PICOSECOND FLUORESCENCE RELAXATION KINETICS FROM ALL-TRANS RETINAL

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The fluorescence relaxation kinetics from all-trans retinal in polar and non-polar solvents have been investigated as a function of temperature. An activation energy of ≈1 kcal/mole has been measured. Our results, in conjunction with previous picosecond absorption measurements made by Hochstrasser et al., strongly suggest that initially excited molecules to Franck–Condon states relax to three singlet excited states.

1. Introduction

The photophysics of retinal is quite complex. The excited state level ordering is complex and may depend on temperature, molecular conformation, and solvent interactions. There is little available information on the early relaxation pathways that retinal undergoes after excitation by light. Hochstrasser et al. [1] have studied retinal's excited state formation and relaxation kinetics at room temperature by picosecond absorption spectroscopy. The fluorescence kinetics of all-trans retinal have been examined by Fugate and Song at 77 K [2]. In this report, we extend this research by obtaining the temperature dependence of the fluorescence relaxation kinetics from retinal, to 77 K in polar and non-polar solvents as well as retinal powder.

2. Methods and materials

The apparatus used in the fluorescence kinetic measurements has been previously described [3]. A single pulse from the output of a mode-locked Nd:glass laser was selected and amplified. The third harmonic at 353 nm was produced by mixing the second harmonic (527 nm) and the fundamental (1054 nm) in a KDP crystal. The 353 nm pulse was used to excite the sample. The exciting beam was collimated to a spot size 5×10^{-2} cm^2 at the sample position. The average energy of a pulse was 30 μJ (3×10^{15} photons/cm^2). The sample was frontally excited and the fluorescence collected and focused onto the 50 μm entrance slit of a Hamamatsu streak camera. Resolution of the system, laser–streak camera, was ≈12 ps. A cut-off filter (3-72 Corning) was placed in the path of the fluorescence to eliminate any scattered light from the exciting pulse. The integrated fluorescence was measured over 450–720 nm. In addition, a combination of cut-off filters was used to determine the fluorescence profile (R62, R64, R66, R68, R72, R74, Hoya Optics and SPF620, SPF660, SPF680, Ditric Optics). The fluorescence profile covers a broad range of the spectrum up to 720 nm, though the maximum intensity is centered in the region of 450–650 nm. The sample was placed in a 2 mm cell inside a glass dewar cooled by liquid nitrogen. The temperature was monitored by a copper–constantan thermocouple and it is accurate to ±1 K. The position of the sample was moved after every two to three shots to avoid accumulation of other retinal isomers.

The sample all-trans retinal was purchased from Sigma and used without any further purification. High-pressure liquid chromatography (Waters Associates) assay of the sample showed that better than 95% of the retinal was the all-trans isomer, ≈4% 13-cis isomer and less than 1% 9-cis and 11-cis isomers. Assays of retinal samples were determined under the following conditions: one column, $\frac{1}{8}'' \times 1'$, μ-Porasil, solvent 10% ether in *n*-hexane by volume, flow rate 1 ml/

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min, detector 370 nm. Samples of all-trans retinal in absolute ethanol (USI) at concentrations of 10^{-3} and 10^{-4} M, in carbon tetrachloride (Fisher Scientific) 10^{-4} M and powder were prepared under dry nitrogen and red dim lights. All solvents were drawn from freshly opened bottles and bubbled with dry nitrogen for one hour before the experiment to ensure dryness of the samples. Even under those conditions, however, the presence of traces of water cannot be completely eliminated. The fluorescence of all three samples was measured as a function of temperature in the range of 298–77 K. The fluorescence from all three samples at room temperature was found to be resolution limited. To ensure that fluorescence is produced by all-trans retinal and not of any impurities we prepared retinal oxime. Hydroxylamine was dissolved in absolute ethanol to form saturated solution. A drop of this solution was mixed with all-trans retinal in absolute ethanol. The formation of retinal oxime was followed in an absorption spectrophotometer. The fluorescence lifetime of all-trans retinal oxime at room temperature was measured to be ≈300 ps.

3. Results

Typical fluorescence kinetic data of all-trans retinal powder at four different temperatures are shown in fig. 1. The fluorescence risetime is unresolved at all temperatures (less than 12 ps). The fluorescence decay is well fitted by a single exponential lifetime in all cases. The solid curves are the fits. The decay lifetime is temperature dependent, ranging from a barely resolved ≈17 ps at room temperature to 190 ps at 93 K. The fluorescence properties of all-trans retinal dissolved in absolute ethanol and carbon tetrachloride are similar.

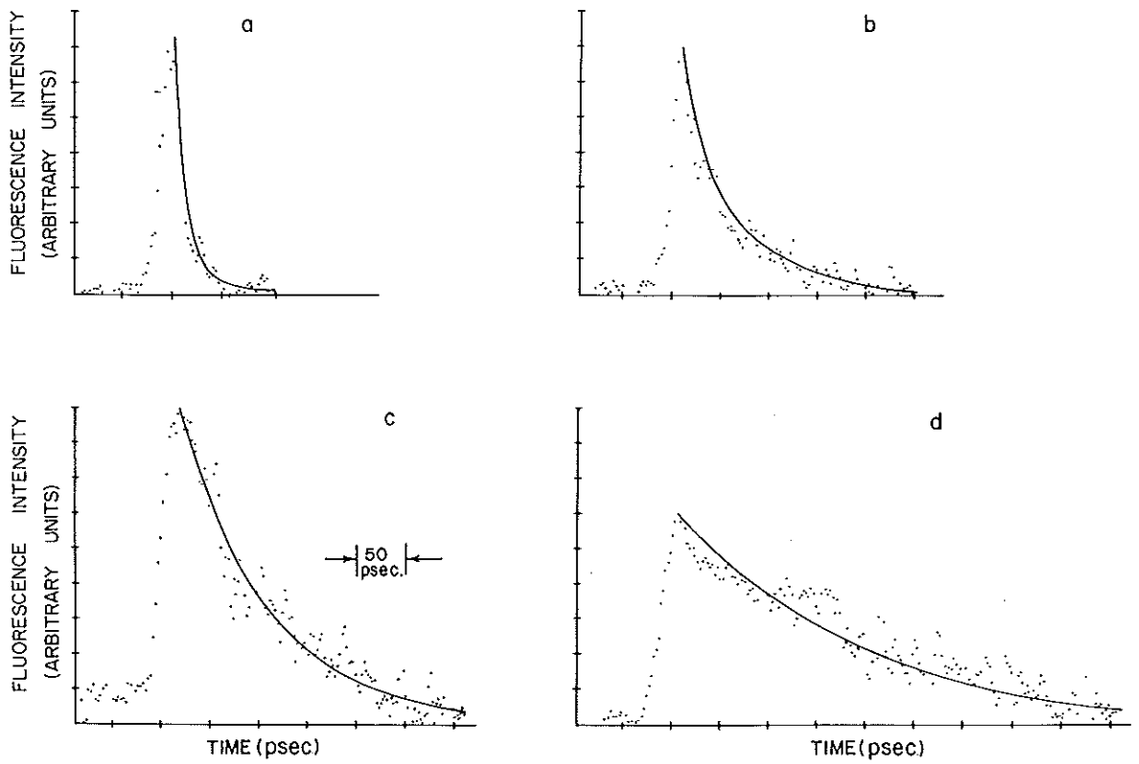

Fig. 1. Fluorescence decay profiles from all-trans retinal powder excited by a single 353 nm pulse at four temperatures: (a) 296 K, τ = 17 ps, (b) 216 K, τ = 40 ps, (c) 156 K, τ = 90 ps, (d) 93 K, τ = 190 ps. The solid lines are the single exponential fits.

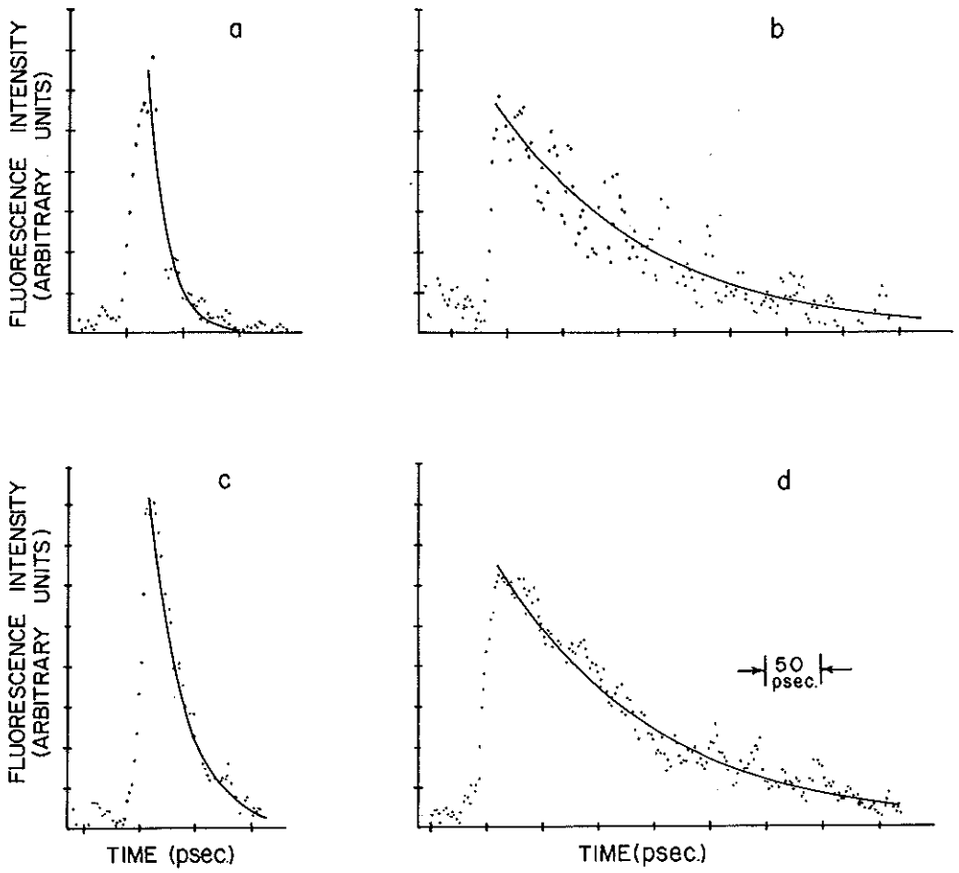

Fig. 2. Fluorescence decay profiles from all-trans retinal excited by a single 353 nm pulse in absolute ethanol (a) and (b); and in carbon tetrachloride (c) and (d) of two temperatures. (a) 295 K, τ = 17 ps, (b) 80 K, τ = 400 ps, (c) 231 K, τ = 30 ps, (d) 85 K, τ = 425 ps.

Representative results for the ethanol and carbon tetrachloride solutions at high and low temperatures are shown in figs. 2a–2d. It appears that the lifetime from dissolved retinal at low temperatures is larger by a factor of 2–3 than the lifetime of the retinal powder.

The fluorescence lifetime as a function of inverse temperature from the powder sample and from retinal in ethanol and in carbon tetrachloride is shown in figs. 3a, 3b, and 3c, respectively. The fluorescence decay shows quite good Arrhenius behavior for all three samples down to ≈140 K for the samples of retinal powder and retinal in carbon tetrachloride and ≈110 K for the samples of retinal in ethanol. At this temperature the decay becomes temperature independent, within our signal to noise. The activation energies obtained from these curves are 1.2 kcal/mol for the retinal powder, 0.98 kcal/mol for the ethanol solution, and 0.93 kcal/mol for the carbon tetrachloride solution.

Fugate and Song [2] previously measured the fluorescence lifetime of all-trans retinal in ethanol at 77 K using phase-modulation fluorimetry methods. Their result is in good agreement with our result. We have found that the fluorescence quantum yield is some 5–10 times smaller for the carbon tetrachloride samples compared to the ethanol samples. Steady state fluorescence measurements comparing the quantum yield of alkane solution of retinal to hydrogen bonding solvents like ethanol give a factor of ≈50 [4,5]. It

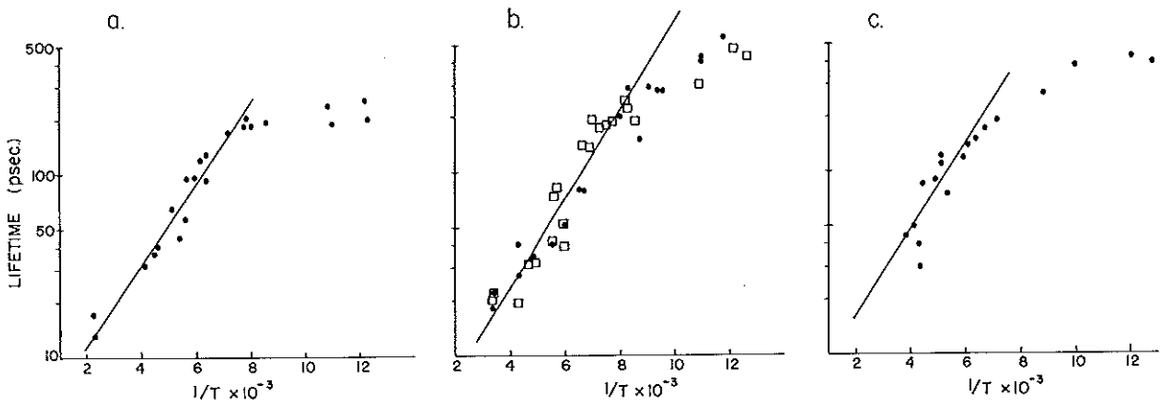

Fig. 3. Fluorescence lifetime as a function of inverse of temperature. (a) All-trans retinal powder, (b) all-trans retinal in absolute ethanol at two concentrations: □, 10^{-4} M, ●, 10^{-3} M, and (c) all-trans retinal in carbon tetrachloride.

should be noted, however, that the relative quantum yields are preliminary and have not been corrected for the effects of light scattering of the samples at low temperatures. Finally, the results were not dependent on retinal concentration over the 10^{-3}–10^{-4} M range showing that dimer formation plays no role in our fluorescence measurements [6].

4. Discussion

Hochstrasser and co-workers have reported on aspects of the picosecond kinetic absorption properties of all-trans retinal [1]. They observed two transients from all-trans retinal in *n*-hexane at room temperature. One corresponded to triplet production with a formation time of 34 ± 5 ps. The second transient formed instantaneously and decayed in ≈20 ps. Preliminary results on EPA rigid glasses containing retinal at 77 K showed essentially the same results. This study suggested that excited molecules relax from this initially excited Franck–Condon state into at least two different excited singlet states, one being the precursor of triplet formation and the second corresponding to the observed 20 ps transient. Hochstrasser et al. also speculated that partitioning of initial excitation to a third excited singlet state species [1] took place.

Our results strongly suggest that a third state is indeed necessary in understanding the photophysics of retinal. While the room temperature fluorescence lifetimes reported here match the observed 20 ps absorption transient at room temperature within experimental error, the temperature dependent fluorescence lifetime is in marked contrast to the reported basically temperature independence of the 20 ps absorption transient and the triplet precursor. Moreover, it is clear that the partitioning into these three states upon excitation occurs within the resolution of our experiments at all temperatures since the fluorescent state we observe is formed instantaneously. Also the single exponential behavior of the fluorescence decay, at all temperatures, suggests that the three relaxed excited state species are relatively independent from each other once formed.

There are several candidates for these states. The $^1B^*_{u+}(\pi\pi^*)$, $^1A^*_{g-}(\pi\pi^*)$, and $^1n\pi^*$ states (using linear polyene C_{2h} point group labels) are believed to be very close in energy in retinal [7]. In addition, it is possible that state switching occurs between states as a result of interaction of the retinal with solvent. There has been speculation that the potential energy minima of the three types of states could involve different nuclear configurations, as for example, different single bond conformers. There are clearly not enough data to distinguish amongst these possibilities.

The rather low, ≈1 kcal/mol, activation energies of the fluorescence decay suggest the mediation of a torsional coordinate in the deactivation. In this regard and in general kinetic behavior, our results are quite similar to the fluorescence behavior of Schiff bases of retinal as reported recently by Everaert and Rentzepis [8].

Acknowledgement

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