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Femtosecond pump-probe experiments on trapped flavin: Optical control of dissociation

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Femtosecond pump-probe experiments are performed on flavin biomolecules isolated in an ion trap. Mass spectra of the photoinduced fragments show that the fragmentation pathways can be modified using two-color two-photon excitation. In particular, when an infrared probe pulse (810 nm) is added subsequent to the first excitation step (excitation of the S_1 state of flavin mononucleotide at 405 nm), branching ratios between lumichrome and lumiflavin production are inverted relative to the single excitation case. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2828558]

INTRODUCTION

Flavins are redox-active chromophores which are found in enzymes and photoreceptors. Flavoenzymes are known to play a crucial role in light-mediated signal transduction. For example, in the blue-light photoreceptor phototropin, the photoexcitation of flavin is the initial step in the subsequent long reaction cascade.[1,2] Among flavins, flavin mononucleotide (FMN) is a coenzyme of many electron transferring enzymes.[3] Because of the biological relevance of flavins, their photophysics has been studied extensively,[4] especially in aqueous solution,[5,6] with particular reference to the photodegradation of riboflavin (RF) into lumichrome (LC) and lumiflavin (LF), two of the main photofragments. Furthermore, the photodissociation of LC and LF has been shown to depend strongly on the *p*H of the solution; LC being the major fragment in acidic solutions.[7] The relevance of the photochemical properties of these molecules with the fact that experiments on flavins in the gas phase are relatively easy to perform, together, indicates that flavins are ideal systems for gas-phase dynamics studies. A recent experiment reports on studying the photodegradation of flavins through mass spectrometry of photofragments produced, using flash photolysis of flavins in aqueous solution.[5] The present paper presents femtosecond pump-probe experiments on trapped FMN ions with a setup similar to the one recently developed by Nolting *et al.*[8] The viability of partially controlling dissociation pathways using a pump-probe excitation scheme is shown. In particular, we demonstrate that adding the probe pulse increases the LC production to the detriment of LF. This behavior might be linked to previous results in liquids where the amount of LC is enhanced by low *p*H environments.[7]

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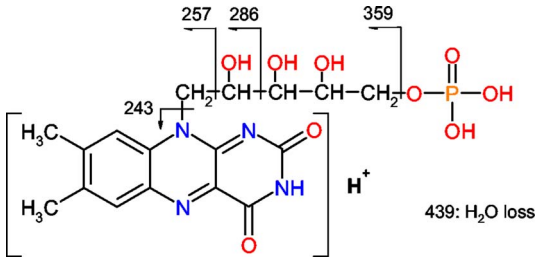
EXPERIMENTAL SETUP

Femtosecond pump-probe experiments were performed using a modified commercial ion-trap mass spectrometer (LCQ DUO with the MSn option; Thermo Electron, San Jose, CA) equipped with an off-axis electrospray ionization source. The ring electrode of the quadrupole ion trap was pierced to allow the introduction of laser beams (see Talbot *et al.*[9] for details). For photodissociation experiments, precursor ions are first isolated in the trap. After isolation, they are irradiated for 50 ms (i.e., one laser shot), but results do not change significantly by increasing the interaction time up to 500 ms. The ions are finally ejected from the trap and the resulting mass spectrum is recorded, with an average subsequently taken over about 200 spectra. For all experiments, a constant number of FMN ions was maintained inside the trap (~600).

The excitation laser is a chirped pulse amplified Titanium Sapphire (Ti:SA) (typically 150 fs around 810 nm) operated at a repetition rate of 22.5 Hz. The laser is frequency doubled through a 1 mm thick beta-barium borate (BBO) crystal. The 810 nm (probe) and 405 nm (pump) beams are split by a dichroic mirror in a pump-probe delay line. Both pump and probe beams are spatially recombined with an adjustable delay before entering the trap. The precise temporal overlap of the pump and the probe pulses (zero delay) is calibrated by observing some frequency mixing in a thin BBO crystal. Both laser beams pass a mechanical shutter that is electronically synchronized with the mass spectrometer. A lens ($f=500$ mm) located around 600 mm from the center of the trap is used to focus the laser beams on the ion packet. MS^2- and MS^3-CID (collision induced dissociation) experiments were performed for comparison using the same experimental setup. In MS^2-CID experiments, the parent ions are selected and fragmented by collisions and the resulting mass spectrum is recorded. In MS^3-CID experiments, the precursor parent ion is first selected, fragmented by collisions; then, one of the fragment is isolated and fragmented by collisions.



SCHEME 1. The protonated FMN (m/z 457) molecule showing branching points for major photofragments and their m/z value.

RESULTS

FMN (Sigma-Aldrich, Scheme 1) is dissolved, with 1% acetic acid in H_2O/CH_3OH 1:1 (v/v), to a concentration of 200 μM. Electrospraying the FMN solution reveals protonated and sodiated FMN in the mass spectrum. Protonated FMN (m/z 457) is then isolated and CID or ultraviolet photodissociation (UVPD) MS^2 spectra are recorded. The pump pulse wavelength (at 405 nm) for UVPD is chosen to occur in one of the main absorption bands of FMN, as shown in Fig. 1, which gives the absorption of FMN in water. Note that optical absorption spectra of flavins in solution do not depend significantly on pH conditions.[10] The spectrum in the gas phase is not known. However, it is important to note that protonation (which does not change the number of electrons in the system), as well as the solvation in liquid, is expected to induce only small shifts (several nanometers) in the absorption wavelengths. This was observed, for example, for tryptophan with different charge states and different environments.[8,9,11–14] This is true if the protonation or the deprotonation does not occur on the chromophore, while a strong shift can be observed when deprotonation occurs on the chromophore.[15–17] CID and UVPD (typical pump intensity=5×10^{10} W/cm^2) spectra are compared in Fig. 2. CID mainly causes the loss of a water molecule (m/z 439) with a small amount of m/z 359 also produced. The latter fragment corresponds to the loss of the phosphate group H_3PO_4, in other words, to riboflavin minus H_2O (RF^O). Photon-induced dissociation yields very different effects [Fig. 2(b)], the most striking feature in UVPD spectra being the major increase in the m/z 359 peak and the appearance of lumichrome (LC, m/z 243), lumiflavin (LF, m/z 257) and

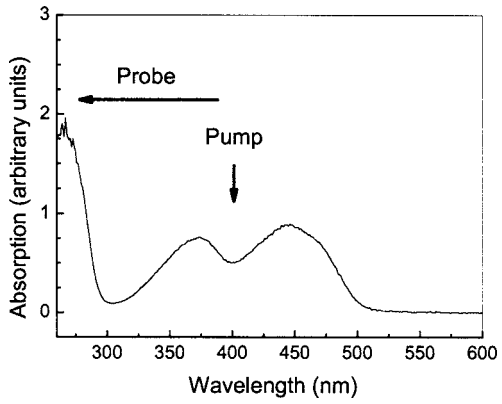


FIG. 1. Absorption spectrum of FMN in water.

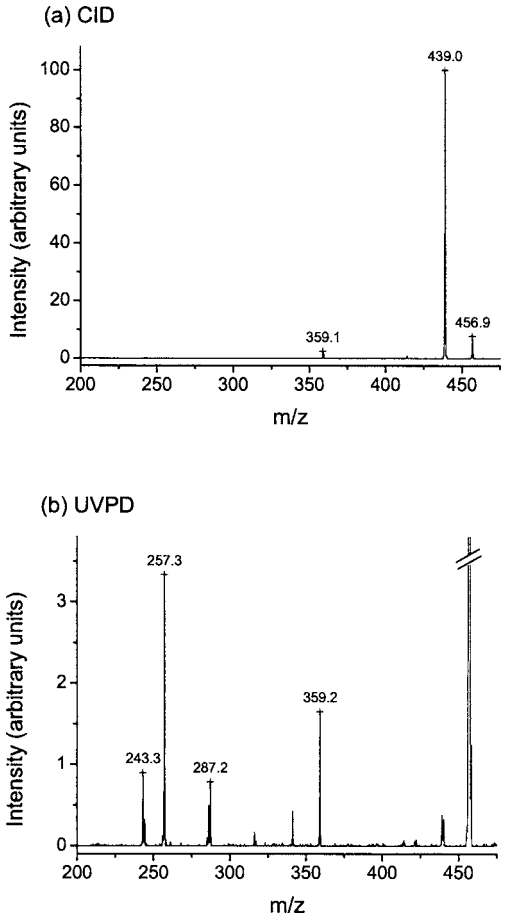
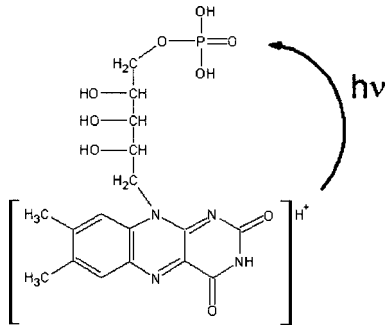


FIG. 2. Mass spectra of trapped protonated FMN (m/z 457). (a) Collision-induced dissociation (CID). (b) Ultraviolet photodissociation (UVPD) for a pump at $\lambda=405$ nm.

formyllumiflavin plus hydrogen(s) (FLF^H, m/z 286 and 287). In contrast, the channel corresponding to the loss of H_2O (m/z 439) is almost suppressed.

The increase in m/z 359 (loss of the phosphate group) observed in Fig. 2(b) may result from a photoinduced proton transfer from the isoalloxazine to the phosphate group (as suggested on Scheme 2) in the excited state following the excitation. This is similar to what was observed by Sobolewski *et al.* on smaller molecules[18] and by Tabarin *et al.* for peptides.[19] This hypothesis is strengthened by mass spectra obtained for deprotonated molecules (the deprotonation occurs on the phosphate group). Figure 3 reveals no major



SCHEME 2. Photoinduced proton transfer from the isoalloxazine group to the phosphate group in FMN.

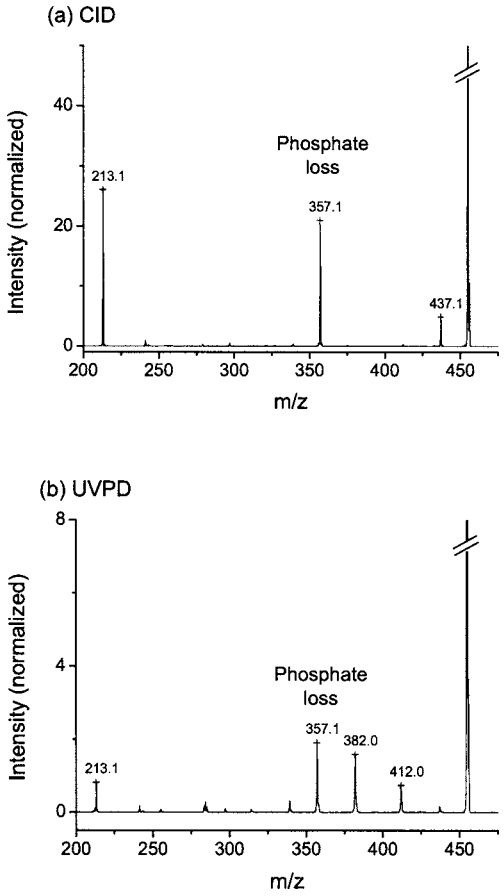


FIG. 3. Mass spectra of trapped deprotonated FMN (m/z 455). (a) Collision-induced dissociation (CID). (b) Ultraviolet photodissociation (UVPD) for a pump at $\lambda=405$ nm.

difference between the CID and UVPD mass spectra; in particular, phosphate loss is observed in both excitation modes. This implies that the protonation state of the molecule plays a role in the loss of phosphate following a UV excitation. Note that for deprotonated molecules, photodetachment should also be considered in the argumentation although from a mononegatively charged ion, it is impossible to detect neutral species after neutral detachment. However, in our experiments, the number of ions after irradiation (parents +fragments) is similar to the number of ions prior to irradiation (precursor parent ions); this means that we do not significantly lose ions after irradiation. Therefore, if detachment occurs, it is not a major channel.

When a probe pulse (at 810 nm) with a typical intensity of 2.8×10^{11} W/cm^2, and superimposed spatially with the pump beam, follows the pump, branching ratios ($I_{\text{fragment}}/\Sigma I_{\text{fragments}}$) corresponding to the different fragmentation pathways are changed, as plotted in Fig. 4. The addition of the IR pulse decreases the production of LF and RF^{O} to the benefit, in particular, of LC. Furthermore, the radical form of FLF^{H} (m/z 286) increases, whereas the other form (m/z 287) surprisingly decreases. The decrease in the branching ratio observed for the production of LF and RF^{O} is not only due to the increase in the other fragments but also reflects a decrease in the intensity of these ions in the mass spectra when the probe laser is added. Note that in the pump-probe delay range from 0 to 100 ps, no specific changes in

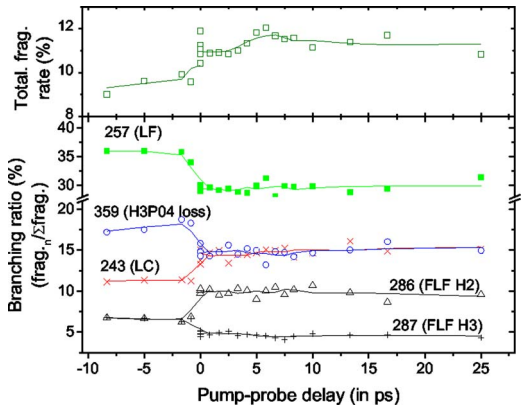


FIG. 4. (Color online) Branching ratio measured for the main fragments of FMN as a function of the pump-probe delay. The total fragmentation rate (Σfragments/(parent+Σfragments)) is also plotted (top curve). Solid lines are smoothed curves of the experimental data.

the dynamics have so far been observed. In particular, no short time scale dynamics, such as vibronic wave packet motions, are observed at the time resolution provided by the experiment (150 fs). As a control, it is observed that the probe pulse alone induces negligible dissociation.

In a second set of experiments, we recorded the dependence of LC, LF, and RF^{O} branching ratios as a function of the probe pulse intensity, with a fixed delay $\Delta t=0$. Figure 5 compares the behavior of these fragments with increasing probe pulse intensity (I_{probe}). While the amount of LC rises with I_{probe}, the percentage of LF decreases from 48% to 36%, which corroborates the previous observations (Fig. 4). At low I_{probe}, the increase in LC (respectively, decrease in LF) appears linear. The decrease in the phosphate-loss fragment as the probe laser intensity increases may reflect a diminution in the proton transfer rate.

DISCUSSION AND CONCLUSION

The main result arising from these experiments is that it is possible to change the branching ratios in the photodissociation of FMN using a pump-probe excitation scheme. As shown in the absorption spectrum (Fig. 1), the pump pulse

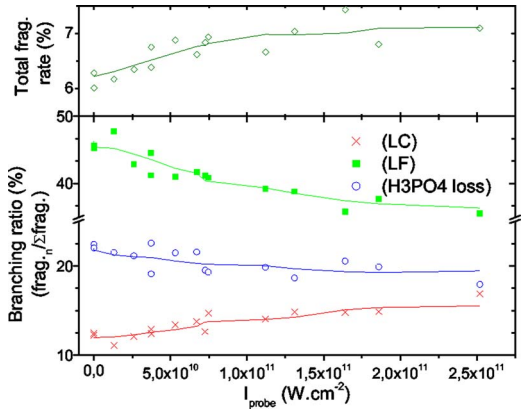
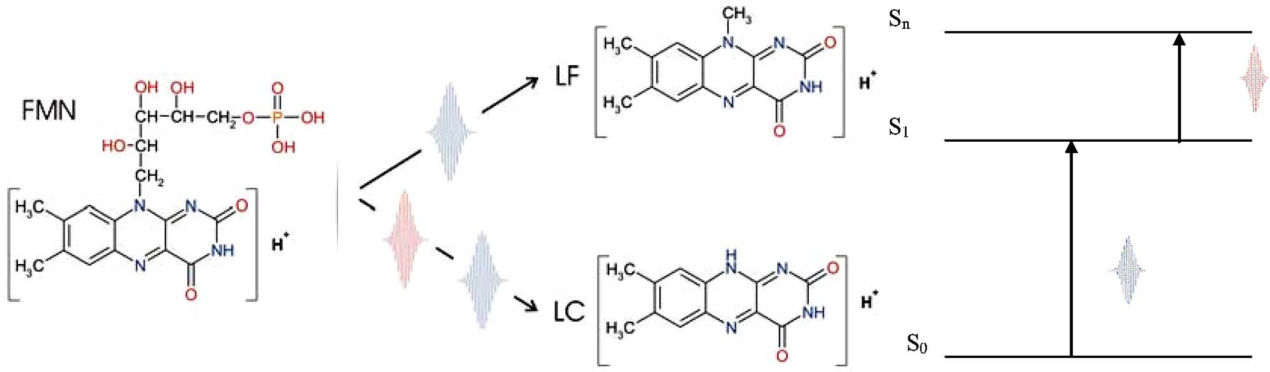


FIG. 5. (Color online) Power dependence on the branching ratio of the fragments LF, LC, and phosphate loss. The total fragmentation rate (Σfragments/(parent+Σfragments)) is also plotted (top curve). Note that $I_{\text{probe}}=0$ means that the probe pulse is off. Solid lines are smoothed curves of the experimental data.



SCHEME 3. Left: When an infrared probe pulse (810 nm) is added subsequent to the first excitation step (excitation of the S_1 state of flavin mononucleotide (FMN) at 405 nm), branching ratios between lumichrome (LC) and lumiflavin (LF) production are inverted relative to the single excitation case. Right: A pictoral diagram of energy levels of FMN is given to summarize our pump-probe experiment.

energy (405 nm, i.e., 24 700 cm^{-1}) lies in the first absorption band of FMN, corresponding to the transition S_0-S_1 (π-π^*). The probe pulse at 810 nm promotes the excited molecules to a higher excited state S_n in the second absorption band (around 270 nm). New dissociation pathways are thus available resulting particularly in an increase in the amount of LC, and corresponding decrease in LF (see Scheme 3). It is important to note that neither the collision-induced nor the photoinduced dissociation of LF (MS^3 spectra not shown) shows formation of LC. Moreover, the photofragments arising from the photodissociation of LC and LF differ. Both observations are against a two-step photodissociation from FMN to LF and then from LF to LC.

In the following, we will mainly focus on the photodegradation of FMN into LC and LF because they are the main products studied in liquids, and into FLF^H because of its striking behavior (see Fig. 4). The photodissociation mechanism is rather complex but an attempt can be made to explain the observed behavior. It is interesting to compare this laser-induced preferential dissociation to the photodegradation of flavins in liquids studied in several previous works. In liquids, if the *p*H of the solution is acidic or neutral, FMN and RF tend to photodissociate into LC rather than into LF, whereas if the *p*H rises above 8, LF is also produced.[7] In our experiment, the decrease in LF (and in the phosphate-loss fragment) in favor of LC might be connected to a change in the proton transfer rate which would reflect different dynamics in the S_1 and S_n excited states. Here, proton transfer, which is in competition with other mechanisms (IVR, direct fragmentations processes, etc.), leads to a deprotonation of the isoalloxazine group. In the first excited state (corresponding to the absorption band around 400 nm), the proton transfer may be faster than in higher excited states; this would be in favor of LF production as observed in basic solutions, for which the isoalloxazine moiety is not protonated. Thus, in the higher excited states, proton transfer may be slower, which would lead to an increase in the LC channel, as observed in acidic solutions for which the isoalloxazine moiety is protonated. To summarize, changes in the LC/LF ratio may be triggered by the ionization state of the isalloxazine group, which can be modified either by *p*H conditions in solution or by proton transfer dynamics in the gas phase.

Another possibility would imply different relaxation pathways between singlet and triplet states, as described in the liquid phase by Heelis.[20] In our experiment, changing the branching ratio of LC and LF might reflect different coupling mechanisms between singlet and triplet states after the absorption of one or two laser pulses. Different triplet states could be reached from S_1 or S_n, leading to different fragmentation schemes. Besides, in the liquid phase, FLF^H is known to be formed via combined photoreduction and dealkylation,[20,21] which involve distinct states (singlet/triplet). This could explain, in our case, the opposite behavior of the two forms of the ions when adding the second pulse.

In conclusion, CID and UVPD lead to different fragmentation pathways of protonated FMN. Moreover, it is possible to use a pump-probe excitation scheme to modify branching ratios in the photodissociation of gas-phase protonated FMN. A tentative explanation relating these results to solution *p*H was proposed; proton transfer is thought to be reduced by adding the probe pulse, leading to an increase in the LC photofragment, which is the main product in acidic solutions. This experiment is a preliminary step toward the control of reactivity in gas-phase biological compounds and opens interesting perspectives for optimal control experiments.

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