Pure & Appl. Chem., Vol. 67, No. 1, pp. 117–126, 1995.
Printed in Great Britain.

The photochemistry of sensitisers for photodynamic therapy

David Phillips

Imperial College, Department of Chemistry, London SW7 2AY

Abstract. The preparation and photophysical properties of a number of water-soluble metallated phthalocyanines are described. These compounds have been shown to be effective photosensitizers for the destruction of tumours. The photophysics of various aluminium phthalocyanines of different degrees of sulphonation, from mono (S_1) to tetra (S_4); and of various disulphonated (S_2) regioisomers was shown to be broadly similar, although the compounds varied greatly in terms of lipo solubility, and phototoxicity.

The effect of change of axial ligand, and of binding to the serum protein human serum albumin upon the photophysics of the dyes is also reported.

Introduction

Apart from radical surgery, the two major techniques used for the treatment of cancer are radiotherapy and chemotherapy. Whilst combatting tumour growth with some success, both methods can also induce disabling and life threatening side effects mainly because they destroy indiscriminately both normal and tumour tissue. Selective tumour destruction has thus become a major goal in oncology research. Photodynamic therapy (PDT) is a technique that uses light activated sensitisers, ie. compounds that are non-toxic until irradiated with light of an appropriate wavelength tuned to the absorption band of the sensitiser. Specific tumour targetting with PDT may be achieved firstly via using photosensitisers that accumulate preferentially in neoplastic tissue and secondly by confining and controlling the irradiation area using fibre-optically delivered laser light. Body tissue is relatively transparent in the red and near-infrared spectral regions with the consequence that PDT sensitisers absorbing at these longer wavelengths are able to produce a greater depth of tumour kill.

The ideal properties of a photosensitiser are easily summarised, although the assessment of a sensitiser in these terms is not as straightforward as might be supposed because the heterogeneous nature of biological systems can profoundly affect the properties. Ideally, a sensitiser should be red or near infrared light absorbing; non-toxic, with low skin photosensitising potency; selectively retained in tumours relative to normal adjacent tissue; an efficient generator of cytotoxic species, usually singlet oxygen; fluorescent, for visualisation; of defined chemical composition, and preferably water soluble, although with use of liposome delivery systems, the last is not essential.

The sequence of events in PDT are shown in Fig. 1, from which it can be seen that complete establishment of the protocol requires wider study of biochemical and photochemical phenomena. Here we concentrate upon the photochemical, with the following questions in mind.

1) What structure - efficacy correlates in 'good' sensitisers can be established?
2) What is the effect of protein binding upon photochemical properties?
3) What is the photochemical mechanism of primary damage?

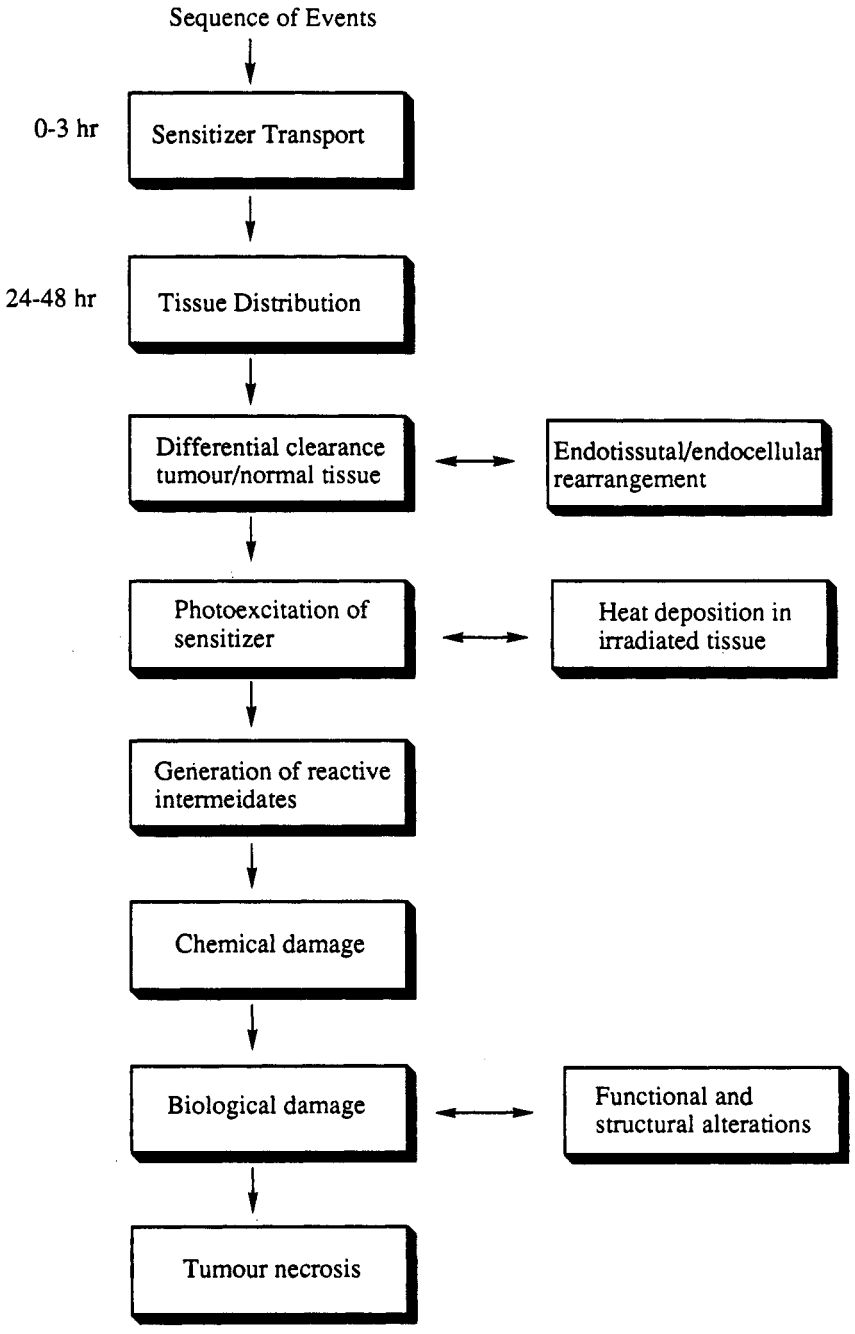
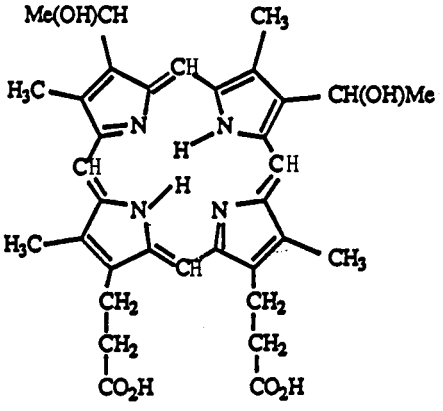


Fig. 1 Sequence of events in PDT (after Jori)

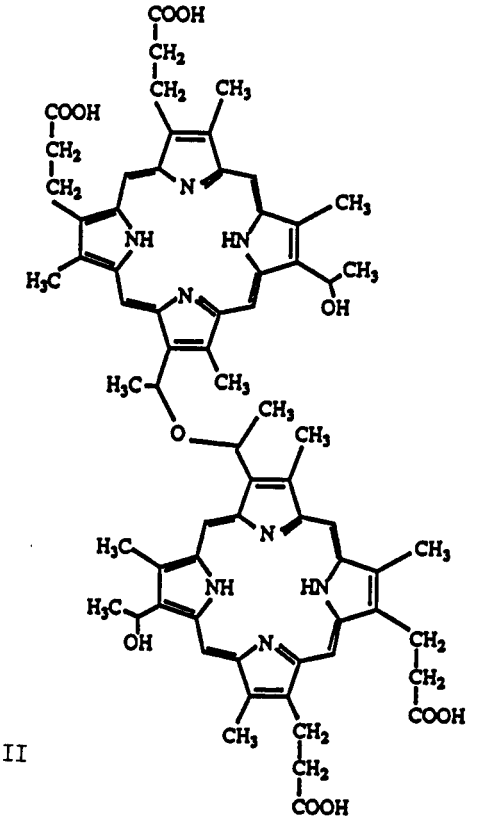
Structure of Sensitisers

All sensitisers to date are based upon porphyrin-like molecules e.g. porphyrins, chlorins, bacteriocholins and phthalocyanines. The in-vivo photodynamic properties of tetrapyrrolic pigments such as porphyrins has been known since the early 1900's when Meyer-Betz self-administered haematoporphyrin (Hp) to determine its biological effect.[1] To date the water soluble haematoporphyrin derivative (HpD) I, and its

purified form commercialised under the trade name Photofrin II, have been used extensively in clinically treating a variety of malignancies. HpD is formed by the treatment of haematoporphyrin with a mixture of acetic and sulphuric acids[2] to give a complex mixture of dimers and oligomers. The active component of HpD is believed to be either the dihaematoporphyrin ether II or di-haematoporhyrin ester (DHE).[3] Clinical trials using HpD have proven PDT to be an effective cancer therapy[4,5] and has shown considerable success in many human tumors.[6,7]



I



II

Although HpD has a low intrinsic toxicity at therapeutic doses and shows selective retention in tumor masses it consists of a complex mixture of porphyrins, the composition of which varies with different preparations and storage times. The disadvantage of attempting to work clinically with a complex mixture of substances are compounded by the fact that HpD may aggregate to different extents in different environment with resultant alterations in biological efficacy.[7-11] Furthermore, HpD absorbs red light only weakly. This is a major disadvantage in a treatment where it is better to use red or near infra-red light (λ_{abs} = 600-1000nm) for optimal tissue penetration.[12]

Finally, an additional complication is that patients receive HpD for PDT often suffer from photosensitive reactions to daylight as a consequence of the retention of appreciable amounts of dye in the skin.[12]

In order to overcome many of the disadvantages associated with HpD, a variety of alternative 'second-generation' photosensititsers have been investigated. Chlorins[13-16], purpurins[17-18], benzoporphyrins[19-20], verdins and rhodins[21], pheophytins[22], pheophorbides[23], tetra-aza-tetra-benzo porphyrins or phthalocyanines[24] and alkoxy phthalocyanines and the siloxy phthalocyanines[25-30] are all compounds with potential use in PDT. We concentrate here upon phthalocyanines, with which the Imperial College group has been long associated.

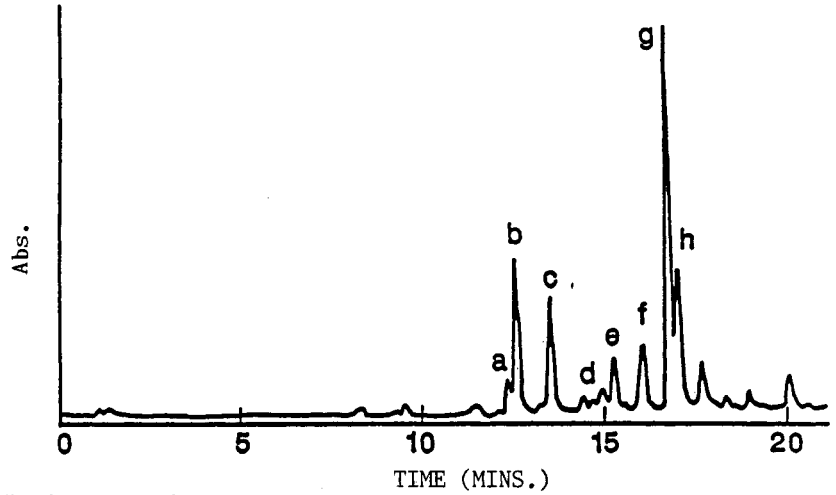


Fig. 2 HPLC trace of 'Clinical S2' disulphonated aluminium phthalocyanine mixture

Aluminium phthalocyanines

"Clinical" $AlPcS_2$ is material synthesised by sulphonation and medium pressure reverse phase chromatography by the Imperial College group. The HPLC chromatogram in Fig. 2 indicates its composition.[31,32] Traces for different batches of "Clinical" $AlPcS_2$ synthesised for biological and medical use show similar ratios of major component peaks, indicated in Fig. 2. The major peak, **g**, is the most lipophilic component. 16 disulphonated regio isomers could result from the synthetic method used, Fig. 3; component **g** has been identified as one of the three possible 3,3 isomer (structures 1, 2, and 3 in Fig. 3).

Photophysics of components

The absorption, fluorescence emission and excitation spectra of component **c,d,e,g,g/h** and **j** in methanol and aqueous solutions were analogues to the bulk $AlPcS_2$ material and thus typical of monomeric metallophthalocyanines.

In aqueous solution, the Q-band of all species was seen to be broader in comparison to that in methanolic solution and the maxima peaks were less sharp.[33] For component **e** in methanol a shoulder on the blue side of the Q-band was observed which became more pronounced in aqueous solution. An analogous profile was also seen in the fluorescence excitation spectra of both solutions. Additionally, evidence of dimerisation of this component in water was provided by the presence of a significant peak at 630 nm that was not observed in the fluorescence excitation spectrum. Components **a/b** and **f** displayed a splitting of the Q-band absorption in both solvents, and difference between the two peaks being more pronounced in water.

It is tentatively suggested that this Q-band splitting with components **a/b** and **f** is caused by different transition dipoles along the x and y direction of the phthalocyanine structure. For unmetallated phthalocyanines the absorption Q band is split into two bands labelled Q_y (lower wavelength) and Q_x. These signify electronic transitions in which the transition dipole lines along the y and x axes of the molecule as depicted in Fig. 4.

In symmetrical metallophthalocyanines the dipole moments are identical and so the Q-band appears as a single peak.[34] Compounds 11-16 in Fig. 3 have different symmetry with respect to the x and y molecular axes, and could thus give Q_x and Q_y transitions of different energies. Compound **f** is either isomer 15 or 16. **a** and **b** have not been identified.

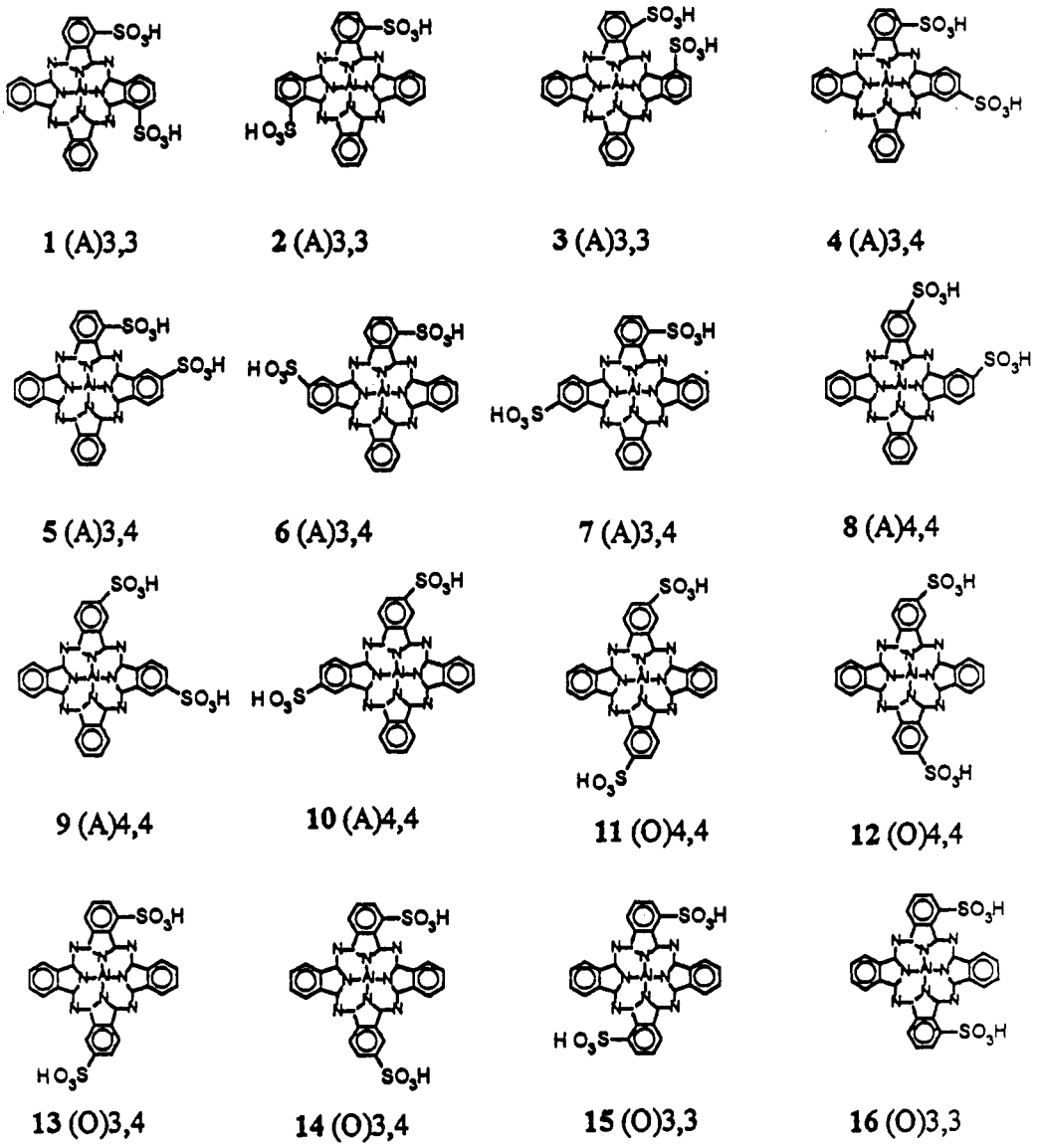


Fig. 3 Regioisomers of disulphonated aluminium phthalocyanine

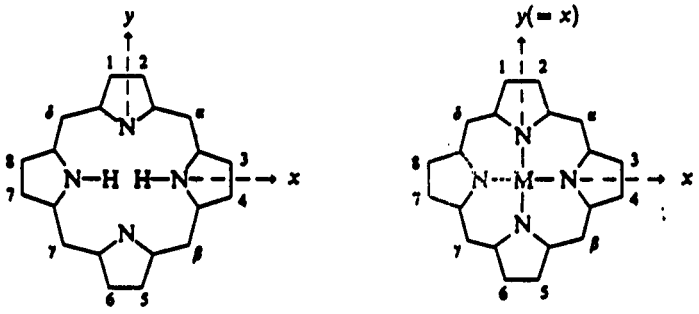


Fig. 4 Electronic transition dipoles of unmetallated and metallated porphyrin based molecules including phthalocyanines [from ref. 34]

Photophysical properties of components **a-j** are summarised in Table 1,[33] and are seen to be very similar. However, the photocytotoxicity is markedly different as can be seen from Fig. 5.

The results of the *in vitro* work with V79 cells shows that photocytotoxicity is generally related to component lipophilicity. Analogous results have been reported for aluminium, gallium and zinc phthalocyanines sulphonated to varying degrees[35-37] in photocytotoxic experiments with V79 cells and a correlation between increasing lipophilicity, as measured by partition coefficients, and cell uptake with differently substituted sulphonated aluminium phthalocyanines has also been shown.[31] The value of LD_{90} determined in this work for the bulk $AlPcS_2$ material, i.e. = 2.1 μM, is consistent with those reported in the literature for $GaPcS_2$ [3] and $AlPcS_2$,[4] i.e. 1.6 μM and 1.7 μM respectively (both used 4 minutes irradiation with a source emitting light at wavelengths greater than 590 nm with an energy fluence of 55 kJ m^{-2}). Component **g** is seen to have a slightly greater photocytotoxicity than the bulk material, whilst all other components are poorer agents.

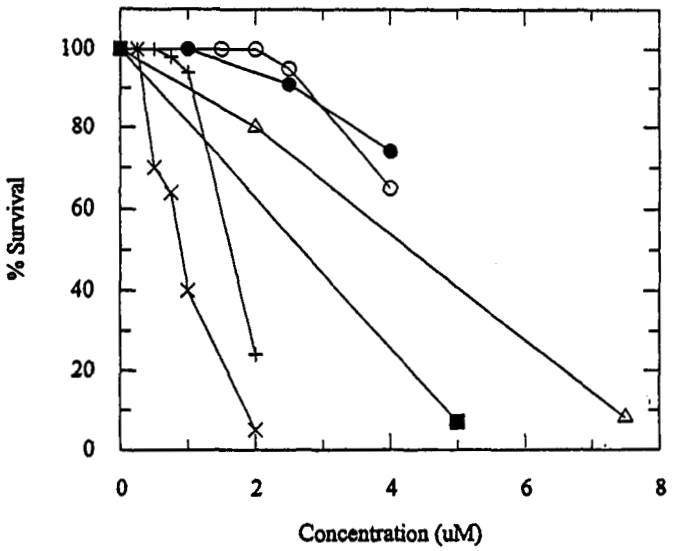


Fig. 5 Survival of V79 cells incubated for 1 hr with $AlPcS_2$ (+) and its isolated HPLC components **a/b** (●), **c** and **d**(Δ), **f**(■), **g**(X) and **g/h**(O) followed by exposure to a red light of intensity 0.71Wm^{-2} for 10 minutes.

The bulk $AlPcS_2$ material, which is at present undergoing tests as a photosensitiser for use as a potential clinical PDT drug, is a mixture of the above components and consequently has reduced photocytotoxic action compared to the isolated lipophilic component **g**. It may therefore be reasonably argued that this isolated species would be more efficacious. However, as demonstrated by Chan et.al.[38], the situation as regards uptake and efficacy *in vivo* is far more complex and less well understood than the *in vitro* case, i.e. *in vitro* the cytotoxicity was seen to increase with decreasing sulphonate substitution of aluminium phthalocyanines whereas *in vivo* the opposite effect was seen such that $AlPcS_4$ was taken up by the tumour to a greater extent than $AlPcS_1$. Recent *in vivo* studies by Peng et.al.[39] have shown that tumour damage is induced by the lipophilic species $AlPcS_{2a}$ (sulphonates on adjacent rings of the phthalocyanine) and the highly hydrophilic compound $AlPcS_4$. Importantly, they showed that the tumour uptake and distribution of each compound was different, i.e. $AlPcS2_a$ locating in tumour cells and causing damage to organelles and nuclear structure and $AlPcS_4$ residing in the vascular stroma and inducing tumour destruction *via* damage to vascular structures. Therefore a PDT treatment of a combination of these two species may prove to be far more efficacious *in vivo*.

Mechanism of phototoxicity

Sensitisers can in general operate via a Type I (radical/radical ion) or Type II (singlet oxygen) mechanism. We have measured the singlet oxygen yields in vitro, and the photophysics, of a variety of sensitisers. Data are collated in Table II, from which it can be see that the addition of fluoride to an aqueous or methanolic solution of $AlPcS_2$ results in a Q-band absorption spectral shift, increase in τ_T, Φ_T and Φ_Δ and a concomitant decrease in τ_T and Φ_F. The fluoride ions are likely to be acting as axial ligands which, through strong complexation to the metallophthalocyanine, perturb the excited state electronic energy levels resulting, in an increase in the intersystem crossing rate. Q-band absorption shifts for methanolic solutions of $GaPcS_2$ + fluoride and $ZnPcS_2$ + cyanide, which both should form strong complexes, were also observed but with no detectable effect upon excited state photophysics. An increase in Φ_T and Φ_Δ as seen with $AlPcS_2$ and fluoride produces a species with increased photodynamic effect; however the AlPc and F^- system has been shown in biological experiments to have a protecting PDT effect. The potential for

TABLE 2 **The photophysics of $AlPcS_2$, $ZnPcS_2$ and $GaPcS_2$ in methanol in the absence and presence of 10^{-2}M fluoride or cyanide ions.**

Pc	A_{max} (nm)	τ_F (ns)	τ_T[#] (μs)	Φ_T	Φ_Δ	S_Δ	k_F (10^8 s^{-1})	k_{ISC} (10^7 s^{-1})	k_{IC} (10^7 s^{-1})
$AlPcS_2$	668	5.9±0.1	775±80	0.24± 0.03	0.27± 0.03	1.13± 0.06	1.0±0.1	4.1±0.1	2.7±0.1
$AlPcS_2$ + F^-	662	4.6±0.1	935±90	0.33± 0.03	0.40± 0.04	1.21± 0.07	1.2±0.1	7.2±0.1	2.4±0.1
$AlPcS_2$ + CN^-	668	—	815±80	0.24± 0.03	0.27± 0.03	1.13± 0.06	—	—	—
$ZnPcS_2$	665	3.0±0.1	270±30	0.46± 0.05	0.52± 0.05	1.13± 0.10	1.2±0.1	15±1.0	5.5±0.1
$ZnPcS_2$ + F^-	665	3.0±0.1	210±20	0.46± 0.05	0.52± 0.05	1.13± 0.10	1.2±0.1	15±1.0	5.5±0.1
$ZnPcS_2$ + CN^-	671	—	230±20	0.46± 0.05	0.52± 0.04	1.13± 0.10	—	—	—
$GaPcS_2$	675	4.2±0.1	390±40	0.36± 0.04	0.38± 0.04	1.06± 0.08	—	8.6±0.1	—
$GaPcS_2$ + F^-	672	4.3±0.1	400±40	0.36± 0.04	0.38± 0.04	1.06± 0.08	—	8.4±0.1	—
$GaPcS_2$ + CN^-	675	—	360±40	0.36± 0.04	0.38± 0.04	1.06± 0.08	—	—	—

[#] for A = 0.4 AU

increased photodynamic action through axial ligand interactions may exist for other metal (or metalloid)/ligand combinations. We have reported elsewhere on the effect of deuterium substitution upon the photophysics of $AlPcS_2$.[40]

Photophysical parameters for some sulphonated gallium phthalocyanines are given in Table 3. In general all gallium species possessed the same photophysics as $GaPcS_2$.

TABLE 3 Some photophysical parameters of $GaPcS_n$ and $GaPcS_n{}^tBu_m$ in methanol.

Pc	A_{max} (nm)	τ_F (ns)	χ^2	DW	τ_T (μs)	Φ_T	Φ_Δ
$GaPcS_2$	675	4.22	0.95	1.91	390*	0.36	0.38
$GaPcS_3$	677	4.26	1.13	1.82	425*	0.36	0.38
$GaPcS_4$	677	4.43	1.01	1.78	420*	0.36	0.38
$GaPcS_1{}^tBu_3$	681	4.13	1.12	1.81	440**	0.36	0.38
$GaPcS_2{}^tBu_2$	679	4.14	1.23	1.66	360**	0.36	0.38
$GaPcS_3{}^tBu_1$	679	3.90	0.99	2.15	300**	0.36	0.38

* measured for A = 0.4 ** measured for A = 0.6

Binding to serum proteins

A complete study has been carried out upon the binding of $AlPcS_n$ (n =1-4) to serum proteins, as exemplified by human serum albumin, HSA.[41]

The HSA equilibrium association constants K_a for $AlPcS_n$ (n = 1,2, & 3) are of the order of 10^5 $mol^{-1}dm^3$, in agreement with previous determinations. The fluorescence decay profiles of $AlPcS_n$ as a function of [HSA] can be fitted to a three exponential decay function associated with the macrocycle experiencing three limiting microenvironments, (a) an interfacial region with a lifetime $\tau_1 \approx 1$ ns, (b) an aqueous environment with a lifetime τ_2 = 5 ns and (c) a non-aqueous environment with a lifetime τ_3 = 6.8 ns. The relative contributions of these environments to the overall decay is dependent on the degree of sulphonation. The contribution (a) A_1 associated with the lifetime τ_1 decreases as the degree of sulphonation increases (b) A_2 and A_3 associated with the lifetimes τ_2 and τ_3 increase and decrease respectively as the degree of sulphonation increases (for $AlPcS_2$, $AlPcS_3$ and $AlPcS_4$). The single exponential triplet state decay kinetics can be described in terms of a dynamic equilibrium between the free and bound forms of the macrocycle during the time span of the long lived triplet state. The limiting triplet lifetime of $AlPcS_2$, $AlPcS_3$ and $AlPcS_4$ are τ_t = 1350 μs, 950 μs and 720 μs respectively. These kinetic models together with triplet state and quenching studies indicate that the site(s) of $AlPcS_n$ (n = 1,2,3 & 4) association are dependent on the degree of sulphonation.

$AlPcS_1$. $AlPcS_1$ partitions to an interfacial site where quenching of the singlet state occurs resulting in a decrease in the fluorescence and triplet yields. The fluorescence and triplet lifetimes measured originated predominantly from the unbound form.

$AlPcS_2$. $AlPcS_2$ is bound at a site which is protected from the aqueous phase resulting in an increase in the fluorescence and triplet lifetimes and a significant reduction in the rate of quenching by BQH_2. On addition of fluoride ions the fluorescence decay kinetics indicate that $AlPcS_2$ is displaced from the protective protein microenvironment to the bulk aqueous phase. The decreased association of AlSPc with HSA has been attributed to the formation of the fluoro species $AlPcS_2$ (F) which would imply that the axial ligands play a role in determining the site and mode of association. While the formation of the shorter lived fluoro complex is observed in the fluorescence decay kinetics it is proposed that fluoride binds to the protein and displaces $AlPcS_2$ (>90%) from the site of binding. The remaining $AlPcS_2$ (~8%, τ_1 = 1 ns) which is not affected by fluoride ions is attributed to the presence of an interfacially bound impurity, presumably $AlPcS_1$.

AlS_3Pc. $AlPcS_3$ is bound to the protein resulting in significant changes in the profile of the Q band absorption. The changes in the Q band absorption are dependent upon the conformational structure of the protein. The fluorescence and triplet state studies indicate that $AlPcS_3$ is bound at a site which offers some protection from the bulk aqueous phase, but to a lesser extent than that experienced by $AlPcS_2$.

$ALPcS_4$. $AlPcS_4$ is irreversibly bound to acid soluble collagen by electrostatic interactions. In solution with HSA this hydrophilic species experiences the bulk aqueous phase. The rates of reductive quenching of the singlet and triplet excited state by BQH_2 and the observation of the long lived radical anion indicate that AlS4Pc is bound to the protein surface. The rate of quenching of the triplet state of $AlPcS_n$ (5μM) bound to HSA (30 μM) by O_2 is reduced by a factor of ten.

Conclusion

$AlPcS_2$ had been shown to be very effective sensitizer for PDT. The effects of axial ligand, of pattern, of sulphonation, and of binding to serum proteins have been found not to perturb greatly the basic photochemistry.

Acknowledgements

Financial assistant from Imperial Cancer Research Fund, the Waldburg Foundation, and the Science and Engineering Research Council is gratefully acknowledged. I am grateful to Sandy MacRobert, Andrew Beeby, and particularly Steve Bishop and Mary Foley, whose work this paper summarises. Financial support from the PDT Euronet [Contract ERB4050PL922071] is gratefully acknowledged.

References

1. F. Meyer-Betz, *Deut. Arch. Klin. Med.*, **112**, 476-503 (1913).
2. R.L. Lipson, E.J. Blades and A.M. Olsen, *J. Natl. Cancer Inst.*, **26**, 1-11, (1961).
3. T.J. Dougherty, W.R. Potter and K.R. Weishaupt, In *Porphyrin Localisation and Treatment of Tumours*, 301-314, Editited by D.J. Doiron, and C.J. Gomer, A.R. Liss, New York, (1984).
4. C.J. Gomer, N. Rucker, A. Ferrario and S. Wong, *Radiation Res.*, **120**, 1-18 (1989).
5. B.C. Wilson, and W.P. Jeeves, In *Photomedicine*, 127-177, Edited by E. Ben-Hur and I. Rosenthal, CRC Press, Boca-Raton, Florida, (1987)
6. A.H. Kaye, In *Photosensitising Compounds: Their Chemistry, Biology and Clinical Use*, 209-224, Edited by G. Bock, and S. Harnett, Ciba Foundation Symposium **146**, Wiley, Chichester, (1989).
7. T.J. Dougherty, in Porphyrin Photosensitisation, 3-13, Edited by D. Kessel, and T.J. Doherty, Plenum Press, New York, (1983).
8. D. Kessel, *Photochem. Photobiol.*, **39**, 851-859 (1984).
9. J. Moan, *Photochem. Photobiol.*, **43**, 681-690 (1986).
10. D. Kessel, Int. *J. Radiat. Biol*, **49**, 901-907, (1986).

11. T.J. Dougherty, *Photochem. Photobiol.*, **45**, 879-889, (1987).
12. B.C. Wilson, M.S. Patterson and D.M. Burns, *Laser Med. Sci.*, **1**, 235-244, (1986).
13. Ch., Grewer, G. Schermann, R. Schmidt, A. Völker, H.D. Brauer, A. Meier, and F.P. Montforts, *J. Photochem. Photobiol. B:Biol.*, **11**, 285-293, (1991).
14. H.B. Ris, H.J. Altermatt, R. Inderbitzi, R. Hess, B. Nachbur, J.C.M. Stewart, Q. Wang, C.K. Lim, R. Bonnett, M.C. Berenbaum and U. Althaus, *Br. J. Cancer*, **64**, 1116-1120, (1991).
15. T.G. Truscott, *J. Chem. Soc., Faraday Trans. 2*, **82**, 2177-2181 (1986).
16. J.K. Hoober, T.W. Sery, and N. Yamamoto, *Photochem. Photobiol*, **48**, 579-582, (1988).
17. C.F. Borland, D.J. McGarvey, A.R. Morgan and T.G. Truscott, *Photochem. Photobiol. B: Biol.*, **2** 427-434, (1988).
18. A.R. Morgan, G.M. Garbo, R.W. Keck, and S.H. Selman, *Cancer Res.*, **48**, 194-198, (1988).
19. A.M. Richter, S. Cerruti-Sola, E.D. Sternberg, D. Dolphin and J.G. Levy, *J. Photochem. Photobiol. B: Biol.* **5**, 231-244, (1990).
20. A.R. Morgan, V. Scherrer Pangka, and D. Dolphin, *J. Chem. Soc. Chem. Commun.*, 1047-1048 (1984).
21. A.R. Morgan, A. Rampersaud, R.W. Keck, and S.H. Selman, *Photochem. Photobiol. B:Biol.*, **46**, 441-444, (1987).
22. D. Kessel, and D.M. Smith, *Photochem. Photobiol.*, **49**, 157-160 (1989).
23. R.K. Pandey, D.A. Bellnier, K.M. Smith, T.J. Dougherty, *Photochem. Photobiol.*, **53**, 65-72, (1991)
24. N. Brasseur, H Ali, R. Wagner, R. Langlois, J. Rousseau and J.E. van Lier, *Photochem. Photobiol*, **45**, 583-586 (1987).
25. P.A. Firey, and M.A.J. Rodgers, *Photochem. Photobiol.*, **45**, 535-538, (1987).
26. N.C. Yates, J. Moan and A. Western, *J. Photochem. Photobiol. B: Biol.*, **4**, 379-390, (1990).
27. W. Ford, B. Rihter, M. Kenney and M.A.J. Rodgers, *Photochem. Photobiol*, **50**, 227, (1989).
28. V. Commo, G. Jori, B. Rihter, M.E. Kenney, and M.A.J. Rodgers, *Br. J. Cancer*, **62**, 966-970, (1990).
29. J. Sounik, L. Schechtman, B. Richter, W. Ford, M.A.J. Rodgers and M.E. Kenney, *Proc. SPIE, Int. Soc. Opt. Eng.*, **1203**, 293-300, (1990).
30. I. Syed, A. Zaidi, R. Agarwal, G. Eichler, B.D. Rihter, M.E. Kenney, and H. Mukhtar, *Photochem. Photobiol.*, **58(2)**, 204-210, (1993).
31. M. Ambroz, A. Beeby, A.J. MacRobert, M.S.C. Simpson, R.K. Svenson and D. Phillips,. *J. Photochem. Photobiol B: Biol.* **9**, 87, (1991).
32. S.M. Bishop, B.J. Khoo, A.J. MacRobert, M.S.C. Simpson and D. Phillips, *J. Chromatography* **646**, 345, (1993).
33. S.M. Bishop, Ph.D. Thesis, University of London 1994.
34. K. Berg, J.C. Bammer, J.W. Winkelman and J. Moan, *Photochem. Photobiol.*, **52**, 775, (1990).
35. N. Brasseur, H. Ali, R. Langlois and J.E. van Lier, *Photochem. Photobiol.*, **47**, 215-200, (1987).
36. B. Paquette, H. Ali, R. Langlois and J.E. Van Lier, *Photochem. Photobiol.*, **47**, 215-220 (1988).
37. N. Brasseur, H. Ali, R. Langlois and J.E. van Lier, *Photochem. Photobiol.* **47**, 705-711, (1988).
38. W.-S. Chan, J.F. Marshall, R. Svenson, J. Bedwell and I.R. Hart, *Canc. Res.* **50**, 4533-4538, (1990).
39. Q. Peng, J. Moan, J.M. Nesland and C. Rimington, *Int. J. Cancer*, **46**, 719-726, (1990).
40. A. Beeby, A.W. Parker, M.S.C. Simpson, and D. Phillips, *J. Photochem. Photobiol. (B) Biol*, **17**, 203, (1993).
41. M.S.C. Foley (nee Simpson), Ph.D. Thesis, University of London, 1994.