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Control of Bond-Cleaving Reactions of Free Protonated Tryptophan Ion by Femtosecond Laser Pulses

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The excited-state dynamics of protonated tryptophan ions is investigated by photoinduced fragmentation in the gas phase. In contrast to the neutral molecule that decays on the nanosecond time scale, the protonated species exhibits an ultrafast decay with two time constants of about 400 fs and 15 ps. In addition, after UV excitation by a pump photon at 266 nm, specific photofragments, and in particular the NH_3-loss channel, can be enhanced by the absorption of a probe photon at 800 nm. The bond-cleaving reactions can thus be controlled by a variation of the pump/probe delay.

Introduction

Although widely used in many molecular biology techniques,[1,2] the interpretation of the strong dependence of the tryptophan fluorescence upon a local protein environment has been long and difficult.[3] Beside the two well-known electronic states of indole, L_a and L_b of $\pi\pi^*$ nature, recent quantum calculations have invoked a nearby $\pi\sigma^*$ excited state, dissociative along the indole N−H stretch coordinate.[4] An alternative mechanism has also been proposed, where the quenching of the $\pi\pi^*$ state is due to charge transfer to the peptide backbone.[5] In protonated tryptamine,[6] an excited state similar to the above-mentioned $\pi\sigma^*$ state of indole, dissociative along the NH coordinate of the protonated amino group, has also been suggested to explain the very fast decay of the excited state and specific observation of an H-atom loss channel after photoexcitation. Quantum chemistry calculations[7,8] indicate that protonation also takes place on the terminal amino group for tryptophan, this structure being more stable by 1.0 eV than protonation on the nitrogen atom of the indole side chain.

In this paper, we present direct experimental evidence of a fast biexponential decay of photoexcited protonated tryptophan ($TrpH^+$). The optical properties of this protonated amino acid have been obtained recently,[8] as well as its photofragmentation properties.[9] In the present work, we use a femtosecond pump−probe technique to follow the fragmentation dynamics. As described in the following, this technique is able to provide information about the lifetimes of the excited states and it also allows for a control of the fragmentation processes. In particular, the NH_3-loss channel can be enhanced by further absorption of a probe photon, i.e., by an increase of the internal energy, even though this fragmentation channel is the lowest-energy channel in the electronic ground state.

Experimental Section

The experimental setup consists of an electrospray ionization source coupled with a hexapole ion guide, an electrostatic lens and a time-of-flight mass spectrometer.[9] A 50/50 water−methanol solution of L-tryptophan (500 μmol/L) is injected in a commercial electrospray setup (Analytica). After desolvation, the protonated ions are trapped for 1 ms in the ion guide by the bias of the exit electrode, set a few volts above that of the hexapole. An ion bunch is ejected from the guide, by a lowering of the voltage of this exit electrode during 800 ns. However, the first electrode of the einzel lens is first maintained at a repulsive voltage (+100V) during 500 ns before being shifted down to −100 V, to compress the ion bunch before it is focused into a 1 mm pinhole at the exit of the einzel lens. This produces an ion packet of 200 ns width, which then intersects perpendicularly the femtosecond laser pulses just after the pinhole. This procedure ensures better overlap between the ion packet and the laser pulses and enhances the S/N ratio of the pump−probe signal. Therefore, the dynamics reported here exhibit a second fast decay that could not be extracted from the previous experiments on $TrpH^+$.[10] Both parent and photofragment ions enter the region between the grids of the linear time-of-flight mass spectrometer, in which they are mass-analyzed. Pump and probe laser beams are the 3rd harmonic (266 nm, 50 μJ) and the fundamental (800 nm, 150 μJ), respectively, of a Ti:sapphire femtosecond laser (150 fs) operating at 1 kHz. The light is linearly polarized and we did not attempt to study the effect of the polarization of the laser on the photodissociation signal. The zero-delay time and cross-correlation of the lasers have been

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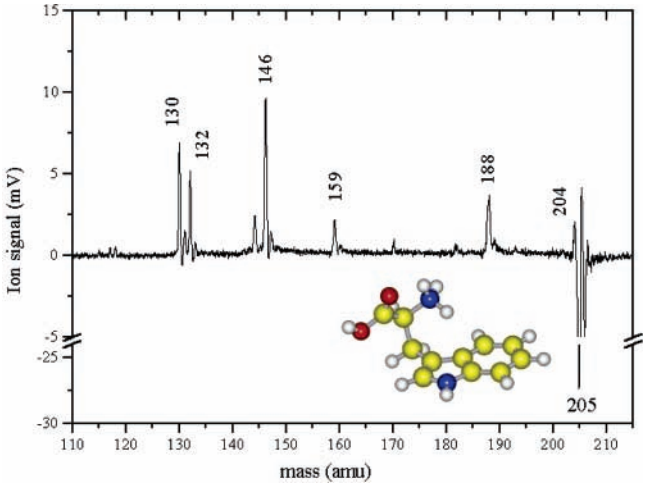

Figure 1. Difference mass spectrum (laser on minus laser off) obtained after excitation of protonated tryptophan at 266 nm. The parention peak ($m/z = 205$) is depleted and comes out as a negative signal, while the corresponding photofragments appear as positive mass peaks.

monitored through the resonant ionization of neutral toluene that provides a temporal laser width of 250 ± 50 fs. The laser pulses are delayed by an optical delay line which is scanned in 50 fs steps, and the spectra are averages of 10 scans with 100 laser shots per scan step.

Results

The photoinduced dissociation (PID) mass spectrum presented in Figure 1 is obtained as a difference between the mass spectra recorded with and without the UV laser. The $TrpH^+$ negative peak at $m/z = 205$ thus indicates a depletion, whereas the positive peaks at m/z= 204, 188, 159, 146, 132, and 130 arise from fragmentation induced by the 266 nm photodissociation laser. As previously reported,[9] the $m/z = 204$ fragment is the signature of the H-atom loss reaction and corresponds to the Trp^+ radical cation that further dissociates into its specific $m/z = 130$ fragment.[11] The other fragments are thought to be produced after internal conversion to the ground state as commonly observed in CID experiments.[12]

In Figure 2 are reported the dependence of the yield on the delay between the pump (266 nm) and probe (800 nm) pulses for three different fragments. The $m/z = 130$, 146, and 188 fragments exhibit clear dynamics, whereas no clear time evolution could be recorded on the $m/z = 132$, 159, and 204 fragments, at least within the signal-to-noise ratio of our experiment. The dynamics are quite different for the three cases: the probe laser leads to an increase of the signal for $m/z = 130$, to a depletion of the signal for $m/z = 146$ and to a depletion followed by an increase for $m/z = 188$. It should be noticed that, again within the experimental uncertainty, the sum of the depleted and the populated signals on all the fragments is a constant signal: the population lost at one mass is found at other masses. The pump−probe signal at $m/z = 130$ can be fitted by a biexponential function with time constants of 400 ± 100 fs and 15 ± 1.5 ps plus a plateau. The depleted signal observed at $m/z = 146$ can be fitted by a biexponential with similar time constants (400 fs and 15 ps) but with different preexponential factors. The signal observed at $m/z = 188$ (ammonia loss) is quite surprising: a depletion during 400 fs and a population gain that vanishes in 15 ps. All of these time evolution curves can be fitted with the same two time constants, which should thus reflect the lifetimes of two different excited

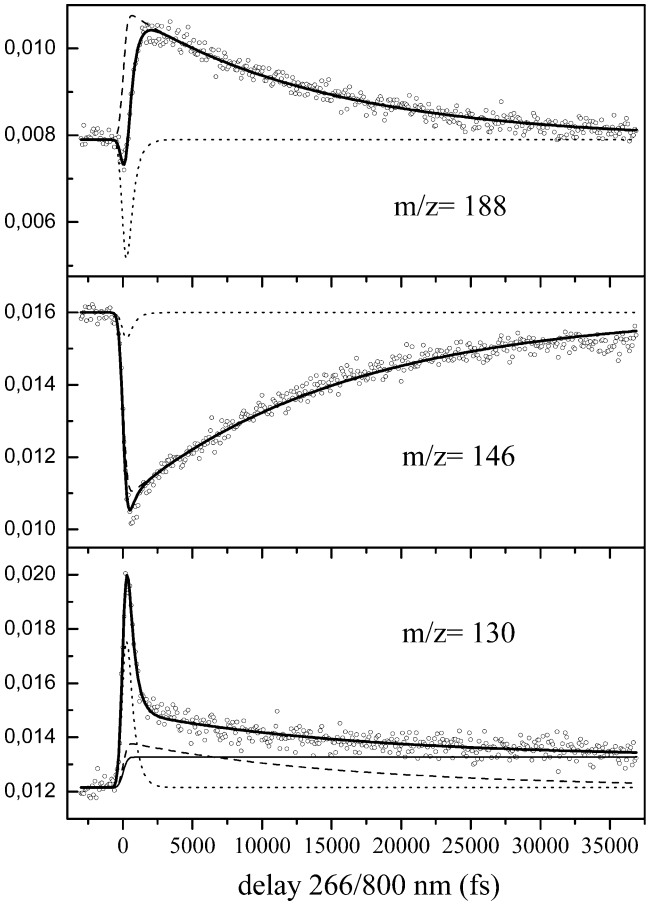

Figure 2. Evolution for 35 ps of the fragment ion signals (open circle) as a function of the pump (266 nm)/probe (800 nm) delay, for different masses. The fitted curves (heavy solid line) are a combination of two exponential functions of 400 fs (dotted line) and 15 ps (dashed line) time constants. For the tryptophan side chain cation at $m/z = 130$, a step function (thin solid line) is added that corresponds to the absorption of the probe photon by the nascent $m/z = 204$ radical cation (under the assumption that the H atom loss reaction is very fast after crossing to the repulsive $\pi\sigma^*$ state).

states. Which can be these two states and how can the pump−probe excitation give such different fragment ion signals?

Discussion

The present data can be understood considering the potential curves of Figure 3 and comparing with previous results obtained on protonated tryptamine,[6] using the same pump−probe scheme. In both cases, UV excitation of the protonated ion induces the H-atom loss reaction with formation of the radical cation that subsequently fragments to the $m/z = 130$ ion.[13]

In the dissociation of protonated tryptamine, the enhancement of the yield of the $m/z = 130$ fragment ion due to the probe pulse exhibits a single exponential decrease with a time constant of 250 fs followed by a plateau. The short time constant has been interpreted as the signature of the predissociation of the initially $\pi\pi^*$ excited state via crossing to a nearby $\pi\sigma^*$ state, dissociative along the N−H coordinate of the protonated amino group. This $\pi\sigma^*$ state corresponds to an electron transfer from the indole ring to the NH_3^+ group resulting in the formation of a hypervalent species (like NH_4) that should be highly unstable. The $\pi\sigma^*$ state crosses the ground state at longer N−H distances (see Figure 3a), and the evolution of the H atom on this $\pi\sigma^*$ dissociative surface should be very fast.[14] The observed short time constant should then reflect to a large extent the lifetime

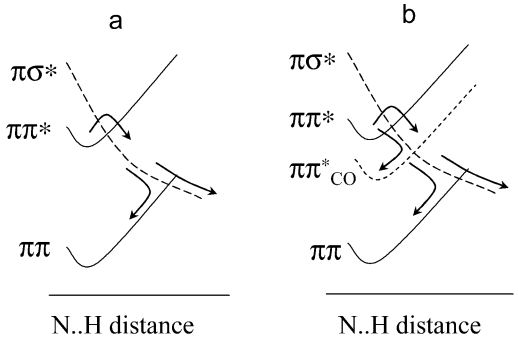

Figure 3. Schematic representation of the potential energy curves of protonated tryptophan along the NH (amino group) coordinate (according to the TD/DFT calculations presented in ref 9). The lifetime of the $\pi\pi^*$ excited state is controlled by the crossing between the $\pi\pi^*$ and the $\pi\sigma^*$ states, the branching ratio between H-atom loss and internal conversion is determined by the crossing between the $\pi\sigma^*$ and the $\pi\pi$ ground state. In a first case depicted on scheme a, the two time constants of 400 fs and 15 ps would be due to two isomers that have a different $\pi\pi^*$ state lifetime: the relative position of the $\pi\sigma^*$ and $\pi\pi^*$ states depends on the isomer (unspecified). When the $\pi\sigma^*$ is higher in energy, the lifetime of the $\pi\pi^*$ excited is longer. The second possibility, presented in scheme b, is that the two time constants correspond to the dynamics from two different electronic states: the long time constant may reflect the lifetime of a lower $\pi\pi^*_{CO}$ state. The two states $\pi\pi^*$ and $\pi\pi^*_{CO}$ do not have the same lifetime and the excitation of each state by the probe photon does not lead to the same fragmentation pattern.

of the initially excited $\pi\pi^*$ state. At the crossing, the H atom can either dissociate leading to a radical cation or recombine on the ground-state potential energy surface (internal conversion), leading to hot protonated ions that will undergo fragmentation on this surface. It should be stressed that the nascent hot protonated species cannot be further excited through absorption of a 800 nm photon since it has an electronic closed shell structure with the first electronic transition in the UV ($\pi\pi \rightarrow$ pp* transition). On the other hand, the radical cation obtained by the H-atom loss can be probed by absorption of the 800 nm photon because it possesses low lying electronic states in the near-IR The main fragmentation channel of the radical cation being the $m/z = 130$ fragment via a C_α-C_β bond rupture, the constant enhancement of the $m/z = 130$ ion signal at long times thus corresponds to the excitation of the radical cation formed after the H-atom loss.

In the photodissociation of $TrpH^+$, the pump probe signal on the $m/z = 130$ fragment also presents a fast exponential decrease (400 fs) and a plateau at long times but, in addition, there is a second exponential decrease with 15 ps lifetime. Due to the similar molecular structures, excited-state potential energy surfaces, and similar ultra-short lifetime measured with the same pump−probe photofragmentation scheme, the analysis of the excited state dynamics of protonated tryptophan should be essentially the same as for protonated tryptamine: the very short time constant reflects the $\pi\pi^*$ lifetime and the plateau observed on mass 130 reflects the absorption of the probe laser by the radical cation after H dissociation.

The longer decay component (15 ps) may have different origins that will be discussed below, but the most striking result of the present experiment is that, after 400 fs, the absorption of a probe photon can induce an increase of the $m/z = 188$ fragment (NH_3 loss) and a simultaneous depletion of the $m/z = 146$ daughter ion ($NH_3 + CH_2CO$ loss). The NH_3 loss channel corresponds to the lowest energy channel after internal conversion in the parent protonated ion.[12,15] In a statistical picture, an increase of the parent ion internal energy due to the probe photon absorption should thus lead to a depletion of this low-energy channel and to a symmetric increase of its daughter ions, in particular that at $m/z = 146$.[16] The reverse is observed and this implies that a nonstatistical decay is induced by the probe photon. We suggest that the mechanism could be selective C_α−N bond rupture in the higher excited state, leading to an increase of the ammonia loss channel.

The first way of interpreting the observed biexponential decay for the photoexcited protonated tryptophan is to involve two different conformers. Such an interpretation has been raised for tryptophan in liquid water in the rotamer model[17] and would mean that the lifetime of the $\pi\pi^*$ excited state is 400 fs for one conformer and 15 ps for the other, the longer lifetime arising from a higher energy gap between the $\pi\pi^*$ and $\pi\sigma^*$ states (Figure 3a). However, the effect of the probe photon must be opposite for the two conformers: for the short lifetime conformer, it leads to a depletion of the two internal conversion channels at $m/z = 188$ and 146, whereas for the 15 ps lifetime conformer, there is a depletion of the $m/z = 146$ fragment but an increase of the $m/z = 188$ fragment. In the latter case, as suggested above, an excitation by the probe photon to a higher dissociative state selectively leading to the NH_3 loss should be involved.

A second interpretation involves the presence of two electronic states that are probed by the 800 nm laser. Because of its absence in protonated tryptamine, the second 15 ps time constant could be specific from the presence of the carboxylic group (Figure 3b). The existence of an excited state where the electron is located on a π^* orbital of the CO group has already been postulated by Callis et al.[5] to explain the quenching of tryptophan fluorescence in proteins. In this scheme, the probe laser excitation toward an antibonding C_α−N orbital may have a larger oscillator strength from the $\pi\pi^*_{CO}$ state than from $\pi\pi^*$ state, due to a larger orbital overlap. The 15 ps decay would then be the lifetime of the $\pi\pi^*_{CO}$ state.

Conclusion

The excited state $\pi\pi^*$ of protonated tryptophan has a very short lifetime and seems to decay through a dissociative $\pi\sigma^*$ excited state. The biexponential decrease observed for the protonated tryptophan could be due either to two conformers or to the presence of a low $\pi\pi^*_{CO}$ excited state. To address this issue, high-level quantum chemistry calculations are highly needed. However, the main result of the present experiment is that the absorption of a probe photon can enhance dissociation along the C_α−N and N−H bonds, with a branching ratio that can be controlled by the time delay between the pump and probe photon.

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