PHOTOPHYSICS OF THE ACID AND BASE FORMS OF RHODAMINE B

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Fluorescence lifetime measurements of rhodamine B in ethanol solution have confirmed the presence of two distinct species dependent on acidity. Rotational diffusion times of these species are identical and we propose that they are the acid and base forms of the dye. Species having identical spectra to these forms have also been obtained in concentration dependent studies and we believe that here, too, the equilibrium is acid-base rather than monomer-dimer as had been suggested.

1. Introduction

Rhodamine dyes have been extensively investigated since the advent of the dye laser. Of these, rhodamine B and rhodamine 6G are now arguably the most widely used laser dyes and yet an anomaly seems to exist in their reported properties.

Although these two dyes have almost identical structures it has been reported that in ethanol solution rhodamine B forms dimers at quite low concentrations \(\approx 1 \times 10^{-4} \text{ M}\) while rhodamine 6G does not aggregate except in concentrated solutions \(\approx 1 \times 10^{-2} \text{ M}\) \[1\]. As dimers of these molecules are believed to form by \(\pi-\pi\) mixing of the xanthene ring orbitals, the slight change in substitution between these two dyes would not be expected to give rise to such a discrepancy in their properties.

Absorption and fluorescence spectra of rhodamine B in ethanol are shown in fig. 1. Selwyn and Steinfeld \[1\] attribute absorptions (a) and (b) to the dimer and monomer of the dye respectively, (a) being observed at concentrations of around \(1 \times 10^{-4} \text{ M}\) and (b) at concentrations of around \(1 \times 10^{-6} \text{ M}\) at 22°C. Ferguson and Mau \[2,3\] on the other hand propose an acid-base equilibrium as the cause of these spectral shifts attributing absorption and fluorescence spectra (a) to the acid form of the dye (I) and (b) to the base form (II).
We have applied the technique of kinetic fluorescence depolarisation to this system in order to determine rotational diffusion time constants for the species involved in the equilibrium process. Excited state lifetimes of the two species have also been obtained.

2. Experimental

Rhodamine B, laser grade, was obtained from Lambda Physik and used without further purification. 2 × 10⁻⁵ M solutions of the dye in ethanol were used in a 1 mm sample cell to preclude the possibility of stimulated emission of fluorescence. One drop of 1 M HCl or NaOH was added to 1 ml of sample solution to provide the spectra in fig. 1.

A complete description of the laser system has been given elsewhere [4]. A 530 nm pulse of 6 ps (fwhm) duration from a mode-locked neodymium/glass laser is used to excite the sample. Fluorescence emitted parallel and perpendicular to the plane of excitation is then time resolved by a streak camera.

An MPF4 spectrofluorimeter (Perkin-Elmer) was used to record steady state polarisation ratios, the inherent polarisation of the fluorimeter being corrected for. The single photon counting apparatus used to measure fluorescence lifetimes was that described by Beddard et al. [5] except that the emission photomultiplier was a Phillips 56 TVP/03, cooled by dry nitrogen to 4°C.

The theory of kinetic fluorescence depolarisation has been described fully elsewhere [6]. Measurements are expressed in terms of the time dependent polarisation anisotropy  

\[ r(t) = \frac{[I_1(t) - I_{||}(t)]}{[I_{\perp}(t) + 2I_1(t)]} \]

and  and  are the values of the fluorescence emission monitored parallel and perpendicular to the plane of excitation at the time . Due to variations in laser intensity from shot to shot the  and  decay curves are not directly comparable and are normalised by tail matching [7].

3. Results and discussion

The distinct absorption and emission maxima and different excited state lifetimes given in table 1 indicate the presence of two distinct species. However, the rotational diffusion times of the two species are the same within our experimental error. This would be very unlikely if significant amounts of dimer were present in the acidified solution, since by volume considerations alone the dimer should rotate 50% more slowly than the monomer, assuming 7 × 7 × 5 Å semi-

<table>
<thead>
<tr>
<th></th>
<th>Acid form (RhB COOH)</th>
<th>Base form (RhB COO⁻)</th>
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<tbody>
<tr>
<td>⁴λ_max absorption (nm)</td>
<td>553</td>
<td>542</td>
</tr>
<tr>
<td>⁴λ_max emission (nm)</td>
<td>576</td>
<td>565</td>
</tr>
<tr>
<td>⁴τF single photon counting (ns) ± 2%</td>
<td>2.48</td>
<td>3.01</td>
</tr>
<tr>
<td>⁴τF streak camera (ns) ± 5%</td>
<td>2.5</td>
<td>3.1</td>
</tr>
<tr>
<td>⁴ΦF [3]</td>
<td>0.49</td>
<td>0.71</td>
</tr>
<tr>
<td>⁴k_non-radiative (s⁻¹)</td>
<td>2.06 × 10⁸</td>
<td>0.97 × 10⁸</td>
</tr>
<tr>
<td>⁴k_radiative (s⁻¹)</td>
<td>1.97 × 10⁸</td>
<td>2.35 × 10⁸</td>
</tr>
<tr>
<td>⁴τ_or steady state (ps) ± 10%</td>
<td>217</td>
<td>208</td>
</tr>
<tr>
<td>⁴τ_or kinetic (ps) ± 10%</td>
<td>228</td>
<td>213</td>
</tr>
</tbody>
</table>
radii for the dimer versus $7 \times 7 \times 2$ Å for the monomer [6]. Thus our results strongly support the conclusion of Ferguson and Mau [3] that the spectral shifts observed with both concentration and acidity changes in ethanolic solutions of rhodamine B are due to acid–base reactions of the carboxylic acid group of the dye. The results of Selwyn and Steinfeld [1] can be explained by the concentration dependence of the degree of dissociation provided that the pK lies in the range 4.5 to 6.5. For example, if the pK were 5, the degree of dissociation to give form II would be almost 1 at $10^{-6}$ M but only 0.3 at $10^{-4}$ M. In contrast, the carboxylic acid function in rhodamine 6G is esterified and so such an equilibrium is not possible.

Applying the Förster cycle [8] to the spectral data in table 1 we obtain from

\[ \Delta \rho (S_0) = \rho (S_1) + 2.1 \times 10^{-3} \Delta \nu, \]

that \( (\Delta \nu = -3.52 \times 10^2 \text{ cm}^{-1}) \)

\[ \Delta \rho (S_1 - S_0) = 0.74. \]

Thus the $S_1$ state is a slightly weaker acid than the ground state. However, the change in pK is very small and the equilibrium will be essentially unchanged upon excitation. The related xanthene dyes, fluorescein and dichlorofluorescein, show similar small increases in pK in their $S_1$ states [9]. The small effect of electronic excitation upon pK argues for little interaction between the carboxylic acid group and the xanthene ring chromophore, supporting a similar conclusion based on spectral evidence [10]. This would appear reasonable since molecular models show that the phenyl ring is sterically constrained to be roughly perpendicular to the xanthene ring.

In table 1 we also give radiative and non-radiative decay rates for the $S_1$ states of the two forms of rhodamine B. The literature values of the fluorescence yield of rhodamine B [11] vary widely, perhaps due to the presence of different amounts of the acid and base forms of the dye in different experiments. We use the values of Ferguson and Mau [3] and have confirmed that the ratio of the yields for the acid and base forms of the dye is very similar to that obtained with their values.

The radiative rate is the same for both forms of the dye within experimental error but the non-radiative rate increases by a factor of two between the base and the acid form. The insensitivity of the radiative rate to the state of protonation of the carboxylic acid function again implies little interaction between this and the xanthene chromophore. Considering the result of Chibisov et al. [12,13] that the triplet yield of rhodamine B in ethanol is very low (0.006) and that the main unimolecular decay process of the excited state is internal conversion, we suggest that the change in non-radiative rate is due primarily to a change in the relative $S_1 - S_0$ energy gap in the two forms of the molecule. A scheme consistent with the spectral data is shown schematically, below, where the ground state of the basic form of the dye has greater solvent stabilisation than the $S_1$ state relative to the acid form.

![Scheme](image)

This would seem reasonable as the identical rotation times for the $S_1$ states of both forms of the dye imply a similar degree of solvation of these states and hence any difference in stabilisation energy might be expected to be small for the two cases. Even though the increase in the $S_1 - S_0$ energy gap in the basic form of the dye is small, relative to the acidic form (≈ 2%), non-radiative rates generally decrease exponentially with increasing energy gap [14] and so this may be sufficient to reduce internal conversion by the observed factor in the basic form of the dye.

The experimental rotational diffusion time for rhodamine B is consistent with the hydrodynamic value for an oblate rotor of $7 \times 7 \times 2$ Å semi-radii with a stick boundary condition ($\tau_{\text{form}} = 220$ ps) [6]. A similar agreement has been found for rhodamine 6G in a series of alcohols [7,15,16]. However, the fact that the doubly charged base form of the dye does not rotate more slowly than the singly charged acid form is rather surprising since the doubly charged fluorescein anions rotate roughly twice as slowly in the same solvent as rhodamine B although their molecular shape and size is essentially identical to that of
rhodamine [6]. More work with different molecules is required to clarify this point.

4. Conclusion

We have shown that the rotational diffusion times of the species involved in the equilibrium undergone by rhodamine B under certain conditions in ethanol are the same within experimental error. We therefore conclude that the equilibrium is not monomer–dimer in nature as has been previously reported.

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References