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Photoacoustic and photothermal detection of photosensitized oxygenations

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Abstract: Study of relaxation processes of excited states of hematoporphyrin IX (HP) solutions (in ethanol and methanol) in the presence of tetramethyl ethylene chemical acceptor using both the photoacoustic (PA) - microphone - and photothermal (PT) - PZT - detection, is described. A significant difference between PA and PT signal behaviour has been observed when extremely low (down to 1.25 μM , PT measurements) and high (up to 2.5 mM, PA measurements) concentrations of sensitizer were used.

1. INTRODUCTION

Recently there has been a growing interest in quantitative analysis of singlet oxygen, $\text{O}_2(^1\Delta_g)$, production and deactivation mechanisms, as it is generally recognised that tumor destruction in photodynamic therapy is achieved via singlet oxygen formation and its reactions with cellular substrates ⁽¹⁾. We had demonstrated the efficiency of the photoacoustic (PA) method when applied to the measurements of photophysical properties of molecules in highly concentrated solutions which relax through a metastable state ⁽²⁾, in particular when singlet oxygen relaxation pathways are altered by chemical acceptors ⁽³⁾. In this paper, results of both, PA and photothermal (PT) - PZT - studies of relaxation processes of hematoporphyrin IX (HP) sensitizer solutions in two, very different, concentration ranges : [μM (PT) and mM (PA)] are presented and initial attempts to quantitate data discussed.

2. THEORY

The ratio Γ of the signal amplitude with chemical acceptor to the signal amplitude without acceptor has been calculated for PA and PT detection. For PA method, which is sensitive to all prompt and delayed heat, Γ could be presented by ^(2,3) :

$$\Gamma = 1 - \frac{\gamma_{\Delta}\gamma_4 E_4}{E_{\text{ex}} - \gamma_f E_f} \quad \text{or} \quad \frac{1}{1-\Gamma} = \alpha(1 + \frac{\beta}{[C]}) \quad (1)$$

where E_{ex} is absorbed photon energy, γ_f is the quantum yield of fluorescence, E_f is the mean fluorescence photon energy, γ_{Δ} is the quantum yield of singlet oxygen production, γ_4 is the quantum yield for chemical reaction : $\gamma_4 = k_r[C]/(k_d + k_r[C])$. Here, $[C]$ is the acceptor concentration, k_d represents the conversion rate of singlet oxygen by physical reaction with the solvent, $k_r[C]$ is the conversion rate of singlet oxygen through chemical reaction with the acceptor and E_4 is the energy of the product. Quantities α and β in

Eq(1) are defined as : $\alpha = (E_{ex} - \gamma_f E_f) / \gamma_\Delta E_4$ and $\beta = k_d / k_r$.

The above relations between Γ and concentration of the acceptor can be used to adjust experimental results. A three parameter fit used for PA experiment analysis, amplitude at $[C] = 0$, α and β was found to give more accurate results than a linear fit. For PT, which is sensitive to prompt heat and to the part of the delayed heat produced in a time shorter than the acoustic transit time τ_a ⁽⁴⁾, the ratio Γ is a complex expression of above parameters, the energy of singlet oxygen E_Δ and the ratio of acoustic transit time τ_a to lifetime of singlet oxygen in the presence of acceptor $\tau_\Delta = 1 / (k_d + k_r[C])$. At high acceptor concentration ($\tau_a > \tau_\Delta$ and $\gamma_4 = 1$) Γ_∞ , a limit of ratio Γ , can be expressed as :

$$\Gamma_\infty = 1 + \frac{\gamma_\Delta (E_\Delta e^{-\tau_a k_d} - E_4)}{E_{ex} - \gamma_f E_f - \gamma_\Delta E_\Delta e^{-\tau_a k_d}} = 1 + \frac{1}{\alpha'} \quad (2)$$

In order to measure Γ_∞ , the model for PA was used to fit our results. The fitted parameters comprise amplitude at $[C] = 0$, α' , and β' which are depending on k_d/k_r and $\tau_a k_r$. The coefficient α' depends on the part of energy stored in the singlet oxygen state and converted into heat by physical quenching during the acoustic transit time. If $\tau_a k_d \gg 1$, Eq(1) can be used for PT experiments ($\alpha' = \alpha$) whilst in the opposite case, ($\tau_a k_d \ll 1$), $\alpha' = (E_{ex} - \gamma_f E_f - \gamma_\Delta E_\Delta) / \gamma_\Delta (E_4 - E_\Delta)$.

3. MATERIALS AND METHODS

The PA spectrometer with Xe-arc lamp and gas-microphone PA-cell used in this study has been slightly modified in comparison to that reported earlier ⁽²⁾. Experiments with various acceptor concentrations have shown that Γ is independent of the modulation frequency (up to 500 Hz) and that the phase of the signal does not depend on $[C]$. Spectral analyses were performed at 40 Hz. A dye laser (384 nm) pumped by a nitrogen laser and a cell with ceramic PZT-detector (Max Planck Institut für Strahlenchemie, Mülheim an der Ruhr, Germany) have been used in the PT study. A beam 2 mm in diameter was used, giving an acoustic transit time τ_a of about 2 μ s. The HP was dissolved to reach concentration of 2.5 μ M (PZT) and 2.5 mM (PA) both in ethanol and methanol without any additional purification. The $O_2(^1\Delta_g)$ specific acceptor - tetramethyl ethylene (TME) - was added at concentrations ranging from 5 to 400 mM. Optical density measurements (transmission spectroscopy) were performed in order to control optical absorption coefficient.

Figure 1 : The normalized PA spectra of HP in ethanol (2.5 mM) with and without TME acceptor.

Figure 2 : Experimental PZT waveforms at 384 nm for HP solution in ethanol (2.5 μM) with and without TME.

4. EXPERIMENTAL RESULTS

4.1 PA measurements

Fig. 1 shows the PA spectra of HP in ethanol, without acceptor, and in the presence of TME for two different acceptor concentrations. Drops in PA amplitude (as a function of increasing acceptor concentration) are observed in the absorption bands. For other wavelengths precision of our measurements did not allow calculation of ratio Γ . A strong decrease in Γ was obtained as the concentration of acceptor increased from 5 mM to 400 mM, when the PA cell was filled with air. For a cell loaded with nitrogen Γ remained equal to 1, demonstrating that the acceptor reacts with singlet oxygen. Experiments with different HP concentrations (down to 0.3 mM), and other dye (methylene blue) or chemical acceptor (furfuryl alcohol) produced similar results.

4.2 PZT measurements

Fig. 2 shows the PZT waveforms for pure HP solution in ethanol and in presence of TME at two different concentrations, the value of Γ was calculated from the amplitude corresponding to the first maximum. Larger Γ was obtained as the TME concentration increased, in contrast to PA results, where a decrease in Γ was observed at 395 nm. Identical PT results were obtained when working with the HP solutions in methanol, or with other dyes or chemical acceptors.

Figure 3 : Experimental results and regression fits (lines) showing the photoacoustic number $1/(1-\Gamma)$ versus $[C]$ for HP in ethanol measured with PT (HP 2.5 μM) and PA (HP 2.5 mM) detection.

Table1 : Results of adjusting experimental results obtained with the PA and PT methods for some solutions in alcohol.

Sample	Detection	α	α'	β (mM)	β' (mM)
HP, 2.5 μ M (methanol)	PZT		- 2.21		109
HP, 2.5 μ M (ethanol)	PZT		- 2.17		210
HP, 2.5 mM (ethanol)	PA	3.36		15	

5. DISCUSSION

The linear variations of the photoacoustic number $1/(1-\Gamma)$ versus the inverse of acceptor concentration for both PT and PA studies are shown in Fig.3 together with the results of the adjustments to the theoretical model given by Eq(1). The adjusted parameters are presented in Table 1. For PT experiments, only the fitted coefficient α' can be interpreted. Using the known values for photophysical parameters : $E_{ex} - \gamma_f E_f = 3.05$ eV for HP and assuming acetone as the final product of TME photooxydation : $E_4 = -1.8$ eV, one can estimate the quantum yield of singlet oxygen production γ_Δ . Assuming $\tau_a k_d \gg 1$, γ_Δ calculated from Eq(1) or Eq(2) were 0.42 for HP in methanol and 0.43 for HP in ethanol. Assuming $\tau_a k_d \ll 1$, the quantum yields γ_Δ calculated from Eq(2) were 0.76 in methanol and 0.78 in ethanol. The accepted (0.5) value for γ_Δ (⁵) corresponds to $\tau_a k_d = 0.43$ for methanol and 0.36 for ethanol. Using the known values for k_d : 10^5 s⁻¹ in methanol and $6.6 \cdot 10^4$ s⁻¹ in ethanol (⁶), it is possible to estimate the effective acoustic transit time (4.3 μ s and 5.5 μ s in methanol and ethanol respectively). This approach can be considered as a way to measure the time response of the spectrometer.

The experimental results from PA experiments yielded positive values for α corresponding to negative values of $\gamma_\Delta E_4$ product and cannot be interpreted with the presently accepted theoretical model. The difference observed between PA and PT signal behaviour- i.e., a decrease and an increase in Γ with increasing acceptor concentration, can be related to significant difference in concentrations, leading to oxygen consumption and diffusion processes not taken into account here. More complex relaxation pathways like type I and type II photosensitizations and singlet oxygen - sensitizer interactions should also be considered. The first PT experiments reported above seem to provide a new approach for observation of singlet oxygen production and relaxation. Quantitative measurements of photophysical parameters (γ_Δ , E_4 and k_f) require more precise theoretical studies. These, and the experimental verifications, are currently being considered.

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