An Iron(III) Porphyrin that Exhibits Minimal Dimerization in Aqueous Solution

P. HAMBRIGHT*, A. TURNER

Department of Chemistry, Howard University, Washington, D.C. 20059, U.S.A.

J. S. COHEN, R. C. LYON, A. KATZ

Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Md. 20892, U.S.A.

P. NETA

National Bureau of Standards, Gaithersburg, Md. 20899, U.S.A.

and A. ADEYEMO

Department of Chemistry, University of Ibadan, Ibadan, Nigeria

(Received October 27, 1986)

Certain porphyrins and metalloporphyrins concentrate in malignant tissue [1, 2] and magnetic resonance (MR) imaging [3] and relaxometry [4] techniques have been used to examine tumors and organs of animals which have been injected with paramagnetic water soluble metalloporphyrins. Such complexes acting as paramagnetic contrast agents should have a high relaxivity value, where the relaxivity is the slope of the longitudinal spin lattice relaxation rate of water protons ($1/T_1$) *versus* the concentration of the agent. Since $1/T_1$ is proportional to the square of the effective magnetic moment of the complex [5], high spin d^4 manganese(III) and f^7 gadolinium(III) porphyrins have been studied [3, 4]. While Gd(III)–tetrakis(4-sulfonatophenyl)-porphyrin (Gd–TPPS) has about twice the relaxivity as does Mn-TPPS, Gd^{3+} is removed from the macrocycle under *in vitro* conditions. Preliminary work on the two water soluble d^5 iron(III) porphyrins, tetrakis(*N*-methyl-4-pyridyl)porphyrin (Fe–TM-PyP) and Fe–TPPS showed relaxivity parameters that decreased from a common high value at pH 1 to undesirably lower values near physiological pH [4]. This was due to the formaton of mu-oxo bridged Fe–O–Fe dimers around pH 7, where the antiferromagnetically coupled iron centers produce low moments [6]. Under the same conditions, the iron(III) complexes of the picket fence type tetrakis(α,α,α,β-*ortho*-(*N*-methyl-isonicotinamidophenyl))porphyrin (Fe–PF) showed less change in relaxivity with pH than did either of the less sterically hindered derivatives. We report the preparation and solution properties of Fe–PF, which is the least dimerized of any reported porphyrin in aqueous solution. The use of relaxivity data as a complement to the usual spectral and magnetic measurements on paramagnetic complexes is described.

Experimental

The free base of the tetrakis(α,α,α,β-*ortho*-(*N*-methyl-isonicotinamidophenyl))porphyrin (Fig. 1) was synthesized by literature methods [7]. To prepare the Fe(III)–PF, a twenty fold molar excess of cadmium nitrate was added to an aqueous solution of H_2-PF to preform Cd–PF, and this solution was warmed at 40 °C for twenty minutes with excess $FeCl_2$. The absorption spectra indicated complete transmetallation [8]. The solution was filtered, and the porphyrin precipitated with aqueous sodium iodide, and washed with small portions of ice water. The metalloporphyrin was redissolved in water, passed through a chloride ion exchange column, and lyophilized by the freeze-dry technique. *Anal.* Calc. for $C_{72}N_{12}O_4H_{44}FeCl_5 \cdot 11H_2O$: C, 54.6; N, 10.6; H, 5.0; Fe, 3.5; C/N, 6.0. Found: C, 54.7; N, 10.6; H, 5.4; Fe, 3.4; C/N, 6.0%.

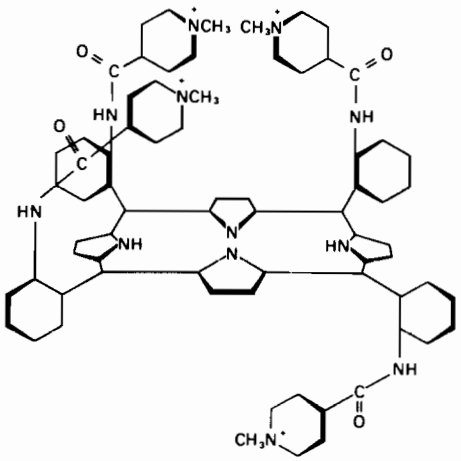

Fig. 1. Structure of the water soluble picket fence porphyrin.

The magnetic moments were determined by the Evans PMR method [9] at 20 °C on 3–7 mM solutions of the porphyrins in D_2O. A Gammacell 220 ^{60}Co source was used for steady state radiolytic reductions [10] of N_2O saturated aqueous solutions of the iron porphyrins containing 2% isopropanol. Longitudinal spin lattice relaxation times (T_1) were measured at 10.7 MHz (0.25 Tesla) using a Praxis II pulse spectrometer equipped with a 10 mm probe

*Author to whom correspondence should be addressed.

0020-1693/87/$3.50

and temperature controlled at 37 °C. Solutions of 0.5–4 mM porphyrin were prepared in H_2O at various pHs. The solutions for pK_a measurements were buffered with MES, PIPES and TRIS buffers at 10^{-2} M concentrations.

Results

The equilibrium studies at ionic strength 0.1 ($NaNO_3/HNO_3$) were run at 25 °C. At pH 2 and 7, Fe–PF obeyed Beers law in the 3×10^{-4} M to 5×10^{-7} M range. The major bands and molar extinction coefficients at pH 2, attributed to $(H_2O)_2$–Fe–PF were 685 nm (1.7×10^3 M^{-1} cm^{-1}), 580 nm (2.1×10^3), 532 nm (7.9×10^3) and 400 nm (7.3×10^4). In the 10 μM concentration range, a spectrophotometric pH titration from pH 2 to 8 (Fig. 2) showed isosbestic points at 720, 687, 555 and 512 nm. The reaction monitored was mono-hydroxy–Fe–PF fomation

$$(H_2O)_2\text{–Fe–PF} = HO(H_2O)\text{–Fe–PF}^- + H^+ \quad K_{a1} \tag{1}$$

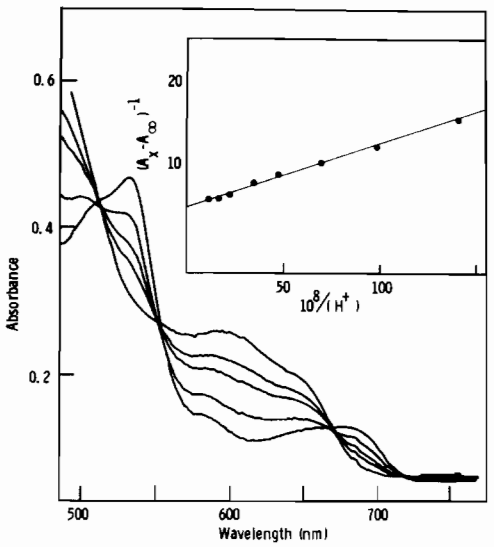

Fig. 2. A typical spectrophotometric pH titration of Fe–PF in the visible region. The band at 532 nm decreases and that at 600 nm increases with an increase in pH. The insert shows a typical double reciprocal plot used to calculate pK_{a1}.

Analysis of the spectral curves in Fig. 2 by standard methods gave $pK_{a1} = 6.0 \pm 0.1$. For $HO(H_2O)$–Fe–PF^-, the major peaks were at 600 nm (4.0×10^3) and 420 nm (5.6×10^4). Between pH 8 and 12, much smaller absorbance changes than those shown in Fig. 2 were found. Isosbestic points were noted at 555, 625 and 740 nm, with a small decrease in absorbance at 600 nm and an increase at 675 nm,

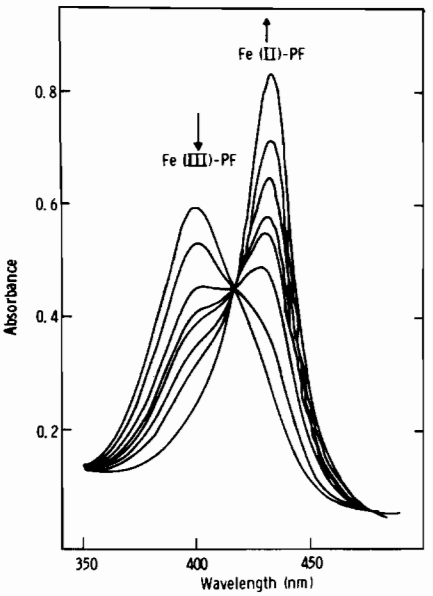

Fig. 3. Soret spectra at pH 1.8 of the reduction of Fe(III)–PF to Fe(II)–PF by $(CH_3)_2COH$ radicals.

as the pH increased. Using differential spectrophotometric methods, a $pK_{a2} = 10.5 \pm 0.2$ was found for the reaction

$$HO(H_2O)\text{–Fe–PF}^- = (HO)_2\text{–Fe–PF}^{2-} + H^+ \quad K_{a2} \tag{2}$$

Figure 3 shows the spectra in the Soret region of the reduction of Fe(III)–PF to Fe(II)–PF by the radiolytically produced $(CH_3)_2CHOH$ radicals at pH 1.8. The isosbestic point at 417 nm indicates that the reduced and oxidized iron porphyrins are the major absorbing species. The Fe(II)–PF at 432 nm has $\epsilon = 1.0 \times 10^5$ M^{-1} cm^{-1}, and addition of O_2 rapidly reforms Fe(III)–PF. While the latter is stable in 0.1 M acid, the Fe(II)–PF slowly solvolyzes into the di-acid form under such conditions, as was found previously for the Fe(II)–tetrakis-(4-*N*,*N*,*N*-trimethylanilinium)porphyrin [11].

The magnetic moments of Fe–TMPyP, Mn–TPPS and Fe–PF obtained by us are shown in Table I. Related data by others are also listed. The relaxivity values of these metalloporphyrins in solution at the corresponding pHs are given in Table II.

Discussion

In the course of evaluating water soluble paramagnetic metalloporphyrins as potential organ contrast agents for MR imaging [3, 12], we noticed that the relaxivity of Fe–PF was much less dependent on pH than that of Fe–TPPS or Fe–TMPyP (Table II). The pH dependence presumably arises [13, 14] by the formation from iron monomers of the antiferro-

TABLE I. Magnetic Moments (BM) of Metalloporphyrins in Solution[a]

	pH			Reference
	2.0	7.0	10.0	
Mn(III)–P				
Fe–PF[b]	5.9	5.6	5.4	tp[c]
Fe–TMPyP	5.9	2.4	2.2	tp
	6.1	2.8	2.9	21[d]
	6.0	2.5		17
Fe–TPPS	6.0	2.8	2.7	21
Mn–TPPS[e]	4.8	4.9	4.8	tp

[a]T = 20 °C, 3–7 mM solutions, $\mu \pm 0.1$ BM. [b]μ = 2.3 BM at pH 10 with excess cyanide. [c]tp is this paper. [d]pHs are 2.5, 7.2 and 9.2 in ref. 21. [e]μ = 3.8 BM for the green form at pH 13.

TABLE II. Proton Relaxivity Values (mM s^{-1}) of Metalloporphyrins in Solution[a]

	pH			
	1.0	4.0	7.0	10.0
Mn(III)–P				
Fe–PF	4.0	3.9	2.7	3.8
Fe–TMPyP	4.3[b]	4.2	1.3	1.3
Fe–TPPS	3.9	3.3	0.08	0.05
Mn–TPPS	7.6		7.7	6.4

[a]T = 37 °C, 0.05–4 mM solutions. [b]pH = 1.5.

magnetically coupled iron(III)mu-oxo dimers in the neutral pH region

$$2H_2O\text{–Fe–PF} = P\text{–Fe–O–Fe–}PF^{2-} + 2H^+ + H_2O \qquad K_3 \qquad (3)$$

For both Fe–TMPyP [15] and Fe–TPPs [16], $K_3 \sim 10^{-8}$ M. Solid state Mössbauer [17], solution kinetic [15–18], electrochemical [19] and EPR [20] studies support the existence of water soluble Fe–O–Fe dimers, and related work has appeared on water insoluble iron porphyrin dimers [13].

Goff and Morgan [21] found that Fe–TPPS and Fe–TMPyP had typical $S = 5/2$ high spin moments of *ca.* 6 BM for the aquo iron monomers at pH 2.5 (at total porphyrin concentrations of about 5×10^{-3} M), which dropped to *ca.* 2.8 BM due to Fe–O–Fe formation by pH 7.2. In agreement with the relaxivity data, the solution magnetic moments of Fe–PF were less pH dependent than found with other porphyrins: 5.9 BM at pH 2, 5.6 BM at pH 7, and 5.4 BM at pH 10. The $(NC)_2$–Fe–PF has μ = 2.3 BM at pH 10 in the presence of excess cyanide, a value typical for an $S = 1/2$ dicyano iron(III) porphyrin [22]. Mn(III)–TPPS has a relaxivity (Table II) and absorption spectra [23] independent of pH between 1 and 7, and Table I shows that the magnetic moments of Mn–TPPS are also pH invariant in this range. The spectra of Mn–TPPS begin to change above pH 10 due to hydroxy or dimeric species, and this is also reflected in the relaxivity parameter.

Wilkins and co-workers have done extensive solution studies on Fe–TPPS [16] and Fe–TMPyP [15]. For Fe–TMPyP which is similar in charge to Fe–PF, they found that a dilution of a 10 mM solution of the porphyrin to 100 μM at pHs between 7 and 8 gave rise to a slow relaxation, as the porphyrin dimer transformed into the hydroxy-monomer

$$2HO\text{–Fe–}PF^- = P\text{–Fe–O–Fe–}P^{2-} + H_2O \qquad K_4 \qquad (4)$$

$K_4 = 2 \times 10^3$ M^{-1} for Fe–TMPyP and 8×10^5 M^{-1} for Fe–TPPS. A similar rapid dilution study on Fe–PF in the same pH range showed no spectral changes with time. The pK_{a1} for the Fe–PF reaction was 6.0, indicating that $(H_2O)_2$–Fe–PF is intermediate in acidity between Fe–TMPyP (5.5 [15], 5.5 [24], 5.7 [25]), and Fe–TPPS (7.0 [16]). pK_{a2} for Fe–PF was 10.5, less than that of Fe–TMPyP (11.5 [15], 12 [24], or 12.5 [25]). The second hydroxy group might be in the hydrophobic cavity on the tri-positively charged side of Fe–PF. The positive environment of this negative group and possible hydrogen bonding stabilization with the amide functions might indicate why $HO(H_2O)$–Fe–PF^- is more acidic than the open faced Fe–TMPyP. The absorption spectra of the aquo and mono-hydroxy forms of Fe–TMPyP and Fe–PF are similar, and no spectral evidence for a mu-oxo Fe–PF dimer could be obtained. We were unable to reproduce the pK_{a1} of 3.9 found by other workers [26] for Fe–PF.

The somewhat lower than $S = 5/2$ spin-only moments found for Fe–PF at pHs 7 and 10 could be interpreted [17] as a high spin–low spin equilibrium for the $HO(H_2O)$–Fe–PF^- species in solution. For example, hydroxymethemoglobin [27] is 69% in the high spin form at 20 °C. However, a HO–Fe(III)–tetra alpha picket fence porphyrin [28] with the hydroxy group presumably in the cavity gives μ = 5.9 BM in the solid state between 300 and 40 K, and another hydroxy porphyrin that is water insoluble shows μ = 5.7 BM in solution [29]. Thus the spin equilibria phenomena found in hemoproteins may not apply to mono-hydroxy iron(III) porphyrins. The lower moments of Fe–PF could be due to about 10% dimerization of Fe–PF at 5×10^{-3} M concentration. This would imply that $K_4 \sim 10$ M^{-1}, and since $K_3 = K_4 K_1^2$, a $K_3 \sim 10^{-11}$ M can be estimated for Fe–PF. This value is not inconsistent with the spectral Beers law work below 3×10^{-4} M,

where the porphyrin should be monomeric. Since both Fe–TPPs [16] and Fe–TMPyP [15] have $K_3 \sim 10^{-8}$ M, the Fe–PF is the most monomeric of all current water soluble iron(III) porphyrins.

Several non water soluble sterically protected iron porphyrins have been reported to form only HO–Fe–P and not the mu-oxo bridged dimer. Thus the tetra(5-anthryl)porphyrin [30], and porphyrins substituted in the 2,6-phenyl positions with methyl or methoxy [31], phenyl [32] or chloro [33] groups do not dimerize. Young and Chang [34] used derivatized *meso*-diphenyl porphyrins to show that only one bulky *ortho* group appropriately placed on each side of the macrocycle was sufficient to prevent Fe–O–Fe formation. This is apparently the case here. The three large positive, substituents on one face and the fourth on the other side of Fe–PF, coupled with the fact that positively charged substituents cause less dimerization than compounds containing negative groups, cause Fe–PF to show minimal dimerization. It is not clear at this stage whether the steric effects of the large groups, or the possibility that such groups make the porphyrin nucleus less flexible [7], is responsible for the monomeric behavior. Such water soluble picket fence types show promise as MR contrast agents with a variety of metal centers.

Acknowledgements

The work at Howard University was supported by the NIH Biomedical Research Grant 5-SO-6-RR-08016-9 and at NBS by the Office of Basic Energy Sciences, Department of Energy.

References

1 D. R. Doiron and C. J. Gomer (eds.), 'Porphyrin Localization and Treatment of Tumors', A. Liss, New York, 1984.
2 P. Hambright, R. Fawwaz, P. Valk, J. McRae and A. J. Bearden, *Bioinorg. Chem., 5,* 87 (1975).
3 N. J. Patronas, J. S. Cohen, R. H. Knop, A. J. Dwyer, D. Colcher, J. Lundy, F. Mornex, P. Hambright, M. Sohn and C. E. Myers, *Cancer Treat. Rep., 70,* 391 (1986).
4 R. C. Lyon, P. J. Faustino, J. S. Cohen, A. Katz, F. Mornex, D. Colcher, C. Baglin, S. H. Koenig and P. Hambright, *Magn. Reson. Med.,* (1986) in press.
5 N. Bloembergen, E. M. Purcell and R. V. Pound, *Phys. Rev.., 73,* 679 (1948).
6 J. H. Helms, L. W. ter Haar, W. E. Hatfield, D. L. Harris, K. Jayaraj, G. E. Toney, A. Gold, T. D. Mewborn and J. R. Pemberton, *Inorg. Chem., 25,* 2334 (1986).
7 A. Valiotti, A. Adeyemo, R. F. X. Williams, L. Ricks, J. North and P. Hambright, *J. Inorg. Nucl. Chem., 11,* 2653 (1981).
8 S. E. Haye and P. Hambright, *Inorg. Chem., 23,* 4777 (1984).
9 D. F. Evans, *J. Chem. Soc.,* 2003 (1959).
10 M. C. Richoux, P. Neta, A. Harriman, S. Baral and P. Hambright, *J. Phys. Chem., 90,* 2462 (1986).
11 A. Valiotti, A. Adeyemo and P. Hambright, *Inorg. Nucl. Chem. Lett., 17,* 213 (1981).
12 C. Chen, J. S. Cohen, C. E. Myers and M. Sohn, *FEBS Lett., 168,* 70 (1984).
13 L. Fielding, G. R. Eaton and S. S. Eaton, *Inorg. Chem., 24,* 2309 (1985).
14 E. B. Fleischer, J. M. Palmer, T. S. Srivastava and A. J. Chatterjee, *J. Am. Chem. Soc., 93,* 3162 (1971).
15 G. A. Tondreau and R. G. Wilkins, *Inorg. Chem., 25,* 2745 (1986).
16 A. A. El-Awady, P. C. Wilkins and R. G. Wilkins, *Inorg. Chem., 24,* 2053 (1985).
17 R. F. Pasternack, H. Lee, P. Malek and C. Spencer, *J. Inorg. Nucl. Chem., 39,* 1865 (1977).
18 F. L. Harris and D. L. Topen, *Inorg. Chem., 17,* 71, 74 (1978).
19 P. A. Forshey and T. Kuwana, *Inorg. Chem., 20,* 693 (1981).
20 J. D. Strong and C. R. Hartzell, *Bioinorg. Chem., 5,* 219 (1976).
21 H. Goff and L. O. Morgan, *Inorg. Chem., 15,* 3180 (1976).
22 L. Bullard, R. N. Panayappan, A. Thorpe, P. Hambright and G. Ng., *Bioinorg. Chem., 3,* 161 (1974).
23 R. Langley and P. Hambright, *Inorg. Chem., 24,* 1267 (1985).
24 H. Kurihara, F. Arifuku, I. Ando, M. Saita, R. Nishino and K. Ujimoto, *Bull. Chem. Soc. Jpn., 55,* 3515 (1982).
25 N. Kobayashi, M. Koshiyama, T. Osa and T. Kuwana, *Inorg. Chem., 22,* 3608 (1983).
26 M. Faraggi, P. Peretz and D. Weinraub, *Int. J. Radiat. Biol., 49,* 951 (1986).
27 J. Beetlestone and P. George, *Biochemistry, 3,* 707 (1964).
28 M. J. Gunter, G. M. McLaughlin, K. J. Berry, K. S. Murray, M. Irving and P. E. Clark, *Inorg. Chem., 23,* 283 (1984).
29 K. Jayaraj, A. Gold, G. E. Toney, J. H. Helms and W. E. Hatfield, *Inorg. Chem., 25,* 3516 (1986).
30 J.-M. Cense and R-M. LeQuan, *Tetrahedron Lett.,* 3725 (1979).
31 R. Cheng, L. Latos-Grazynski and A. L. Balch, *Inorg. Chem., 21,* 2412 (1982).
32 M. M. Fox and K. S. Suslick, *J. Am. Chem. Soc., 105,* 3507 (1983).
33 P. S. Traylor, D. Dolphin and T. G. Traylor, *J. Chem. Soc., Chem. Commun.,* 279 (1984).
34 R. Young and C. K. Chang, *J. Am. Chem. Soc., 107,* 898 (1985).