Evaluation of Photodynamic Therapy Agents through Transient Grating Measurements

Andrew C. Beveridge, Barbara A. Bench, Sergiu M. Gorun,* and Gerald J. Diebold*

Department of Chemstry, Brown University, Providence, Rhode Island 02912

Received: December 31, 2002

The diffracted-light signals produced in transient grating experiments by phthalocyanine dyes, candidates for photodynamic therapy agents, are shown to be strongly dependent on the presence of oxygen in solution. Data indicate a facile transfer of energy from the excited dye to oxygen in solution to form excited $^1\Delta_g$ oxygen. The efficiency of excited-oxygen production can be determined by recording the thermal mode of wave motion in a transient grating experiment. The triplet-state lifetimes and quantum efficiencies for the formation of $^1\Delta_g$ oxygen determined from the experiments are found to depend on structural details of the dyes, which can be rationalized by considering π-orbital interactions. The salient features of the transient grating method for evaluating type II photodynamic therapy agents are that the method requires no chemical species to be added to the dye solution that complicate the photochemistry and that the method is sensitive enough that experiments can be carried out at micromolar concentrations.

Introduction

Photodynamic therapy refers to the optical excitation of a dye to kill living tissue, typically a tumor. In practice, the photodynamic therapy dye can be injected intravenously or sometimes directly into a tumor. When tissue is to be destroyed, a specific site is irradiated with a directed beam of optical radiation, which excites the dye and has the effect of killing only those cells at the site of the tumor. Over time, the body slowly digests the dead tissue in much the same way as bruised tissue is healed. Photodynamic therapy has advantages over surgical tumor removal in that there is little chance of infection, no bleeding, reduced disfigurement, and less trauma to the patient. There are two mechanisms ascribed to the action of the excited dyes. In the “type I” mechanism, the dye binds to a critical component of the cell, resulting in cell death through chemical reaction or energy transfer, whereas in the “type II” mechanism, the optical energy of the photoexcited dye is transferred to ground-state oxygen, raising it to its $^1\Delta_g$ state, which, in turn, kills cells through chemical or energy-transfer processes.

Here we describe the application of the transient grating method for determining the efficiency of producing $^1\Delta_g$ O_2 by a new photodynamic therapy agent, 1,4,8,11,15,18,22,25-octafluoro-2,3,9,10,16,17,23,24-octa-perfluoro-isopropyl zinc phthalocyanine,[1] referred to here as F_{64}PcZn, and two other dyes, (protio) zinc phthalocyanine, abbreviated PcZn, and 1,2,3,4,8,9,10,11,15,16,17,18,22,23,24,25-hexadecafluoro-29H,31H-zinc phthalocyanine, abbreviated F_{16}PcZn. The structures of the PcZn, F_{16}PcZn, and F_{64}PcZn dyes are shown in Scheme 1.

As shown in this scheme, the three dyes constitute a systematic series of isoelectronic Zn complexes in which only peripheral modifications are performed to alter the electronic density of the common aromatic macrocycle and metal center. These complexes produce singlet oxygen and thus may act via a type II mechanism. As described elsewhere,[1] the electronic deficiency of the metal center increases in the order PcZn < F_{16}PcZn < F_{64}PcZn. The increased level of fluorination, moving across this series, is particularly beneficial both in enhancing the hydrophobicity of the dyes and in inducing a potentially favorable partition coefficient in hydrocarbon-based cellular components such as membranes or fatty tissues. The fluorine-rich environment may favor O_2 binding and activation, necessary for a type II mechanism to be operative. Another significant advantage of fluorination, if this mechanism is operational, is that the replacement of C−H by C−F bonds gives the molecule higher chemical and thermal stability and thus renders the dye resistant to oxidation by the singlet oxygen it produces. The higher level of aliphatic C−F bonding in F_{64}PcZn, compared with that in F_{16}PcZn, also lessens the susceptibility of the dye to nucleophilic attack.

We report application of the transient grating method, configured with an optical fringe spacing that places the decay of the thermal mode of wave motion on a time scale commensurate with the lifetime of $^1\Delta_g$ O_2, to measurements of the excited-state lifetimes of the dyes and to the determination of the quantum efficiencies for $^1\Delta_g$ O_2 production. The Theory section describes the experimental work and method of data analysis based on a three-level model for the energy levels of the dye. The Experiments section reports data for the triplet-state lifetimes and the efficiency of excited O_2 production for the three dyes. Control experiments are carried out with dyes previously shown to generate $^1\Delta_gO_2$, to provide a comparison with the results of measurements using conventional methods. The Discussion section comments on both the usefulness of the transient grating method for the evaluation of photodynamic therapy agents on the kinetics of energy transfer found in the experiments. The salient features of the transient grating method for evaluating photodynamic therapy agents are that no additional chemical species that typically complicate the photochemistry are required to perform the measurements and that the measurements can be carried out at micromolar concentrations.

Theory

Briefly stated, the transient grating technique[2] employs two pulsed, phase-coherent light beams, referred to as pump beams,

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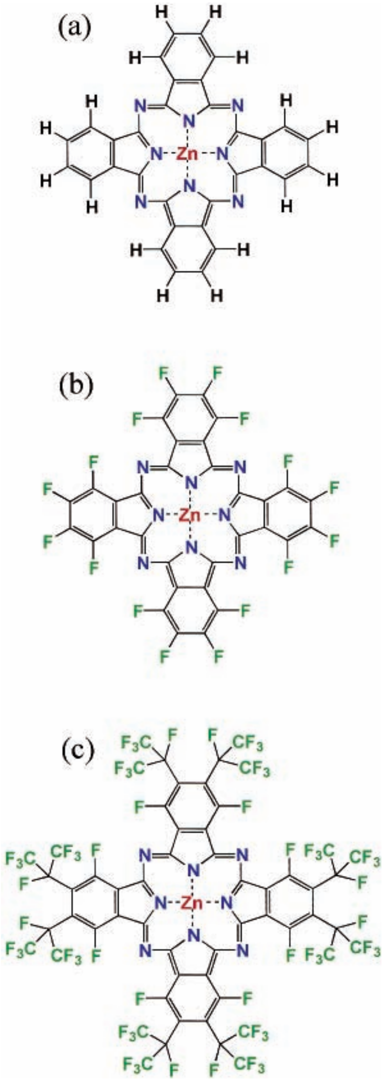
* Corresponding authors. E-mail: gerald_diebold@brown.edu and sergiu_gorun@brown.edu.

10.1021/jp022646e CCC: \$25.00

SCHEME 1: (a) PcZn; (b) F_{16}PcZn; (c) F_{64}PcZn



that are directed to intersect inside a cuvette, forming a series of nodes and antinodes in the electrical field in space. If the beams intersect in a weakly absorbing medium energy is deposited only in the antinodal regions, creating a sinusoidal excitation of dye molecules in space with a fringe spacing governed by the wavelength of the radiation and the angle at which the beams cross. Deactivation of excited molecular states results in heat deposition that launches a standing photoacoustic wave as well as a sinusoidal temperature elevation. The slow decay of the temperature back to its ambient value through heat conduction is referred to as the thermal mode of wave motion. Both the acoustic and thermal modes are accompanied by density fluctuations,[3] which produce spatially periodic changes in the index of refraction of the fluid and act as a phase grating for a light beam from a probe laser directed into the cuvette. Note that the absorption of radiation from the pump beams can give rise to a spatially periodic change in both the index of refraction and the absorption spectrum of the solution as a result of the transfer of population of the absorbing molecule from its ground state to a long-lived excited state. In the experiments reported here, a probe laser is directed at the Bragg angle to the grating. A photodetector records the diffracted light intensity that results from the thermal and acoustic mode density changes, as well as from contributions from the index of refraction change that takes place as a result of population transfer to a molecular excited state.

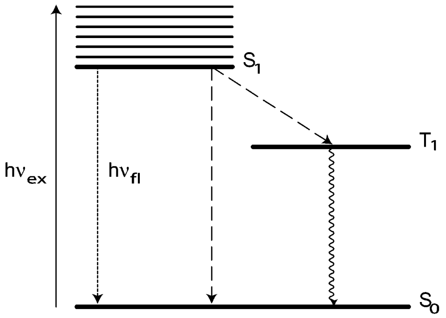


Figure 1. Energy-level diagram for sensitizer dyes. The photon energy of the pump laser is given by $h\nu_{\mathrm{ex}}$; that of the fluorescence photon is given by $h\nu_{\mathrm{fl}}$.

In the experiments, the pump beams are provided by a tunable dye laser pumped by the 532-nm frequency-doubled output of a Q-switched Nd:YAG laser. The dye laser is operated at the absorption maxima of the three photodynamic therapy dyes in the vicinity of 680 nm and has an output energy at the cuvette of approximately 5 mJ. Because the pulse width of the laser is roughly 15 ns long and because the acoustic mode is generated only if the period of the acoustic wave is longer than the laser pulse width, the laser beams were positioned to intersect at relatively small crossing angles to produce gratings with fringe spacings from 65 to 150 μm. The sound speed in acetone is 1174 m/s, giving acoustic frequencies from 8 to 18 MHz for fringe spacings in this range. The grating was probed with the output of a 786-nm, continuous, infrared diode laser operating at a power of 30 mW. The probe laser wavelength was selected to be longer than the longest wavelength absorption of the dyes used in the experiments. The diffracted light was detected with the conventional side-on a photomultiplier tube whose output was fed to an inverting amplifier. The amplifier signal was recorded by a digital oscilloscope with a bandwidth of 250 MHz and a sampling rate of 1 GHz. Signals were averaged over a large number of firings of the laser to improve the signal-to-noise ratio.

The simplified energy-level scheme for the dyes shown in Figure 1 was used to model the flow of energy in the dyes when no O_2 is present in solution. The dye molecules are excited by the pump laser from the ground S_0 state to the first excited singlet state S_1, where a fraction of the population decays to the ground state both nonradiatively and through fluorescence, while the remaining population decays to a long-lived triplet state T_1. The triplet state, in turn, decays on a much longer time scale to the ground state. The density changes arising from the thermal mode and acoustic modes can be found by solving the 1D heat-diffusion equation and the wave equation for pressure given by

$$\frac{\partial T}{\partial t} - \chi \frac{\partial^2 T}{\partial z^2} = \frac{(1 + \cos Kz)}{\rho c_P}\left[Q_1\, \delta(t) + \frac{Q_2}{\tau} \mathrm{e}^{-t/\tau}\right] \tag{1}$$

and

$$\left(\frac{\partial^2}{\partial z^2} - \frac{1}{c^2}\frac{\partial^2}{\partial t^2}\right) p = -\frac{\beta Q_1}{c_P}(1 + \cos Kz)\frac{\partial \delta(t)}{\partial t} \tag{2}$$

respectively, where T and P are the temperature and pressure variations from their ambient values; χ, c_P, ρ, c, and β are the thermal diffusivity, specific heat capacity, density, sound speed, and thermal expansion coefficient of the fluid, respectively; z

is the coordinate, and t is the time. The source terms for both differential equations are taken as being proportional to $(1 + \cos Kz)$, which describes the sinusoidal heat deposition with wavenumber K and contains δ functions describing the deposition of heat from the nonradiative decay of population in S_1 to both S_0 and T_1 with an amplitude Q_1 and a term describing the exponential decay of T_1 to S_0 with a time constant τ and amplitude Q_2. The dimensions of Q_1 and Q_2 are energy per volume. The solution of eq 1 gives the thermal-mode temperature induced by heat deposition, which is related[3] to the density change δ through the relation $\delta = -\rho\beta T$. The solution to the wave equation gives a sinusoidal function for the pressure that contributes to the overall density, as determined by the acoustic mode expression, $\delta = p/c^2$, which describes the relation between density and pressure for a purely adiabatic wave.[4] The amplitude of the acoustic wave is damped exponentially in time by a factor γ_a, which in liquids depends primarily on the fluid viscosity, as can be found from the wave equation for pressure that includes losses.[4] An approximate expression for γ_a is $\gamma_a = ({}^1/_{2}\rho c)(\eta + {}^{4\mu}/_{3})K^2$ where η, μ, c, and ρ are the bulk and shear viscosities, speed of sound, and ambient density, respectively.

When population of the dye is shifted from the ground to an excited state, both the absorption spectrum and the index of refraction of the solution are changed,[5] resulting in additional mechanisms for the diffraction of light through the creation of an absorption grating and a phase grating.[2] For the above three dyes, the effect of excited-state absorption was found to be statistically insignificant; however, the change in the index of refraction on shifting population to T_1 had an easily discernible effect on the time profile of the signal appearing as a function of time decaying faster than the thermal mode. If the decay of the triplet is taken as an exponential in time with a decay rate of ${}^1/_{\tau}$, then the index of refraction change generated by the absorption of the pump beams is described by a function of the form $A\exp(-t/\tau)$, where A is a constant depending on the change in the index of refraction of the solution on transfer of population to the triplet state.

The intensity of light diffracted by the phase grating I_D is proportional to the square of the density variation under the experimental conditions[6] employed here. Thus, the expression for the photomultiplier signal that follows from the energy flow in a molecule with the energy levels shown in Figure 1, which is essentially that obtained by Miller and co-workers[7] using somewhat different means, is given by

$$I_D = k_e\left[Ae^{-t/\tau} + Q_1e^{-K^2\chi t} + \frac{Q_2}{1 - K^2\chi\tau}(e^{-K^2\chi t} - e^{-t/\tau})\right]^2 \tag{3}$$

where k_e is an experimental constant dependent on instrumental parameters such as the collection efficiency for the optical radiation and the photodetector gain. The first term in eq 3 describes the change in the index of refraction of the solution via the transfer of population from S_0 to T_1. The term containing Q_1 arises from rapid heat deposition corresponding to the nonradiative decay of S_1 to S_0 and T_1. The term containing Q_2 describes a thermal-mode density change caused by the slow liberation of heat in the decay of T_1 to S_0 that takes place simultaneously with the transfer of heat from the antinodal to the nodal regions of the grating. It is noteworthy that the term in Q_2 could be obtained by a convolution of the exponential function describing the decay of T_1 with the delta function response of the density given by the Q_1 term. Note also that the sinusoidal functions describing the acoustic standing wave generated by the rapid deposition of heat are not included in eq 3 because the acoustic mode undergoes a substantial decay in its amplitude before the thermal mode changes significantly from its initial amplitude; the acoustic-mode density changes are not used in the data analysis.

Thus, the quantities Q_1 and Q_2 can be expressed as $Q_1 = N(h\nu - E_f\Phi_f - \Phi_{isc}E_T)$ and $Q_2 = N\Phi_{isc}E_T$, where N is the number of molecules per unit volume excited by the pump laser, $h\nu$ is the photon energy of the pump beam, Φ_{isc} is the quantum efficiency for intersystem crossing (i.e., the fraction of the population of S_1 transferred to T_1), E_f is the mean energy per photon lost to fluorescence, Φ_f is the quantum efficiency for fluorescence, and E_T is the energy of the triplet state above the ground state. The quantum efficiency for the production of T_1 is thus given by

$$\Phi_{isc} = \frac{h\nu - E_f\Phi_f}{E_T}\left[\frac{Q_2/Q_1}{1 + Q_2/Q_1}\right] \tag{4}$$

Consider a dye solution saturated with O_2, whose concentration in the case of acetone at room temperature in air is 2 mM.[8] Following absorption of radiation, the S_1 state is populated, resulting in both rapid heat deposition and population of the T_1 state through intersystem crossing. Dye molecules in the T_1 state can decay to the ground state, as described above, or react with ground-state O_2 to produce ${}^1\Delta_g$ O_2, which in turn decays back to its ground state, liberating heat at a rate determined by the lifetime of the ${}^1\Delta_g$ state. The initial analysis of data from solutions of $F_{64}PcZn$ in O_2-saturated acetone was based on a three-level model for the dye molecule using a kinetic scheme for the bimolecular reaction of the T_1 state with ground-state O_2 to produce ${}^1\Delta_g$ O_2, followed by the decay of the excited ${}^1\Delta_g$ O_2 to its ground state. In fitting the experimental data to this kinetic model it was found that the parameters describing the reaction were indeterminate in the sense that the bimolecular reaction rates for all three dyes were too fast to be resolved on the time scale of the experiment. Additionally, the diffracted light signals showed no evidence of a contribution from an index of refraction modulation, indicating as well that the transfer of population from the T_1 state to the ${}^1\Delta_g$ state of O_2 was too rapid to be resolved on the time scale of the experiment. Data analysis for the dyes was therefore carried out using what amounts to, insofar as the mathematics is concerned, a three-level energy scheme with optical excitation of the dye from the ground-state S_0 to S_1, followed by the nonradiative decay of S_1 into both the S_0 state and the ${}^1\Delta_g$ state of O_2, with a quantum efficiency of Θ for population transfer from S_1 to T_1, followed by the decay of the excited O_2 to its ground state. The qualitative change in the transient grating signal when O_2 is added to solution, with no evidence of population in the T_1 state, followed by the production of a state that releases energy with a single lifetime corresponding to accepted values of that for ${}^1\Delta_g$ O_2, is strong evidence for the above kinetic scheme for energy transfer.

Experiments

Dye solutions were made with reagent-grade acetone and degassed using three cycles of freezing and thawing. Solutions were then capped and stored under argon before use. To reduce the effects of quenching of the excited triplet states of the dyes by either ground-state dye molecules or other triplet state molecules, the concentrations of the dyes used were made as small as possible, consistent with obtaining a reasonable signal-to-noise ratio in the diffracted light signal. To introduce O_2 into solution, balloons filled with high-purity O_2 were placed over the degassed solutions in the cuvettes used in the transient

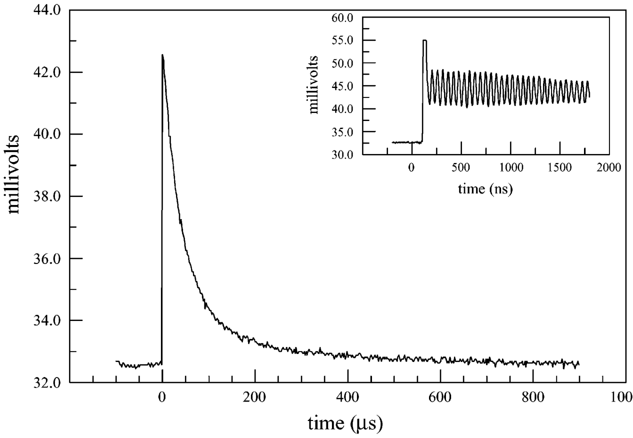


Figure 2. Diffracted-light signal from $F_{64}PcZn$ in acetone under Ar. The offset in the baseline is caused by the amplifier. The rapidly decaying waveform is characteristic of an index of refraction change decaying with the lifetime of the excited triplet state. Inset: The same waveform recorded on a shorter time scale showing the acoustic-mode signal. The initial peak in the waveform is due to fluorescence from the dye not rejected by the band-pass filters preceding the photomultiplier. The offset of the acoustic mode from the baseline is a consequence of the contribution of the refractive index change arising from the population of the T_1 state.

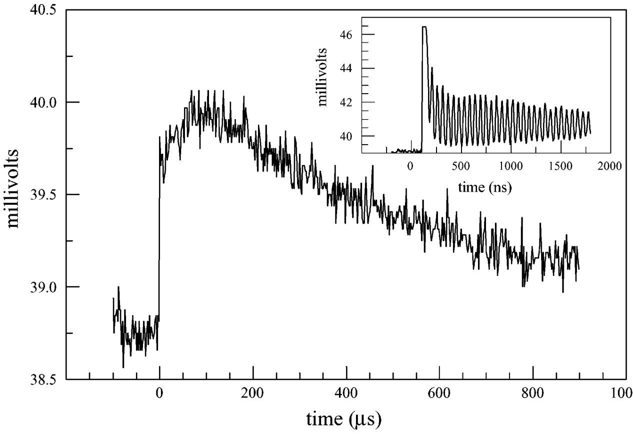


Figure 3. Diffracted-light signal from $F_{64}PcZn$ in acetone saturated with O_2. The offset in the baseline is caused by the amplifier. The slowly rising waveform is characteristic of energy transfer from the dye to O_2 to form $^1\Delta_g$ O_2. Inset: The same signal displayed on a shorter time scale showing the acoustic mode.

grating apparatus. Diffusion, with the help of occasional agitation, resulted in the saturation of the solutions. Experiments were conducted on $F_{16}PcZn$ and PcZn at the same concentrations and laser fluences as those for $F_{64}PcZn$ so that the efficiencies of the dyes for $^1\Delta_g$ O_2 production could be compared under nearly identical conditions. Methylene blue was purchased from Aldrich, Inc. and was used without further purification.

Typical experimental data are shown in Figures 2 and 3 for $F_{64}PcZn$ in degassed solution and in solution saturated with O_2, respectively. The profound effect of O_2 on the kinetics of heat release is evidenced by a comparison of the waveforms shown in these two Figures. The rapid decay in the initial signal amplitude from $F_{64}PcZn$ under argon, which is substantially faster than that dictated by thermal diffusion, is a distinct marker for a nonthermal process—in the case of the present experiments, an index of refraction change that is dependent on the transfer of population into T_1. The insets in Figures 2 and 3 show the transient grating signals on a short time scale and display the acoustic mode, which is not used in the data analysis given here.

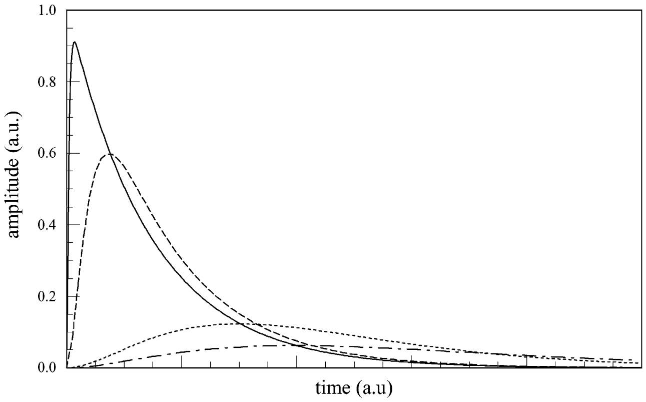


Figure 4. Diffracted-light signal versus time calculated from the convolution term in eq 3 for $K^2\chi\tau$ equal to (—) 0.01, (- - -) 0.1, (···) 1.1, and (— · —) 2.0.

TABLE 1: Table of Excitation Wavelengths λ, Flourescence Quantum Yields Φ_f, Triplet Lifetimes τ, and Singlet Oxygen Quantum Yields Θ for the Three Phthalocyanines[a]

compound	λ (nm)	Φ_f	τ (μs)	Θ
PcZn	666	0.17	77 ± 3	0.17 ± 0.02
$F_{16}PcZn$	664	0.04	≪1	0.13 ± 0.01
$F_{64}PcZn$	686	0.39	131 ± 3	0.21 ± 0.03

[a] Errors are from the least-squares fitting.

The inset in Figure 2 distinctly shows an offset from the baseline characteristic of the presence of the signal arising from the index of refraction change that is dependent on the population of a long-lived excited state.

For the data taken with $F_{64}PcZn$ under Ar, it was possible to determine the triplet-state lifetime; however, a least-squares fit to the data gave the parameters Q_1 and Q_2 with errors of the same magnitude as the parameters themselves, so it was not possible to determine statistically significant values of the parameter Φ_{isc}. Values for the fluorescence quantum efficiency were determined with a conventional spectrofluorometer using PcZn in propanol,[9] $\Phi_f = 0.45$ as a standard. Values for the fluorescence quantum efficiencies of the three dyes are reported in Table 1. Values of the parameter Θ derived from fits to the data for the dyes are reported in Table 1 as well.

The wavelengths of excitation listed in Table 1, were selected to excite the dyes at their absorption maxima. The diffracted light waveforms recorded from $F_{16}PcZn$ under Ar showed no evidence of either a rapidly decaying signal from an index of refraction change or a signal increasing in time characteristic of a long-lived triplet state; thus, neither the triplet state lifetime nor Φ_{isc} could be determined from the experiment. In the presence of O_2, both PcZn and $F_{16}PcZn$ solutions gave diffracted light signals similar to that shown in Figure 3, indicating substantial production of $^1\Delta_g$ O_2. For both dyes, it was possible to determine Θ, values of which are given in Table 1.

To test the validity of the data analysis and the three-level kinetic model, experiments were carried out on methylene blue, a well-characterized sensitizer whose photophysical parameters Θ and Φ_f have been previously reported.[10] Solutions of methylene blue in reagent-grade ethanol were prepared in the 5 to 6 μM concentration range. Experiments were carried out with the laser operating at 633 nm with a relatively low energy of 1.4 mJ per pulse to avoid photobleaching, which was seen in methylene blue but not in the other dyes. Balloons of oxygen were placed over the cuvette to ensure that the solution was saturated with gas. Experiments performed with the cuvette open to the atmosphere, however, yielded identical results. Values for Q_2/Q_1 were determined from data fits to 10 experiments. The value of Φ_f from ref 10, 0.04, was used together with the experimental data to give Θ equal to 0.49 ± 0.02, in excellent agreement with the previously determined values of 0.52 and 0.50 reported in refs 11 and 12, respectively. In addition, the data taken with the transient grating apparatus yielded a lifetime of 15 ± 2 μs for $^1\Delta_g$ O_2 in ethanol. Although there is some variation in the literature values for the lifetime of $^1\Delta_g$ O_2 in ethanol, the lifetime determined here is in excellent agreement with the recently determined values of 14 and 15 μs reported in refs 13 and 14, respectively.

Discussion

This study has been directed toward the exploration of the transient grating technique as a general method for determining the production of $^1\Delta_g$ O_2 by photodynamic therapy agents, with a focus on the properties of the new compound, F_{64}PcZn. One of the important findings is that the time scale for the decay of the thermal mode, determined by the grating fringe spacing, must be adjusted appropriately to determine the efficiency of $^1\Delta_g$ O_2 production. That is, in the case of chemical kinetics where there is rapid energy transfer for the production of excited oxygen, whether a statistically significant value for Θ can be found, depends critically on the parameter $K^2\chi\tau$. This parameter is the ratio of the lifetime of the state depositing heat into solution to the decay time of the thermal mode of the grating and appears explicitely in the denominator of the last term in eq 3. As shown in Figure 4, as the parameter $K^2\chi\tau$ is varied, the time dependence of the diffracted-light waveform from the convolution term varies substantially, with that for $K^2\chi\tau \ll 1$ giving a waveform with a fast rise time and a decay that approximates an exponential function with a lifetime of $1/K^2\chi$, while that for $K^2\chi\tau \gg 1$ giving a singly peaked waveform delayed in time from the firing of the pump beams with a small amplitude. In both cases with extreme values of $K^2\chi\tau$, the extraction of statistically significant values for Q_1 and Q_2 is problematic. In the first case, if $K^2\chi\tau$ is too small, the initial fast-rising part of the signal is masked by the signal arising from rapid heat deposition, which is almost universally found in the excitation of any molecule, while the remaining decay takes place with approximately the same time constant as the usual decay of the thermal mode, $1/K^2\chi$. Thus, the initial part of the trace would appear as a single rising signal with a sharp peak and with the remaining part of the trace decaying at a rate of $1/K^2\chi$, giving the waveform the appearance of what would be recorded with a dye that simply released rapidly the energy from S_1. In the case where $K^2\chi\tau \gg 1$, the signal amplitude from the slow heat release is necessarily small and takes place over a long time, resembling a slight shift in the baseline, adding insignificantly to that from fast heat release. In this case, the extraction of photophysical parameters is strongly contingent on a high signal-to-noise ratio in the detector signal. It is for intermediate values of $K^2\chi\tau$ that a signal of sufficient amplitude, having a functional form that differs from the simple exponential decay of the thermal mode, makes the extraction of statistically significant values for parameters Q_1 and Q_2 possible. However, even in cases where $K^2\chi\tau$ has intermediate values, the signal from the change in the index of refraction can be so large that it overwhelms the signal from a density change, so the determination of Q_1 and Q_2 is again not possible. In the present experiments with F_{64}PcZn under Ar, a three-parameter fit of the waveforms to give Φ_{isc} was not possible, owing to the large index of refraction change from population transfer that masks the initial rise of the signal from the density change required to determine Q_1 and Q_2. However, a signal dominated by the index of refraction change makes possible the determination of the lifetime of T_1 with high precision. In the present experiments with $^1\Delta_g$ O_2, the parameter $K^2\chi\tau$ was in the range of 0.02 to 0.1. It is noteworthy that the unusually low value of $K^2\chi\tau$ that characterizes the experiments here with large grating fringe spacings contrasts greatly with what would be seen in experiments with the small fringe spacings employed in typical picosecond transient grating experiments arranged to give maximal time resolution, and is precisely what makes the recording of the thermal-mode signal from the decay of $^1\Delta_g$ O_2 possible: in experiments set up to give GHz acoustic mode signals, the values of $K^2\chi\tau$ would be so large that the amplitude of the diffracted-light signal from the decay of $^1\Delta_g$ O_2 would be so small as to be virtually undetectable.

It is interesting that the $^1\Delta_g$ O_2 quantum yield for the phthalocyanines increases with the degree of fluorination, the Θ value for F_{64}PcZn being significantly higher than that for F_{16}PcZn, but only marginally higher relative to that of PcZn (Table 1). Both Φ_f and τ follow the same pattern. The relative values of Φ_f and τ for PcZn and F_{16}PcZn indicate that halogenation decreases the lifetime of the excited triplet state and the fluorescence quantum yield. This observation is consistent with the notion that the aromatic fluorine groups are part of the phthalocyanine π system and thus increase the reactant−product intersystem crossing. As a result, intersystem crossing is favored.[15] An increase in both the excited triplet-state lifetime and fluorescence quantum yield is noticed when aromatic fluorogroups in F_{16}PcZn are replaced by aliphatic groups to yield F_{64}PcZn (Scheme 1b and c). The fluoro-aliphatic substituents, as we have shown previously,[1] are not able to participate in π back-bonding, unlike the aromatic fluorogroups; thus, they are not conjugated with the π ring system. In the absence of quantitative information about changes in energy levels and nonradiative coupling between them, the increases in Φ_f and τ of F_{64}PcZn relative to those of F_{16}PcZn can be tentatively ascribed to a change in intersystem crossing resulting from changes in the conjugation of the π ring system.

In summary, transient grating apparatus set up to act as a photothermal monitor of heat evolution from photodynamic therapy agents can be used to determine quantum efficiencies for the production of $^1\Delta_g$ O_2. A number of methods[16,17] have been developed for measuring quantum yields for the production of $^1\Delta_g$ O_2 from photosensitive dyes including photoacoustic,[18] calorimetric,[19] spin[20] and chemical[21] trapping, and luminescence.[22] The transient grating method is most closely related to photothermal beam deflection, where heat evolution is monitored by thermal lensing. Both the transient grating, recorded on a long time scale, and beam deflection methods are photothermal in origin; perhaps the distinguishing feature of the former is its superior time resolution. The salient features of the transient grating method for studying photodynamic therapy agents as shown in this work are that it does not require the addition of reagents that can complicate the photochemistry, its data analysis is straightforward, and it is highly sensitive, permitting measurements to be carried out at low concentrations, thus reducing the effects of intermolecular interactions such as quenching. The profound effect of O_2 on the signals recorded from sensitizer dye solutions, the small statistical variation found for the values of the quantum efficiency for the production of $^1\Delta_g$ O_2, and the relative ease of carrying out experiments at concentrations in the micromolar range suggest that the transient grating method is a powerful tool for the evaluation of $^1\Delta_g$ O_2 production by type II photodynamic therapy agents in general.

Acknowledgment. We are grateful for the support of this research by the U.S. Department of Energy under grant ER-13220 and the Salomon Foundation.

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