

## Doubly N-Confused Porphyrins as Efficient Sensitizers for Singlet Oxygen Generation

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The high singlet oxygen quantum yield (ca. 0.8) for the Ag(III) complex of doubly N-confused porphyrin, a potential PDT photosensitizer, was observed according to its favourable intersystem crossing and the triplet lifetime characteristics.

Nowadays, there is a great interest in finding new molecules that can be used as sensitizers in photodynamic therapy (PDT).<sup>1</sup> Porphyrins and their analogues are being extensively studied, aiming this kind of application because of their favourable photophysical and photochemical properties such as long excited triplet state lifetime and high quantum yield for photoinduced generation of singlet oxygen.<sup>2</sup> However, the majority of porphyrins have low absorptivities in the phototherapeutic window (about 600 to 1000 nm), where light can be more effectively used, because it can penetrate deeper in the tissues. Recently, we have reported a new type of N-confused porphyrin (NCP) isomer, doubly N-confused porphyrin ( $H_2N_2CP$ ) and its Cu(III) and Ag(III) complexes,  $MHN_2CP$  (Figure 1), exhibiting relatively intense absorption bands around 660 nm.<sup>3</sup> Now the photophysical properties of the doubly N-confused porphyrins have been investigated in order to evaluate their potentiality as sensitizers in photodynamic therapy. A remarkable discovery is that the Ag(III) complex constitutes a very efficient singlet oxygen generator in solution.



**Figure 1.** Structures of the core framework of N-confused porphyrin, free base doubly N-confused porphyrin, and its metal complexes.

$H_2N_2CP$  and  $MHN_2CP$  in  $CH_3CN$  solution exhibited absorption band profiles similar to those found previously in dichloromethane,<sup>3</sup> namely, the lowest energy absorption bands were found at 710 and 665 nm, respectively.  $H_2N_2CP$  shows an emission band with a shoulder at lower energy side around 720 nm. On the other hand, no detectable fluorescence could be observed for the Ag(III) and Cu(III) metal complexes, suggesting that relatively rapid nonradiative relaxation pathways are competing with the radiative process for the singlet excited state species.

The triplet state lifetimes were measured by flash-photolysis

**Table 1.** Triplet state lifetime ( $\tau_0^T$ ), singlet oxygen quantum yields ( $\phi_\Delta$ ) and bimolecular quenching rate constants ( $k_{sup}$ ) for the doubly N-confused porphyrins in acetonitrile solution

	$\tau_0^T/\mu s$	$\phi_\Delta$	$k_{sup}/ \times 10^{-8}$
$H_2N_2CP$	1.2	0.19	9.8
$AgHN_2CP$	1.8	0.81	1.9
$CuHN_2CP$	1.4	0.48	11.7

technique and are summarized in Table 1. A strong bleaching of the absorption bands of the doubly N-confused porphyrin species could be observed, particularly in the range of 400 to 500 nm. This behavior is consistent with a triplet excited state localized on the macrocyclic ligand.<sup>4</sup> Interestingly the triplet state lifetimes of the Ag(III) and Cu(III) complexes are slightly longer than of the free base, suggesting that the metal coordination is reducing the number or the efficiency of the thermal quenching pathways. Presumably, the vibrational coupling of the solvent with the three inner ring hydrogen atoms constitutes the main thermal relaxation pathway suppressed by the coordination of the metal ion.

The most striking results were obtained when the doubly N-confused porphyrins were used as photosensitizers for singlet oxygen generation. This species is very reactive<sup>5,6</sup> and responsible for the photodynamic effects of Type II sensitizers. The development of new molecules able to efficiently transfer energy to molecular oxygen is of great interest from both the scientific and technological points of views. The free base  $H_2N_2CP$  was found to be poor sensitizers, exhibiting quantum yield, 0.19. In contrast, the Ag(III) complex is highly efficient ( $\phi_\Delta = 0.81$ ), even more than the methylene blue used as standard ( $\phi_\Delta = 0.5$ ). In fact, it is similar to the quantum yield of the monoacidic benzoporphyrin ( $\phi_\Delta = 0.76$ ) which is the active species of Visudyne®, the phototherapeutic drug recently approved by FDA for treatment of macular neovascularization.<sup>7</sup> The copper complex exhibited intermediary quantum yields. Less than 15% decrease in absorbance was observed after 6000 laser shots of 3 mJ, at 532 nm, showing that all species are reasonably stable.<sup>8</sup>

Interestingly, the slope of the singlet oxygen phosphorescence decay curves monitored at 1270 nm, increased as a function of the sensitizer concentration. This behavior is consistent with a second order mechanism, where the photogenerated  $^1O_2$  is quenched by the sensitizer itself. Accordingly, the rate constants for this process were determined by using a Stern–Volmer plot and listed in Table 1. The most efficient quenchers were found to be the free base and the copper complex, in contrast with the silver complex.

The excited singlet state has too much short lifetime (1.0 ns)

to effectively participate in bimolecular events. Consequently, the higher efficiency of the metal complexes should be associated with the heavy atom effect, which is known to increase the intersystem crossing quantum yield to the triplet state. In fact, the free base is the less efficient sensitizer. Two other factors, the lifetime and the suppression constant ( $k_{\text{sup}}$ ) of the singlet oxygen by the sensitizer may also play a role. Consequently, the silver derivative has the highest  $\phi_{\Delta}$  value, reflecting its high intersystem crossing yield, longest lifetime and smallest  $k_{\text{sup}}$  value, as shown in Table 1.

In conclusion, from the photophysical properties of free base, Ag(III) and Cu(III) doubly N-confused porphyrin complexes, as examined by means of electronic and time-resolved spectroscopy, one can infer that the fluorescence of the metal complexes is being suppressed by the enhancement of the intersystem crossing, induced by the heavy atom effects rather than by a redox mechanism. The high singlet oxygen quantum yield for the silver derivative (ca. 0.8) can be explained on the basis of the favourable intersystem crossing and lifetime characteristics. Because of the relatively high absorption bands in the 600 to 750 nm range and good thermal and photochemical stability, the  $\text{Ag}^{\text{III}}\text{HN}_2\text{CP}$  species turns out to be the first unusual valence metal ion macrocycle complex with suitable features as PDT photosensitizer. The further details of the photochemistry of doubly N-confused porphyrins will be reported in due course.

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## References and Notes

- 1 "Photodynamic Therapy of Neoplastic Disease," ed. by D. Kessel, CRC Press, Boca Raton (1990), Vol. 2.
- 2 a) R. Bonnett, *Chem. Soc. Rev.*, **24**, 19 (1995). b) F. Stewart, P. Baas, and W. Star, *Radiother. Oncol.*, **48**, 233 (1998). c) E. D. Sternberg, D. Dolphin, and C. Brückner, *Tetrahedron*, **54**, 4151 (1998).
- 3 a) H. Furuta, H. Maeda, and A. Osuka, *J. Am. Chem. Soc.*, **122**, 803 (2000). b) K. Araki, H. Winnischofer, H. E. Toma, H. Maeda, A. Osuka, and H. Furuta, *Inorg. Chem.*, **40**, 2020 (2001).
- 4 K. Araki, P. Losco, F. M. Engelmann, H. Winnischofer, and H. E. Toma, *J. Photochem. Photobiol. A*, **142**, 25 (2001).
- 5 R. W. Redmond and J. N. Gamlin, *Photochem. Photobiol.*, **70**, 391 (1999).
- 6 P. Dimascio, E. J. H. Bechara, M. H. G. Medeiros, K. Briviba, and H. Sies, *FEBS Lett.*, **355**, 287 (1994).
- 7 J. W. Miller, U. Schmidt-Erfurth, M. Sickenberg, C. J. Pournaras, H. Laqua, I. Barbazetto, L. Zografas, B. Piguet, G. Donati, A. M. Lane, R. Birngruber, H. Van den Berg, A. Strong, U. Manjuris, T. Gray, M. Fsadni, N. M. Bressler, and E. S. Gragoudas, *Arch. Ophthalmol.*, **117**, 1161 (1999).
- 8 Singlet oxygen decay kinetics and its formation quantum yields were monitored by its phosphorescence at 1270 nm using a time-resolved NIR fluorimeter (Edinburgh Analytical Instruments), in which a Nd:YAG laser from Continuum (Surelite III) excites the samples (30–300  $\mu\text{M}$  in  $\text{CH}_3\text{CN}$ ) at 532 nm and light emission was collected into a NIR-PMT (R5509 from Hamamatsu Co.) after passing to a silicon filter and a monochromator.