three of them with ionic character since they involve the CO_2^- group of $[R - H]^-$. By means of infrared multiphoton dissociation (IRMPD) spectroscopy, this biologically active structure has been found to be conserved for deprotonated V + R complexes in the gas phase[31] but not for the doubly protonated complex,[32] in line with early low-energy collision-induced dissociation experiments.[33] Indeed, in the protonated complex, R is neutral and the native H-bonding network is lost; thus, noncovalent binding is not specific anymore.[32] The advantages of the $[V + R - H]^-$ complex as a test case for this study are the following:

1. The 3D geometrical structure of the complex in the gas and condensed phases is known, establishing that vancomycin is neutral and R is deprotonated in the complex. Moreover, the noncovalent interactions do not involve the aromatic chromophores of vancomycin,

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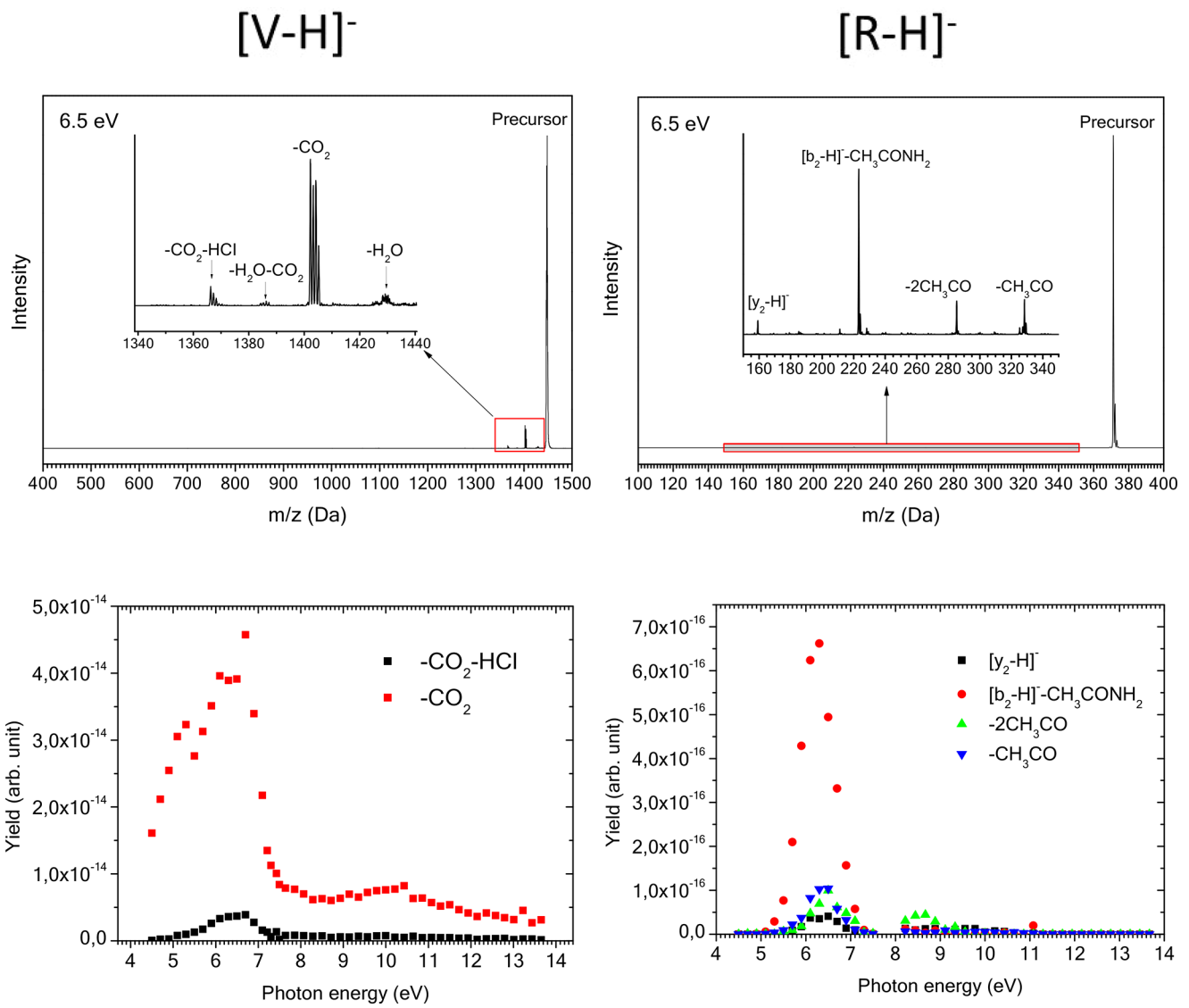

Figure 2. Mass spectra after single-photon absorption at 6.5 eV (top) and partial fragmentation yields in the UV−VUV range 4.5−13.8 eV (bottom) of $[V - H]^-$ (left) and $[R - H]^-$ (right) for the main fragmentation channels observed (for $[R - H]^-$, the lack of data between 7.6 and 8.2 eV is due to a technical problem). The arbitrary units for the yields are the same for both $[V - H]^-$ and $[R - H]^-$, and the yields can thus be directly compared.

namely, modified side chains of the amino acid tyrosine, indicating that condition 2 above is fulfilled.

2. R does not contain any aromatic UV chromophore, and it is relatively small compared to vancomycin; we thus expect its absorption cross section to be lower than that of vancomycin (condition 1 above), with little contribution in the UV range.
3. Photoabsorption of $[V - 2H]^{2-}$ as well as of the $[V + R - 2H]^{2-}$ complex in the gas phase has been studied previously, notably with synchrotron radiation in a complementary VUV range of higher energy.[34] Thus, the reported fragmentation channels will provide a basis for our present investigation.

METHODS

Vancomycin hydrochloride and $Ac_2{}^{L}K^{D}A^{D}A$ have been purchased from Sigma-Aldrich as powders of over 80% purity and used without further purification. Electrospray solutions have been prepared in 50:50 (volume ratio) water/methanol at 50 μM concentration with 1% of ammonia to deprotonate the molecules. For the experiments conducted with a UV−visible spectrophotometer, the concentration of the solution sample was 6.7 μM in deionized water. The UV−visible spectroscopy experiments in the solution phase were conducted at CIMAP using a Varian Cary 300 spectrophotometer in the 190−900 nm range, with a 1 nm step. The solution was located in a quartz cell with an optical path of 10 mm. The blank solution was made of deionized water, and the "blank spectrum" was subtracted from the spectrum of vancomycin in deionized water.

The UV−VUV gas-phase tandem mass spectrometry experiments were performed using a commercial mass spectrometer (Thermo Finnigan LTQ XL) coupled to the DESIRS beamline at the SOLEIL synchrotron radiation facility.[35] The setup has previously been described in detail;[36] only a brief description is given here. The molecular ions were put in the gas phase from the solution by electrospray ionization using a syringe pump with a flow rate of about 5 μL/

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min and a needle biased at 5 kV in front of a capillary held at 35 V. Ions were then guided through a quadrupole and an octopole before being injected, thermalized by helium buffer gas, and trapped in a commercial linear triple quadrupole ion trap. In this trap, the ions of interest were selected by mass-to-charge ratio (m/z) with an isolation width of 6−10 depending on the ion; for example, for deprotonated vancomycin (m/z 1446.42) all ions with m/z between 1443.42 and 1449.42 were trapped in order to obtain the main isotopic pattern. After isolation, the ions were irradiated by photons from the DESIRS VUV beamline of the SOLEIL synchrotron facility in Saint-Aubin (France).[35] Product anions from photoabsorption as well as the remaining precursors were then analyzed with respect to m/z in the ion trap, and these mass spectra were accumulated until sufficient statistics were reached (typically 30 spectra).

Mass spectra were recorded for different photon energies, using 0.2 eV steps between 4.5 and 7.5 eV and between 7.2 and 13.8 eV. The UV/VUV beam was free of high harmonics owing to the use of a Kr gas filter (7.2−13.8 eV) and a quartz window (4.5−7.5 eV). The photon flux was in the 10^{12} −10^{13} photons/s range and was measured independently using an AXUV100 photodiode (International Radiation Detectors) under the measurement conditions. The total number of photons for a given ion and energy was tuned to obtain a maximum of 10% conversion of the precursor ions to photoproducts in order to limit the occurrence of sequential two-photon processes. It was regulated by adjusting the exit slit (100−600 μm) of the monochromator and a mechanical shutter that controlled the irradiation time of the trapped ions between 200 and 1000 ms.

Results were obtained in the form of one mass spectrum for each photon energy. To quantify the formation of photoproducts as a function of photon energy, action spectra were constructed. These spectra show the yield of each photoproduct as a function of photon energy. This yield has been calculated from the intensity of the peaks in the mass spectra and corrected for the number of incident photons (to account for photon flux variations upon scanning) and for the precursor ion intensity (to account for fluctuations of the number of precursor ions in the ion trap). The yield of photoproduct A can thus be calculated for a given photon energy according to the following:

$$I_{A(rel)} = \frac{I_A}{I_{pre} \cdot N_{phot}}$$

where I_A is the abundance of photoproduct A, measured by the sum of the peak intensities of all isotopes. I_{pre} is the abundance of the precursor ion peak (sum of all isotopes). The incident number of photons N_{phot} is given by

$$N_{phot} = \Phi_q \cdot \Delta t \cdot \frac{w_{exp}}{w_{ref}}$$

where Φ_q is the photon flux measured in the reference scan with the photodiode, Δt is the irradiation time, w_{exp} is the slit width during the experiment, and w_{ref} is the slit width during the reference scan.

RESULTS AND DISCUSSION

First, we explain our method to obtain the UV−VUV photofragmentation spectra and demonstrate that in this photon energy range, the probability of fragmentation following photoabsorption of the deprotonated R peptide ($[R - H]^-$) is negligible compared to that of deprotonated vancomycin ($[V - H]^-$). In Figure 2, the mass spectra of these two anions after single-photon absorption at 6.5 eV are shown: the peaks observed can only be due to photofragmentation, not to photodetachment of one electron, because the latter process creates a neutral that is not detected with the present mass spectrometer. For $[R - H]^-$, the most intense fragment is detected at m/z 223, which we attribute to cleavage of the peptidic backbone to give the $[b_2 - H]^-$ fragment of m/z 282, together with loss of CH_3CONH_2 from either the N terminal or the lysine side chain after H transfer from the fragment. We also observe loss of one as well as two CH_3CO radicals from the precursor but also backbone cleavage leading to the $[y_2 - H]^-$ fragment ion, as minor channels. Backbone cleavage and loss of neutrals are very common in photofragmentation[37] or collision-induced dissociation (CID) of deprotonated peptides.[38] Loss of radicals is less common, although Brunet et al.[37] reported the loss of radical tryptophan and tyrosine side chains from deprotonated peptides upon photoabsorption in the same photon energy range as the one studied here. The most abundant process detected after photoabsorption by $[V - H]^-$ is loss of CO_2, which has already been reported for mass spectrometry activation techniques such as CID,[33] surface-induced dissociation (SID),[39] and IRMPD,[31] where fragmentation occurs in the electronic ground state of the system. This channel is also common in organic molecules containing a deprotonated carboxyl group, which is an indication of the deprotonation site of vancomycin. HCl can also be lost in addition to CO_2 but to a much lower extent. Other minor channels are loss of H_2O and $H_2O + CO_2$.

We plot the photofragmentation yield of the main channels for $[V - H]^-$ and $[R - H]^-$ as a function of photon energy in Figure 2 in the UV−VUV range 4.5−13.8 eV. One can see that for both molecules, the fragmentation yield is much higher in the low photon energy region, which might be explained by a higher absorption cross section in this region and/or the increase of electron detachment cross section with photon energy. The spectrum for $[R - H]^-$ features a maximum around 6.3 eV (~197 nm), which is consistent with the peak observed around 6 eV in the action spectra reported for $[WVVVV - H]^-$ and $[YVVVV - H]^-$ deprotonated peptides.[37] Electronic π−π^* transitions involving the peptide backbone have been predicted around this energy by *ab initio* quantum chemical calculations on peptides.[40] In the case of $[V - H]^-$, the peak is broadened toward low photon energy, and the fragmentation yield is still high at 4.5 eV. This is due to the presence of five aromatic rings coming from modified tyrosine side chains (cf. Figure 1): indeed, tyrosine strongly absorbs light around 275 nm (4.5 eV) in the gas phase due to π−π^* transitions located in the phenol moiety.[15] Note that these rings also contribute to absorption at higher photon energy since phenol has excited states with very high oscillator strength around 6.8 eV and also Rydberg states at even higher energy but with lower oscillator strength,[41] the latter probably being responsible for the still high level of CO_2 loss well above 6 eV in Figure 2. The crucial conclusions here are the following: first, in the whole photon energy range, the probability of photoexcitation leading to fragmentation of $[R - H]^-$ is clearly negligible compared to that of $[V - H]^-$ (by about 2 orders of magnitude), and second, the fragmentation signal of these singly charged species becomes much lower for photon energies over 7 eV, notably due to the increasing yield

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of electron photodetachment processes as compared to photofragmentation, as we will demonstrate in the next paragraph.

Second, we confirm that the charge state influences the UV−VUV spectrum of deprotonated vancomycin, and we present the electron detachment-related processes. In Figure 3, the

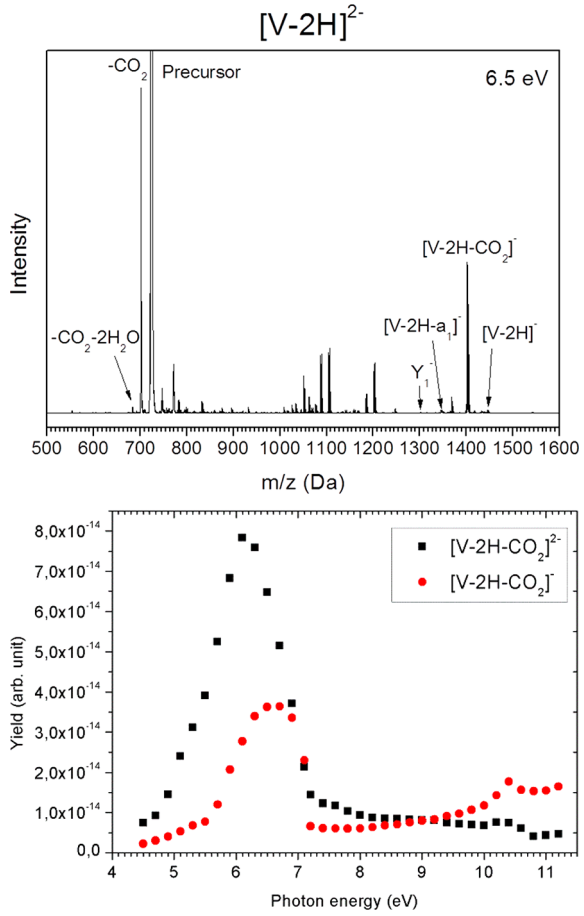

Figure 3. Top: mass spectrum of $[V - 2H]^{2-}$ after single-photon absorption at 6.5 eV. The unassigned peaks are unaffected by the photon beam. Bottom: partial fragmentation yields of $[V - 2H]^{2-}$ in the UV−VUV 4.5−11.2 eV range for the main fragmentation channels observed.

mass spectrum of $[V - 2H]^{2-}$ after absorption of a single 6.5 eV photon is depicted: loss of CO_2 from the precursor anion is a major channel (and much weaker subsequent loss of $2H_2O$ is also present), like for $[V - H]^-$, but here loss of CO_2 is also abundant from the radical anion $[V - 2H]^-$ formed as a consequence of single-electron photodetachment. We have already observed the latter process from the same molecular system after single-photon absorption at 16 eV,[34] as well as formation of the Y_1^- fragment after glycosidic bond cleavage, which is also detected in the present lower-energy range but as a minor process. Other low-intensity peaks are attributed to nondissociative electron detachment and subsequent peptidic backbone cleavage leading to loss of the a_1 fragment. In Figure 3, we show the evolution of the yield of channels associated with loss of CO_2 as a function of photon energy in the 4.5−11.2 eV range. Although the yield of CO_2 loss is dominant in the low photon energy region, its evolution looks different from the case of $[V - H]^-$: there is only one maximum around 6.2 eV, and the shoulder around 5.2 eV is almost vanishing. This shows that charge state has an effect on the UV−VUV excitation spectra of gas-phase vancomycin. CO_2 loss associated with electron detachment has a lower yield between 4.5 and 9 eV but progressively increases from 8 eV and dominates at high photon energy (see Figure 3). This is consistent with our previous mass spectrum at 16 eV photon energy[34] and confirms our hypothesis of photodetachment quenching the photofragmentation of singly deprotonated vancomycin at high photon energy (see previous paragraph). At low photon energy, the yield of $[V - 2H - CO_2]^-$ shows a maximum at 6.6 eV and then falls with the same yield as CO_2 loss from the precursor. It is unusual for the yield of electron detachment-related processes to show a peak falling to almost zero in this low photon energy range since the cross section for ejection of one valence electron usually rises monotonically with photon energy from the detachment threshold energy, as electrons from deeper valence orbitals can be involved in the photodetachment process. Therefore, the peaked shape of the CO_2 loss associated with photodetachment might be the signature of photoinduced resonant transitions to electronic states of $[V - 2H]^{2-}$ followed by CO_2 loss and subsequent delayed electron detachment. Such an assumption is supported by the fact that the peaks for the yield of CO_2 loss both without and with electron detachment are observed in the same photon energy range (5−7 eV). Delayed electron detachment has only been reported once, in the doubly deprotonated peptide gramicidin, by photoelectron spectroscopy.[42] Furthermore, this process most probably occurs after internal conversion to the ground state because it is associated with CO_2 loss, the main fragmentation channel of $[V - H]^-$ and $[V - 2H]^{2-}$ after UV photofragmentation but also CID at low collision energy.

In order to show that the UV−VUV spectrum of neutral vancomycin can be obtained by recording, as a function of photon energy, the photofragmentation of the $[V + R - H]^-$ noncovalent complex between neutral vancomycin and the deprotonated Ac_2KAA receptor, we have to investigate the mass spectra of $[V + R - H]^-$ after photoabsorption. In Figure 4, the mass spectra of $[V + R - H]^-$ at 6.5 and 12 eV photon energies are visible and appear to be strongly different. After absorption of a single photon of 6.5 eV, the mass spectrum is dominated by the loss of neutral molecules from the precursor (H_2O, HCl, and CO_2), which implies covalent bond cleavage (and rearrangement for HCl and H_2O, see Figure 1) without breaking of the H bonds linking the complex. This process is highly unusual for closed-shell noncovalent complexes, in contrast to the case of radicals: indeed, we have already observed CO_2 loss from $[V + R - 2H]^-$ after electron photodetachment from $[V + R - 2H]^{2-}$ in the 16−24 eV range.[34] Loss of H_2O, HCl, and CO_2 has also been observed from deprotonated vancomycin (see Figure 2), whereas CH_3CO loss occurs from $[R - H]^-$ (cf. Figure 2), which strongly indicates that H_2O, HCl, and CO_2 are lost from neutral vancomycin in the $[V + R - H]^-$ complex. These losses are much less abundant after photoabsorption at 12 eV, while $[V - H - CO_2]^-$ is the most abundant species, like in other activation techniques such as CID,[33] SID,[39] and IRMPD[31] where it is formed in the electronic ground state of the system. This is somehow surprising since we would expect $[V - H - CO_2]^-$ to dominate at *low* photon energy after internal conversion. This behavior indicates a key role played by excited electronic states of vancomycin in the loss of

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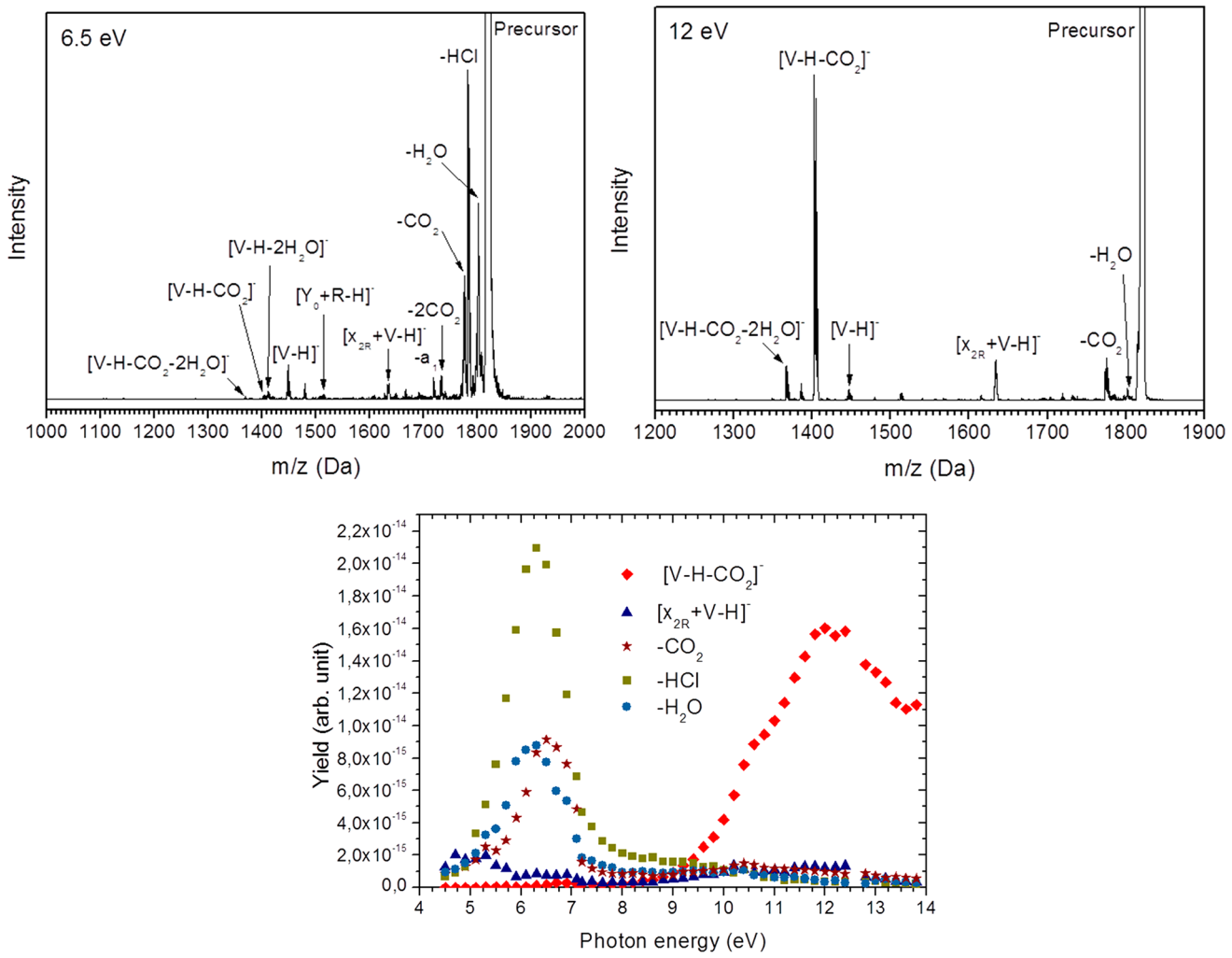

Figure 4. Top: mass spectra of $[V + R - H]^-$ after single-photon absorption at 6.5 eV (left) and 12 eV (right). Bottom: partial fragmentation yields of $[V + R - H]^-$ in the UV–VUV 4.5–13.8 eV range for the main fragmentation channels observed.

neutral molecules observed at low photon energy. On the contrary, observing $[V - H - CO_2]^-$ at 12 eV photon energy implies dissociation of the complex and thus substantial redistribution of the available energy into the vibrational modes of the system, in line with dissociation occurring in the electronic ground state, as in CID, SID, and IRMPD.

Moreover, we know from previous studies (see the Introduction) that vancomycin is initially neutral in the $[V + R - H]^-$ noncovalent complex; thus, the negative charge of $[V - H - CO_2]^-$ means that intermolecular charge transfer has been triggered upon photoabsorption. $[V - H - CO_2]^-$ can be formed by proton transfer from neutral vancomycin to $[R - H]^-$, followed by CO_2 loss, which would imply photoabsorption by vancomycin. $[V - H - CO_2]^-$ may also come from electron transfer from $[R - H]^-$ to vancomycin, followed by CO_2H loss, which would mean that the photon has been absorbed by $[R - H]^-$. However, we have shown earlier in this Article that the photofragmentation yield of $[R - H]^-$ is negligible compared to that of vancomycin in the whole photon energy range studied here (see Figure 2). Therefore, we attribute the formation of $[V - H - CO_2]^-$ to the sole photoexcitation of neutral vancomycin followed by proton transfer to $[R - H]^-$, associated with the formation of $[V - H]^-$ and CO_2 loss. This is supported by the presence of $[V - H]^-$ in the mass spectra of $[V + R - H]^-$ (see Figure 4). To our knowledge, proton transfer within a negatively charged molecular system in the gas phase has never been reported; additional work is thus needed to confirm this interpretation. A reasonable hypothesis for the origin of the proton relies on the observation of massive CO_2 loss from deprotonated vancomycin in our mass spectra: it is the lowest-energy decay channel of deprotonated vancomycin in its electronic ground state.[31] Vancomycin is deprotonated at the C-terminus of the peptidic moiety, and CO_2 loss occurs from this carboxylate group. Therefore, the proton most probably comes from the carboxyl group of vancomycin. A low-intensity peak present at 6.5 and 12 eV photon energy is observed at m/z 1633: from the isotopic pattern, we assign it to bond cleavage in $[R - H]^-$ to form the x_2 fragment, without dissociation of the complex: we call this species $[x_{2R} + V - H]^-$. Again, the negligible photofragmentation yield of $[R - H]^-$ implies photoabsorption in vancomycin followed by energy transfer and fragmentation of $[R - H]^-$ within the complex. Note that the x_2 fragment has not been observed after photoabsorption by the isolated $[R - H]^-$, consistent with this mechanism. This is also supported by the crucial fact that none of the fragmentation channels observed for the isolated $[R - H]^-$ is present for $[V + R - H]^-$.

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Now, let us investigate the evolution of the photofragments from $[V + R - H]^-$ as a function of photon energy (cf. Figure 4). The low-energy part is dominated by the loss of neutral molecules from the complex, the shape of their peaks being similar to the cases of $[V - H]^-$ and $[V - 2H]^{2-}$ (cf. Figures 2 and 3). It supports our conclusion that these losses come from vancomycin in the complex (see the previous paragraph). In the high-energy range (9−13.8 eV), the most abundant species is $[V - H - CO_2]^-$, and from our measurements, we can extract its appearance energy at 8.2 ± 0.4 eV. Its yield smoothly increases up to 12 eV and then falls. This kind of behavior has been reported for a protonated peptide after photoabsorption in the same energy range[22] and has been attributed to excitation in high-lying electronic states: we can assume that the same process occurs here. Moreover, electronic transitions over 8 eV have been predicted in peptides, formamide, and *N*-methylacetamide by *ab initio* calculations.[40,43] These transitions are not visible in $[V - H]^-$ (cf. Figure 2), probably because they are associated with electron detachment (which does not apply to neutral vancomycin): this is supported by the spectra measured for $[V - 2H]^{2-}$ (see Figure 3).

After having commented on the main channels after photofragmentation of $[V + R - H]^-$ in the 4.5−13.8 eV range, we now present in Figure 5 the overall UV−VUV photofragmentation spectrum of $[V + R - H]^-$, obtained by summing the yield of all fragments. $[V + R - H]^-$ is a noncovalent complex composed of neutral vancomycin and $[R - H]^-$, and we have shown that in this photon energy range, the photofragmentation probability of $[R - H]^-$ is negligible compared to that of vancomycin (see Figures 2 and 3). Moreover, photoabsorption by vancomycin does not seem to massively induce electron detachment from $[R - H]^-$ since we still detect abundant negative ions up to 14 eV (cf. Figure 4), even if electron detachment dominates for negatively charged molecules in the high photon energy range (see Figure 3). Therefore, the spectrum of Figure 5 can be considered as the UV−VUV photofragmentation spectrum of neutral vancomycin, to a first approximation. Strictly speaking, some of the photoinduced electronic transitions within vancomycin are, of course, influenced by the presence of $[R - H]^-$, but the H-bonds between the two molecules do not involve the aromatic chromophores of vancomycin, and these H-bonds are expected to induce only modest shifts in the energy of electronic states relative to the ground state. For instance, for neutral serotonin (an important neurotransmitter), UV−UV hole-burning spectroscopy experiments have shown that the transition from the ground state to the first excited state is red-shifted by only 0.1 eV upon H-bonding to two water molecules (that would induce a shift of about 3 at 200 nm).[44] The limited resolution of our measurements made at room temperature makes our spectrum insensitive to such small shifts. One might also argue that the proton transfer process observed here (from neutral vancomycin to $[R - H]^-$ in the complex) can change the photofragmentation spectrum of neutral vancomycin. However, it is most probably not the case here. Indeed, this proton transfer process occurs in the electronic ground state of the system and is therefore decoupled from the formation of the excited states of vancomycin. As soon as photoexcitation eventually leads to fragmentation in a hot ground state, which is the case for photons of 8−14 eV absorbed by these systems,[34] the VUV action spectrum is unaffected by the subsequent proton transfer. That said, to our knowledge, Figure 5 displays the first photofragmentation spectrum of such a large neutral organic molecule in the gas phase. Moreover, the wavelength range extends down to 90 nm, which is unreachable by absorption spectroscopy in solution. An example of such a spectrum is given in Figure 5 for vancomycin in aqueous solution: in the 290−200 nm region, the absorbance rises, in good agreement with the gas-phase spectrum. The latter allows recording the whole band peaking around 195 nm, in addition to another band with a maximum around 100 nm, attributed to high lying excited states of neutral vancomycin (see the previous paragraph).

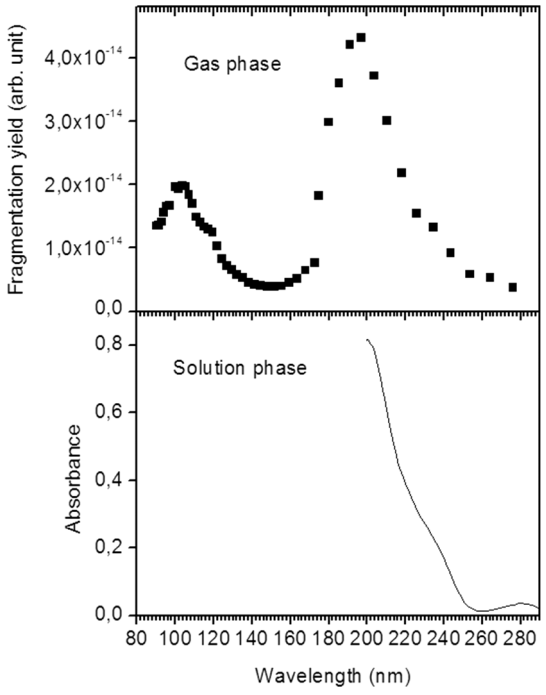

Figure 5. Top: total fragmentation yield of isolated $[V + R - H]^-$ in the UV−VUV 4.5−13.8 eV range. The yields of all channels have been added, and the photon energy has been converted to wavelength. Bottom: UV spectrum of a solution of vancomycin in deionized water.

CONCLUSIONS

In this Article, we have presented data from experiments consisting in irradiating trapped gas-phase molecular anions with a synchrotron beam in the UV−VUV range and recording the mass spectrum of negatively charged photoproducts as a function of photon energy. By taking advantage of the fact that electrospray ionization is a soft technique that allows bringing fragile noncovalent complexes into the gas phase, we recorded the photofragmentation spectrum of the complex between neutral vancomycin and the singly deprotonated $Ac_2{}^LK^DA^DA$ peptide. We show that the recorded spectrum can be considered as the UV−VUV photofragmentation spectrum of neutral vancomycin in the gas phase because in this wavelength range (90−275 nm), the probability of photofragmentation of the singly deprotonated $Ac_2{}^LK^DA^DA$ is negligible compared to that of vancomycin. Furthermore, noncovalent binding does not induce sufficient spectral shifts to have an influence on our spectrum, given the experimental resolution.

This proof-of-principle of charge-tagging action spectroscopy opens up new possibilities regarding UV−VUV spectros-

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copy of gas-phase neutral organic/biological molecules, thanks to the versatility of the coupling of electrospray ionization and mass spectrometry techniques. The next step is, first, to test our method with other complexes composed of fragile neutral molecules noncovalently bound to small tag anions. Then, it would be worth exploring the soft X-ray energy range, even if electron detachment is expected to be even more dominant over photofragmentation than in the UV−VUV range. Another perspective takes advantage of the enantiospecific aspect of noncovalent binding of vancomycin to $Ac_2{}^{L}K^{D}A^{D}A$: it has been shown that the $^{D}A^{D}A$ termination is required for the receptor to locate in the binding pocket of vancomycin in the condensed phase,[29] in line with the dissociation energy of the doubly deprotonated complex being much larger for $Ac_2{}^{L}K^{D}A^{D}A$ than for $Ac_2{}^{D}K^{L}A^{L}A$ in the gas phase.[33] Thus, it would be interesting to record the action spectrum of the singly deprotonated noncovalent complex between vancomycin and $Ac_2{}^{D}K^{L}A^{L}A$ with the same method as applied here: we expect major differences with the results presented here.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Schermann, J. P.*Spectroscopy and Modelling of Biomolecular Building Blocks*; Elsevier: Amsterdam, 2007.

(2) Ahmed, M.; Kostko, O. From Atoms to Aerosols: Probing Clusters and Nanoparticles with Synchrotron Based Mass Spectrometry and X-Ray Spectroscopy. *Phys. Chem. Chem. Phys.* **2020**, *22* (5), 2713−2737.

(3) Baer, T.; Tuckett, R. Advances in Threshold Photoelectron Spectroscopy (TPES) and Threshold Photoelectron Photoion Coincidence (TPEPICO. *Phys. Chem. Chem. Phys.* **2017**, *19* (15), 9698−9723.

(4) *VUV and Soft X-Ray Photoionization*; Becker, U., Shirley, D. A., Eds.; Springer: New York, 1996.

(5) Berden, G.; Peeters, R.; Meijer, G. Cavity Ring-down Spectroscopy: Experimental Schemes and Applications. *Int. Rev. Phys. Chem.* **2000**, *19* (4), 565−607.

(6) Schwell, M.; Hochlaf, M.Photoionization Spectroscopy of Nucleobases and Analogues in the Gas Phase Using Synchrotron Radiation as Excitation Light Source. In *Photoinduced phenomena in nucleic acids I: Nucleobases in the gas phase and in solvents*; Barbatti, M., Borin, A., Ullrich, S., Eds.; Springer: Cham, Germany, 2015; Vol. 355, pp 155−208. DOI: 10.1007/128_2014_550.

(7) Prince, K.; Bolognesi, P.; Feyer, V.; Plekan, O.; Avaldi, L. Study of Complex Molecules of Biological Interest with Synchrotron Radiation. *J. ELECTRON Spectrosc. Relat. Phenom.* **2015**, *204*, 335−344.

(8) Meinert, C.; Garcia, A. D.; Topin, J.; Jones, N. C.; Diekmann, M.; Berger, R.; Nahon, L.; Hoffmann, S. V.; Meierhenrich, U. J. Amino Acid Gas Phase Circular Dichroism and Implications for the Origin of Biomolecular Asymmetry. *Nat. Commun.* **2022**, *13* (1), 502.

(9) Tia, M.; Cunha de Miranda, B.; Daly, S.; Gaie-Levrel, F.; Garcia, G. A.; Powis, I.; Nahon, L. Chiral Asymmetry in the Photoionization of Gas-Phase Amino-Acid Alanine at Lyman-α Radiation Wavelength. *J. Phys. Chem. Lett.* **2013**, *4* (16), 2698−2704.

(10) Levola, H.; Kooser, K.; Rachlew, E.; Nommiste, E.; Kukk, E. Fragmentation of Thymidine Induced by Ultraviolet Photoionization and Thermal Degradation. *Int. J. Mass Spectrom.* **2013**, *353*, 7−11.

(11) Hadidi, R.; Božanić, D. K.; Ganjitabar, H.; Garcia, G.; Powis, I.; Nahon, L.Conformer-Dependent Vacuum Ultraviolet Photodynamics and Chiral Asymmetries in Pure Enantiomers of Gas Phase Proline. *Commun. Chem.***2021**, *4* (1).

(12) Gaie-Levrel, F.; Garcia, G.; Schwell, M.; Nahon, L. VUV State-Selected Photoionization of Thermally-Desorbed Biomolecules by Coupling an Aerosol Source to an Imaging Photoelectron/Photoion Coincidence Spectrometer: Case of the Amino Acids Tryptophan and Phenylalanine. *Phys. Chem. Chem. Phys.* **2011**, *13* (15), 7024−7036.

(13) Wilson, K. R.; Jimenez-Cruz, M.; Nicolas, C.; Belau, L.; Leone, S. R.; Ahmed, M. Thermal Vaporization of Biological Nanoparticles: Fragment-Free Vacuum Ultraviolet Photoionization Mass Spectra of Tryptophan, Phenylalanine-Glycine-Glycine, and Beta-Carotene. *J. Phys. Chem. A* **2006**, *110*, 2106−2113.

(14) Giuliani, A.; Milosavljevic, A. R.; Canon, F.; Nahon, L. Contribution of Synchrotron Radiation to Photoactivation Studies of Biomolecular Ions in the Gas Phase. *Mass Spectrom. Rev.* **2014**, *33* (6), 424−441.

(15) Antoine, R.; Dugourd, P. Visible and Ultraviolet Spectroscopy of Gas Phase Protein Ions. *Phys. Chem. Chem. Phys.* **2011**, *13*, 16494−16509.

(16) Milosavljevic, A. R.; Canon, F.; Nicolas, C.; Miron, C.; Nahon, L.; Giuliani, A. Gas-Phase Protein Inner-Shell Spectroscopy by Coupling an Ion Trap with a Soft X-Ray Beamline. *J. Phys. Chem. Lett.* **2012**, *3* (9), 1191−1196.

(17) Giuliani, A.; Milosavljevic, A. R.; Hinsen, K.; Canon, F.; Nicolas, C.; Refregiers, M.; Nahon, L. Structure and Charge-State Dependence of the Gas-Phase Ionization Energy of Proteins. *Angew. Chem.-Int. Ed.* **2012**, *51* (38), 9552−9556.

(18) Hendricks, N.; Julian, R. Leveraging Ultraviolet Photodissociation and Spectroscopy to Investigate Peptide and Protein Three-Dimensional Structure with Mass Spectrometry. *ANALYST* **2016**, *141* (15), 4534−4540.

(19) Bari, S.; Gonzalez-Magana, O.; Reitsma, G.; Werner, J.; Schippers, S.; Hoekstra, R.; Schlatholter, T. Photodissociation of Protonated Leucine-Enkephalin in the VUV Range of 8−40 EV. *J. Chem. Phys.* **2011**, *134* (2), 024314.

(20) González-Magaña, O.; Reitsma, G.; Tiemens, M.; Boschman, L.; Hoekstra, R.; Schlathölter, T. Near-Edge X-Ray Absorption Mass Spectrometry of a Gas-Phase Peptide. *J. Phys. Chem. A* **2012**, *116* (44), 10745−10751.

(21) Schwob, L.; Dörner, S.; Atak, K.; Schubert, K.; Timm, M.; Bülow, C.; Zamudio-Bayer, V.; von Issendorff, B.; Lau, J. T.; Techert, S.; Bari, S. Site-Selective Dissociation upon Sulfur L-Edge X-Ray Absorption in a Gas-Phase Protonated Peptide. *J. Phys. Chem. Lett.* **2020**, *11* (4), 1215−1221.
(22) Canon, F.; Milosavljevic, A. R.; Nahon, L.; Giuliani, A. Action Spectroscopy of a Protonated Peptide in the Ultraviolet Range. *Phys. Chem. Chem. Phys.* **2015**, *17*, 25725−25733.
(23) Boyarkin, O. Cold Ion Spectroscopy for Structural Identifications of Biomolecules. *Int. Rev. Phys. Chem.* **2018**, *37* (3−4), 559−606.
(24) Wang, X.; Rathnachalam, S.; Bijlsma, K.; Li, W.; Hoekstra, R.; Kubin, M.; Timm, M.; von Issendorff, B.; Zamudio-Bayer, V.; Lau, J. T.; Faraji, S.; Schlathölter, T. Site-Selective Soft X-Ray Absorption as a Tool to Study Protonation and Electronic Structure of Gas-Phase DNA. *Phys. Chem. Chem. Phys.* **2021**, *23* (20), 11900−11906.
(25) Rosu, F.; Gabelica, V.; De Pauw, E.; Antoine, R.; Broyer, M.; Dugourd, P. UV Spectroscopy of DNA Duplex and Quadruplex Structures in the Gas Phase. *J. Phys. Chem. A* **2012**, *116* (22), 5383−5391.
(26) Paizs, B.; Suhai, S. Fragmentation Pathways of Protonated Peptides. *Mass Spectrom. Rev.* **2005**, *24*, 508−548.
(27) Milosavljevic, A. R.; Nicolas, C.; Rankovic, M. L. J.; Canon, F.; Miron, C.; Giuliani, A. K-Shell Excitation and Ionization of a Gas-Phase Protein: Interplay between Electronic Structure and Protein Folding. *J. Phys. Chem. Lett.* **2015**, *6* (16), 3132−3138.
(28) McKay, A.; Sanz, M.; Mooney, C.; Minns, R.; Gill, E.; Fielding, H. Development of a New Photoelectron Spectroscopy Instrument Combining an Electrospray Ion Source and Photoelectron Imaging. *Rev. Sci. Instrum.* **2010**, *81* (12), 123101.
(29) Nieto, M.; Perkins, H. R. Modifications of the Acyl-D-Alanyl-D-Alanine Terminus Affecting Complex-Formation with Vancomycin. *Biochem. J.* **1971**, *123*, 789−803.
(30) Nitanai, Y.; Kikuchi, T.; Kakoi, K.; Hanamaki, S.; Fujisawa, I.; Aoki, K. Crystal Structures of the Complexes between Vancomycin and Cell-Wall Precursor Analogs. *J. Mol. Biol.* **2009**, *385* (5), 1422−1432.
(31) Poully, J. C.; Lecomte, F.; Nieuwjaer, N.; Manil, B.; Schermann, J. P.; Desfrancois, C.; Calvo, F.; Gregoire, G. Probing the Specific Interactions and Structures of Gas-Phase Vancomycin Antibiotics with Cell-Wall Precursor through IRMPD Spectroscopy. *Phys. Chem. Chem. Phys.* **2010**, *12* (14), 3606−3615.
(32) Poully, J. C.; Lecomte, F.; Nieuwjaer, N.; Manil, B.; Schermann, J. P.; Desfrancois, C.; Gregoire, G.; Ballivian, R.; Chirot, F.; Lemoine, J.; Calvo, F.; Antoine, R.; Dugourd, P. Combining Ion Mobility Mass Spectrometry and Infrared Multiphoton Dissociation Spectroscopy to Probe the Structure of Gas-Phase Vancomycin-Ac(2)(L)K(D)A(D)A Non-Covalent Complex. *Int. J. Mass Spectrom.* **2010**, *297* (1−3), 28−35.
(33) Jørgensen, T. J. D.; Delforge, D.; Remacle, J.; Bojesen, G.; Roepstorff, P. Collision-Induced Dissociation of Noncovalent Complexes between Vancomycin Antibiotics and Peptide Ligand Stereoisomers: Evidence for Molecular Recognition in the Gas Phase. *Int. J. Mass Spectrom.* **1999**, *188*, 63−85.
(34) Abdelmouleh, M.; Lalande, M.; Vizcaino, V.; Schlatholter, T.; Poully, J.-C. Photoinduced Processes within Noncovalent Complexes Involved in Molecular Recognition. *Chem.- Eur. J.* **2020**, *26* (10), 2243−2250.
(35) Nahon, L.; de Oliveira, N.; Garcia, G. A.; Gil, J.-F.; Pilette, B.; Marcouillé, O.; Lagarde, B.; Polack, F. DESIRS: A State-of-the-Art VUV Beamline Featuring High Resolution and Variable Polarization for Spectroscopy and Dichroism at SOLEIL. *J. Synchrotron Radiat.* **2012**, *19* (4), 508−520.
(36) Milosavljevic, A. R.; Nicolas, C.; Gil, J.-F.; Canon, F.; Refregiers, M.; Nahon, L.; Giuliani, A. VUV Synchrotron Radiation: A New Activation Technique for Tandem Mass Spectrometry. *Journal of Synchrotron Radiation* **2012**, *19*, 174−178.
(37) Brunet, C.; Antoine, R.; Allouche, A.-R.; Dugourd, P.; Canon, F.; Giuliani, A.; Nahon, L. Gas Phase Photo-Formation and Vacuum UV Photofragmentation Spectroscopy of Tryptophan and Tyrosine Radical-Containing Peptides. *J. Phys. Chem. A* **2011**, *115*, 8933−8939.
(38) Bowie, J. H.; Brinkworth, C. S.; Dua, S. Collision-Induced Fragmentations of the (M-H)− Parent Anions of Underivatized Peptides: An Aid to Structure Determination and Some Unusual Negative Ion Cleavages. *Mass Spectrom. Rev.* **2002**, *21* (2), 87−107.
(39) Yang, Z.; Vorpagel, E. R.; Laskin, J. Influence of the Charge State on the Structures and Interactions of Vancomycin Antibiotics with Cell-Wall Analogue Peptides: Experimental and Theoretical Studies. *Chem.—Eur. J.* **2009**, *15*, 2081−2090.
(40) Serrano-Andrés, L.; Fülscher, M. P. Charge Transfer Transitions in Neutral and Ionic Polypeptides: A Theoretical Study. *J. Phys. Chem. B* **2001**, *105* (38), 9323−9330.
(41) Jones, D. B.; da Silva, G. B.; Neves, R. F. C.; Duque, H. V.; Chiari, L.; de Oliveira, E. M.; Lopes, M. C. A.; da Costa, R. F.; Varella, M. T. do N.; Bettega, M. H. F.; Lima, M. A. P.; Brunger, M. J. An Experimental and Theoretical Investigation into the Excited Electronic States of Phenol. *J. Chem. Phys.* **2014**, *141* (7), 074314.
(42) Matheis, K.; Joly, L.; Antoine, R.; Lépine, F.; Bordas, C.; Ehrler, O. T.; Allouche, A.-R.; Kappes, M. M.; Dugourd, P. Photoelectron Spectroscopy of Gramicidin Polyanions: Competition between Delayed and Direct Emission. *J. Am. Chem. Soc.* **2008**, *130* (47), 15903−15906.
(43) Besley, N. A.; Hirst, J. D. Ab Initio Study of the Effect of Solvation on the Electronic Spectra of Formamide and N-Methylacetamide. *J. Phys. Chem. A* **1998**, *102* (52), 10791−10797.
(44) LeGreve, T. A.; James, W. H., III; Zwier, T. S. Solvent Effects on the Conformational Preferences of Serotonin: Serotonin−(H2O)n, n = 1,2. *J. Phys. Chem. A* **2009**, *113* (2), 399−410.

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J. Phys. Chem. A 2022, 126, 9042−9050