Diffusion-controlled sensitization of photocleavage reactions on surfaces†

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The kinetic rate equation for the photosensitized cleavage reaction of surface-bound photolabile chromophores with free diffusion of sensitizer molecules from the bulk of a solution to the surface is derived by determining the stationary solution of a diffusion equation with suitable boundary conditions. The relation between the phenomenological rate constant for the photosensitized reaction at the surface and in the bulk is established. Applying the result to the analysis of an experimental example, the origin of the quasi zeroth-order kinetics of the sensitized reaction is revealed. A theoretical comparison of intramolecular sensitization in photocleavable protecting groups with a molecular antenna and sensitization with the freely diffusing sensitizer shows that in a typical case sensitization with free diffusion is more effective than intramolecular sensitization for sensitizer concentrations higher than 5 mM.

Introduction

In photolithographic DNA chip technology, photocleavable protecting groups caging the reactive mononucleotide building blocks play a key role because they allow for a controlled step-wise and spatially addressable assembly of oligonucleotides in an efficient manner.^{1–3} Apart from DNA chips, the fabrication of microarrays of other biomolecules at surfaces has also been reported.^{4–7} In a recent paper by San Miguel *et al.*,⁸ the photodeprotection of caged surfaces was investigated with seven different photocleavable groups and their photosensitivity at different wavelengths were quantified, and wavelength selectivity was demonstrated. Besides others, the *o*-nitrobenzyl (oNB) group and its derivatives have figured most prominently as photoremovable protecting groups in the photolithographic synthesis of oligonucleotide arrays.^{9,10} The photolabile 2-(2-nitrophenyl) propoxycarbonyl (NPPOC) group introduced by Pfeleiderer and coworkers¹¹ has been in the focus of mechanistic investigations in our group.¹² As for many protecting groups of the oNB class, one of its drawbacks is a weak absorption in the first UV band that limits the rate of its light-induced photocleavage. To overcome this problem we took advantage of triplet sensitization as an efficient method to populate the photoreactive state of the

NPPOC chromophore.¹³ For this purpose, thioxanthone, an efficient triplet sensitizer, proved to be most suitable.

As for triplet–triplet energy transfer processes in general, the Dexter exchange mechanism of the process requires close contact of triplet energy donor and acceptor. In terms of spatial restrictions, three different scenarios have been investigated: (i) homogeneous bulk solutions containing free donor and acceptor in random 3D distribution,¹³ (ii) solutions of free donor in contact with a surface loaded with a monomolecular layer of the acceptor,¹³ and (iii) donor/acceptor dyads with flexible covalent linkage of donor and acceptor.^{14–16} In homogeneous solution (i), the calculation of the quantum yield of sensitization is straight forward by applying the usual Stern–Volmer kinetics:

$$\phi_{\text{sens}}(3\text{-D}) = \phi_{\text{isc}} \frac{k_{\text{ET}}^{(2)} c_{\text{A}}}{k_{\text{ET}}^{(2)} c_{\text{A}} + \sum_i k_{\text{des},i}} \quad (1)$$

Here ϕ_{isc} is the intersystem crossing quantum yield of the sensitizer, $k_{\text{ET}}^{(2)}$ the second order rate constant of triplet energy transfer, c_{A} the acceptor concentration and the $k_{\text{des},i}$ account for all possible deactivation channels of the sensitizer triplet apart from energy transfer. In the case of intramolecular linked dyads of donor and acceptor (iii), the energy transfer can be described as a first-order rate process. Hence:

$$\phi_{\text{sens}}(1\text{-D}) = \phi_{\text{isc}} \frac{k_{\text{ET}}^{(1)}}{k_{\text{ET}}^{(1)} + \sum_i k_{\text{des},i}} \quad (2)$$

In this paper, we consider the situation (ii) of a sensitizer homogeneously distributed in a solution film in contact with a bound monolayer of a substrate caged with a photocleavable protecting group as it is schematically depicted in Fig. 1. The analogous kinetic problem for thermal reactions between a reactant in

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Fig. 1 Schematic representation of intermolecular triplet sensitization of photolabile groups on a surface.

the bulk volume and another reactant bound at a surface in contact with the bulk solution,^{17,18} and in other restricted spaces¹⁹ has been treated before. The scenario described by the equation

is of interest for many biochemical applications such as reactions with surface-immobilized enzymes or antigens, but also cell adhesion processes in living systems.¹⁸ However, in case of thermal reactions there is only the non-stationary kinetics of re-establishing an equilibrium after a perturbation, *e.g.* the transient bleaching of a surface adsorbed fluorescing chromophore.¹⁸ In our case of a triplet sensitized cleavage reaction at a surface, we are interested in the photokinetics under conditions of continuous illumination. Thus we will derive a stationary solution for the rate of sensitization and present the theoretical justification of a kinetic analysis employed in our earlier work.¹³

Results

Theory

The situation to be simulated is represented in Fig. 1. The photocleavable protecting groups (PG) caging the termini of the surface-bound molecules are exposed to the attack of the excited sensitizer molecules in the supernatant solution. The surface density of unreacted PGs is denoted as σ_{PG} , the volume concentration of the sensitizer and excited sensitizer as c_{sens} and c_{sens^*} , respectively. The diffusion of the sensitizer molecules in solution is characterized by a diffusion constant D . They are continuously excited by constant monochromatic illumination with a photon irradiance E_p and the excited triplet states are characterized by a lifetime τ_0 . In the horizontal direction, the size of the chip is regarded as infinite and uniform, variations of concentrations occur only in the z -direction perpendicular to the surface.

The excited sensitizer can only transfer energy if it reaches the photolabile protecting group on the chip surface ($z = 0$), whereby the rate at which the energy is transferred can be

assumed proportional to the concentration $c_{\text{sens}^*}(0)$ of excited sensitizer at the surface and to the surface density σ_{PG} of photocleavable protecting groups.

$$\dot{\sigma}_{\text{PG}}(t) = \eta_c k_{\sigma} \sigma_{\text{PG}} c_{\text{sens}^*}(0) \quad (4)$$

The proportionality constant k_{σ} represents a rate constant of the same dimension as a second order rate constant for a homogeneous bulk reaction. However, its value cannot be the same as for k_{ET} , the rate constant of energy transfer between donor and acceptor in the bulk. We will come back to the relation between these two rate constants at the end of our derivation. The parameter η_c denotes the efficiency of the cleavage reaction after quenching of an excited sensitizer molecule by energy transfer or any other interaction with the acceptor has taken place.

The spatial dependence of the concentration c_{sens^*} of excited triplet sensitizer can be expressed as follows

$$\frac{\partial c_{\text{sens}^*}(z, t)}{\partial t} = D \frac{\partial^2 c_{\text{sens}^*}(z, t)}{\partial z^2} - \frac{1}{\tau_0} c_{\text{sens}^*}(z, t) + I \quad (5)$$

The first term on the right hand side describes the diffusional motion of the excited triplet sensitizers according to Fick's second law. The second term describes the decay rate of the excited triplets and refers to relaxation of excitation through all deactivation channels except for energy transfer or any other quenching by the PGs. The third term accounts for continuous formation of excited triplets through excitation of ground state sensitizer molecules. It is assumed that the solution layer is sufficiently thin as to neglect weakening of the light on its way through the liquid film. Under such conditions, the pump term is given by

$$I = \ln(10) \epsilon_{\text{sens}} \phi_{\text{isc}} E_p c_{\text{sens}, 0} \quad (6)$$

where ϵ_{sens} is the molar absorption coefficient of the sensitizer at the irradiation wavelength and ϕ_{isc} is the quantum yield of triplet formation.

A general solution of eqn (5) can only be obtained numerically. However, for a given surface density σ_{PG} of protecting groups, a quasi-stationary concentration profile of c_{sens^*} can be assumed, and the time-differential in eqn (2) can be set to 0.

$$D \frac{\partial^2 c_{\text{sens}^*}(z)}{\partial z^2} - \frac{1}{\tau_0} c_{\text{sens}^*}(z) + I = 0 \quad (7)$$

For $z \rightarrow \infty$, the boundary condition to eqn (7) is

$$\lim_{z \rightarrow \infty} \frac{\partial^2 c_{\text{sens}^*}(z)}{\partial z^2} = 0 \quad (8)$$

under which condition a stationary flux is attained. Combining eqn (7) and (8), one obtains for the stationary concentration of excited sensitizers far from the reactive surface

$$\lim_{z \rightarrow \infty} c_{\text{sens}^*}(z) = I \tau_0 \quad (9)$$

For $z \rightarrow 0$, the boundary condition for eqn (7) is derived from the fact that under stationary conditions the diffusional flux density of excited sensitizer molecules at the surface must equal the rate $\dot{\sigma}_{\text{PG}}$ as given in eqn (4) divided by the cleavage efficiency η_c , *i.e.*

$$D \left(\frac{dc_{\text{sens}^*}(z)}{dz} \right)_{z=0} = k_{\sigma} \sigma_{\text{PG}} c_{\text{sens}^*}(0) \quad (10)$$

The inhomogeneous differential eqn (7) is transformed to a homogeneous one by introducing the substitution:

$$c_{\text{sens}^*} = \tilde{c}_{\text{sens}^*} + I\tau_0 \quad (11)$$

where $\tilde{c}_{\text{sens}^*}$ represents the deviation between the actual concentration of excited sensitizer and the stationary value $I\tau_0$ in the bulk of the solution. Substituting eqn (11) into eqn (7) yields

$$D \frac{\partial^2 \tilde{c}_{\text{sens}^*}(z)}{\partial z^2} - \frac{1}{\tau_0} \tilde{c}_{\text{sens}^*}(z) = 0 \quad (12)$$

with the general solution

$$\tilde{c}_{\text{sens}^*}(z) = C_1 \exp(-\kappa z) + C_2 \exp(\kappa z) \quad (13)$$

where the parameter κ is defined as

$$\kappa = (D\tau_0)^{-1/2} \quad (14)$$

Using the transformed boundary conditions

$$\lim_{z \rightarrow \infty} \tilde{c}_{\text{sens}^*}(z) = 0 \quad (15)$$

and

$$D \left(\frac{d\tilde{c}_{\text{sens}^*}(z)}{dz} \right)_{z=0} = k_{\sigma} \sigma_{\text{PG}} \tilde{c}_{\text{sens}^*}(0) + k_{\sigma} \sigma_{\text{PG}} I\tau_0 \quad (16)$$

we obtain from eqn (15)

$$C_2 = 0 \quad (17)$$

and from eqn (16)

$$-\kappa C_1 = \frac{k_{\sigma}}{D} \sigma_{\text{PG}} C_1 + \frac{k_{\sigma}}{D} \sigma_{\text{PG}} I\tau_0 \quad (18)$$

yielding

$$C_1 = -\frac{k_{\sigma} \sigma_{\text{PG}} I\tau_0}{\kappa D + k_{\sigma} \sigma_{\text{PG}}} \quad (19)$$

Thus, combining eqn (11), (13), (17) and (19), we obtain

$$c_{\text{sens}^*}(z) = -\frac{k_{\sigma} \sigma_{\text{PG}} I\tau_0}{\kappa D + k_{\sigma} \sigma_{\text{PG}}} \exp(-\kappa z) + I\tau_0 \quad (20)$$

From the last equation we obtain for $c_{\text{sens}^*}(0)$ the concentration of the excited sensitizer in contact with the surface

$$c_{\text{sens}^*}(0) = \frac{\kappa D}{\kappa D + k_{\sigma} \sigma_{\text{PG}}} I\tau_0 \quad (21)$$

Finally, substituting eqn (21) into eqn (4) yields the rate equation for the surface density of protecting groups

$$\dot{\sigma}_{\text{PG}} = -\eta_c k_{\sigma} \sigma_{\text{PG}} \frac{\kappa D}{\kappa D + k_{\sigma} \sigma_{\text{PG}}} I\tau_0 \quad (22)$$

Eqn (22) comprises two interesting limiting cases.

$$\begin{aligned} \text{(i) If } k_{\sigma} \sigma_{\text{PG}} \gg \kappa D \\ \dot{\sigma}_{\text{PG}} = -\eta_c \kappa D I\tau_0 = -\eta_c I \sqrt{D\tau_0} \end{aligned} \quad (23)$$

In this case, the reaction rate is constant, *i.e.* the order of the reaction with respect to the unreacted protecting groups is zero. Since the quantity $\sqrt{D\tau_0}$ corresponds to the average length an excited sensitizer molecule can diffuse during its lifetime, the

rate given by eqn (23) is equivalent to the production rate of excited sensitizer molecules created up to a depth of $\sqrt{D\tau_0}$ over a unit surface area multiplied by the reaction efficiency η_c after energy transfer.

$$\begin{aligned} \text{(ii) If } k_{\sigma} \sigma_{\text{PG}} \ll \kappa D \\ \dot{\sigma}_{\text{PG}} = -\eta_c k_{\sigma} I\tau_0 \sigma_{\text{PG}} \end{aligned} \quad (24)$$

In this case, the reaction is of first order with respect to the unreacted protecting groups. The effective first order rate constant $\eta_c k_{\sigma} I\tau_0$ implies a concentration of excited sensitizer triplets at the surface that is identical to that far away from the surface.

It is of basic interest to relate the rate constant k_{σ} to the rate constant k_V of triplet–triplet energy transfer for the case that both donor and acceptor are freely diffusing in the bulk volume. Under conditions of low surface concentration of the acceptor (*cf.* Fig. 2) it was derived by Burlatskii^{17,20} that

$$k_{\sigma} = \frac{2\pi D_D R_{\text{DA}} N_A}{4\pi D_D R_{\text{DA}} N_A + k_1} k_1 \quad (25)$$

Here k_1 is the second order rate constant of energy transfer in the bulk that would be measured for infinitely fast diffusion. D_D is the diffusion coefficient of the donor, R_{DA} the distance at which energy transfer takes place. This expression may be compared with the normal Collins and Kimball result²¹ for the rate constant k_V of a reaction with both reactants in the volume of the solution.

$$k_V = \frac{4\pi (D_A + D_D) R_{\text{DA}} N_A}{4\pi (D_A + D_D) R_{\text{DA}} N_A + k_1} k_1 \quad (26)$$

As a rationale for eqn (25) one can argue, that if the acceptor molecules were fixed but accessible from all directions, the standard Collins–Kimball eqn (26) with $D_A = 0$ would result, which gives just twice the value as for the case when they are fixed on a surface and half of the space were inaccessible for the donor molecules (*cf.* Fig. 2).

Fig. 2 Top: Situation considered in the derivation of rate constant k_{σ} by Burlatskii.^{17,20} Bottom: Situation leading to the Collins–Kimball result, *i.e.* twice the value given by eqn (25). The acceptor molecules (circles or semicircles, respectively) are considered as fixed. The excited donor molecules (stars) can diffuse freely only in the upper half space (top) or in the full space (bottom).

Assuming that $D_A = D_D = D$ the following general relation between k_{σ} and k_V can be derived:

$$k_{\sigma} = \frac{1}{2} \frac{k_V}{1 + \frac{k_V}{k_{V,d}}} \quad (27)$$

where the diffusion controlled rate constant $k_{V,d}$ in the volume is given by

$$k_{V,d} = 8\pi DR_{DA}N_A \quad (28)$$

We will come back to this relation when discussing an experimental example in the light of the present theory.

Comparison with experiment

Fig. 3 shows the photokinetic data of experiments conducted with surface bound thymidine caged by the 2-(2-nitrophenyl) propoxycarbonyl (NPPOC) protecting group.¹³ The diagram includes both the results for photocleavage by direct irradiation and by sensitized photocleavage with thioxanthone as a triplet sensitizer in solution. In terms of the notation used in the present paper the full rate equation for combined direct and triplet sensitized reaction is given by:

$$\dot{\sigma}_{PG} = -E_P \ln(10) \times \left(\varepsilon_{PG} \phi_{dir} \sigma_{PG} + \eta_C k_{\sigma} \sigma_{PG} \frac{\kappa D}{\kappa D + k_{\sigma} \sigma_{PG}} \varepsilon_{sens} \phi_{isc} c_{sens,0} \tau_0 \right) \quad (29)$$

Here ε_{PG} is the molar absorption coefficient of the photocleavable protecting group at the wavelength of irradiation and ϕ_{dir} the quantum yield of photocleavage by direct excitation. Using the substitutions

$$x = \frac{\sigma_{PG}}{\sigma_{PG,0}} \quad (30)$$

where $\sigma_{PG,0}$ is the surface density of photocleavable groups for full surface coverage at $t = 0$, and

$$\theta = E_J t = f_{PJ} E_P t \quad (31)$$

where E_J is the irradiance in $J\ cm^{-2}\ s^{-1}$ and f_{PJ} the conversion factor relating E_P to E_J , eqn (29) is reduced to the form

$$\frac{dx}{d\theta} = -\tilde{a}_{dir} x - \tilde{a}_{sens} \frac{bx}{1 + bx} \quad (32)$$

wherein the parameters $\tilde{a}_{dir}$, $\tilde{a}_{sens}$ and b are given by

$$\tilde{a}_{dir} = \frac{\ln(10) \varepsilon_{PG} \phi_{dir}}{f_{PJ}} \quad (33)$$

$$\tilde{a}_{sens} = \frac{\ln(10) \varepsilon_{sens} c_{sens,0} \phi_{sens}^0 \sqrt{D\tau_0}}{\sigma_{PG,0} f_{PJ}} \quad (34)$$

and

$$b = \frac{k_{\sigma} \sigma_{PG,0}}{\kappa D} \quad (35)$$

The product $\eta_C \phi_{isc}$ has been contracted to the parameter ϕ_{sens}^0 which is more directly accessible from experiment than the individual factors.²²

From the present derivation, it is possible to relate the phenomenological rate constant k_{σ} in b to the kinetic constant k_V in bulk solution. Using eqn (27) one obtains:

$$b = \frac{1}{2} \frac{k_V}{1 + \frac{k_V}{k_{V,d}}} \frac{\sigma_{PG,0}}{\kappa D} \quad (36)$$

Fig. 3 Experimental results for direct and triplet-sensitized photokinetics of photocleavage of the NPPOC group from a chip surface illuminated at 366 nm. The solid lines represent best fits using the theoretical rate equations given in the text. (1) For unsensitized illumination ($b = 0$). (2) With $b = 57$. Adapted from ref. 13 by converting relative fluorescence intensities into the unreacted fraction of photolabile surface groups.

Integrating eqn (32) allowed determination of the parameters $\tilde{a}_{dir}$ and $\tilde{a}_{sens}$ by fitting the photokinetic curves.¹³ From the unsensitized kinetics curve, a value of $0.25\ J^{-1}\ cm^2$ followed for $\tilde{a}_{dir}$, and fitting the sensitized curve with the use of the latter value yielded $0.24\ J^{-1}\ cm^2$ for $\tilde{a}_{sens}$. Whereas the parameter $\tilde{a}_{dir}$ completely determines the unsensitized kinetic curve, the combination of $\tilde{a}_{dir}$ and $\tilde{a}_{sens}$ only determine the initial slope of the sensitized curve where its linear behavior indicates that the limiting kinetic case of zeroth order (*cf.* eqn (23)) is prevailing. The transition region between zeroth order and first order is determined by the parameter b . For high values of b , the reaction remains of zeroth order up to residual values of $\sigma_{PG}/\sigma_{PG,0}$ close to the detection uncertainty. This is the behavior shown by the experimental results. Thus a value of infinity was assumed for b in ref. 13. The present theoretical treatment allows the estimation of a finite value of b according to eqn (36). The following values can be taken from ref. 13: $\varepsilon_{PG}(366\ nm) = 2.47 \times 10^5\ cm^2\ mol^{-1}$, $\varepsilon_{sens}(366\ nm) = 4.9 \times 10^6\ cm^2\ mol^{-1}$, $c_{sens,0} = 5 \times 10^{-7}\ mol\ cm^{-3}$, $D = 5 \times 10^{-6}\ cm^2\ s^{-1}$, $\phi_{dir} = 0.14$. According to a recent determination of ϕ_{dir} and ϕ_{sens}^0 in bulk DMSO, their values are 0.39 and 0.46, respectively.²² Assuming, as in ref. 13, that the ratio of the two quantum yields is the same on the surface and in the bulk, we obtain $\phi_{sens}^0 = 0.165$ for the quantum yield on the chip. As a correction to ref. 13, we note that the triplet lifetime in the experiment was much longer than assumed previously. In air-saturated DMSO solution the triplet lifetime is about $4\ \mu s$, whereas for oxygen-free DMSO it was found to be $20\ \mu s$.²² The solutions in the maskless array synthesizer system¹³ were flushed with argon. However, the oxygen content of the solutions was probably not zero. Thus assuming an intermediate triplet lifetime of $10\ \mu s$ appears reasonable. Then, from eqn (34) we obtain $\sigma_{PG,0} = 8.3 \times 10^{-11}\ mol\ cm^{-2}$ corresponding to 5×10^{13} molecules per cm^2 . The experimental value of k_V in DMSO is $1.2 \times 10^{12}\ cm^3\ mol^{-1}\ s^{-1}$.²²

Finally, substituting all known parameter values into eqn (36) yields $b = 57$. This value represents an order of magnitude, where, according to eqn (32), the rate contribution of the sensitized reaction is in fact constant down to almost complete cleavage of the photolabile protecting groups. According to eqn (36), the parameter b is proportional to the surface density σ_{PG} and to the factor $1/\kappa = \sqrt{D\tau_0}$. Note that the further dependence on D in the denominator is approximately cancelled by the proportionality of k_V to D in case of nearly diffusion-controlled energy transfer. Thus it is the high surface density of protecting groups on the chip and the long distance of diffusion during the lifetime of the sensitizer triplets that lies at the basis of the effective zeroth-order kinetics.

Discussion

The quasi zeroth-order kinetics (*cf.* Fig. 3) obtained under sensitization by freely diffusing energy donors from bulk solution to the surface may be of particular advantage in the practical chip synthesis process because the completion of the cleavage reaction exhibits a rather sharp end as opposed to the exponential tail resulting from first order kinetics. The limiting rate is essentially determined by the number of photons that can be harvested in a zone of width $\sqrt{D\tau_0}$ and is proportional to the concentration $c_{sens,0}$ of the sensitizer in the solution (*cf.* eqn (34)). On the other hand, large sensitivity enhancements have been achieved by photolabile protecting groups with a sensitizing chromophore covalently linked as an intramolecular antenna to the photocleavable chromophore.¹⁴ With these compounds, the photocleavage kinetics is essentially a first order rate process. Thus it is an interesting question if there are conditions under which free diffusion of the sensitizer can beat intramolecular sensitization.

The rate equation for reaction by intramolecular sensitization is

$$\dot{\sigma}_{PG} = -\ln(10)E_P\phi_{sens}^0\epsilon_{sens}\sigma_{PG} \quad (37)$$

from which we obtain in analogy to eqn (32)

$$\frac{dx}{d\theta} = -k_J x \quad (38)$$

with the rate constant k_J given by

$$k_J = \frac{1}{f_{PJ}}\ln(10)\phi_{sens}^0\epsilon_{sens} \quad (39)$$

The resulting kinetics curve is shown in Fig. 4 together with the kinetics of free sensitization at various concentrations of the sensitizer thioxanthone. As can be seen for the system under consideration, equal initial rates in the cases of intermolecular and intramolecular sensitization is achieved for $c_{sens,0} = 10$ mM, a concentration which is not unpractical. In case of free sensitization with this concentration, the reaction is completed at *ca.* 0.2 J cm^{-2} where, however, the curve for intramolecular sensitization is still 30% uncompleted. Due to the zeroth-order behavior even the case of $c_{sens,0} = 5$ mM would be advantageous over the intramolecular sensitization case. These results should find due consideration in the practical process of high density DNA chip synthesis.

Fig. 4 Kinetic comparison of photocleavage by intramolecular and intermolecular sensitization. Dotted curve: intramolecular sensitization, solid curve: intermolecular sensitization with $c_{sens,0} = 10$ mM, dashed curve: 5 mM, dashed-dotted line: 2 mM.

Conclusions

A surprising finding of a previous experimental investigation of diffusion dependent sensitization of a surface reaction from the bulk,¹³ namely an apparent zeroth-order kinetics, can be explained in the light of the present derivation of the rate law in terms of diffusion theory. A parameter b which is proportional to the surface density of reacting groups and the average distance of the sensitizer triplets during their lifetime has been identified as the critical quantity. Zeroth-order kinetics ensue if $b \gg 1$. It should be of practical interest that for high concentrations of sensitizer in solution, the diffusion controlled sensitization process is even more effective than intramolecular sensitization by chemically linked antenna groups.

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