Direct monitoring of photon induced isomerization, dissociation and electron detachment of the green fluorescent protein chromophore anion

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Synopsis We present the first experimental demonstration of Z↔E photoisomerization the GFP chromophore anion, $HBDI^-$, in the gas phase. In the single photon absorption regime, the photoisomerization action spectra show two maxima at 480 nm and 455 nm. In the multiphoton absorption regime, photodissociation and photodetachment channels modify the appearance of the photoisomerization band. This work provides a new approach to characterize photoisomerization pathways in biomolecular ions.

The green fluorescent protein (GFP) is one of the most widely used species for imaging biological processes *in vivo* [1]. Its optical absorption properties rely on a chromophore based on p-hydroxybenzylidene-2,3-dimethylimidazolinone (HBDI), which is found inside the folded structure of the protein. Many groups have performed gas phase experiments on $HBDI^-$ in order to characterize the main deactivation pathways in GFP following photoexcitation [2, 3, 4]. While many insights into the nature and dynamics of photoinduced fragmentation and electron emission have been gained, the possibility of Z↔E photoisomerization in $HBDI^-$ (see Figure 1, up), a deactivation pathway competing with photodissociation and photodetachment, has received less attention, mainly due to the challenges associated with its experimental detection.

Combining ion mobility mass spectrometry with laser spectroscopy, we have been able to separate the two isomers of the HBDI anion and demonstrate for the first time Z→E and E→Z photoisomerization in the gas phase. The Z→E photoisomerization action spectrum (Figure 1(a)) presents two maxima at 480 nm and 452 nm at low laser intensity conditions (0.4 mJ/pulse) and shows that photoisomerization dominates over detachment at energies above the electron detachment continuum (454 nm). Nearly equivalent features are observed for the photoexcitation of the E isomer, however a slight blue-shift in the E→Z photoisomerization action spectrum is observed. At increasing laser intensities (1.5 mJ/pulse) photoisomerization competes with multiphoton induced dissociation and detachment. The photodissociation maximum lies at 485 nm, coinciding with a relative decrease of photoisomerization intensity if compared with the low laser power conditions (Figure 1(b)). While this feature is ascribed to a multiphoton induced process involving internal conversion to the ground state, as suggested in a recent work [5], the spectra at low laser intensity conditions show that photoinduced action at the origin of the $S_0 \rightarrow S_1$ band does not only correspond to multiphoton induced dissociation but also to photoisomerization. We will present branching ratios for the three main photoinduced channels at different wavelegths and discuss the effect of photoisomerization on the deactivation efficiency of the GFP protein. These findings provide direct insight into the molecular rearrangement of the GFP chromophore anion upon photoexcitation that could only be indirectly inferred to date.

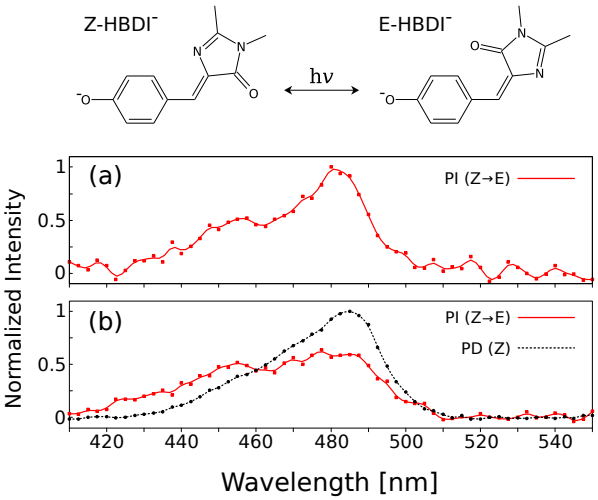

Figure 1. **(a)** Z→E photoisomerization action spectrum (PI) at low light intensity permitting only single photon processes. **(b)** Photoisomerization and photodissociation (PD) action spectra of the Z-HBDI anion at high light intensity.

References

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