Determination of the triplet state energies of a series of conjugated porphyrin oligomers

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Introduction

There is currently much research interest in the utilisation of multiphoton processes for biological and medical applications, such as multiphoton imaging[1,2] and photodynamic therapy (PDT).[3,4] The important advantage of two photon excitation (TPE) stems from the fact that biphotonic absorption depends on the square of the light intensity, therefore it is confined to the focal volume of the laser. The latter factor provides better spatial resolution for TPE imaging due to reduced out-of-focus blur.

PDT uses light activation of a sensitizer for eradication of neoplastic disease.[5] Therefore, TPE PDT has potential advantages over conventional one-photon PDT in the treatment of sensitive tissues such as those found in the eye in the wet form of age related macular degeneration by reducing out-of-focus damage to adjacent healthy tissue. In addition, since TPE uses near infrared light of twice the wavelength of the absorption band, deeper tissue penetration can be afforded due to the minimised absorption and scattering of useful light by tissue components.

The short pulses of high energy in the desired spectral range (700-900 nm) can be provided by commercial solid state ultrafast lasers (e.g. Ti:Sapphire). The current challenge is in creating molecules combining both desirable biological and photophysical properties with high two photon absorption cross sections for efficient biphotonic excitation.

The most commonly used PDT sensitizers are porphyrin-like molecules that permit near IR wavelengths to be used that are away from tissue absorption. Unfortunately, commercial PDT drugs have extremely low TPE absorption cross sections, e.g. 10 GM (1 GM = 10^{-50} cm^4 s/photon) at 800 nm for photofrin,[6] and thus are not useful as efficient TPE PDT sensitizers.

Recently several porphyrin-containing molecules with high two photon absorption cross sections have been reported.[7,8] In the series of conjugated porphyrin oligomers synthesised at Oxford University nonlinear properties increase with increasing length of conjugation as confirmed by two-photon excited fluorescence.[9] These measurements have given cross section values of 7.5, 22, 37 and 83 ($\times 10^3$ GM per molecule) for the porphyrin dimer, tetramer octamer and *ca* 13-mer respectively.[9] The combination of a large TPA cross section with the possibility of modifying the porphyrin periphery to give aqueous solubility or to introduce other biological functionality makes conjugated porphyrin oligomers promising candidates for TPE PDT and imaging applications. In addition, the marked red shift of both absorption and fluorescence bands due to conjugation opens up the possibility of using red light in the 670-800 nm region for single photon excited PDT and imaging, which would benefit from deeper tissue penetration.

The prerequisite for a potent PDT sensitizer is high efficiency of production of singlet oxygen, $^1O_2$, the species widely accepted to be responsible for cytotoxic response in cells and tissues.[5] The efficiency is characterised by the $^1O_2$ quantum yield, ϕ_Δ. $^1O_2$ production occurs via energy transfer from the sensitizer triplet excited state and requires at least 94 kJ/mol, the energy of the $^1\Delta_g$ electronic excited state of molecular oxygen.[10] Generally, increasing the conjugation of a molecular system leads to lowering of the excited state energy. Therefore in a series of porphyrin oligomers the extended conjugation can compromise their ability to produce singlet oxygen, as the triplet state energy approaches the 94 kJ/mol barrier. Herein we report the study of the photophysical properties relevant to PDT for a series of conjugated porphyrin oligomers.

Results and discussion

The structure of the conjugated porphyrin oligomers under study is given in Figure 1, N = 2 (**2**), 4 (**3**), 8 (**4**).
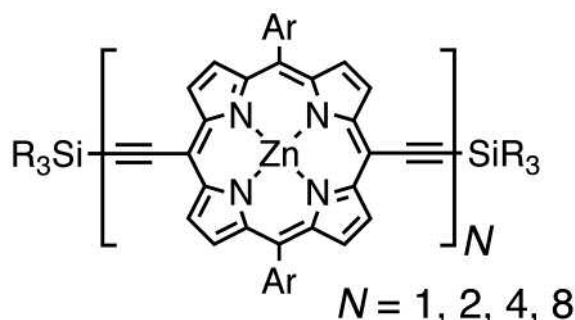

Figure 1. The structure of the conjugated porphyrin oligomers.

A marked red shift is observed in the UV/vis absorption and fluorescence spectra upon going from monomeric porphyrin to dimer **2** in both Soret and Q bands together with band splitting, indicative of conjugation. Absorption spectra of **3** and **4** are characterised by further red shifts and broadening. Likewise, the fluorescence spectra show significant red shifts and a decrease in fluorescence quantum yield with increased oligomer length, consistent with lowering of singlet excited state energy.

Following excitation of deaerated solutions, the singlet ground state absorption is bleached and the triplet-triplet absorption bands appear, Figure 2. The triplet-triplet absorption spectra of **2-4** are typical of porphyrins. The triplet state lifetimes gradually decrease on going from the monomer (600 µs) to dimer (400 µs), tetramer (350 µs) and octamer (300 µs) and this is suggesting increased non-radiative deactivation caused by either lowering of the triplet state energy in the series or increased flexibility of the core of the molecule. Triplet yields are high for monomer (ϕ_T = 1) and dimer (ϕ_T = 0.75) but decrease significantly for **3** and **4** (ϕ_T = 0.45 and 0.3) giving evidence for fast non-radiative decay of the excited singlet state.

In order to establish that the energy of the lowest triplet excited state in the series of porphyrin oligomers exceeded the 94 kJ/mol barrier, essential for singlet oxygen production and effective PDT, the energy of the lowest triplet state was estimated using the triplet quenching method.

Triplet quenching method measures the rate of quenching of the triplet excited state of the molecule in question by known triplet energy acceptors. The quenching constant k^{en}_q is correlated with E_T(quencher)=E(Acceptor*) according to equation (1)[11]:

$$k_q^{en} = \frac{k_d}{1 + \exp\left(\frac{\Delta G}{RT}\right) + \frac{k_{-d}}{k_{en}^0}\exp\left\{\frac{\Delta G + \frac{\Delta G^{\#}(0)}{\ln 2}\ln\left[1 + \exp\left(-\frac{\Delta G \ln 2}{\Delta G^{\#}(0)}\right)\right]}{RT}\right\}}$$

Equation (1)

where ΔG=-E_{00}(Donor*)+E_{00}(Acceptor*) is the free energy change of the reaction; $\Delta G^{\#}(0)$ is the reorganisation energy, the intrinsic barrier for the forward energy transfer; k_d is the diffusion reaction rate (6.44×10^9 s^{-1} in toluene[12]); k_{-d} is the dissociation rate constant; k^0_{en} is the preexponential factor.

The complete set of quenching constants obtained for **1-4** using β-carotene (75 kJ/mol), bacteriochlorophyll (95), Zn phthalocyanine (109), rubrene (111), all-trans-retinal (123), chlorophyll a (123), tetracene (124), 1,8-diphenyl-octatetaene (132), tetraphenyl porphyrin (137) and perylene (146) as triplet acceptors was measured. The concentration of the quencher [Q] was chosen such that it was sufficient to significantly reduce the triplet lifetime of the chromophore to allow determination of k^{en}_q according to the equation $k_{observed}$ = k_0 + k^{en}_q[Q] where k_0 is the observed decay constant in the absence of the quencher, $k_0 = 1/\tau_0$.
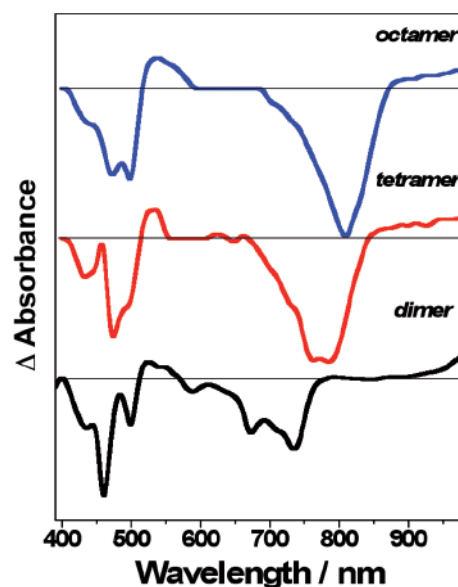

Figure 2. Triplet minus singlet absorption spectra of 2, 3 and 4 obtained following 700 nm excitation of deoxygenated toluene solutions containing 1% pyridine at 298 K.

The corresponding curves of $\log(k^{en}_q)$ vs E_{00}(Acceptor*) are shown in Figure 3. On all plots two regions are clearly visible: the first region where $\log(k^{en}_q)$ is proportional to the Gibbs free energy of the process and a second region where the diffusion controlled rate is observed independent of overall free energy change of the process.

These data were used to determine energy transfer parameters, such as $\Delta G^{\#}$, k_{-diff} and E_T using least-squares fitting based on expectations of equation (1). We find that $\Delta G^{\#}$ is the single most important parameter affecting the shape of the curve and hence the resulting triplet energy. We also found that simultaneously iterating both $\Delta G^{\#}$ and k_{-diff} introduces considerable uncertainty to the fitting procedure. It is known that k_{-diff} is strongly dependent on molecular size ($\sim r^{-3}$)[13] thus it cannot be forced to be a constant for the fitting of data for **1-4**, whose size varies dramatically. On the other hand, the reorganisation energy $\Delta G^{\#}$ for the energy transfer process for different oligomers **1-4** is not expected to vary dramatically.
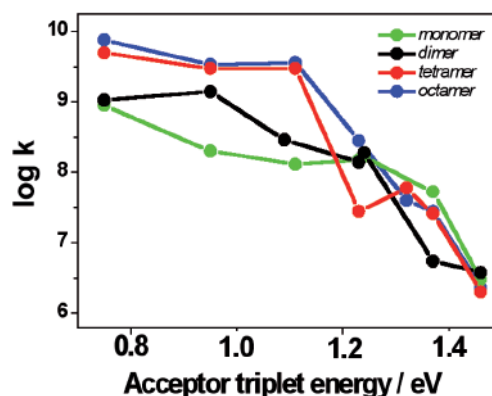

Figure 3. Results of quenching of triplet states of 1-4 with a series of triplet acceptors in deoxygenated toluene solutions containing 1% pyridine at 298 K.

In addition, energy transfer does not incur much reorganisation thus the $\Delta G^{\#}$ value is expected to be relatively small. We fit the experimental data for all compounds using two fixed values of $\Delta G^{\#}$, 10 kJ/mol and 16 kJ/mol, both slightly above the reorganisation energy values typical for the solvent. These were chosen as limiting values since $\Delta G^{\#} < 10$ kJ/mol and $\Delta G^{\#} > 16$ kJ/mol did not yield suitable fits. The triplet energy values (eV) for **1-4** obtained from the fits using $\Delta G^{\#} =10 / 16$ kJ/mol are 1.59/1.48 eV (**1**), 1.46/1.40 eV (**2**), 1.39/1.35 eV (**3**) and 1.34/1.32 eV (**4**). While the fitting procedure produces a range for the triplet energy levels of **1-4**, the progressive decrease in the energy from **1** to **4** is clear, consistent with the trend observed in other spectroscopic parameters in the series.

It is clear that for all oligomers the triplet energies exceed 94 kJ/mol (0.98 eV) required for singlet oxygen production. We note that even though the triplet energies of longer oligomers **3** and **4** are sufficient for $^1O_2$ production, the triplet state yields are relatively low. We expect that this factor will limit the usefulness of tetramer and octamer for PDT. Thus conjugated porphyrin dimer is deemed to be the most promising core structure for TPE PDT applications. We are currently developing versions of these chromophores with aqueous solubility for efficient TPE PDT function.

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