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Ultraviolet transient absorption, transient grating and photon echo studies of aqueous tryptophan

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ABSTRACT

We compare UV transient grating (TG) experiments of aqueous tryptophan with transient absorption (TA) and fluorescence up-conversion measurements. The TG and TA signals show a bi-exponential rise with sub-ps and ps time constants, which are consistent with the fluorescence studies. Using experimental data, we provide an equation for the homodyne-detected TG signal, taking into account the sub-100 fs internal conversion of tryptophan after excitation. In addition, we measure a sub-100 fs homogeneous electronic dephasing time for tryptophan in water by the photon echo (PE) technique. These measurements provide a consistent picture of excited state dynamics of aqueous tryptophan that may serve as a basis for coherent 2D-UV spectroscopy of biosystems.

1. Introduction

Interrogating protein dynamics on the femtosecond to picosecond time scales has been extensively carried out with ultrafast optical spectroscopic techniques. Most studies [1–7] have consisted in impulsively exciting the bio-active centre of the protein (or artificially modified structures [8]), and monitoring its evolution in time using a time-delayed probe pulse. From its response, conclusions were drawn about the role of the protein environment. However, these studies do not probe the structure of the chromophore nor of its environment. For this X-ray studies are more useful as was demonstrated for the case of myoglobin by diffraction [9] or by X-ray absorption spectroscopy [10]. However, at present these techniques are limited in time resolution to the 50–100 ps pulse width of the synchrotron X-ray sources.

In recent years, multidimensional spectroscopy has emerged as a powerful tool that allows probing the dynamics of the native protein peptide chains by monitoring the IR absorption bands of specific chromophores (e.g. amide bands) that are part of it [11–15]. Pushing multidimensional spectroscopy into the visible range was pioneered by Fleming and co-workers [16–18] allowing to detect electronic couplings (e.g. excitonic effects) between chromophores within biological systems. In addition to probing electron or energy transfer among these chromophores, the analysis of the couplings can deliver information about the structure of the system as was recently demonstrated [19].

Probing the response of specific amino-acid residues upon excitation of the bio-active centre is another way to detect the protein response during a biological function. This necessitates probe pulses below 300 nm, which is the region where amino-acid residues absorb. Chergui and co-workers [20–22] used visible pump/UV probe transient absorption to monitor the response of tryptophan (Trp, which is the most abundant amino-acid residue in biological systems, has the highest absorption coefficient and is highly sensitive to the environment [23]) around photoexcited retinal in bacteriorhodopsin, while Mizutani et al. [24–26] used 220 nm probe pulses to monitor by resonance Raman spectroscopy the response of Trp in myoglobin after ligand photodissociation. Over the past few years, as complements to the above mentioned UV transient absorption tools, we have implemented ultrafast UV fluorescence up-conversion [27], which was used to study the relaxation dynamics of aqueous Trp [28], or tryptophan embedded in proteins, e.g. Cytochrome c [29,30].

Photon echo (PE) and transient grating (TG) experiments [31–36] hold the potential for increased sensitivity to environment effects, e.g., solvation dynamics, where the amplitude of the solvent-induced changes in absorption and/or the Stokes shift can be small. These techniques are also sensitive to both the ground and the excited state dynamics. While being widely used in the

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visible and IR spectral regions [31–35,37–42], PE and TG techniques have rarely been attempted in the UV due to, in particular, the significant non-resonant contributions from the solvent. Following the two-pulse PE experiments by Zimdars et al. [43], we implemented the three-pulse UV PE [44–46]. The implementation of these techniques is a necessary ingredient for the development of 2D coherent UV spectroscopies, which have been recently demonstrated on adenine [47]. A more detailed discussion about the ongoing developments in this area are found in Refs. [48] and [49]. 2D UV transient absorption spectroscopy has recently been implemented with very broad continua (>60 nm) [50] and used to investigate electron and energy transfer involving Trp in myoglobins [51]. Because of the importance of Trp in biological systems and in view of 2D coherent UV spectroscopy on them, the present paper compares the UV transient grating and photon echo experiments of aqueous tryptophan, with transient absorption and fluorescence up-conversion studies.

2. Experimental

The setup developed for the UV pump/broadband UV probe measurements was previously described [52]. Briefly, the output of a non-collinear optical parametric amplifier (NOPA) was doubled in a BBO crystal using the achromatic doubling scheme to obtain the broadband UV probe. The bandwidth of the generated UV light ranges between 20 nm and 60 nm, depending on the spectral window. Three measurements with different spectral windows were performed to cover the spectral range of interest (260–380 nm). The pump beam was generated by frequency doubling the output of a second NOPA in a 0.25 mm thick BBO crystal. The generated pump pulses have energies of around 100 nJ. The excitation wavelength was set at 287 nm and the measurements were performed under parallel polarisation. The temporal resolution of the set-up (cross-correlation of the pump and probe pulses) is about 160 fs.

The experimental set-ups for the Transient Grating (TG) and three-pulse photon echo peak shift (3PEPPS) measurements in the UV were presented in Refs. [44–46]. Briefly, after frequency doubling, we generate a central wavelength of 285–290 nm with a bandwidth of about 4 nm, at an average power of 1.6–2 mW at 30 kHz repetition rate (pulse energy of 50–66 nJ). A prism pair compression stage was used to compress the UV pulses before sending them to the photon echo set-up. UV pulses of 50–60 fs FWHM (Full Wave at Half Maximum) were used on the sample, yielding a temporal resolution of about 85 fs. In the photon echo set-up we divide the input beam into three beams of equal intensity, using thin beam splitters, with a time delay between them and focus them into a spot of about 100 μm. The photon echo signal is generated in the phase-matched direction ($k_s = -k_1 + k_2 + k_3$) where it is detected after blocking the incident beams. Detection of the generated signal is done in the two phase-matching directions simultaneously by means of two avalanche photodiodes.

Once the PE signal is detected, we do serial scans to get the PEPS (photon echo peak shift) results by scanning t_{12} for a sequence of t_{23} times. PEPS is defined as the value of t_{12} in which the maximum of the PE signal (for a given value of t_{23}) is detected. By detecting simultaneously the signals in the two directions $-k_1 + k_2 + k_3$ and $k_1 - k_2 + k_3$, we determined the photon echo peak shift as half the delay between the maxima (t_{12}^{max}) of the two curves. We can also perform the transient grating (TG) experiment which is the signal from a scan of t_{23} while $t_{12} = 0$.

The peak powers of the UV pulses on the sample for both photon echo and pump–probe set-ups are in the order of 10^8 W/cm^2, which is at least one order of magnitude weaker than the photoionization limit of Trp solution.

The solution of tryptophan was circulated with a mechanical pump to avoid sample degradation. The sample was a flow jet (for TG and PE experiments) and a flow cell (for TA experiments) with a thickness of 100 μm (or 200 μm). The concentration of Trp was chosen so as to have a sample absorbance of around 0.3 OD. For a sample thickness of 100 μm, this corresponds to a tryptophan concentration of around 8 mM in water.

3. Results

3.1. Transient absorption experiments

Fig. 1 shows a selection of transient spectra at different time delays. The transient absorption (TA) signal is positive over the entire observed spectral range, indicating the presence of a dominant contribution from excited state absorption (ESA). We observe a minimum of the signal between 265 and 290 nm. As shown in Fig. 1, this feature reflects the shape of the static absorption of the sample and is due to ground state bleaching (GSB). Similar measurements were already reported by Haacke and co-workers [53], with identical dynamics, who probed a wider spectral window ranging from 300 to 700 nm.

The time evolution of the signal at different wavelengths is shown in Fig. 2. It is described by a linear combination of exponential rise and decay components convoluted with the instrumental response function, and analysed with a global fit algorithm. The overall time evolution can be described in terms of four exponential contributions of characteristic times $\tau_1 = 250 \pm 20$ fs, $\tau_2 = 1.28 \pm 0.05$ ps, $\tau_3 = 31 \pm 6$ ps and $\tau_4 >> 100$ ps. In particular, we observe a bimodal rise of the signal with τ_1 and τ_2 for wavelengths between 280 nm and 350 nm and a decay with the same time constants at $\lambda < 280$ nm and $\lambda > 350$ nm. A Singular value decomposition (SVD) analysis confirms a very similar spectral evolution. In their study, Haacke and co-workers reported similar time scales for the τ_2–τ_3 components [53].

3.2. Transient grating and photon echo experiments

Since the TA signal is dominated by ESA, the higher states will most likely contribute to the Transient Grating (TG) signal. The TG experiments are performed with three identical pulses at 287 nm with a bandwidth of about 4 nm.

Fig. 3a and b shows, respectively, the long and short time evolution of the TG signal of tryptophan in water, normalised in

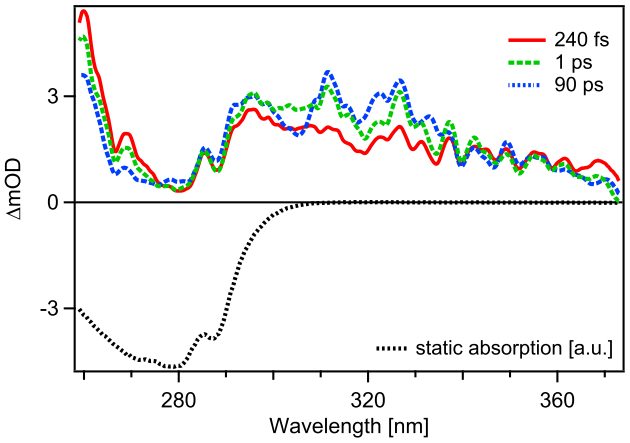

Fig. 1. Transient absorption of aqueous tryptophan excited at 287 nm at different delays after the excitation. The static absorption of the sample (dotted line, reversed sign) shows the expected shape of ground state bleaching in absence of excited state absorption.