LUMINESCENCE OF PORPHYRINS AND METALLOPORPHYRINS IX: DIMERIZATION OF *meso*-TETRAKIS(*N*-METHYL-4-PYRIDYL)-PORPHINE

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Summary

meso-Tetrakis(*N*-methyl-4-pyridyl)porphine ($H_2TMPyP(4)^{4+}$) undergoes dimerization in aqueous solution. The dimer is believed to possess a stacked face-to-face configuration with the two porphyrin rings held about 1 nm apart. Fluorescence from the dimer is red shifted relative to the monomer but it is not quenched. The other isomeric H_2TMPyP^{4+} compounds together with metal chelates and the conjugate diacids exist only as monomeric species in aqueous solution (below 10^{-4} mol dm^{-3}).

1. Introduction

Water-soluble metalloporphyrins have found prominent use as photosensitizers in solar-energy storage devices based upon the photodissociation of water [1]. In particular the zinc complexes have been used widely in sacrificial photosystems which evolve H_2 and O_2 [2 - 6]. In order to render the porphyrin moiety water soluble, it is necessary to attach ionic groups (*e.g.* SO_3^-, CO_2^- or $N(CH_3)_3^+$) at the porphyrin ring and, in general, it has been found that negatively charged substituents induce a rather facile dimerization of the metalloporphyrin [7]. For example, *meso*-tetrakis(4-sulphonatophenyl)porphine and many of its metal complexes aggregate in aqueous solution at modest concentrations [8] and the aggregates have no tendency to function as photosensitizers. In contrast, positively charged metalloporphyrins show no tendency to dimerize in water. Thus, from nuclear magnetic resonance studies [9] it was found that zinc *meso*-tetrakis-(*N*-methyl-4-pyridyl)porphine ($ZnTMPyP(4)^{4+}$) had a dimerization constant of less than 7 dm^3 mol^{-1} whilst from absorption and temperature jump studies [10] it was concluded that the metal-free compound $H_2TMPyP(4)^{4+}$ was monomeric throughout the concentration range 10^{-6} - 10^{-4} mol dm^{-3}. In recent absorption and fluorescence studies with various isomeric H_2TMPyP^{4+} complexes [11] there was no mention of aggregation and this

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implied that all the compounds were monomeric in aqueous solution. However, on the basis of fluorescence experiments, Kano *et al.* [12] have reported that $H_2TMPyP(4)^{4+}$ exists in water in an aggregated form even at very low concentration (10^{-7} mol dm^{-3}). These authors made a convincing argument in support of aggregation and because of the importance of these compounds as photosensitizers and as labels for DNA and other biological cells [13, 14] we have investigated the fluorescence properties of $H_2TMPyP(4)^{4+}$. Our studies have confirmed that aggregation occurs at low concentrations with $H_2TMPyP(4)^{4+}$ but show that the other isomers are monomeric in water.

2. Experimental details

The various isomeric H_2TMPyP^{4+} compounds (chloride salts) were prepared and purified by conventional methods [15]. Water was deionized and doubly-distilled from a quartz still. Absorption measurements were made using a Perkin–Elmer 554 spectrophotometer with optical cells of path length 1 - 100 mm. Fluorescence studies were carried out using a Perkin–Elmer MPF4 spectrofluorometer and all reported fluorescence spectra were corrected fully for the wavelength response of the instrument [16]. Fluorescence quantum yields were determined by employing the optically dilute method, as was described previously [17], using air-equilibrated water. Singlet excited-state lifetimes were measured by means of the single-photon counting technique (time resolution, 100 ps or better). Aqueous solutions of the porphyrin (10^{-6} mol dm^{-3}) were excited at 580 nm by pulses from a mode-locked dye laser. Fluorescence was detected at right angles and the 660 nm region was isolated by bandpass filters. A fast photomultiplier tube was used to detect the fluorescence and the signals were averaged. Both the ortho and meta isomers gave single-exponential fluorescence decay curves ($\psi^2 < 1.20$) but the para isomer gave a poor fit to one exponential. A much better fit was obtained for dual-exponential decay ($\psi^2 = 1.18$).

3. Results and discussion

The various isomeric H_2TMPyP^{4+} compounds (chloride salts) were prepared by conventional methods and purified by ion-exchange chromatography. Their absorption spectra were in good agreement with those reported previously [10, 11, 15] although the extinction coefficients differed. Other workers have reported linear Beer's law plots for $H_2TMPyP(4)^{4+}$ in the concentration range 10^{-6} - 7×10^{-5} mol dm^{-3} [10] and 10^{-5} - 10^{-3} mol dm^{-3} [12] and Fig. 1 shows a similar plot for the concentration range 10^{-7} - 10^{-4} mol dm^{-3}. The Beer's law plot is linear over most of the concentration range, but it is curved at the lower concentration end. The behaviour

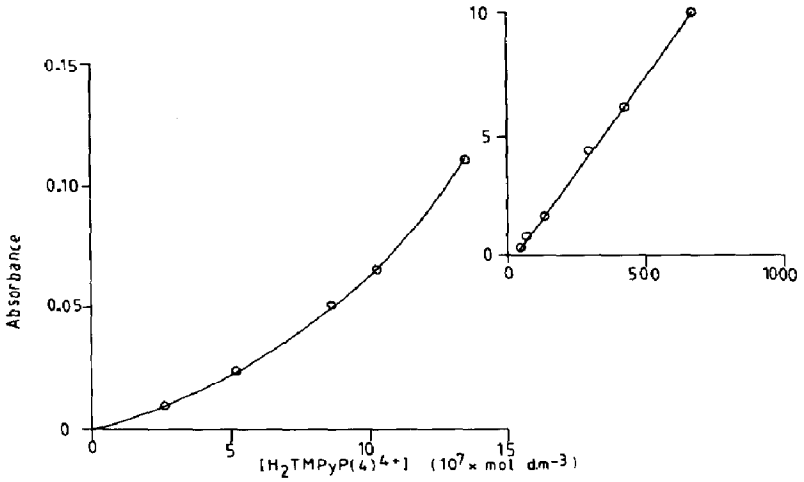

Fig. 1. Beer's law plot for $H_2TMPyP(4)^{4+}$ in aqueous solution as monitored at 424 nm (slits, 0.5 nm) using cells of different optical path length.

is consistent with very efficient dimerization and shows that at porphyrin concentrations greater than 10^{-6} mol dm^{-3} the dimer is the predominant species. Below 10^{-6} mol dm^{-3}, the porphyrin exists as an equilibrium mixture of dimer and monomer but we were unable to observe a system with only monomer species present. Analysis of the data gave a molar extinction coefficient of $(1.49 \pm 0.08) \times 10^5$ dm^3 mol^{-1} cm^{-1} at 424 nm for the dimer but no reliable estimate of the monomer extinction coefficient could be made. This prevented an accurate calculation of the dimerization constant K_D. With other water-soluble metal-free porphyrins, K_D varies [7] between 10^5 and 10^2 dm^3 mol^{-1}.

At the lowest concentration studied (10^{-7} mol dm^{-3}), the B band was blue shifted 1.5 - 2.0 nm relative to that found at 10^{-5} mol dm^{-3}. A similar spectral shift was observed in the fluorescence excitation spectra recorded for the above solutions. Thus dimerization is accompanied by a very slight red shift of the B band which suggests that there is minimal exciton coupling in the dimer [18]. Presumably, the strong electrostatic repulsion between the charged porphyrin moieties precludes the close approach of the two porphyrin rings. The linear Beer's law plot observed above 10^{-6} mol dm^{-3} suggests that association is restricted to dimerization rather than higher order aggregation. For the 2- and 3-isomers there is no evidence for dimerization and both the five-coordinated zinc chelate ($ZnTMPyP(4)^{4+}$) and the conjugate diacid ($H_4TMPyP(4)^{6+}$) [19] exhibit only monomeric species at concentrations below 10^{-4} mol dm^{-3}. Thus, of the compounds studied, only $H_2TMPyP(4)^{4+}$ shows evidence of dimerization.

This suggests that the dimer has a stacked face-to-face orientation with the two porphyrin rings held about 1 nm apart. (This distance is roughly compatible with a B band blue-shifted by 2 nm.) The non-planar diacid [19] and $ZnTMPyP(4)^{4+}$ complexes are prevented from adopting such

structures whilst steric hindrance prevents the stacking of the 2- and 3-isomers. However, the very high estimated K_D value shows that there is a large driving force for dimerization in $H_2TMPyP(4)^{4+}$ which must be connected with the electron density on the porphyrin ring. For the simple diamagnetic $TMPyP(4)^{4+}$ complexes [20] it is believed that the positive charges on the pyridinium nitrogen atoms are somewhat delocalized over the porphyrin ring via resonance structures of the type

The 3-isomer cannot pull electron density from the porphyrin ring and it can only delocalize the positive charge around the pyridyl group. In principle, the 2-isomer can push the positive charge onto the porphyrin ring but it is prevented from doing so by steric hindrance to rotation of the pyridyl group around the plane of the porphyrin ring [21]. Consequently, only the 4-isomer can push the positive charge onto the porphyrin ring via resonance isomerism and this positive character seems to be responsible for the very efficient dimerization found for $H_2TMPyP(4)^{4+}$.

As reported by Kano *et al.* [12] the dimerization of $H_2TMPyP(4)^{4+}$ has a pronounced effect upon the fluorescence spectrum. Fluorescence spectra of the isomeric H_2TMPyP^{4+} compounds, recorded at 10^{-6} mol dm^{-3} in aqueous solution, are given in Fig. 2. The spectra are in poor agreement with those reported previously [11, 12] because the latter were uncorrected and consequently possess little significance. A comparison of the various spectra in Fig. 2 shows that the 2- and 3-isomers give spectra with two well resolved bands that look similar to the reported [22] fluorescence spectra of water-insoluble metal-free porphyrins. These spectra are assigned to monomer species and the relevant fluorescence quantum yields ϕ_F and excited singlet state lifetimes τ_s are given in Table 1. The corrected fluorescence spectrum recorded for $H_2TMPyP(4)^{4+}$ at 10^{-6} mol dm^{-3} is fairly broad with a maximum at about 730 nm and a poorly defined shoulder at about 680 nm. Diluting the solution to about 10^{-8} mol dm^{-3} leads to a marked change in the spectral profile (Fig. 2(d)). In very dilute solution, the fluorescence spectrum shows the expected two bands (at 650 and 720 nm) characteristic of the monomer. Thus, the fluorescence spectrum observed from $H_2TMPyP(4)^{4+}$ at 10^{-6} mol dm^{-3} is assigned to the dimeric species. Changing the counterion (Cl^-, I^-, SO_4^{2-} or tosylate) had no effect upon the fluorescence spectra of either monomer or dimer and for all the compounds there was a good correlation between the corrected excitation spectrum and the ground-state absorption spectrum.

Normally, the consequence of dimerization is the quenching of the fluorescence since internal conversion is more efficient in the dimer than in the monomer [23]. However, the extent of fluorescence quenching depends upon the amount of exciton coupling in the dimer [24] and upon the struc-

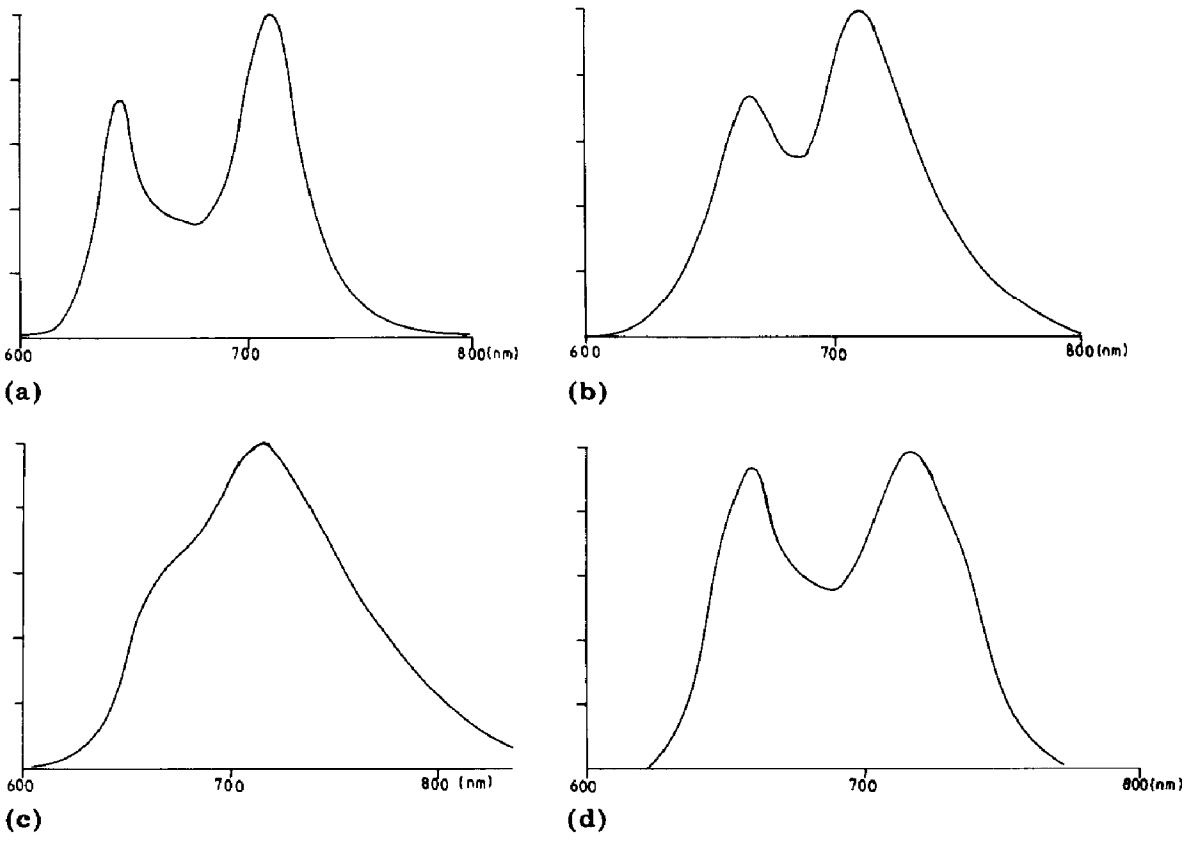

Fig. 2. Corrected fluorescence spectra recorded for the various isomeric H_2TMPyP^{4+} compounds in aqueous solution (y axis, relative intensity): (a) $H_2TMPyP(2)^{4+}$ (10^{-6} mol dm^{-3}); (b) $H_2TMPyP(3)^{4+}$ (10^{-6} mol dm^{-3}); (c) $H_2TMPyP(4)^{4+}$ (10^{-6} mol dm^{-3}); (d) $H_2TMPyP(4)^{4+}$ (10^{-8} mol dm^{-3}).

TABLE 1

Fluorescence properties of the isomeric H_2TMPyP^{4+} compounds recorded in air-equilibrated aqueous solution at 10^{-6} mol dm^{-3} (and at 10^{-8} mol dm^{-3} for monomeric $H_2TMPyP(4)^{4+}$)

Compound	*Species*	ϕ_F [a]	τ_s [b] (ns)	τ_s [c] (ns)	τ_s [d] (ns)
$H_2TMPyP(2)^{4+}$	Monomer	0.082	10.4	13.8	—
$H_2TMPyP(3)^{4+}$	Monomer	0.057	5.3	7.9	—
$H_2TMPyP(4)^{4+}$	Monomer	0.048	3.7	—	—
$H_2TMPyP(4)^{4+}$	Dimer	0.056	6.1	6.0	4.1

[a] ±5%.
[b] ±1 ns (this work).
[c] From ref. 11.
[d] From ref. 12.

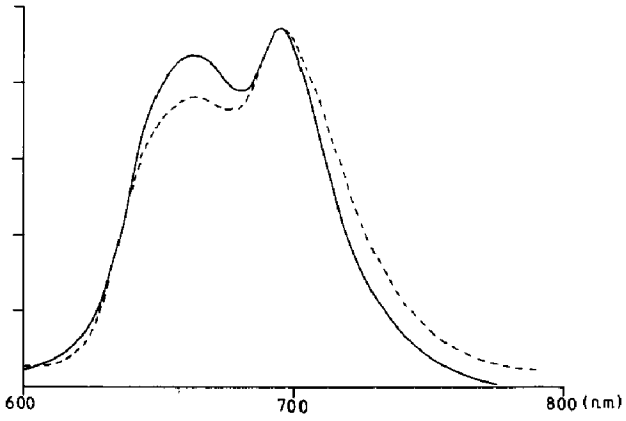

Fig. 3. Uncorrected time-resolved fluorescence spectra observed with $H_2TMPyP(4)^{4+}$ (10^{-7} mol dm^{-3}) in aqueous solution (*y* axis, relative intensity): ———, recorded 0 - 0.8 ns after the pulse; — — —, recorded more than 4 ns after the pulse. The spectra have been normalized at 700 nm.

ture of the dimer [25]. For $H_2TMPyP(4)^{4+}$, the fluorescence properties of the dimer are almost identical with those of the monomer (Table 1). This is to be expected in view of the minimal exciton coupling in the dimer and, in fact, our work shows that the dimer is slightly more fluorescent than the monomer. This is apparent not only from the values of ϕ_F but also from the time-resolved fluorescence spectra shown in Fig. 3: the fluorescence spectrum recorded at early times (0 - 0.8 ns after the pulse) is somewhat blue shifted relative to that recorded at longer times (more than 4 ns after the pulse). Thus, the dimer appears to possess the longer lifetime.

In accordance with the known properties of porphyrin dimers [7] the monomer–dimer equilibrium can be perturbed by environmental changes. Thus, addition of pyridine or ethanol to an aqueous solution of $H_2TMPyP(4)^{4+}$ (10^{-6} mol dm^{-3}) results in the appearance of the characteristic monomer fluorescence spectrum. Similarly, adsorbing the porphyrin onto the surface of colloidal TiO_2 or onto sodium dodecylsulphate micelles restores the monomer fluorescence. Increasing the temperature to 80 °C brings about a progressive increase in the concentration of monomer, as monitored by means of fluorescence spectroscopy. Finally, addition of KCl (1 mol dm^{-3}) to a very dilute solution of $H_2TMPyP(4)^{4+}$ (about 10^{-8} mol dm^{-3}) causes dimerization.

Overall, there is strong spectroscopic evidence to show that $H_2TMPyP(4)^{4+}$ undergoes efficient dimerization in aqueous solution whereas the other isomeric H_2TMPyP^{4+} compounds together with the non-planar chelates and diacids do not dimerize in aqueous solution at concentrations below 10^{-4} mol dm^{-3}. The dimerization of $H_2TMPyP(4)^{4+}$ does not really affect its fluorescence properties, although the spectrum of the dimer is markedly different to that of the monomer. These findings show that the use of $H_2TMPyP(4)^{4+}$ as a fluorescent label for DNA and other biological materials is not to be recommended.

Acknowledgments

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