Excited-state Reactions of 4-methylumbelliferone Studied by Nanosecond Fluorometry

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Excited-state protolytic reactions of 4-methylumbelliferone were studied using pulse and phase fluorometry.

A concept of internal convolution which simplifies the data analysis was applied. The fluorescence decay of the excited neutral species was found to be monoexponential, while the decay of the long-wavelength emitting species (most probably a zwitterion) is biexponential.

An excited-state reactions model is proposed, in which the rapidly dissociating cation is an intermediate between the neutral and zwitterionic species.

1. Introduction

4-methylumbelliferone (4-MU) is a laser dye with an exceptionally broad tuning range (390–570 nm) due to the excited-state reactions occurring on the nanosecond time scale and yielding several emitting species. The kinetics of these reactions was studied by many authors [1–15]. It is generally agreed that three forms of the dye can exist in the ground state: neutral N, anionic A and cationic C (Fig. 1) having specific fluorescence bands. Dienes et al. [1] found one more emission band at $\lambda_{max} = 485$ nm with a shoulder at 530 nm. This band appears in solutions where the absorbing species is the neutral form, so they ascribed this emission to an excited-state reaction product.

Bergman and Jortner [2] suggested that the fluorescence in the 485 nm and 530 nm bands is emitted by two protonated forms $(4\text{-MUH}^+)_1$ and $(4\text{-MUH}^+)_2$, while others [3–7] tend to ascribe this emission to photoautomers or zwitterions. In addition, Lippert [8] suggested the possibility of the ring-opening reaction giving rise to the long-wavelength emission. The broad 485/530 nm band is present in weakly acidic aqueous ethanolic solutions and its intensity increases with increasing water content [9, 10]. Dienes et al. [11] investigated the influence of laser action at one of these wavelengths on the amplified fluorescence at the other one. They found that the laser action at 485 nm quenched the fluorescence at 530 nm, but not vice versa. This might suggest that the emission at 485 nm originates from an intermediate species which undergoes some transformation to yield the species emitting at 530 nm.

Kindt and Lippert [12] found solvent dependent variations of the intensity ratio at the two wavelengths, which they associate with the presence of two emitting species in the 485/530 nm spectral range.

Kowalczyk [13], however, has shown that apparent changes in the intensity ratio of the two bands are due to the varying contribution of the 445 nm anionic band. Other authors [5, 7, 14, 15] also support the view that only one species is responsible for the 485/530 nm emission.

Since spectral data alone did not provide sufficient understanding of the excited-state behaviour of 4-MU, nanosecond pulse [6, 8, 14, 15] and phase [5] fluorometry techniques were also applied. However, the data obtained in such experiments have been either incomplete or analyzed in a qualitative manner. We found it necessary to reexamine the problem of 4-MU excited-state reactions using in-

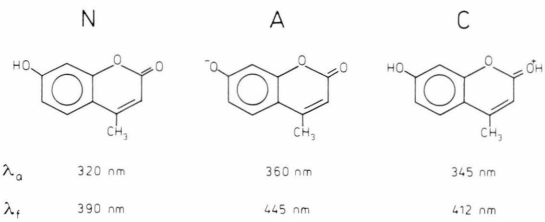

Fig. 1. Ground-state species of 4-MU and wavelengths of their absorption and fluorescence maxima.

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strumentation and data handling methods which allow for the analysis of multiexponential decay functions. These methods had been used before in the studies of excited-state reactions of acridine and 2-naphthol [16–18, 21].

2. Experimental

We prepared three series of solutions of the dye 4-MU (from Koch-Light, three times recrystallized from ethanol) in ethanol (absolute-ethanol, spectral pure from POCh-Poland)-water (twice distilled) mixtures, all with the dye concentration $5 \cdot 10^{-5}$ M.

In each of the three series the water content grew in 6 steps (0.1, 0.5, 1.1, 2.2, 4.4 and 8.9 M). The series differed one from the other by the addition of $HClO_4$ (from Ferak, Berlin) in concentrations 0, 10^{-3} and 10^{-1} M, respectively. All solutions were saturated with air and measured at 293 K. The absorption spectra, measured with a double-beam spectrophotometer, were found to be identical for all studied solutions. The emission spectra were recorded using a home-made photon-counting spectrofluorimeter and corrected for the sensitivity of the detecting system.

The fluorescence phase shift angles were measured by means of a phase fluorometer with a modulation frequency of 11.8 MHz (a modified version of the instrument described in [19]).

The time-resolved fluorescence decays were obtained with a single-photon nanosecond fluorometer built in our laboratory (Figure 2). The maximum repetition frequency of the N_2 excitation lamp was 30 kHz. The overall instrumental function for an XP 2020Q photomultiplier had a FWHM duration of about 2.5 ns. A deconvolution using a non-linear least-squares procedure assuming an n-exponential true fluorescence decay, with $n = 1$, 2 and 3, was performed with a MERA 60 microcomputer.

Both in the phase- and pulse-measurements special attention was paid to the elimination of a "color effect" [19, 20] i.e. a wavelength dependence of the transit time between the photocathode and the first dynode of the photomultipliers. This was done using an ethanolic solution of 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene (dimethyl-POPOP) as a lifetime standard [20].

The excitation wavelength was 334 nm for steady-state and phase shift measurements and 337 nm for pulse measurements.

3. Theory

In a reversible one-step excited-state reaction (Fig. 3) the initially excited A* molecules can either return to the ground state (rate constant k_A) or undergo a forward reaction with a rate k_1 forming B* molecules. k_2 and k_B denote the reverse reaction rate and the intrinsic decay rate of the B* molecules, respectively. Only the A molecules are supposed to have a stable ground state.

Then the decay of the fluorescence intensities I_A and I_B following a δ-pulse excitation is biexponential [22]:

$$I_A(t) = \alpha_1 e^{-t/\tau_1} + \alpha_2 e^{-t/\tau_2}, \quad (1)$$

$$I_B(t) = \beta(e^{-t/\tau_1} - e^{-t/\tau_2}), \quad (2)$$

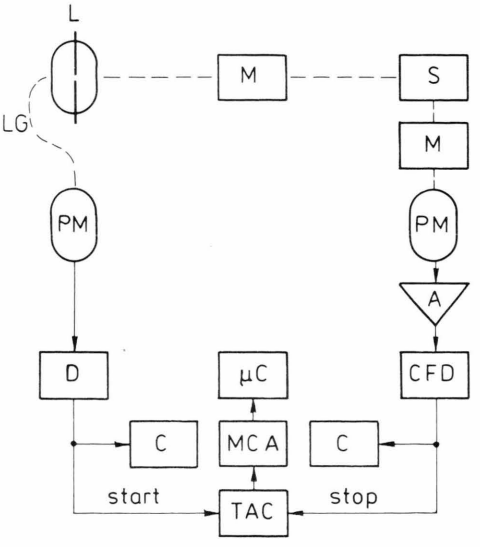

Fig. 2. Block diagram of the single-photon pulse fluorometer. L: N_2 lamp, LG: light guide, M: monochromators, S: sample, PM: photomultipliers, A: amplifier, D: Ortec 436 discriminator, CFD: Ortec 463 constant-fraction discriminator, C: counters, TAC: Polon 1701 time to amplitude converter, MCA: EMG NTA 1024 multichannel analyzer, μC: Mera 60 microcomputer.

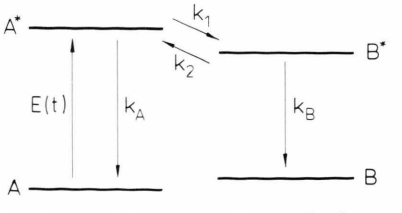

Fig. 3. Model for a reversible excited-state reaction.

where α_1, α_2, β, τ_1 and τ_2 are functions of the rate constants in the Fig. 3 scheme. For an irreversible reaction ($k_2 = 0$) α_1 equals zero and the decay of the A* fluorescence is monoexponential. The product B* fluorescence decay is biexponential with equal amplitudes but with opposite signs. The detection of such temporal behaviour proves that the observed emission is originating from a species formed in an excited-state process.

In the case of a two-step excited-state reaction the decay of fluorescence is in general triple-exponential.

The analysis of multiexponential decays can be substantially simplified by using the concept of internal convolution [17]. This reduces the number of exponents in the analysis by one. For instance, using the decay of the A* fluorescence instead of the instrumental response function in the deconvolution procedure for the B* fluorescence one gets an intrinsic monoexponential decay $\exp[-(k_B + k_2)t]$. This corresponds to the decay of B* molecules excited directly.

The internal convolution principle is also applicable for multiexponential ($n > 2$) decays, but the irreversibility of the consecutive reaction steps is required in this case [17].

In a scheme shown in Fig. 4 the fluorescence intensity of the final product C* decays according to the formula

$$I_C(t) = \alpha\left[(1-\beta)\, e^{-k_C t} + \beta\, e^{-(k_B + k_{BC})\, t} - e^{-(k_A + k_{AB})t}\right], \tag{3}$$

where α is a constant and $\beta = (k_A + k_{AB} - k_C)/(k_B + K_{BC} - k_C)$. Application of the internal convolution principle in this case [17] gives

$$I_C(t) \propto I'_C(t) \otimes I'_B(t) \otimes I_A(t), \tag{4}$$

where $I'_C(t)$ and $I'_B(t)$ are the intrinsic decays of the C* and B* species and the symbol $\otimes$ denotes the convolution operation. As a result one gets

$$I_C(t) \propto I_A(t) \otimes [e^{-k_C t} - e^{-(k_B + k_{BC})t}]. \tag{5}$$

This means that, if the deconvolution versus the decay of the initially excited species gives a term with a negative amplitude on the long-wavelength side of the emission, one may confidently infer the existence of the intermediate species B*. If, however, k_{BC} is much larger than the other rate constants, one may erroneously conclude that there is no such an intermediate species.

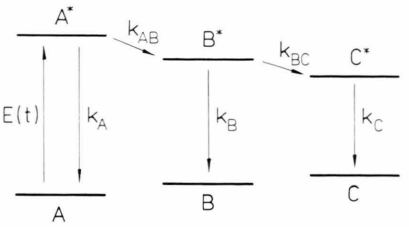

Fig. 4. Model for an irreversible two-step excited-state reaction.

The phase fluorometry can also be used to study multiexponential fluorescence decays [16, 18, 22]. This somewhat less direct method involves measurements of the phase shift angles φ between the harmonically modulated excitation and emission.

The internal convolution principle is also applicable here [16, 18]. For the scheme in Fig. 3 the lifetime of A* is $\omega^{-1} \tan \varphi_A$ and the lifetime of the reaction product B* can be calculated from the relative phase shift $\varphi_B - \varphi_A = \varphi_R$ as $\omega^{-1} \tan \varphi_R$ [18].

4. Results and discussion

Three overlapping emission bands were beyond any doubt identified in the ethanol-water solutions of 4-MU (Fig. 5) with maxima at 390, 445 and 485 nm. It is commonly accepted that the first two bands are due to the neutral and anionic species, respectively (Fig. 1). The most favoured assignment of the 485 nm band is a tautomer or a zwitterion. Views on the nature of the 530 nm shoulder in this band are conflicting. We found no difference in the nanosecond behaviour of the 485 and 530 nm emission. Reports in the literature claiming the existence of such differences [6, 12] must be treated with some doubt as they may be due to the photomultiplier "color effect" or the overlap of the emission bands. We are therefore convinced that our assignment of a single excited-state species to the 485/530 nm band is justified.

Results of our pulse measurements are presented in Table 1. The fluorescence decay was measured at two wavelengths, 380 nm and 580 nm. This large spectral separation was necessary to avoid complications due to the overlapping of the bands. The fluorescence decay at 380 nm (the neutral form of 4-MU) is monoexponential, while at 580 nm (the excited-state reaction product) we obtained a bi-

exponential decay $\exp(-t/\tau_1) - \exp(-t/\tau_2)$. We also applied the internal convolution principle to find the differential decay at 580 nm versus 380 nm. This was found to be monoexponential. A fitting procedure with a larger number of exponential components (a maximum of three) also converged to those presented in Table 1. Our results will be discussed in the framework of the scheme shown in Figure 6. In the excited neutral molecules of 4-MU the phenolic group becomes more acidic and the carbonyl group becomes more basic relative to the ground state. Since water can act as a proton donor and an acceptor as well, in aqueous solutions two reaction pathways are possible, namely N* → A*

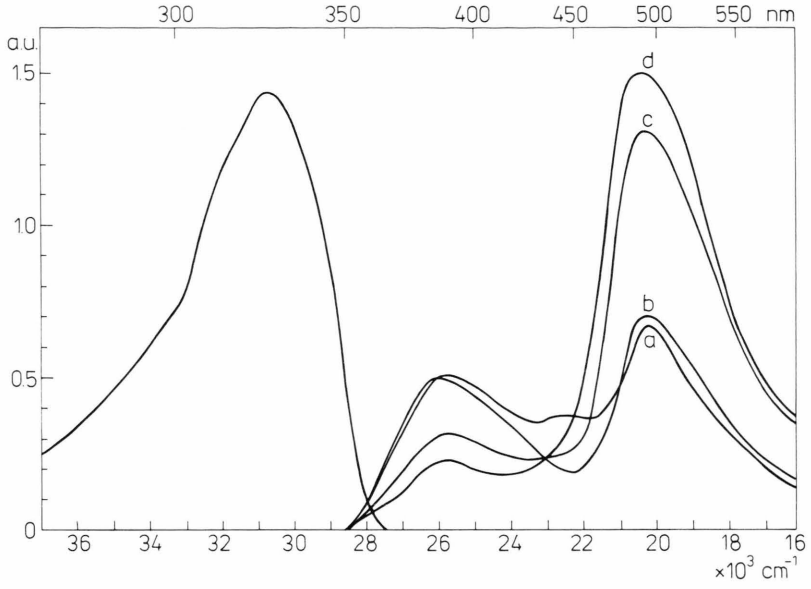

Fig. 5. Absorption and fluorescence spectra of aqueous ethanolic solutions of 4-MU. a: 0 M $HClO_4$, 1.1 M H_2O, b: 0.1 M $HClO_4$, 0.1 M H_2O, c: 0.1 M $HClO_4$, 2.2 M H_2O, d: 0.1 M $HClO_4$, 8.9 M H_2O.

Table 1. Fluorescence decay times (in ns) for aqueous ethanolic solutions of 4-MU at two emission wavelengths.

$[HClO_4]$	$[H_2O]$	380 nm	580 nm		580 vs. 380 nm
(M)	(M)	τ_N	τ_1	τ_2	τ_R
0	0.1	1.5	3.2	1.6	3.2
	0.5	1.0	3.8	1.1	4.0
	1.1	1.0	5.9	1.2	5.4
	2.2	1.0	5.5	1.0	5.2
	4.4	0.8	5.4	0.9	5.3
	8.9	0.6	5.3	0.7	5.3
10^{-3}	0.1	1.6	5.1	1.6	5.2
	0.5	1.3	5.3	1.3	5.2
	1.1	1.2	5.2	1.3	5.2
	2.2	1.1	5.2	1.1	5.2
	4.4	0.7	5.2	0.7	5.1
	8.9	0.5	5.3	0.6	5.1
10^{-1}	0.1	1.1	5.6	0.5	5.1
	0.5	0.9	5.5	0.3	5.5
	1.1	0.8	5.5	0.8	5.5
	2.2	0.7	5.4	0.3	5.0
	4.4	0.6	5.5	0.3	5.2
	8.9	0.5	5.3	1.1	5.2

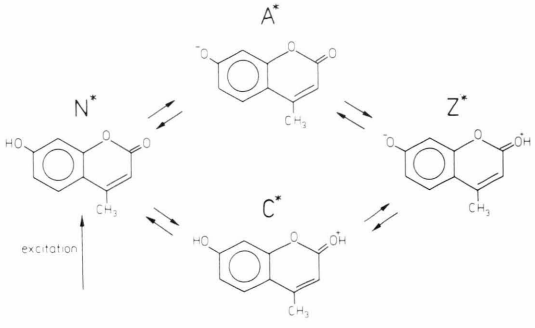

Fig. 6. Possible excited-state protolytic reactions for the 4-MU molecule.

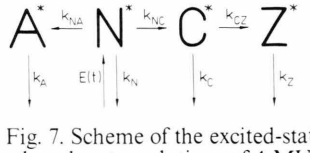

Fig. 7. Scheme of the excited-state reactions for the acidified ethanol-water solutions of 4-MU.

and N* → C*. We ascribe the 485/530 nm band to the zwitterion Z* which may be created from the neutral form N* either via A* or C*.

In our acidified ethanol-water solutions this general scheme can be considerably simplified. First, the monoexponential decay of the neutral species means that back reactions A* → N* and C* → N* can be neglected. Since no cation emission has been observed in our solutions, we conclude that the C* → Z* dissociation rate is very high compared with other reaction rates and no detectable back reaction occurs. Our final model of the excited-state reactions of 4-MU must also be consistent with the observation of the biexponential decay of the Z* species and the presence of the anionic emission. This eliminates the A* → Z* and Z* → A* reactions from our scheme. In this way we finally obtain the scheme shown in Figure 7. The following set of kinetic equations can be written for this scheme:

$$dN^*/dt = -(k_N + k_{NC} + k_{NA})\,N^* + E(t), \quad (6)$$

$$dA^*/dt = -k_A A^* + k_{NA} N^*, \quad (7)$$

$$dC^*/dt = -(k_C + k_{CZ})\,C^* + k_{NC} N^*, \quad (8)$$

$$dZ^*/dt = -k_Z Z^* + k_{CZ} C^*. \quad (9)$$

Assuming a δ-pulse excitation at $t = 0$ and $N^*(0) = 1$ one gets

$$N^*(t) = e^{-\alpha t}, \quad (10)$$

$$A^*(t) = \frac{k_{NA}}{\alpha - k_A}(e^{-k_A t} - e^{-\alpha t}), \quad (11)$$

$$C^*(t) = \frac{k_{NC}}{\alpha - \gamma}(e^{-\gamma t} - e^{-\alpha t}), \quad (12)$$

$$Z^*(t) = \frac{k_{NC}\,k_{CZ}}{(\alpha-\gamma)(\alpha-k_Z)}[(1-\beta)\,e^{-k_Z t} + \beta e^{-\gamma t} - e^{-\alpha t}], \quad (13)$$

where $\alpha = k_N + k_{NA} + k_{NC}$, $\beta = (k_Z - \alpha)/(k_Z - \gamma)$ and $\gamma = k_C + k_{CZ}$. Although in general the decay of Z^* contains three exponential terms, it becomes biexponential when $k_{CZ} \to \infty$. Then

$$Z^*(t) = \frac{k_{NC}}{\alpha - k_Z}(e^{-k_Z t} - e^{-\alpha t}). \quad (14)$$

Thus the measured decay times (Table 1) should have the following meaning: $\tau_N = 1/\alpha$, $\tau_2 = 1/\alpha$, $\tau_1 = 1/k_Z$ and $\tau_R = 1/k_Z$. Indeed, $\tau_N \approx \tau_2$ and $\tau_1 \approx \tau_R$. For the 10^{-1} M concentration of $HClO_4$ there is, however, a discrepancy between the values of τ_N and τ_2.

The lifetime τ_N of the neutral form will be shortened when either the water or the acid concentration is increased. Since $1/\tau_N = k_N + k_{NA} + k_{NC}$, this may be caused by

(i) the increase of k_{NC} with increasing acid concentration,

(ii) the increase of both k_{NA} and k_{NC} with increasing water content.

The lifetime of Z^* $(1/k_Z)$ appears to be constant, except for the water content of 0.1 M and 0.5 M at $[HClO_4] = 0$. For these two solutions the emission at 580 nm is faint enough to make the results unreliable. The average value of $1/k_Z$ for the remaining solutions is (5.3 ± 0.2) ns.

Results of the pulse measurements (Table 1) can be compared with those obtained using the phase fluorometer (Table 2).

The values of $\omega^{-1}\tan\varphi$ at 380 nm represent the lifetimes of the neutral form (τ_N in Table 1). At 580 nm we get values which cannot be related directly to the lifetimes [18].

However when the phase shift angles are measured versus 380 nm, the $\omega^{-1}\tan\varphi_R$ values are equal to $1/k_Z$. The average value of $1/k_Z$ obtained in this way is (5.4 ± 0.3) ns.

Our scheme presented in Fig. 7 is thus practically reduced to the scheme $A^* \leftarrow N^* \rightarrow Z^*$. In order to confirm or deny the role of the cation further studies

Table 2. $\omega^{-1}\tan\varphi$ (in ns) for aqueous ethanolic solutions of 4-MU at two emission wavelengths.

$[HClO_4]$ (M)	$[H_2O]$ (M)	380 nm	580 nm	580 vs. 380 nm
0	0.1	1.3	6.3	4.8
	0.5	1.1	6.1	4.9
	1.1	1.1	6.4	5.4
	2.2	1.2	6.5	5.1
	4.4	0.9	6.2	5.2
	8.9	0.8	6.1	5.1
10^{-3}	0.1	1.3	6.8	5.3
	0.5	1.1	6.8	5.5
	1.1	0.9	6.9	5.8
	2.2	1.1	6.5	5.2
	4.4	0.6	6.7	6.0
	8.9	0.8	6.3	5.4
10^{-1}	0.1	1.1	6.2	4.9
	0.5	0.8	6.3	5.4
	1.1	0.8	6.5	5.6
	2.2	1.0	6.3	5.1
	4.4	0.7	6.5	5.6
	8.9	0.7	6.4	5.5

are needed with higher (picosecond) time resolution. The nature of the long-wavelength emitting species should also be further investigated, perhaps by means of quantum-chemical calculations, to decide whether our zwitterion hypothesis is correct.

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