Ultrafast non-radiative decay of neutral amino acids: Phenylalanine and Tryptophan

Contact *sdecamillis01@qub.ac.uk, j.greenwood@qub.ac.uk*

S. De Camillis, J. Miles, G. Alexander, I.D. Williams, J.B. Greenwood

Centre for Plasma Physics

School of Mathematics and Physics
Queen's University Belfast

Introduction

The molecular building blocks of biological systems strongly absorb at wavelengths in the range 250-280 nm, due to the presence of DNA nucleobases and the aromatic amino acids tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe). When excited these chromophores can fluoresce allowing them to be exploited as markers in protein research. The quantum yield for Trp and Tyr in water is around 15% but varies dramatically depending on the environment [1], while for Phe the yield is much weaker and therefore less useful for applications [2]. Given these molecules are well known for their photo-stability, it is clear that there must be additional de-excitation mechanisms in these molecules much like that found in DNA nucleobases that can dissipate the absorbed energy through non-radiative relaxation processes [3,4].

Theoretical calculations and experimental results suggest that the low fluorescence quantum yields of indole, phenol and pyrrole, which are the chromophores of the amino acids Trp, Tyr and histidine, are due to a dissociative property of the potential energy curve of a highly excited state [5,6]. Indeed, the excited singlet $\pi\sigma^*$-state energy, with a repulsive profile with respect to the OH or NH bond stretching, intersects not only the $\pi\pi^*$ states but also the electronic ground state, triggering an ultrafast internal-conversion (IC) process. However, unlike the other two aromatic amino acids, Phe is not predicted to have the analogous repulsive excited state along the C–H bond. A possible alternative decay mechanism could be through C_α–C_β bond stretching which plays a predominant role in the photophysics of neutral Phe [7] and has been reported as the most prominent relaxation channel in the protonated form [8]. Very recently a theoretical result showed the presence of a conical intersection (CI) in the potential energy profile of neutral Phe along the same stretching profile [9]. Although ultrafast IC has been experimentally observed for highly excited toluene [10], to the best of our knowledge, no well resolved pump-probe results of the first excited state of the neutral amino acid Phe have been reported in the literature so far. In this report we present pump-probe results of non-radiative de-excitation mechanisms from the first excited states of the neutral amino acids Trp and Phe, and their chromophores, indole and toluene.

Experimental technique

In order to produce neutral gas-phase samples, the amino acids were evaporated from a thin metallic foil (10μm thick stainless steel) heated to ~150-200 °C. This thermal desorption process was produced by irradiating the back side of the foil with a CW laser (455 nm). The foil holder was integrated into the repeller of the time-of-flight mass spectrometer KEIRA [11] so that the distance between the desorption point and the laser interaction region was minimized. Figure 1 gives a schematic representation of the interaction region.

For studying the ultrafast relaxation processes of amino acids, we used a two-colour pump-probe scheme. The femtosecond laser pulses for exciting and probing the electronic dynamics were produced by a Coherent Libra Ti:Sapphire laser system which generates 800 nm, 1 mJ pulses with a repetition rate of 1 kHz. The beam was converted into the second and third harmonics, 400 nm and 267 nm, for use as probe and pump

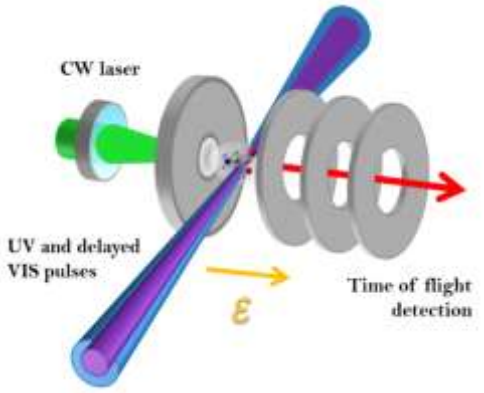

Figure 1: Schematic representation of the pump-probe experiment: a CW laser irradiates the reverse side of a stainless steel foil, producing a plume of neutral molecules in gas phase. A femtosecond 267 nm laser pulse and a time delayed 400nm pulse are focused in the interaction region, where the charged products are accelerated by a static electric field and collected in a time-of-flight mass spectrometer.

pulses respectively. The two pulses were separated by means of a beam splitter in order to introduce a temporal delay between the two pulses. This delay was computer controlled by a linear motor stage placed along the 267-nm optical path. Subsequently, the laser pulses were collinearly recombined and focused down into the interaction chamber where they intersected the molecular plume at a distance of about 4 mm from the metallic foil. The charged products of the laser interaction were accelerated by a static electric field, separated by mass along a drift region and detected by a channel electron multiplier.

The gas-phase biomolecules were photo-excited by the 267 nm pump pulse with a peak intensity of 1×10^{12} W cm^{-2}. The subsequent dynamics were probed by the 400-nm laser pulse with an intensity of 2×10^{12} W cm^{-2} at the interaction region. Mass spectra were acquired for delays between the pump and the probe pulses of up to 8 ps at increments of 20 fs. Xe gas was introduced into the interaction chamber to determine the zero delay from the cross-correlation signal, from which both pulses were determined to have a length of about 130 fs.

Results and discussion

The total ion yield as a function of the pump-probe delay for indole and Trp is displayed in Figures 2 (a) and (b). The contribution from fragment ions (mainly the side chain ion at m/z = 130) was added to the parent yield since the fragment signal showed the same dynamics as the parent ion. A best fit of the experimental data (black line) is the result of several contributions: (i) a Gaussian centred at zero delay (green line), which is due to signal enhancement from the temporal overlap of the two pulses, (ii) a long-lived exponential decay convolved with the cross-correlation of the laser (blue line), and (iii) a fast-decay (red line) for the ultrafast dynamics.

For indole, Figure 2(a), an ultrafast decay process with a lifetime of $\tau_{Ind} = 330 \pm {}^{70}_{40}$ fs and a long lived component ($\gg 100$ ps) can be observed. Indole has two singlet $\pi\pi^*$ electronic states, named L_b (S_1) and L_a (S_2), relatively close in energy, with a gap of 1800 cm^{-1} (0.22 eV) at the Franck-

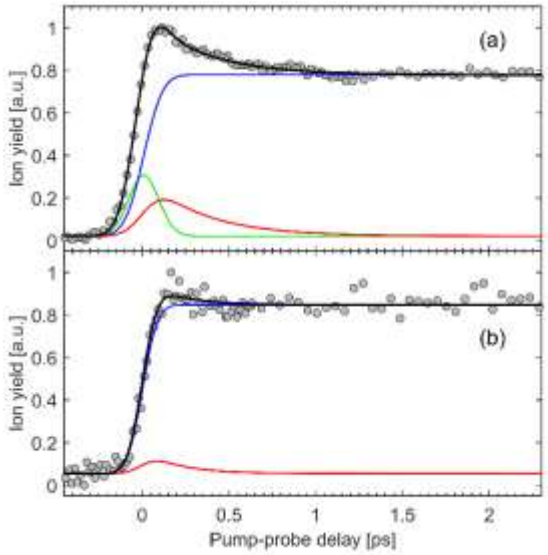

Figure 2: Total ion yield for Indole (a) and Trp (b) as function of the pump-probe delay. Curve fitting: total fit – black, zero delay Gaussian – green, relaxation dynamics – red, long lived component – blue. Fitting decay time: $\tau_{Ind} = 330 \pm {}^{70}_{40}$ fs. The fitted relaxation dynamics of Trp has a large uncertainty.

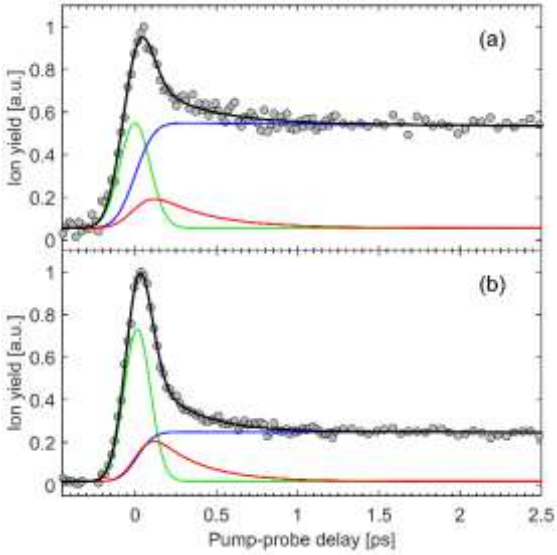

Figure 3: Total ion yield for Toluene (a) and Phe (b) as function of the pump-probe delay. Curve fitting: total fit – black, zero delay Gaussian – green, relaxation dynamics – red, long lived component – blue. Fitting decay times: $\tau_{Tol} = 330 \pm {}^{150}_{80}$ fs and $\tau_{Phe} = 270 \pm 40$ fs.

Condon region [6]. Vertical excitation at 267 nm strongly populates the L_a state, which was previously observed to decay to L_b in 50 fs [6]. The slow decay component (blue line) in our data can be attributed to this population of the L_b state which is long lived. However, the L_a state also couples to the dissociative $\pi\sigma^*$ state (S_3) along the N–H bond stretching coordinate which, in turn, has a CI with the ground state promoted by out-of-plane vibrations of the ring. Our measured lifetime τ_{Ind} therefore corresponds to re-population of the ground state and is in good agreement with the results of Livingstone et al. [12], who determined a threshold for this decay at 273 nm.

Figure 2(b) shows pump-probe results of the amino acid Trp. In this case, although the transient yield is also characterized by the nanosecond lifetime of the L_b state, the ultrafast de-excitation process appears to be much weaker than indole but with a similar lifetime. Although this relaxation dynamics along the $\pi\sigma^*$ state was observed more strongly by Ovejas et al. [13], they used a much longer probe wavelength of 1365 nm. Since further absorption of two probe photons in our experiment significantly exceeds the ionization potential of Trp (7.8 eV), this reduces the probe sensitivity for our results (see also Supplementary Information of [13]). Overall, our results for indole and Trp are in excellent agreement with previous studies and therefore provide a good benchmark for our measurements.

In contrast, to the best of our knowledge, no ultrafast pump-probe experiment has been published for Phe so far. Our measurements of the time evolution of the total ion yields for toluene and Phe are presented in Figures 3 (a) and (b). Both molecules show a weakly changing component indicating that the S_1 state is long lived ($\tau \gg 50$ ps). This is consistent with previous measurements for toluene by Malis et al. [14] and explains why toluene has a high fluorescence quantum yield. However, there is also a component with a lifetime of about 300 fs for both toluene and Phe. In their results, Farmanara et al. [10] attribute fast de-excitation due to population of higher vibrational levels of S_1 which decay to the ground state. Recent theoretical calculations of Phe by Tseng et al. [7] identify a relaxation pathway from S_1 via C_α–C_β bond stretching which brings the system to a CI with the ground state. As this potential energy curve has a shallow minimum near the equilibrium position, only higher vibrational states can escape from the potential well and hence access the CI to the ground state. The similarity of the decay lifetime in toluene and Phe suggests a related de-excitation mechanism, but with the significant difference that the contribution relative to the population remaining in the long lived S_1 state is about 5 times larger for Phe than toluene. This could arise if the energy of the S_1 state is slightly lower for Phe so that a greater proportion of the photo-excited levels are above the potential barrier. This may explain why the quantum yield of toluene is high but very weak in Phe.

Conclusions

Ultrafast pump-probe results of excited state dynamics in the amino acids Trp and Phe have been presented. Although both molecules show a large degree of UV photo-stability, different relaxation mechanisms are involved for each molecule. In particular, the dissociative $\pi\sigma^*$ excited state, which opens up a de-excitation pathway through a conical intersection with the ground state in Trp, has no equivalent in the case of Phe. In Phe and its chromophore toluene, fast de-excitation (300 fs) is possible through a conical intersection of the S_1 state with the ground state. In toluene, excitation with a 267 nm photon appears to be insufficient for most of the population to overcome a small potential barrier in the S_1 state, as opposed to Phe for which a greater proportion of the population undergoes ultrafast decay. These results demonstrate the subtle interplay between excited states makes de-excitation of biological chromophores sensitive to small structural and environmental changes. This can be seen as an increase in the relative importance of fast internal conversion for the excited S_1 state of Phe compared to toluene which results in a much lower fluorescence quantum yield.

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