

PHOTOIONIZATION OF FLUORESCEIN VIA EXCITED TRIPLET AND SINGLET STATES

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Summary

The two-photon ionization of sodium fluorescein in neutral aqueous solution is investigated using laser spectroscopy. Two different pathways are substantiated independently. One proceeds via the first excited singlet state and the subsequent absorption of a photon of UV light (3.8 eV); the other involves the lowest excited triplet state which is ionized by the absorption of a low energy photon of visible light (2.5 eV). For this second pathway the ejection yield, *i.e.* the probability that the electron definitely escapes from the parent ion pair and solvates, is estimated to be 0.4.

1. Introduction

Photoionization, in which light absorption by a molecule in solution leads to the formation of charged species such as solvated electrons, is a primary step of growing importance in molecular photophysics because of the implications of this process in the fields of solar energy conversion and photosynthesis.

Among the different possible pathways, the monophotonic ionization, in which only one photon of high energy is required for the continuum to be reached, has been extensively investigated [1]. However, the consecutive bi-photonic process is still in need of further examination. A two-quantum mechanism which proceeds by the absorption of two photons is now widely recognized [2 - 4]; however, the identification of the photoactive intermediates and the determination of the relative extent of the pertinent transitions leading to the ionization continua still require active investigation.

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With a view towards contributing to this issue, in this work we investigate the photoionization of fluorescein, a staining material of common and intensive use, which could serve as a model compound for further examination of biphotonic processes in dyes of photochemical, electrophotochemical or photobiological interest.

It should be mentioned that, because of the different stages of protonation of the basic molecule and its related transients (the complexity of which has been emphasized [5, 6]), this study is deliberately restricted to the case of fluorescein in buffered aqueous solutions at neutral pH. Under these experimental conditions, fluorescein has a very low triplet quantum yield (0.03) and has a quantum yield of fluorescence close to unity. These conditions are favourable for the characterization of the different pathways which are likely to lead to photoionization. In fact, a study of the ionization process via the first excited singlet state should not be disturbed by the presence of a possible triplet mechanism; these two processes are expected to occur in proportion to the quantum yields of the intermediate states involved.

It should also be noted that, because of the foregoing physical parameters and because of the very high intensities used, an extreme situation is reached with respect to saturation effects. Nevertheless, the experimental results agree satisfactorily with the conclusions drawn by Lachish *et al.* [7] from their theoretical analysis of the photochemical processes induced by consecutive biphotonic absorption.

2. Experimental

Sodium fluorescein (BDH) and iodoethane (Hopkin and Williams Ltd.) were used without further purification. All the solutions were prepared using triply distilled water; the pH was controlled with a phosphate buffer (pH = 6.98). The experimental results were obtained at a wavelength of 337 nm and with a dye concentration [c] of 10^{-4} M in a 1 cm cell.

The nitrogen laser photolysis system and the nitrogen-laser-pumped dye laser (NLPDL) photolysis system have been described in detail previously [8, 9]. At 337 nm the maximum output energy was 0.5 mJ and the pulse duration was 7 ns; at 488 nm these parameters were 100 μ J and 5 ns respectively. The laser intensities which reached the samples were varied by attenuation with a set of Schott-Jena neutral filters calibrated using a spectrophotometer.

3. Results

3.1. Laser photolysis experiments

The spectroscopic data obtained from the laser experiments on sodium fluorescein are reported in Fig. 1. In order to define precisely the experi-

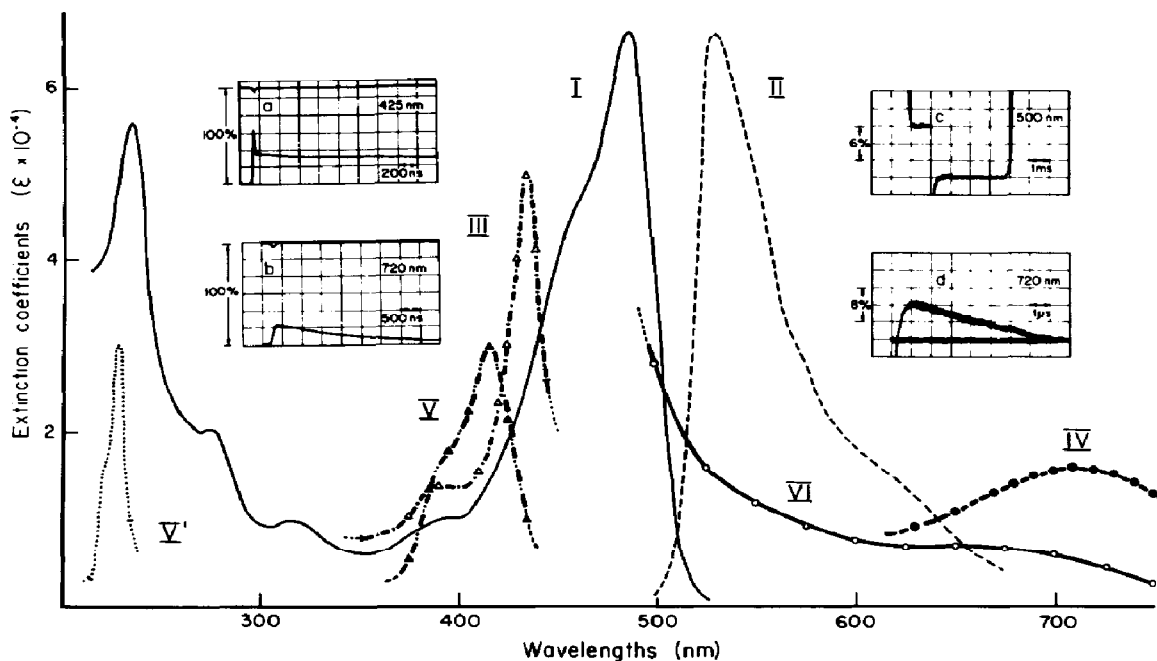


Fig. 1. Signals and spectra recorded for fluorescein: curve I, ground state absorption; curve II, uncorrected fluorescence; curve III, semi-oxidized form absorption; curve IV, solvated electron absorption; curve V, excited singlet state absorption ($\epsilon \times 2$); curve V', as curve V, but with the zero-zero energy of the $S_0 \rightarrow S_1$ transition serving as the zero level energy reference; curve VI, triplet state absorption with ϵ in arbitrary units. The insets correspond to: (a) the absorption at 425 nm on nitrogen laser excitation (337 nm); (b) the absorption at 720 nm on nitrogen laser excitation (337 nm); (c) the bleaching at 500 nm on dye laser excitation (488 nm); (d) the absorption at 720 nm on dye laser excitation (488 nm).

mental conditions under which the transient spectra were recorded, the ground state absorption and the fluorescence spectra of fluorescein are also shown in Fig. 1.

It can be seen from the insets (a) and (b) of Fig. 1 that, on nitrogen laser excitation at 337 nm, two types of transient signals were obtained depending on the detection wavelength. The inset (a) corresponds to the signal of a very fast absorption from the first excited singlet state, which is observed at 425 nm and which is followed by a long-lived transient signal due to a radical species. The assignment of these two absorptions is supported by the following observations.

(1) The fast transient absorption and the fluorescence emission both increase within the laser pulse duration when observed with a 2 ns resolution time. When the power density of the laser beam is decreased to allow only the first transient to be generated (see Section 3.2), the decay times of the transient signal and the fluorescence emission are identical. The spectrum, uncorrected for bleaching, corresponding to this fast absorption is centred at 415 nm (Fig. 1). Therefore this transient signal can be attributed to a transi-

tion between the first excited singlet state S_1 and one of the upper singlet states S_n . This state might also be reached directly from the ground state, since the energy gaps are about $19\,400\text{ cm}^{-1}$ for the $S_0 \rightarrow S_1$ transition and about $43\,000\text{ cm}^{-1}$ for the $S_0 \rightarrow S_n$ transitions. Thus, the energy corresponding to the $S_1 \rightarrow S_n$ transition is in agreement with the curves in Fig. 1. It should be noted that, because the symmetry requirements are not very stringent in such a large molecule, transitions from S_0 and S_1 to an upper singlet state S_n may be simultaneously allowed [10, 11].

(2) The long-lived transient is formed within the resolution time of the detection system; the decay time in aerated or degassed solutions exceeds 10^{-4} s and the absorption spectrum has a maximum at 435 nm (Fig. 1). Therefore this transient is identified with the semi-oxidized form of fluorescein, an ionized form which is known to be generated through an electron ejection. The photochemical behaviour of this species has been studied using conventional flash photolysis [5, 6, 12].

The inset (b) of Fig. 1 shows the transient signal recorded in the near IR in a carefully deaerated solution. This signal also increases within the resolution time of the detection system and the corresponding absorption spectrum is centred around 720 nm ; this species, which is quenched by oxygen and by N_2O [13], is identified with the solvated electron which is generated simultaneously with the semi-oxidized form of sodium fluorescein [12]. By measuring successively the absorptions due to these transients (the semi-oxidized form and the solvated electron) and to bleaching of the ground state, it is possible to evaluate their extinction coefficients. Such an evaluation is not possible for the $S_1 \rightarrow S_n$ spectrum because of the intense fluorescence emission which prevents the concomitant fast bleaching of the ground state from being measured. (However, it was possible to estimate this extinction coefficient when the ground state was almost completely bleached.)

The triplet state absorption spectrum of sodium fluorescein was recorded in the $500 - 750\text{ nm}$ range and is displayed in arbitrary units in curve VI of Fig. 1. It should be noted that a correction for the bleaching corresponding to the formation of semi-oxidized forms and triplet states could not be made owing to the weakness of all the transient signals involved. Therefore, an accurate determination of the true extinction coefficients was not possible. However, the spectrum recorded on the low energy side of the ground state absorption — which in spite of the absence of a bleaching correction increases rapidly at about 500 nm — agrees satisfactorily with the spectrum obtained by Lindqvist [5] under the same pH conditions but in a 60 cm cell. Oxygen and heavy-atom-containing compounds, which are known to alter considerably the lifetime and the degree of conversion to triplet states respectively, have in this instance no significant effect on the overall process for the formation of the semi-oxidized form of sodium fluorescein. This means that there is no active participation of the triplet state in this ionization mechanism.

In the following sections the species which appear after the absorption of light and their subsequent interconversion are surveyed and characterized. The mechanism of their generation is considered.

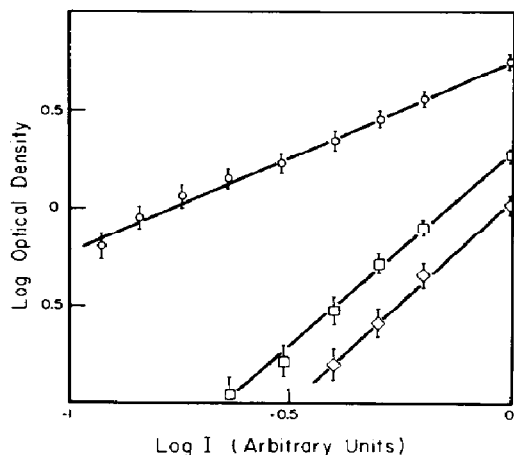


Fig. 2. Intensity dependences of the transients after excitation at 337 nm: \circ , $S_1 \rightarrow S_n$ at 425 nm; \square , radical at 425 nm; \diamond , solvated electron at 720 nm.

3.2. The photoionization mechanism

It was mentioned in Section 1 that fluorescein at neutral pH is a very convenient system for investigating the biphotonic ionization processes. Because of the low triplet quantum yield, a study of the ionization via the singlet fluorescent state can readily be made. Moreover, the singlet and triplet pathways can be studied quite independently because of the significant difference between the ionization potentials of these two transient states.

In order to explore this problem fully, the following strategy was adopted. The nitrogen laser ($\lambda = 337$ nm) was used to induce the ionization via the singlet state and the dependences of the transient state yields on the excitation intensity were recorded. Then the dye laser (NLPDL) was used to locate the ionization threshold via the singlet state and the intensity dependence of the triplet mechanism was studied.

3.2.1. Non-linear ionization at 337 nm

The optical densities, measured at the end of the laser excitation, for the semi-oxidized form of fluorescein, for the solvated electron and for the first excited singlet state, are displayed as functions of the excitation intensity in Fig. 2. It is apparent that the slopes of the straight lines obtained — unity for the excited singlet state and two for the other transients — reflect monophotonic and biphotonic processes respectively. The experimental data were displayed on a log-log scale for convenience; however, it was verified that extrapolations of the straight lines pass through the origin when these extinctions are plotted against I (excited singlet state) and against I^2 (semi-oxidized form and solvated electron) in linear coordinates. This means that saturation effects [7] are not important, and confirms that a two-photon mechanism is involved in which a short-lived excited state is the active species

which absorbs the second photon. This species, which is formed within the resolution time of the detection system and which is insensitive to oxygen and to heavy atom effects, should be the first excited singlet state; this ionization mechanism will be called the singlet mechanism.

In this work the $S_1 \rightarrow S_n$ extinction was employed to measure the concentration of the excited singlet state generated by the different exciting wavelengths used and to ascertain that the excitation term σP (see Section 4) remained almost constant. (It should be noted that the absorption cross sections of the fluorescein ground state are almost constant over the range 320 - 400 nm, which implies an almost constant power density over this range.)

A dramatic reduction of the intensities of the radical and solvated electron transient absorptions is observed when the wavelength is changed from 337 nm (the nitrogen laser wavelength) to 375 nm (the shortest available wavelength of the NLPDL). However, by decreasing the resolution time of the detection system and thereby increasing the signal-to-noise ratio by more than one order of magnitude, the intensities of these weak transient absorptions can be shown to be independent of the exciting wavelength within the range 375 nm (the shortest available wavelength) — 488 nm (the longest wavelength absorbed significantly by the ground state).

Because these two species were still generated at exciting wavelengths (*i.e.* at 488 nm) which were not absorbed at all by the first excited singlet state, any participation of the first singlet state seems to be excluded. Therefore it is concluded that the photoionization threshold of the fluorescent state lies somewhere between $29\,700\text{ cm}^{-1}$ (energy of the nitrogen laser photons) and about $22\,000\text{ cm}^{-1}$ (energy of the NLPDL photons inducing an ionization but not absorbed by the first excited singlet state). It must be noted that the exact position of this threshold within this range is much more delicate; in fact, it could not be decided whether there is a gradual change from the singlet pathway to another pathway at about 375 nm or whether there is a clear threshold located between 337 and 375 nm.

In an attempt to determine the nature of the transient state involved in the photoionization process induced by visible light, Lindqvist [5] first suggested the possibility of obtaining photoionization via the first triplet state to account for an irreversible bleaching of sodium fluorescein solutions occurring within the flash duration in some flash photolysis experiments. This suggestion was based on the fact that the photodecomposition caused by radical formation continuously increases as the incident flash intensity increases, even beyond complete conversion of the ground state to the triplet state.

In view of these observations a specific study was undertaken to decide whether the triplet state of sodium fluorescein could also serve as a relay in a biphotonic ionization process.

3.2.2. Non-linear ionization by visible light

Since, within the resolution of the detection system, the radical generation rate was observed to be independent of the exciting wavelength

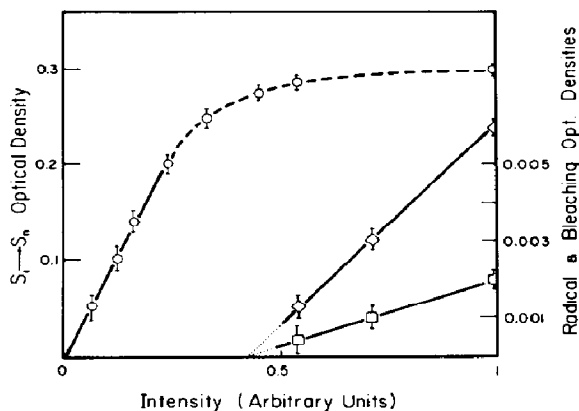


Fig. 3. Intensity dependences of the transients after excitation at 488 nm: \circ , $S_1 \rightarrow S_n$ at 410 nm; \diamond , radical at 425 nm; \square , bleaching at 500 nm.

between 375 and 488 nm, the wavelength 488 nm, which was efficiently absorbed by the ground state, was used throughout this study.

The insets (c) and (d) of Fig. 1 correspond to the ground state bleaching and to the solvated electron absorption on excitation at 488 nm. (Because of the weakness of these signals the vertical scale was expanded, thus shifting the baseline out of the frame. The signal corresponding to the inset (c) of Fig. 1 is limited in time by the pulse duration of the analysing light.) The very weak signal of the radical and the excited singlet absorption were also recorded under the same experimental conditions. The absorbances of these transients are shown as functions of the incident light intensity in Fig. 3. The experiment was carried out in deaerated solutions in order to simplify the photochemical process which was assumed to yield ionization. In the presence of oxygen the triplet state is also known to convert to the semi-oxidized form, whereas in deaerated solutions the semi-oxidized and the semireduced forms appear simultaneously as the result of one of the deactivation pathways. This latter route corresponds to a biphotonic process which involves the interaction between two one-photon-excited molecules. The intersystem crossing rate constant is lower than 10^7 s^{-1} [14] and the bimolecular rate constants of formation and geminate recombination are about $10^7 - 10^8 \text{ M}^{-1} \text{ s}^{-1}$ and $10^9 \text{ M}^{-1} \text{ s}^{-1}$ respectively [5, 15]. Thus the permanent concentration of the semi-oxidized form generated from triplet deactivation is negligible.

Therefore, the transient absorption assigned to the semi-oxidized form can be assumed to correspond only to radicals formed by ionization of an upper triplet state within the laser pulse duration.

4. Discussion

The experimental results obtained for the so-called singlet pathway do not merit many further comments. The expected dependences of the

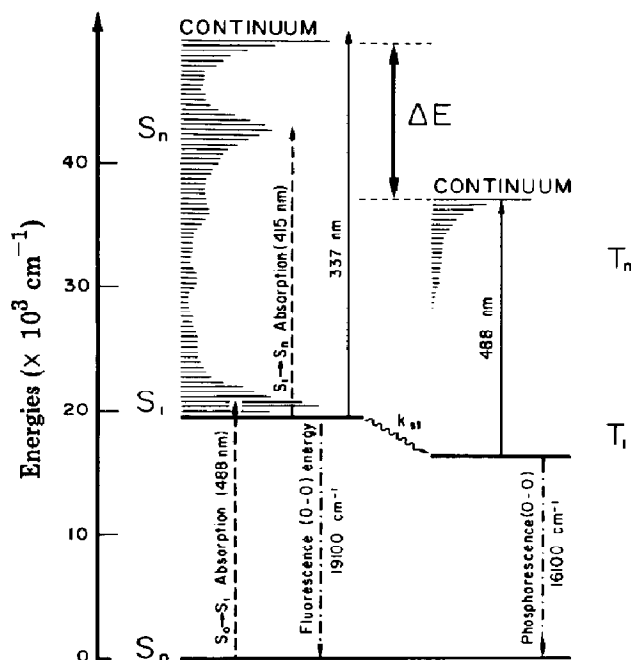


Fig. 4. An energy diagram for the biphotonic ionization of fluorescein.

transient absorbances on the excitation intensity are observed (Fig. 2) and agree with the theoretical analysis [7]. Moreover, the energy of the ionization band attained from the S_1 excited state coincides with an absorption band in the ground state absorption spectrum.

In contrast, the excitation by visible light via the triplet state demands much more attention. By locating the phosphorescence energy level at $16\,000\text{ cm}^{-1}$ [16] (see Fig. 4), it can be deduced that the ionization potential of the triplet lies between $5\,000$ and $12\,000\text{ cm}^{-1}$ lower than the ionization potential of the singlet.

This indicates that the electron is ejected from a chemically different state of the molecule. Since the pK corresponding to the monoanion–dianion equilibrium is about 7, it is possible that a different stage of protonation in the excited singlet and triplet states is responsible for this difference.

Such an ionization process, which involves the absorption of two photons of visible light by an aqueous solution of a common dye at neutral pH, is important provided that the quantum yield ϕ_e of electron ejection from the upper triplet state is not negligible.

In order to estimate this ejection yield we now consider the experimental conditions under which ionization via the triplet state was obtained. As shown in Fig. 3, the solvated electron and the semi-oxidized form become detectable only at laser intensities which are high enough to depopulate the ground state almost completely.

It can be inferred [17] from the shape of this curve that the excitation term σI has a value greater than about 4 (the absorption cross section σ of

the ground state is $2 \times 10^{-16} \text{ cm}^2$ at 488 nm; the time-integrated intensity I of the laser beam at this wavelength was about 10 mJ cm^{-2} or 2×10^{16} photons cm^{-2} in this work). It is interesting to note that this almost complete bleaching of the ground state allows the extinction coefficient of the $S_1 \rightarrow S_n$ transition to be calculated by assuming that the S_1 population is equal to the initial ground state concentration [10]; a maximum value for ϵ of 30 000 is deduced.

However, by assuming that the exciting pulse behaves as a square function ($P(t) = P(0)$) and that the $S_0 \rightarrow S_1$ transition becomes saturated from the very beginning of the excitation process, the population [T] of the triplet state can be given as a function of time:

$$[T] = k_{ST}[S_0]t$$

where k_{ST} is the intersystem crossing rate constant and $[S_0]$ is the concentration of the ground state prior to excitation.

Since the radical concentration [R] varies linearly with the excitation intensity (Fig. 3), the linear approximation

$$\frac{d[e_{aq}^-]}{dt} = \phi_e \sigma_T P(t) [T]$$

is justified. (σ_T is the cross section of the triplet state and $P(t)$ is the laser intensity.) The total electron concentration at the end of the pulse can be evaluated by integration:

$$\begin{aligned} [e_{aq}^-] &= [R] \\ &= \int_0^{\Delta t} \phi_e \sigma_T P(t) k_{ST} [S_0] t dt \\ &= \phi_e \sigma_T P k_{ST} [S_0] \frac{(\Delta t)^2}{2} \end{aligned}$$

Hence the following expression is obtained for ϕ_e :

$$\phi_e = \frac{[R]}{[S_0]} \frac{2}{\sigma_T P k_{ST} (\Delta t)^2}$$

By taking into account the facts that $I = P\Delta t$ (I is the time-integrated intensity) and that Δt is approximately equal to the fluorescence lifetime τ_F ($\Delta t \approx 5 \text{ ns}$ and $\tau_F = 4.5 \text{ ns}$ [16]), this expression can be reduced to

$$\phi_e = \frac{[R]}{[S_0]} \frac{2}{\sigma_T I \phi_T}$$

Substitution of the experimental values used in this work, *i.e.* $I = 2 \times 10^{16}$

photons cm^{-2} , $\phi_T = 0.03$ and $\sigma_T = 4 \times 10^{-17} \text{ cm}^2$, and of $[R]/[S_0] = 0.005$ (as measured experimentally), yields

$$\phi_e = 0.4$$

This result must only be regarded as the order of magnitude of the ejection yield from the triplet state; the experimental conditions — high concentrations and high excitation power densities — exclude a more precise statement [7, 18 - 20].

Nevertheless, this high yield leads to an important conclusion, *i.e.* a significant quantity of the excited triplets actually generate solvated electrons, thereby establishing the effectiveness of a photoionization mechanism by visible light in neutral aqueous solutions of fluorescein.

Moreover, in view of the relatively long lifetime of the triplet transient state of fluorescein compared with the singlet lifetime and in view of the low energy of the photons required to induce ionization, the implications of such a process for steady state applications are obvious.

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