Quantitative comparison of excited state properties and intensity-dependent photosensitization by rose bengal

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Abstract

A quantitative (multistep) excitation–deactivation model of rose bengal (RB) has been developed which includes energy and electron transfer to oxygen and the possibility of photoproduct formation via higher excited triplet–singlet states. The model is based on previous measurements of non-linear absorption (NLA) and emission of RB with picosecond pulses at 532 nm and on NLA measurements with nanosecond pulses. A coupled rate equation and photon transport equation approach for non-linear light–matter interaction is used. The resulting term scheme with all relevant excited state parameters confirms that (i) in the first excited state of RB, relevant absorption at 532 nm takes place only in the triplet, and (ii) the previously reported intensity dependence of RB-sensitized enzyme inhibition is well modelled by the intensity-dependent RB-T1 population and (as the main process) subsequent energy transfer to form singlet oxygen.

Keywords: Photosensitization; Excited states; Non-linear absorption

1. Introduction

There is much current interest in using high intensity pulsed lasers to excite organic compounds to upper excited states that may produce reactive species not formed by the lowest excited states \[1–6\]. Sequential two-photon absorption at a single wavelength is possible when the dye possesses the appropriate absorption cross-sections and the appropriate pulse length is used. Interest in this approach to photodynamic therapy (the treatment of tumours with dyes plus light) is motivated by the possibility that the toxic species produced from upper triplet or singlet states, most likely radicals, will affect cells by a non-oxygen-dependent mechanism and thus allow treatment of oxygen-depleted tissues. A second motivation is the possibility of delivering the high excitation energy necessary for radical formation by two photons of lower energy; this allows use of photons in the red region (the "therapeutic window") with maximum tissue transmission. The more highly energetic upper excited states of certain dyes and drugs have been shown to react to form species which may be toxic to cells \[1–7\].

To apply this approach effectively, it is important to establish the absorption cross-sections and kinetics of the excited states of a candidate dye. In this paper we present such an analysis for the dye rose bengal (RB).

Recently it was shown that one-photon excitation of RB to a higher electronic state \(\lambda_{exc} = 308 \text{ nm}\) resulted in an enhancement of the photoinhibition of red blood cell acetylcholinesterase as compared with excitation to the first excited singlet state \(S_1 (\lambda_{exc} = 532 \text{ nm})\) under comparable excitation conditions \[10\]. The main photoinhibition effect was triggered by singlet oxygen, \(O_2 (1A_g)\), generated by energy transfer from the lowest triplet state of RB \(T_1\). However, an additional effect via a higher excited state was caused by initial formation of a highly reactive radical \[7\]. Trials to obtain a comparable enhancement by two-step excitation \(S_0 \rightarrow S_1 \rightarrow S_x\) (where \(S_0\) is the ground state, \(S_1\) the first excited singlet state, and \(S_x\) a higher excited singlet state) with 532 nm pulses failed. This is rather unexpected because in many organic molecules with \(\pi\) electron systems there is an \(S_1 \rightarrow S_x\) absorption with transition moment and bandshape comparable with those of the \(S_0 \rightarrow S_1\) absorption \(\text{e.g. Refs. [11–13]}\). Moreover, RB has an excited state around 260 nm (corresponding to twice the \(S_1\) energy) in the conventional \(S_0\) absorption spectrum (Fig. 1). Even if the pure electronic transition \(S_1 \rightarrow S_x\) is prohibited by selection rules, a non-vanishing transition probability is expected for \(S_1 \rightarrow S_x\) because of simultaneous vibrational excitation (Herzberg–Teller effect). In addition, there is at least one example that the same radical can be generated via a higher one-photon
excited state as via stepwise two-photon excitation to a higher electronic state [8].

To gain deeper insight into the excited state behaviour of RB, we completed the experimental investigations by additional non-linear absorption measurements at 532 nm using nanosecond pulses. Based on a rate equation system [14], we developed a quantitative excitation–deactivation model for RB (term scheme with relevant excited state parameters). This model describes the experimental results (non-linear absorption, intensity dependent emission, intensity dependence of O2(Ag) generation and photoinhibition) and confirms that the RB singlet excited state absorption at 532 nm is indeed vanishing small.

2. Materials and methods

RB was supplied by Aldrich (UK). It was used without further purification. The solutions were prepared with bidistilled water using phosphate-buffered saline (PBS) tablets, pH 7.4, from Sigma. All measurements were carried out at concentrations $c < 10^{-4}$ M.

Ground state absorption cross-sections (cf. Fig. 1) were calculated from the absorption spectra recorded with a Beckman UV 5270 spectrophotometer. The fluorescence lifetime of RB in PBS was measured using the single-photon counting equipment which was described elsewhere [15].

2.1. Non-linear absorption

Non-linear absorption measurements were carried out utilizing the equipment described in Ref. [16]. The sample was excited by a nitrogen laser pumped tunable dye laser (wavelength region, 510–550 nm; pulse duration, 1.4 ns; pulse energy, 15 μJ). The cross-sectional area of the laser beam at the cell entrance was approximately $3 \times 10^{-5}$ cm$^2$. The maximum available photon flux density was $10^{27}$ cm$^{-2}$ s$^{-1}$. To minimize the influence of photochemical reactions on the measurement, the sample cell (optical pathlength, 0.1 cm) was shifted perpendicular to the optical axis after every laser shot. The relative error of the intensity-dependent transmission measurement was about 2%.

All calculations were carried out on an AT-compatible microcomputer by means of a special software package CALE [14].

3. Model description

3.1. Photoactivation of rose bengal at high excitation intensities

In general, the absorption of photons by an organic molecule causes a cascade of photophysical and photochemical processes. The scheme for photoactivation of the first excited singlet and triplet states of RB is as follows:

$$\text{RB} + h\nu \rightarrow ^1\text{RB}^* \text{ characterized by } \sigma_{01}I$$

$$^1\text{RB}^* \rightarrow \text{RB} + h\nu_F \text{ characterized by } k_r$$

$$^1\text{RB}^* \rightarrow ^3\text{RB}^* \text{ characterized by } k_{ISC}$$

$$^1\text{RB}^* \rightarrow \text{RB} \text{ characterized by } k_{IC}$$

$$^3\text{RB}^* \rightarrow \text{RB} + h\nu_{ph} \text{ characterized by } \tau_{ph}^{-1}$$

Fig. 1. Absorption spectrum of RB–PBS ($c = 10^{-5}$ M).
where $\sigma_{g0}$ is the absorption cross-section of the ground state, $k_r$ is the rate of deactivation of the first excited singlet state by fluorescence, $k_{ISC}$ is the intersystem crossing rate, $k_{IC}$ is the radiationless deactivation rate of the first excited singlet state by internal conversion and $\tau_{ph}^{-1}$ is the phosphorescence lifetime of the triplet state in an oxygen-free solution.

At high photon flux densities a second photon may be absorbed by the molecule. The related excited state absorption cross-sections and relaxation rates are given by $\sigma_{ex}$, $k_s$, or the singlet and $\tau_{TT}$, $k_{TT}$ for the triplet state:

$$\text{RB}^* + h\nu \rightarrow {^1}\text{RB}^{**} \quad \text{characterized by } \sigma_{ex}$$

$$\text{RB}^{**} \rightarrow {^3}\text{RB}^{*} \quad \text{characterized by } k_s$$

$$\text{RB}^* + h\nu \rightarrow {^3}\text{RB}^{**} \quad \text{characterized by } \sigma_{TT}$$

$$\text{RB}^* \rightarrow {^3}\text{RB}^{*} \quad \text{characterized by } k_{TT}$$

As was shown in Ref. [17], the interaction of the lowest RB triplet with molecular oxygen $O_2$ produces singlet oxygen $O_2(\Delta_g)$ with about 75% efficiency and the superoxide anion radical $O_2^-$ with about 25% efficiency:

$$\text{RB}^* + O_2 \rightarrow \text{RB} + O_2(\Delta_g) \quad \text{characterized by } k_{aO_2}$$

$$\text{RB}^* + O_2 \rightarrow \text{RB}^+ + O_2^- \quad \text{characterized by } k_{SO}$$

$\sigma_{O_2}$ is the concentration of molecular oxygen in the solution (in an oxygen-saturated solution $c_{O_2} = 1.2 \times 10^{-3}$ M [18]); $k_{a}$ and $k_{SO}$ are the rates of energy and electron transfer to form singlet oxygen and superoxide radical anion respectively. $\tau_a$ is the luminescence lifetime of singlet oxygen.

As well as the formation of photoproducts via the lowest triplet state, photochemical processes starting from higher excited triplet or singlet states cannot be excluded. For instance, as was shown in Ref. [7], RB undergoes a photochemical reaction (homolytic cleavage of carbon–iodine bonds) if it is excited with the UV radiation of a xenon arc lamp. This process was not observed on irradiation at 514 nm, indicating the involvement of a higher excited state in the photochemical reaction. The formation of photoproducts (e.g. $\text{RB}^*$ and $\Gamma$ as described in Ref. [7]) from higher excited triplet or singlet states at high photon flux densities is considered in our scheme by

$$\text{RB}^{**} \rightarrow \text{PP} \quad \text{characterized by } k_{\text{photo}}$$

$$\text{RB}^{**} \rightarrow \text{PP}' \quad \text{characterized by } k_{\text{photo}}$$

$k_{\text{photo}}$ are the formation rates for the photoproducts PP and PP' respectively.

The reaction scheme given by Eqs. (1)–(4) corresponds to a system of kinetic equations describing the incoherent interaction of organic molecules in solution with a strong laser field. The rate equation system for the normalized population densities $n_i(x, t)$ of the RB ground and excited states is given by

$$\frac{\partial n_1(x, t)}{\partial t} = \sigma_{g0} (n_2 - n_1) I + (k_r + k_{IC}) n_3 + n_3 / \tau_{ph} + k_{SO} c_{O_2} n_7 n_5$$

$$\frac{\partial n_2(x, t)}{\partial t} = \sigma_{ex} (n_1 - n_2) I - n_2 k_{PC}$$

$$\frac{\partial n_3(x, t)}{\partial t} = \sigma_{ex} (n_4 - n_3) I - (k_s + k_{\text{photo}}) n_4$$

$$\frac{\partial n_4(x, t)}{\partial t} = \sigma_{TT} (n_6 - n_5) I + k_{TT} n_6 + k_{IC} n_3 - n_5 / \tau_{ph} - (k_{SO}) + k_{a} c_{O_2} n_7 n_5$$

$$\frac{\partial n_5(x, t)}{\partial t} = n_8 / \tau_a - (k_{SO} + k_{a}) c_{O_2} n_7 n_5$$

$$\frac{\partial n_6(x, t)}{\partial t} = k_{a} c_{O_2} n_5 n_7 - n_8 / \tau_a$$

$$\frac{\partial n_7(x, t)}{\partial t} = k_{SO} c_{O_2} n_7 n_7$$

$$\frac{\partial n_8(x, t)}{\partial t} = k_{SO} c_{O_2} n_7 n_7$$

$$\frac{\partial n_9(x, t)}{\partial t} = n_9 k_{\text{photo}} + n_9 k_{\text{photo}}$$

where the photophysical parameters are the same as described in the reaction schemes (1)–(4) and $k_{PC}$ is the Frank–Condon relaxation rate constant. The normalized population densities are assigned to the energy levels (Fig. 2) as follows: $n_1$, singlet ground state $(S_0)$ of RB; $n_2$, $n_3$, first excited singlet state $(S_1)$ of RB; $n_4$, higher excited singlet state $(S_4)$ of RB; $n_5$, $n_6$, first $(T_1)$ and higher $(T_n)$ excited triplet states respectively of RB; $n_7$, $n_8$, triplet ground state and first excited singlet state respectively of oxygen; $n_9$, $n_{10}$, ground states of the RB cation radical and the superoxide anion radical respectively. The normalized population density $n_{11}$ refers to photoproduct(s) generated from higher excited singlet or triplet states of RB. It holds that $\sum_{i=1}^{11} n_i = 1$ for all $x$ and $t$.

The initial conditions at $t=0$ and $x=0$ are given by

$$n_1 + n_2 = 1$$
Fig. 2. Formal energy level scheme describing excited state absorption and possible pathways of one- and two-photon photochemistry of RB; for the photophysical parameters cf. Table 1.

where

\[ n_1 = \frac{c_{\text{sens}}}{c_{O_2} + c_{\text{sens}}} \]

\[ n_2 = \frac{c_{O_2}}{c_{O_2} + c_{\text{sens}}} \]

c_{O_2} is the molar concentration of molecular oxygen, \( c_{\text{sens}} \) is the molar concentration of RB molecules and \( n_i = 0 \) for \( i \neq 1, 7 \).

The propagation of the laser pulse in the x direction through the sample cell is described by the photon transport equation:

\[ \frac{\partial I(x,t)}{\partial t} = -N I(x,t) \left[ \sigma_{\text{o1}}(n_1 - n_2) + \sigma_{\text{ex}}(n_3 - n_4) + \sigma_{\text{TT}}(n_5 - n_6) \right] \]

where \( I \) (cm\(^{-2}\) s\(^{-1}\)) is the photon flux density of the laser pulse and \( N \) (cm\(^{-3}\)) is the total concentration of RB molecules and molecular oxygen \( (N = N_A(c_{O_2} + c_{\text{sens}}), N_A \) being Avogadro's number). The population densities \( n_i(x,t) \) in Eq. (6) are taken from the solution of Eqs. (5).

The model considers excited state absorption of the RB molecules only and not those of the photoproducts generated by energy and electron transfer to molecular oxygen. This seems to be justified for the following reasons: (i) the superoxide radical anion \( O_2^{2-} \) absorbs strongly in the UV region (250–300 nm) and not in the visible region [18]. (ii) As is known from the literature [17], the absorption spectrum of RB\(^{+}\) has a maximum at 470 nm (\( \sigma_{\text{max}} = 6 \times 10^{-17} \) cm\(^2\)) and appears to decrease sharply at longer wavelengths [17], whereas the absorption coefficient for RB\(^{2+}\) has not been measured at 532 nm. In addition, the rate constant of the electron transfer to molecular oxygen is very slow in comparison with the pulse duration. Thus the probability of absorption of a photon by the radical cation (the second with respect to the first photon which is absorbed by the RB molecule and which generates the triplet state) is very low.

3.2. Determination of excited state formation yield

The classical definition of quantum yield formulated as the ratio of the number of molecules that have reacted or have been formed to the number of photons thereby absorbed should be re-examined for the case of multiphoton processes. In general, in the case of a single-photon-initiated reaction from an excited singlet or triplet level the quantum yield of the process does not depend on the intensity of the excitation source. At high excitation intensities the probability of absorption of a second photon by an excited triplet or singlet state is a function of the excitation intensity itself. That is why Nikogosyan [19] proposed to define the photoreaction quantum yield in this case as the ratio of molecules which have reacted with respect to the total number of photons absorbed. The total number \( Q_{\text{abs}} \) of absorbed photons in an optically thin sample may be expressed by

\[ Q_{\text{abs}} = \int_0^\infty I(x,t) \left[ \sigma_{\text{o1}}(n_1 - n_2) + \sigma_{\text{ex}}(n_3 - n_4) + \sigma_{\text{TT}}(n_5 - n_6) \right] dt \]

where \( n_i \) is the number of molecules in a certain excited state \( i \), determined from the solution of the rate equation system (5).

The relative excited state formation yield is then given by

\[ \eta_i = \frac{\int_0^\infty n_i \, dt}{Q_{\text{abs}}} \]

where \( \tau_i \) is the lifetime of the excited state \( i \). It can be seen easily that the relative excited state formation rate \( \eta_i \) defined...
by Eq. (8) gives at low excitation intensities and under steady state conditions (that means \( n_2 = \sigma_{01} T_{\text{exc}} \ll 1 \) and \( n_3 = 1 \)) the fluorescence quantum yield if \( \tau_3 = k_{-1} \) and the intersystem crossing yield for \( \tau_3 = \tau_{\text{PH}} \).

In our experiments (relative enzyme inhibition yield due to singlet oxygen generation and/or radical formation by RB) instead of the excited state formation yield as defined in Eq. (8), the ratio of the number of molecules in the excited state to the number of incident photons was measured.

4. Results

The intensity-dependent transmission of RB in PBS excited with 1.4 ns laser pulses at 532 nm is shown in Fig. 3. The transmission of the sample of 0.20 at low photon flux densities agrees with the low signal transmission value that was obtained using the Beckman UV 5270 spectrophotometer. As can be seen from Fig. 3, the transmission of the sample increases with increasing photon flux density. It reaches a value of 0.67 at a photon flux density of about \( 10^{27} \text{ cm}^{-2} \text{s}^{-1} \). The increase in transmission from the beginning of non-linear absorption at \( 10^{24} \text{ photons cm}^{-2} \text{s}^{-1} \) to the maximum transmission value covers an intensity range of about 3 orders of magnitude. An analytical treatment of non-linear absorption [14] shows that such a behaviour can be explained only by an energy level scheme containing at least two consecutive absorption steps (cf. also the negative test calculation without any excited state absorption in Fig. 3). Further, because the transmission increases with increasing photon flux density, the relations \( \sigma_{\text{ex}} < (\frac{1}{2}) \sigma_{01} \) and \( \sigma_{\text{TT}} < (\frac{1}{2}) \sigma_{01} \) hold. Because of a high intersystem crossing rate (see Table 1), the triplet state of RB is populated with nearly the same efficiency as the \( S_1 \) state in the case of nanosecond excitation. For this reason it is very difficult to distinguish between a singlet–singlet and a triplet–triplet absorption utilizing only nanosecond excitation pulses. Thus, for the determination of the absorption cross-sections \( \sigma_{\text{ex}} \) and \( \sigma_{\text{TT}} \) from non-linear absorption measurements additional information is necessary, e.g. from experiments using a different excitation pulse duration.

Measurements are already available for the non-linear absorption and emission of RB obtained using picosecond

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<td>( \tau_3 )</td>
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<td>( \sigma_{\text{TT}} )</td>
<td>( (7.4 \pm 0.7) \times 10^{-17} \text{ cm}^2 )</td>
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Fig. 3. Measured (■) and calculated (——) intensity-dependent transmission of RB-PBS (\( c = 1.4 \times 10^{-4} \text{ M} \), \( \lambda_{\text{exc}} = 532 \text{ nm} \), \( \tau_{\text{imp}} = 1.4 \text{ ns} \), \( d = 0.1 \text{ cm} \)). For photophysical parameters cf. Table 1. For comparison the intensity dependent transmission (-----) for the hypothetical case of only one step absorption (\( \sigma_{\text{ex}} = 0 \text{ cm}^2 \), \( \sigma_{\text{TT}} = 0 \text{ cm}^2 \)) is also shown.
pulses [10]. The increase in transmission from the initial value of about 0.8 to a final value of nearly 1.0 covering an intensity range of 2 orders of magnitude (Fig. 4) was attributed to ground state depletion. It was concluded that there is no or only very weak $S_1 \rightarrow S_x$ absorption [10]. Because of the short pulse duration (40 ps) relative to the $S_1$ lifetime (Table 1) formation of the triplet state is not very efficient during the pulse and consequently the intensity-dependent transmission curve is hardly influenced by triplet–triplet absorption.

Both the nanosecond and the picosecond non-linear absorption curves can be fitted using the energy level scheme depicted in Fig. 2 and the rate equation system given by Eqs. (5). The photophysical parameters which were used for curve simulation are listed in Table 1. We tried to fit both curves assuming $\sigma_{ex} = 0$ cm$^2$ and varying $\sigma_{TT}$ and $T_{pe} = (k_{photo} + k_{T_1})^{-1}$. The other parameters were fixed during the fitting procedure. The minimization procedure [14] yields a triplet absorption cross-section $\sigma_{TT}$ of $(7.4 \pm 0.7) \times 10^{-17}$ cm$^2$ and an excited triplet state lifetime $\tau_{T_1} = 50 \pm 15$ ps. The goodness of fit $^1$ is scarcely influenced by the rate $k_{photo}$ of photochemical reactions starting from $T_1$. An upper limit of about $10^{10}$ s$^{-1}$ was found for the parameter.

Taking into account the experimental error, our data fit both curves (Figs. 3 and 4) well. Only the non-linear absorption curve measured with nanosecond pulses is sensitive to variations in $\sigma_{TT}$. The goodness of fit for the non-linear absorption curve measured with picosecond pulses decreases by 1 order of magnitude if the singlet–singlet absorption cross-section $\sigma_{ex}$ exceeds $10^{-18}$ cm$^2$, and more for higher $\sigma_{ex}$ (Fig. 4). Because of the low value of $\sigma_{ex}$, it was not possible to determine from the curve fitting at 532 nm the lifetime $T_{pe} = (k_{S_2} + k_{photo})^{-1}$ of the formally introduced higher excited singlet state $S_2$.

To test the reliability of the model we calculated the relative emission intensity as a function of incident photon flux density of the picosecond laser pulses. For this purpose we solved the differential equation system (5) using the photophysical parameters presented in Table 1 and the experimental data given in Ref. [10]. From this solution the normalized emission intensity was calculated as the number of molecules emitting from $S_2$ ($n_{S_2} = \int_0^\infty n_{S_2}(x = 0, t) \, dt$) divided by the number of incident photons. The shape of the curve calculated this way agrees with the measured non-linear emission curve of RB (Fig. 5) given in Ref. [10].

A further test of the model was to compare the decrease in enzyme inhibition efficiency observed at higher intensities [10] with the calculated yield of toxic species. First we calculated the excited state formation yields for picosecond and nanosecond excitation (Figs. 6 and 7). In both cases a decrease in $\eta_{S_1}$ and $\eta_{T_1}$ at high excitation intensities was found. On the contrary, the higher excited triplet state formation yield $\eta_{T_2}$ increased with increasing intensity. In the

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$^1$ The goodness of fit is defined as

$$\left[ \frac{1}{n} \sum_{i=1}^{n} \left( \frac{T_i - \bar{T}}{\bar{T}} \right)^2 \right]^{-1}$$

where $T_i$, $\bar{T}$, are the measured and calculated transmission values and $n$ is the number of measured points.
The efficiency of a photoreaction \( k_{\text{photo}} \) starting from higher excited triplet state \( T_n \) is lower than that of an energy or electron transfer starting from \( T_1 \). The relative contributions of photoproducts (level 11) generated by the higher excited triplet state, of \( \text{RB}^{\ddagger} \) radicals (level 9), of superoxide radical anions \( \text{O}_2^{\ddagger}^- \) (level 10) and of singlet oxygen (level 8) to the enzyme inhibition...
reported in Ref. [10] will depend on their quantum yields for formation and their reactivity with the enzyme. Photo-products (e.g. RB* and T) formed from an upper excited state of RB have been shown to be about 200-fold more reactive than singlet oxygen [7]. Assuming that the main mechanism leading to enzyme inhibition in the presence of oxygen induced by irradiation of RB at 532 nm using picosecond pulses is the generation of singlet oxygen, we calculated the quotient of the overall number of molecules of level 8 (\( \hat{n}_8 = \int_{\Omega} n_8(x=0, t) \, dt \)) and the number of incident photons.
as a function of the photon flux density. At low intensity, the calculated relative $^1O_2$ yield is 0.75 reflecting the quantum yield for its formation by energy transfer from RB triplet [17]. The relative $^1O_2$ yield decreases with increasing photon flux density reflecting the greater transmission of these samples (cf. Fig. 4). As can be seen from Fig. 8 the calculated curve has the same general shape as the experimental curve obtained with picosecond excitation. A contribution from radicals (RB++, $O_2^-$) or photoproducts generated from higher excited triplet state (levels 9-11) is possible because, as shown in Fig. 8, above a photon flux density of about $10^{27}$ cm$^{-2}$ s$^{-1}$ the enzyme inhibition is greater than that predicted by the calculated relative singlet oxygen production.

However, the contribution by photoproducts to enzyme inhibition is only possible if the photoproducts were formed with 100% efficiency from the $T_r$ state or are more than 100-fold more effective than $^1O_2$ since, in this intensity range, the yield of $T_1$ is about 100-fold greater than that of $T_r$.

### Discussion

From the measurements and calculations it can be concluded that the scheme of RB photoactivation given by Eqs. (1-5) describes the picosecond and nanosecond non-linear absorption behaviour, and the picosecond non-linear emission behaviour as well as earlier experiments concerning the intensity-dependent enzyme photoinhibition mediated by RB.

The picosecond intensity-dependent transmission measurements were fitted well when absorption from $S_1$ to $S_6$ was not permitted to compete with absorption from $S_0$. In contrast, fitting the nanosecond intensity-dependent transmission measurements required inclusion of competing absorption by $T_1$. These results confirm the earlier assumption [10] that there is no or only very weak $S_1 \rightarrow S_6$ absorption for RB in aqueous solution at 532 nm. The relation $\sigma_{ex}(532 \text{ nm}) < 10^{-18} \text{ cm}^2$, which means that $\sigma_{ex} < 10^{-2} \sigma_{01}$, characterizes RB as one of the very few known examples where excited state absorption within the spectral region of the $S_1 \rightarrow S_1$ transition is absent, although there is a transition at twice the $S_1$ energy: $S_0 \rightarrow S_1 \rightarrow S_6$ (532 nm) and $S_0 \rightarrow S_6$ (266 nm).

From our measurements and calculations the lifetime $\tau_{T_1}$ of $T_1$ and the absorption cross-section $\sigma_{TT}$ for the transition $T_1 \rightarrow T_r$ were estimated. The relatively long-living $T_r$ state (50 ps) favours photochemical reactions starting from $T_r$. Unfortunately it was impossible to determine from our experimental data the exact rate constant for these reactions; only an upper limit ($10^{10} \text{ s}^{-1}$) of $k_{\text{phot}}$ could be estimated. From the literature some other molecules exhibiting a relatively long higher excited state lifetime are known. For example, the lifetime of the $T_2$ state of several substituted anthracenes amounts to 20-200 ps [23], and the effective $T_2$ photochemistry of anthracene in acetonitrile [24] was attributed to a long-living excited triplet state.

The absorption cross-section $\sigma_{TT}$ found by our calculations is about 2.4-fold lower than that $\sigma_{01}$ for the $S_0 \rightarrow S_1$ transition. It corresponds to an absorption coefficient of 19 600 M$^{-1}$ cm$^{-1}$. In literature, no data at 532 nm seem to be available; at 580 nm and 590 nm values of 3400 M$^{-1}$ cm$^{-1}$ and 14 500 M$^{-1}$ cm$^{-1}$ respectively have been reported [20].

The approach presented here may be useful for initial evaluation of dyes to be used for intensity-dependent photosensitization [8] because it allows estimation of the cross-sections for absorption by the lowest singlet and triplet excited states in comparison with that for the ground state. Sequential two-photon absorption to upper singlet states will be enhanced as $\sigma_{ex}$ approaches $\sigma_{01}$ and $\tau_F > \tau_{\text{imp}}$. Likewise, sequential two-photon absorption to upper triplet states will be favoured when $\sigma_{TT}$ approaches $\sigma_{01}$ and $\tau_F < \tau_{\text{imp}}$ (whereby $\tau_F$ should be dominated by $k_{\text{ISC}}$). The wavelength to be used for an optimization of the intensity-dependent photochemistry can be selected by measuring the intensity-dependent transmission at several wavelengths, calculating the absorption parameters and choosing the wavelength with optimal values of $\sigma_{01}$, $\sigma_{ex}$ or $\sigma_{TT}$. If the relevant excited state parameters of the molecule have been determined, the laser parameters ($\lambda$, $\tau_{\text{imp}}$, and $I$) for an optimized excitation regime can be calculated.

The capability of predicting the intensity-dependent formation yields of the excited state species is useful for evaluating whether or not sequential two-photon absorption produces a biological effect in cells. For example, if the ratio of calculated singlet oxygen yield to the relative photosensitization by a dye decreases significantly with increasing intensity, it will indicate that reactive, toxic species produced from upper excited states are contributing to the biological effect.

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### References


