The Effect of Overlapping Electronic Excited States on the Subpicosecond
Fluorescence Anisotropy Decay Behavior of Tryptophan in Water.


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Introduction. The fluorescence properties of tryptophan and its derivatives
in solution have been the subject of intense study for several years [1].
This research effort has been motivated primarily by its importance as an
intrinsic fluorescent probe of protein structure and conformational dynamics
[2,3]. The use of frequency conversion optical gating in this work results in
an increase in time resolution of two orders of magnitude over previous mea-
surements of the time resolved fluorescence anisotropy of tryptophan. An
important parameter in the evaluation of time resolved fluorescence depolar-
ization data is the experimental value of the anisotropy at zero time, r(0).
It is the intrinsic polarization anisotropy of the chromophore and is a func-
tion only of the orientation of the absorption and emission transition dipole
moments. The theoretical value for a chromophore with parallel absorption
and emission transition dipoles is 0.4. Observations of experimental values
of r(0) less than the theoretically predicted value generally indicate the
existence of relaxation processes occurring on a time scale shorter than the
time resolution of the experiment. These processes can involve actual reor-
ientational or librational motion of the molecule or purely electronic (vi-
bronic) relaxation mechanisms [4,5,6]. Prior to the measurements presented
here, the theoretical limiting value (r(0)=0.4) for tryptophan had never been
observed. The spectroscopy of tryptophan is complicated by the existence of
two overlapping electronic transitions with roughly perpendicular transition
moments, designated the $^1L_a$ and $^1L_b$ bands [7,8,9]. Theoretical modeling of
fluorescence anisotropy decay of tryptophan including the effects of $^1L_a$, $^1L_b$
level kinetics has shown that the form of the decay and the value of the
initial anisotropy can depend strongly on the ratio of excited states pre-
pared and the observation wavelength of the fluorescence [10].

Experimental. The ultraviolet fluorescence upconversion spectrometer
developed for this work is shown in Fig. 1. The second harmonic of a mode-
locked Nd:YAG laser is used to synchronously pump a cavity dumped dual jet
hybrid R6G dye laser with chirp compensation. The 1 nm bandwidth, output
pulses are cavity dumped and amplified at a 12 KHz repetition rate in a
copper vapor laser based 6 pass amplifier. The second harmonic of the ampli-
fied pulse is used to excite the sample. The fluorescence is collected and
upconverted by type I mixing with the fundamental in a beta-barium borate
crystal. The upconverted signal is detected by gated photon counting with a
solar blind PMT. A 500 fs response function for the system generated by the
cross-correlation of a 578 nm dye laser fundamental with its second harmonic
and detected at 192 nm is shown in the inset on the right hand side of Fig. 1.
The system response function varied between 500 fs and 1 ps depending on the
experimental excitation wavelength. Since the upconversion signal is propor-
tional to the cube of the laser power, small changes in power can have a
Fig. 1. Schematic for ultraviolet fluorescence upconversion spectrometer. Inset: 500 fs cross correlation of the 578 nm dye laser fundamental with its second harmonic. Sum frequency detection at 192 nm.

dramatic effect on the signal level. To insure the integrity of the data, the count period is determined by the product of the intensities of the fluorescence and the fundamental and thereby compensates for laser power drifts over the course of data acquisition.

Results and Discussion. The raw parallel and perpendicular polarization decay components for tryptophan in water excited at 300 nm are shown in Fig. 2. Fluorescence at 330 nm (+/- 5 nm) on the short wavelength edge of the tryptophan emission was mixed with the 600 nm fundamental to produce the upconverted signal near 213 nm. A short decay component with a 2.6 picosecond lifetime is found in both the parallel polarization component of the emission and the total intensity decay of the fluorescence. The detection wavelength and time scale of the decay suggest that it is due to unrelaxed emission from the $^1I_b$ state. The raw anisotropy under these experimental conditions has an initial value of 0.4. The anisotropy decay consists of a 2.6 psec decay component and a slower decay component corresponding to the overall rotational diffusion of the molecule. Data simulated using the Cross-Fleming model for fluorescence depolarization including the effects of level kinetics are shown in the inset. The parameters used in these preliminary simulations were chosen to approximate the experimental conditions i.e. long wavelength mostly $^1L_a$ excitation and detection of short wavelength mostly $^1L_b$ emission. The rate constants used in the simulations were crude order of magnitude estimates based on literature values. The general form of the simulated decays are in good agreement with the raw experimental data. It is interesting to note that the unusual shape of the horizontal emission component is reproduced by this simple model. For purely orientational motion this polarization component would exhibit a simple risetime correlated with the decay of the parallel polarization component. Data collected at higher excitation energies (289 nm, 290 nm, 292 nm, 294 nm and 297 nm) under the same emission detection conditions, all have a short 1 to 4 psec lifetime component in the total fluorescence decay. The raw anisotropies constructed at these wavelengths, however, exhibit different initial anisotropies and short time decay behavior. The initial anisotropies vary from an $r(0)=0.3$ at 297 nm excitation to an $r(0)=0.18$ at 289 nm. Evaluation of these data within the level kinetics model as
Fig. 2. Parallel (top curve) and perpendicular polarization components of the tryptophan emission. [300 nm excitation, 330 nm +/- 5 nm fluorescence, 600 nm gate pulse, upconverted signal detected at 213 nm.] Inset: Simulated data. See text.

well as acquisition of fluorescence anisotropy data on the long wavelength edge of the emission band is currently underway. Detailed simulations of the anisotropy along with refinement of the kinetic model to include the effects of solvent relaxation, will certainly be necessary to understand the complex polarization anisotropy of tryptophan at short times. In conclusion, the initial anisotropy and short time decay behavior of tryptophan are found to be complicated by non-rotational contributions to the anisotropy decay arising from the interaction of the two low lying $1_{B}$ and $1_{D}$ excited states on the one to two picosecond time scale. These initial results indicate that the use of time resolved fluorescence depolarization measurements to obtain information on short time librational motions of tryptophan sidechains in proteins may be severely complicated by contributions to the anisotropy from these electronic (vibronic) relaxation processes.

References.