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Triplet state spectroscopic studies on some 5,10,15,20-tetrakis(methoxyphenyl)porphyrins

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Abstract

Extensive triplet state spectroscopic investigations were carried out with a series of 5,10,15,20-tetrakis(methoxyphenyl)-porphyrins. Triplet absorption spectra, triplet lifetime, triplet quantum yield and quantum yield for singlet oxygen production were determined with different absorption and emission techniques, using the frequency-doubled beam of a Nd:YAG laser. It has been found that these synthetic porphyrins are effective photosensitizers which can be used as model compounds to investigate the theoretical and instrumental aspects of PDT. © 1998 Elsevier Science B.V.

Keywords: Porphyrins; Triplet state; Singlet oxygen; Photosensitizers; Photodynamic therapy

1. Introduction

Since the 1960s porphyrins and related macrocycles have been in the focus of the clinical practice and experimental investigations on photodynamic therapy (PDT) of cancer [1]. The curative activity of PDT arises from the interaction of the visible light with a photosensitizer. The naturally occurring haematoporphyrin and its derivatives were the first commercially available products (first generation photosensitizers) used in PDT. They have several disadvantages [2], so considerable efforts have been made to find more effective tumor killing agents with less phototoxicity.

At present it appears to be generally agreed that singlet oxygen plays an important role in PDT. According to the Type II photosensitized energy transfer process [3] the photosensitizer in its triplet state interacts with ground state (triplet) oxygen, producing reactive singlet oxygen. The latter oxidizes important biomolecules in the cells leading to cellular damage of the tumor tissue. A good PDT photosensitizer should have sufficient triplet energy ($\geq 94\ \mathrm{kJ\ mol^{-1}}$ for adequate interaction with ground state oxygen), high triplet yield, long triplet lifetime and high quantum yield for singlet oxygen formation. Owing to the light absorption and scattering properties of the human tissue, red light is applied in PDT, thus photosensitizers should have strong absorption in the red region of the spectrum. Using amphiphilic porphyrins with hydrophilic moieties better localization in the tumor tissue can be achieved [2].

The aim of this study was to measure the important photophysical and absorption characteristics of four

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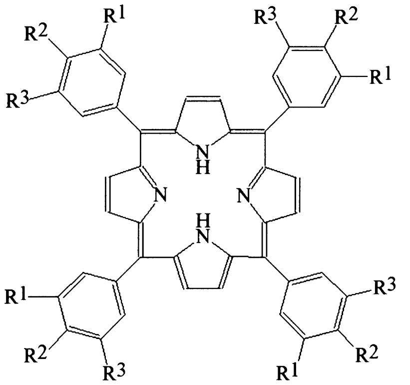
5,10,15,20-tetrakis(methoxyphenyl)porphyrins (**I**)[1] which are the synthetic precursors of the appropriate hydroxy derivatives applied in PDT [4]. Although the triplet state properties investigated by photolysis techniques can be used in the preliminary evaluation of the photosensitizing activity of compounds designed for PDT, these data for (**I**) and related porphyrins have not been reported previously.



(I)

2. Experimental

Compounds (**I**) were synthesized from the corresponding aromatic aldehydes and pyrrole with an improved version of the Rothemund–Adler method [5].

The absorption spectra were recorded on a HP8452A diode array spectrophotometer. Porphyrin concentration was 1 or 5 μM, the optical path length was 1.00 cm.

A laser flash photolysis setup [6] based on a Q-switched frequency-doubled Nd:YAG laser excitation source (Continuum, Surelite I-10, 4–6 ns pulses at 532 nm) was used for the triplet absorption measurements in the range 370–500 nm. Pulse energy was measured by an energy meter (Rj 7100, Laser Precision Corporation). Deoxygenation of the samples was carried out by bubbling helium through the solution. The analyzing light from a xenon lamp (150 W) was set perpendicular to the excitation beam on the sample cell, and focused on a monochromator (Applied Photophysics Ltd. The signal was detected by a photomultiplier tube (RCA, 1P28) and digitized by a PC data acquisition board (Sonix STR*832). A shutter used to protect the sample from the analyzing light, as well as data acquisition and processing were controlled by computer. Porphyrin concentrations were between 5 and 10 μM, the optical path length was 1.00 cm, the energy of the laser flashes was 0.3–1.3 mJ and absorbances in the range 0.02–0.08 were measured at 532 nm.

Determination of triplet lifetime in the presence of oxygen and of triplet quantum yield were carried out on a similar laser instrument based on a Quanta-Ray DCR-1 Nd:YAG laser, a xenon lamp (450 W) and a Tektronix TDS-684A digital oscilloscope (1 GHz bandwidth, 5 GS s^{-1} sampling rate). This setup allowed measurements with a time resolution of the order of 10 ns and of absorbances of the order of 0.001 [7].

For the determination of singlet oxygen quantum yield (Φ_Δ) the emission at 1.27 μm following laser excitation was monitored by a liquid nitrogen-cooled germanium photodiode (EO-817P, North Coast Scientific Co.). The excitation source was the Surelite Nd:YAG laser mentioned above. Two interference filters (centered at 1.27 m, FWHM 40 nm) were placed between the sample and the detector. The absorbances of the reference solution (Rose Bengal sensitizer (Fluka) in ethanol, $\Phi_\Delta = 0.80$, or *meso*-tetraphenylporphyrin (**I**, $R^1 = R^2 = R^3 = H$) in benzene, $\Phi_\Delta = 0.66$ [8]) and the sample solution (at 532 nm) were set equal (between 0.30 and 0.67) by dilution.

The measurements were carried out at room temperature (298 K) in benzene (spectroscopic grade) and ethanol (analytical grade). Naphthacene used in triplet quantum yield measurements was purchased from Fluka. *meso*-Tetraphenylporphyrin was synthesized as described in Ref. [5], and its purity was checked.

3. Results and discussion

The absorption characteristics of compounds (**I**) are shown in Table 1. It is clearly seen that the number

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[1] The compounds (**I**) will be abbreviated as follows: 3-TMPP (5,10,15,20-tetrakis(3-methoxyphenyl)porphyrin ($R^1 = OCH_3$, $R^2 = R^3 = H$)), 3,4-TDMPP (5,10,15,20-tetrakis(3,4-dimethoxyphenyl)porphyrin ($R^1 = R^2 = OCH_3$, $R^3 = H$)), 3,5-TDMPP (5,10,15,20-tetrakis(3,5-dimethoxyphenyl)porphyrin ($R^1 = R^3 = OCH_3$, $R^2 = H$)), 3,4,5-TTMPP (5,10,15,20-tetrakis(3,4,5-trimethoxyphenyl)porphyrin ($R^1 = R^2 = R^3 = OCH_3$)).

Table 1
The visible absorption bands [λ_{max} (nm) (ε (10^3 M^{-1} cm^{-1}))] of porphyrins (**I**)

3-TMPP (benzene)	420 (490)	514 (20.6)	548 (7.0)	592 (5.3)	648 (3.2)
3,4-TDMPP (benzene)	426 (430)	518 (17.8)	556 (10.7)	594 (4.5)	654 (4.9)
3,5-TDMPP (benzene)	422 (520)	514 (22.2)	548 (6.2)	590 (5.8)	648 (2.9)
3,4,5-TTMPP (benzene)	426 (470)	518 (21.0)	554 (9.7)	592 (6.6)	648 (4.7)
3,4,5-TTMPP (EtOH)	418 (570)	514 (24.1)	550 (9.6)	590 (7.4)	646 (4.2)

and position of the methoxy groups do not significantly affect the ground state absorption properties. The strong absorption near 420 nm (Soret band, FWHM 14–18 nm) and the weak bands in the visible region (Q bands, FWHM ≈ 20 nm) suggest typical aetio type [3] spectra. The measured values are in agreement with the results in other solvents [4,9].

The absorption maxima of triplet–singlet difference spectra were determined from the flash photolysis measurements (see Table 2). The triplets have very strong and broad absorption near 450 nm, and only the monomethoxy derivative (3-TMPP) shows a slight shift to 440 nm. The actual triplet–triplet absorption spectra (corrected for ground-state absorption), however, might not show this difference since the intense ground-state Soret band overlaps with triplet absorption between 400 and 435 nm.

Triplet–singlet difference absorption coefficients (ε_T, see Table 2) were obtained by the energy transfer method [10]. The transient absorbance of the porphyrin triplet serving as the donor was compared with that of the naphthacene acceptor triplet used as the standard with known ε_T (31 200 M^{-1} cm^{-1} at 465 nm in benzene) and triplet quantum yield ($\Phi_T = 0.6$) [11]. The triplet energy difference between the donor [12] and the acceptor [13] in apolar solvents is around 10 kJ mol^{-1}. It was not possible to measure energy transfer in polar solvents, probably because of unfavorable changes in the triplet energies. The values of the triplet absorption coefficients are of the same order of magnitude as that of the parent *meso*-tetraphenylporphyrin in benzene (66 600 M^{-1} cm^{-1}) [12].

Triplet quantum yields (Φ_T, Table 3) were also determined by the energy transfer method with respect to the naphthacene standard mentioned. Purging the sample with helium resulted in long (≈0.5 ms) triplet lifetime, obviously influenced by remaining minute traces of oxygen. The triplet lifetimes were around 300 ns in the presence of air, the oxygen quenching rate constants (Table 3) were deduced from these measurements [14].

In the presence of air the emission signal of the singlet oxygen forming after laser excitation showed a first-order exponential decay. The initial singlet oxygen emission intensities were calculated from extrapolation to zero time (determined by the laser shot). These initial signal intensities were then plotted versus laser intensities (between 20 μJ and 2 mJ). The quantum yields of singlet oxygen production (Φ_Δ) were calculated from the slopes of the plots for the porphyrins investigated compared with the corresponding slope obtained for the reference (see Fig. 1). The singlet oxygen quantum yields obtained in this way were very similar for all of the compounds investigated. A higher value (Φ_Δ = 0.87) was published by Zen'kevich et al. [15] for 3-TMPP, using chlorophyll *a* or octaethylporphine as reference compounds.

Table 2
Main triplet–singlet difference absorption maxima and absorption coefficients [a] of compounds (**I**)

Compound (solvent)	Wavelength (nm)	Triplet–singlet difference absorption coefficients ε_T (M^{-1} cm^{-1})
3-TMPP (benzene)	440	45500
3,4-TDMPP (benzene)	450	38000
3,5-TDMPP (benzene)	450	34000
3,4,5-TTMPP (benzene)	450	31000

[a] Estimated accuracy ± 20%.

Table 3
Triplet quantum yields [a], oxygen quenching rate constants [a] and singlet oxygen quantum yields [b]

Compound (solvent)	Φ_T	Oxygen quenching rate constants ($\times 10^9$ M^{-1} s^{-1})	Φ_Δ
3-TMPP (benzene)	0.84	1.4	0.69
3,4-TDMPP (benzene)	0.63	1.5	0.69
3,5-TDMPP (benzene)	0.80	1.3	0.73
3,4,5-TTMPP (benzene)	0.71	1.2	0.66
3,4,5-TTMPP (EtOH)	[c]	1.4	0.67

Estimated accuracy: [a] ± 20%; [b] ± 10%.

[c] Energy transfer could be measured only in apolar solvents.

4. Conclusion

The 5,10,15,20-tetrakis(methoxyphenyl)porphyrins investigated show similar photophysical properties. Reasonable absorption in the red, high triplet quantum yield (0.63–0.84), long triplet lifetime (around 300 ns in the presence of air), and high quantum yield of singlet oxygen production ($\Phi_\Delta \approx 0.7$) show that substituted *meso*-tetraphenylporphyrins are good models to study the most characteristic photophysical features, the knowledge of which is regarded of great importance in the design of novel photosensitizers for PDT. We plan to carry out similar experiments in liposomal vesicles to investigate how the variation of the molecular environment affects these properties.

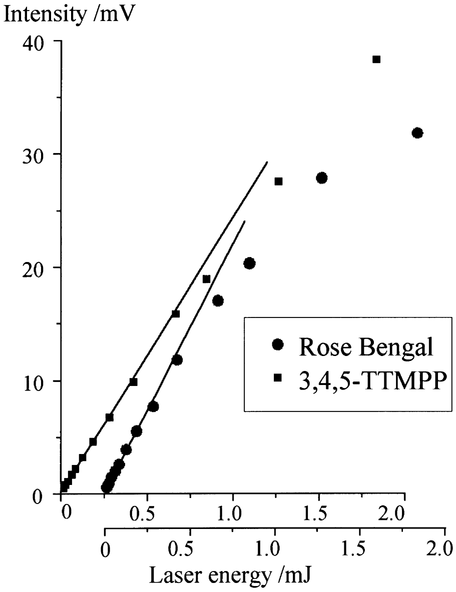

Fig. 1. Initial singlet oxygen signal intensity against pulse energy for 3,4,5-TTMPP: ■, Rose Bengal (standard); ●, in ethanol (absorbances at 532 nm were 0.583). In the linear region the slope of the plots for the porphyrin was compared to the slope obtained for Rose Bengal.

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References

[1] B.W. Henderson, T.J. Dougherty (Eds.), Photodynamic Therapy, Basic Principles and Clinical Applications, Dekker, New York, 1992.
[2] R. Bonnett, Chem. Soc. Rev. 24 (1995) 19.
[3] R. Pottier, T.G. Truscott, Int. J. Radiat. Biol. 50 (1986) 421.
[4] D.A. James, D.P. Arnold, P.G. Parsons, Photochem. Photobiol. 59 (1994) 441.
[5] R.A.W. Johnstone, M.L.P.G. Nunes, M.M. Pereira, A.M.d’A. Rocha Gonsalves, A.C. Serra, Heterocycles 43 (1996) 1423.
[6] J.F. Rabek, Experimental Methods in Photochemistry and Photophysics, Wiley-Interscience, Chichester, 1982.
[7] G. Grabner, G. Köhler, G. Marconi, S. Monti, E. Venuti, J. Phys. Chem. 94 (1990) 3609.
[8] F. Wilkinson, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 22 (1993) 113.
[9] W.J. Albery, P.N. Bartlett, C.C. Jones, L.R. Milgrom, J. Chem. Res. (M) (1985) 3801.
[10] R. Bensasson, E.J. Land, Photochem. Photobiol. Rev. 3 (1978) 163.
[11] B. Amand, R. Bensasson, Chem. Phys. Lett. 34 (1975) 44.

[12] R. Bonnett, D.J. McGarvey, A. Harriman, E.J. Land, T.G. Truscott, U.-J. Winfield, Photochem. Photobiol. 48 (1988) 271.
[13] S.L. Murov, Handbook of Photochemistry, Dekker, New York, 1973.
[14] W. Emmerich, R. Battino, Chem. Rev. 73 (1973) 1.
[15] E.I. Zen'kevich, E.I. Sagun, V.N. Knyukshto, A.M. Shul'ga, A.F. Mironov, O.A. Efremova, R. Bonnett, M. Kassem, Zh. Prikl. Spektrosk. 63 (1996) 599.