Simple dimer containing dissociatively stable mono-imidazole ligated ferrohemes†

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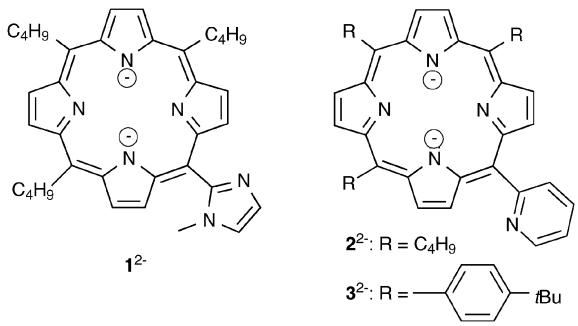
In weakly coordinating solvents Fe^{II} meso-(*N*-methylimidazol-2-yl)porphine 1Fe exists as a stable dimer (K_d = 50 ± 30 nM) that binds ligands without undergoing dissociation and is presently the simplest complex in which the mono-imidazole ligation of a ferroheme is enforced without excess imidazole in solution.

Our objective was to identify simple structural motifs to enforce dissociatively stable axial coordination of a ferroheme to a single heterocyclic base without relying on excess of a sterically hindered ligand (*e.g.*, 2-methylimidazole, 2-MeIm) in solution. The affinity of a 4-coordinate Fe^{II} porphyrin moiety, Fe(por), for imidazoles (Im) is low (dissociation constant, K_d, ~0.1 mM^1) and with the exception of C2-substituted imidazoles, Fe^{II}(por)(Im) is unstable with respect to a mixture of Fe^{II}(por) and Fe^{II}(por)$(Im)_2$.[2] Our long-term objective is to exploit the extensive structure–activity relationship identified in biomimetic studies of O_2 reduction by cytochrome oxidase to develop simple Fe and Co porphyrin complexes as potential Pt-free alternatives for O_2 reduction catalysts for low-temperature fuel cells. Available literature data[3] suggest that, with few exceptions, enforcing the axial coordination of an Fe or Co porphyrin by a heterocyclic base (imidazole or pyridine) throughout the electrocatalytic O_2 reduction cycle is the single most effective strategy to maximize the selectivity, turnover frequency and turnover numbers and to minimize the overpotential. The best reported metalloporphyrin-based electrocatalysts contain an axial imidazole attached covalently to the macrocycle to achieve *intramolecular* chelating coordination to Fe^{II} and require multistep synthesis, which precludes their practical uses. On the other hand, catalytic properties of surface-adsorbed simple Fe or Co porphyrins are not improved by adding imidazole or pyridine to the aqueous electrolyte.[3]

Porphyrins $\mathbf{1}H_2$–$\mathbf{2}H_2$ are obtained by a previously reported one-step mixed condensation.[4,5] Complexes of these porphyrins with many transition metals (but not Fe^{II}) have been reported to form dimers with varying degrees of stability.[6,7] Metallation of free bases with $FeBr_2$ in the presence of 2,6-lutidine in thf proceeded quantitatively. Spectroscopic and ligand-affinity data suggested that **1**Fe existed as a dimer



$(\mathbf{1}Fe)_2$ in C_6H_5Me, $CHCl_3$ or thf, whereas **3**Fe and **2**Fe were present predominantly as monomers.[8]

Solution UV–Vis spectra of **1**Fe manifested a split Soret of a pattern typical for $(\mathbf{1}M)_2$ dimers (Fig. 1a).[4,9,10] The peak at 373 nm is indicative of an Fe^{II} porphyrin with a single imidazole ligand;[11] peaks at 548 nm and 575 nm that are typically observed in such complexes are significantly broadened. From the dilution experiments[8] we determined K_d of $(\mathbf{1}Fe)_2$ to be 50 ± 30 nM. Evans measurements revealed 7 ± 1 unpaired electrons per dimer, in accord with the expected[2] (and calculated) quintet electronic state of imidazole-ligated ferroheme, indicating the absence of electronic communication between the two ferroheme moieties in $(\mathbf{1}Fe)_2$.

Affinities of $(\mathbf{1}Fe)_2$ to *N*-methylimidazole (*N*-MeIm), nitrosophenyl (PhNO), and isopropyl isocyanide (^{i}PrNC) were measured by spectrophotometric titrations of toluene solutions. Titrations with *N*-MeIm and PhNO proceeded with well-defined isosbestic points[8] suggestive of the interconversion between two chromophores: a 5-coordinate and a 6-coordinate ferroheme. Unit slopes of the Hill plots[12] (Fig. 2) indicate that the two binding sites in $(\mathbf{1}Fe)_2$ are independent. The spectral changes in titration of $(\mathbf{1}Fe)_2$ with ^{i}PrNC[8] could only be modeled with a 3-component system:

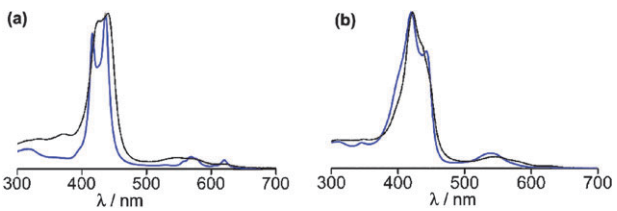


Fig. 1 Absorption spectra of (a) $(\mathbf{1}Fe)_2$ (black) and $(\mathbf{1}Zn)_2$ (blue) and (b) **3**Fe (black) and Fe(tpp) (blue, tpp = tetraphenylporphyrin) in toluene. All spectra are scaled to the same maximum absorption. All solutions were 20 μM in the Fe(por) chromophore.

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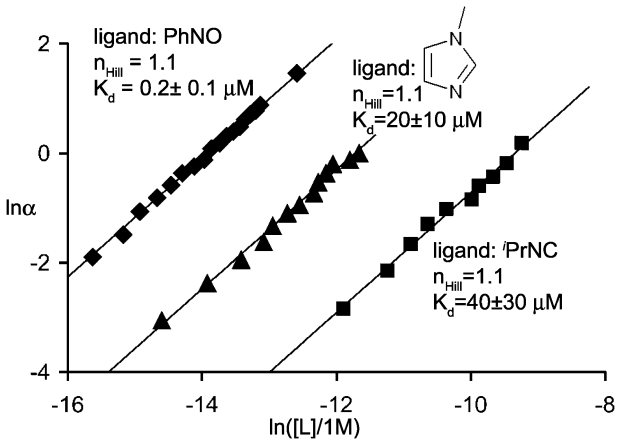

Fig. 2 Thermodynamics of ligand binding to $(\mathbf{1}Fe)_2$ presented as Hill plots;[12] ln([L]/1 M) is the natural log of the total concentration of the indicated ligand (PhNO, *N*-MeIm or ^{i}PrNC) normalized to 1 M; α is the fraction of the 6-coordinate, ImFe(por)L, sites. The data were obtained by spectrophotometric titration of 30 μM solutions of $(\mathbf{1}Fe)_2$ in toluene at 27 ± 1 °C under rigorously anhydrous and anaerobic conditions.

two distinct 5-coordinate and one 6-coordinate ferroheme chromophores. We assigned them to $(\mathbf{1}Fe)_2$, $(\mathbf{1}Fe)(^i PrNC)$ and the 6-coordinate part of $(\mathbf{1}Fe)_2(^i PrNC)$ based on the results of the NMR studies and the UV–Vis spectra of $Fe(tpp)(^i PrNC)_x$ (x = 1, 2). The relationship between these species was adequately described by equilibria (1)–(3) (L = ^{i}PrNC). Because neither $(\mathbf{1}Fe)_2(^i PrNC)_2$ nor $(\mathbf{1}Fe)(^i PrNC)$ absorbs below 400 nm, the disappearance of the peak at 373 nm in $(\mathbf{1}Fe)_2$ (Fig. 1) allowed us to establish that binding of ^{i}PrNC to the two sites of $(\mathbf{1}Fe)_2$ was also independent (Fig. 2). The affinity of Fe^{II} in $(\mathbf{1}Fe)_2$ to ^{i}PrNC is 10^4-fold lower than is typical for a 5-coordinate imidazole-ligated ferroheme[13,14] and 10^2-fold lower than the affinity of 4-coordinate Fe^{II}(por), such as Fe(tpp), **3**Fe or **2**Fe. The dissociation constant of the bisadduct, $(\mathbf{1}Fe)_2(^i PrNC)_2$, K_d^{PrNC} (eqn 3), was 0.5 ± 0.4 mM, *i.e.* 10^4-fold higher than that of $(\mathbf{1}Fe)_2$. From these data and the dissociation constant of $(\mathbf{1}Fe)_2$ the affinity of ^{i}PrNC to 4-coordinate, monomeric, **1**Fe is estimated to be 0.2 μM^{-1}.

$$(\mathbf{1}Fe)_2 + L \rightleftarrows (\mathbf{1}Fe)_2L,\ K \quad (1)$$

$$(\mathbf{1}Fe)_2L + L \rightleftarrows (\mathbf{1}Fe)_2L_2,\ K \quad (2)$$

$$(\mathbf{1}Fe)_2L_2 \rightleftarrows 2\ (\mathbf{1}Fe)L,\ K_d^L \quad (3)$$

^{1}H-NMR spectra of $(\mathbf{1}Fe)_2(PhNO)_2$[8] were consistent with the dimeric formulation of the adduct:[9,15] for example, the chemical shifts of the imidazole protons in $(\mathbf{1}Fe)_2(PhNO)_2$ were 7.5 and 2.7 ppm upfield of those protons in $\mathbf{1}H_2$; the β-pyrrolic protons closest to the imidazole experienced 3.3 ppm upfield shift, whereas those farthest from the imidazole shifted downfield. NMR spectra of $(\mathbf{1}Fe)_2$ in the presence of ^{i}PrNC (1.1–5 equiv.) revealed the presence of two compounds: $(\mathbf{1}Fe)_2(^i PrNC)_2$ and $(\mathbf{1}Fe)(^i PrNC)$, consistent with the results of spectrophotometric titrations. We observed no binding of pyridine or 2-MeIm to $(\mathbf{1}Fe)_2$ consistent with the 'tense' state of Fe^{II} in $(\mathbf{1}Fe)_2$ as suggested by DFT calculations (see below).

In contrast to $(\mathbf{1}Fe)_2$, the spectroscopic and ligand binding properties of **3**Fe and **2**Fe were similar, closely resembling those of 4-coordinate Fe(tpp). Solution UV–Vis spectra of either species in toluene up to 100 μM manifested a split Soret typical of a 4-coordinate ferroheme (Fig. 1). In toluene affinity of **3**Fe and **2**Fe for PhNO was low (K_d = 0.10 ± 0.09 mM and 40 ± 30 μM, respectively); and their affinity for ^{i}PrNC (K_d = 4 ± 3 μM and 0.9 ± 0.7 μM) and 2-MeIm (K_d = 40 ± 30 μM and 16 ± 7 μM) was comparable to that of Fe(tpp).[1] We did not detect bisadducts, $Fe(por)L_2$ (L = PhNO, 2-MeIm or ^{i}PrNC), which typically do not form with these ligands. Like Fe(tpp), solutions of **3**Fe and **2**Fe in C_6D_5N were diamagnetic. The undetectably low dimerization constant of **3**Fe and **2**Fe is a manifestation of the low affinity of pyridine to a 4-coordinate Fe^{II}(por) moiety (K_d > 1 mM).[1]

Four-coordinate monomeric **1**Fe and **3**Fe bind ^{i}PrNC with an identical affinity, suggesting that the affinities of **3**Fe and monomeric **1**Fe to PhNO and 2-MeIm or *N*-MeIm are also similar.‡ With this assumption the stability of the dimeric motif in the presence of RNC, RNO or imidazole can be evaluated. In solution, $(\mathbf{1}M)_2$ dimers (M = Zn, Mg) dissociate upon exposure to even moderate Lewis bases (*e.g.*, MeOH).[16] Whereas $(\mathbf{1}Fe)_2$ is 10^4-fold more stable with respect to the monomers than $(\mathbf{1}Fe)_2(^i PrNC)_2$, we estimate that $(\mathbf{1}Fe)_2(PhNO)_2$ is *more* stable than $(\mathbf{1}Fe)_2$, and stabilities of $(\mathbf{1}Fe)_2(N\text{-MeIm})_2$ and $(\mathbf{1}Fe)_2$ are comparable. The dissociation constants of a bisadduct, K_d^L, and of $(\mathbf{1}Fe)_2$, K_d, are related: $K_d^L = K_d(K_L/K)^2$, where K and K_L are defined by eqns (1) and (4), respectively. K_{PhNO} of $\mathbf{3}Fe^{II}$ is 500-fold lower than that of individual Fe^{II} in $(\mathbf{1}Fe)_2$, giving the dissociation constant of $(\mathbf{1}Fe)_2(PhNO)_2$ to 2 $(\mathbf{1}Fe)(PhNO)$ of 0.2 pM. In contrast, K (eqns (1) and (2), L = *N*-MeIm) and $K_{2\text{-MeIm}}$ (eqn 4, **N** = 3) are identical (within experimental error), suggesting that formation of $(\mathbf{1}Fe)(N\text{-MeIm})_x$ (x = 1, 2) from $(\mathbf{1}Fe)_2$ in the presence of *N*-MeIm is probably unfavorable. Using the literature equilibrium constant[1] for the formation of $Fe(por)(H_2O)_2$ from Fe(por) and the affinity of H_2O to imidazole-ligated 5-coordinate $ImFe^{II}$(por) we estimate that equilibrium (5) will remain unfavorable even in pure water. Based on this analysis, we expect that $(\mathbf{1}Fe)_2$ deposited on a graphite electrode in contact with an aqueous electrolyte will remain intact, thereby enforcing the mono-imidazole ligation of Fe^{II}.

$$\mathbf{N}Fe + L \rightleftarrows (\mathbf{N}Fe)L\ (\mathbf{N} = \mathbf{1}\text{–}\mathbf{3})\ K_L \quad (4)$$

$$(\mathbf{1}Fe)_2(H_2O)_2 + 2H_2O \rightleftarrows (\mathbf{1}Fe)(H_2O)_2,\ K_d^w < 1\ mM^{-1} \quad (5)$$

Despite numerous attempts we were unable to obtain an X-ray diffraction structure of $(\mathbf{1}Fe)_2$ or one of its adducts. To better understand the structural and electronic properties of $(\mathbf{1}Fe)_2$ and its adducts, we optimized dimers **4**, **4**(MeNC) and **4**$(MeNC)_2$ (Fig. 3), as models of $(\mathbf{1}Fe)_2$, $(\mathbf{1}Fe)_2(^i PrNC)$ and $(\mathbf{1}Fe)_2(^i PrNC)_2$, respectively, at the B3LYP/6-31g level.[8] Geometries of relevant 5- and 6-coordinate Fe^{II} porphyrins calculated with B3LYP/6-31g agreed well with experimental data.[8] Replacement of peripheral aliphatic groups with H atoms is known to have an insignificant impact on the computed structural parameters and electronic properties of ferrohemes.[17] The computations revealed the C_{2h} symmetry of **4** and **4**$(MeNC)_2$, in accord with the NMR spectra of $(\mathbf{1}Fe)_2L_2$

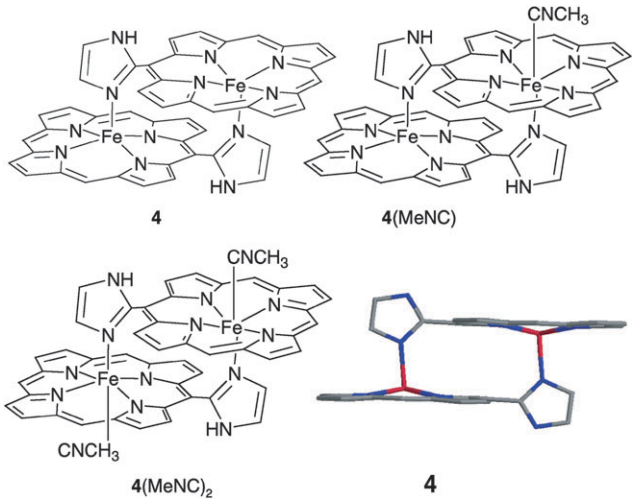

Fig. 3 Chemical structures of dimers **4**, **4**(MeCN) and **4**$(MeCN)_2$ and the minimum energy structure of **4** at the B3LYP/6-31G level. Colors: Fe, red; N, blue; C, gray; hydrogen atoms are omitted for clarity.

(L = PhNO or ^{i}PrNC) and (**1**M)$_2$ (M = Zn, Mg), and an approximate C_s symmetry of **4**(MeNC) and confirmed that the two binding sites in the dimers are structurally and electronically independent.[8] The spin states of the Fe centers in **4** were uncoupled. Upon ligand binding, electronic and structural changes at the *binding* site were pronounced and consistent with known properties of Fe^{II} porphyrins:[2,18,19] the iron ion became low-spin singlet, with a concomitant decrease in its displacement from the porphyrin plane (Fe–Ct distance). In either binding event, the structural and spin state of the spectator site remained unaffected.[8]

The 'tense' state of the 5-coordinate Fe^{II} sites in **4** and **4**(MeNC) (Fe–Ct: 0.355–0.359 Å *vs.* 0.335 Å in (2-MeIm)Fe(porphine)) and unusually small contraction of the Fe–N_{Im} distance upon MeNC binding (<0.010 Å *vs.* 0.039 Å for (2-MeIm)Fe(porphine)) likely result from steric repulsion between the two porphyrins of the dimer. The shortest separation between a pair of carbon atoms in **4**(MeNC) and **4**$(MeNC)_2$ is 3.331 and 3.206 Å, respectively, less than the sum of the van der Waals radii of two sp^2 carbons[20] (3.4 Å). These separations are similar to those observed in the crystal structures of an analog of (**2**Zn)$_2$ (3.28–3.34 Å).[4]

The B3LYP/6-31g method underestimates Fe–Ct distances of 5-coordinate Fe^{II}(por),[8] and the true Fe–Ct value in (**1**Fe)$_2$ may be ~0.37–0.38 Å. Such large displacements of Fe^{II} from the porphyrin core are rare among synthetic imidazole-ligated porphyrins (the two known examples are in refs. 21 and 22) and are comparable to those seen in human deoxyhemoglobin (0.34–0.40 Å).[23] There is evidence that relative energies of electronic states of iron(II) porphyrins (which determine kinetics of ligand binding) are very sensitive to the Fe–Ct distance,[17] and our dimers may be particularly suited for biomimetic studies of ligand binding in T-state hemoglobin.

In summary, absorption spectra, dilution experiments, spectrophotometric titration data, Evans measurements, and MS suggest that in solution simple Fe^{II} meso-(*N*-methylimidazol-2-yl)porphine exists predominantly as a dimer (K_d = 50 ± 30 nM) containing mono-imidazole-ligated ferroheme. The existence of higher oligomers at the working concentrations (<100 μM) was inconsistent with the available data. The molecule is easily accessible synthetically: the free base is available in one step and metallation is quantitative. Binding of PhNO to the dimer increases its dissociative stability and the dimer also binds two molecules of *N*-methylimidazole or ^{i}PrNC. Spectroscopic studies and DFT calculations showed that the two centers bind ligands independently. (**1**Fe)$_2$ provides a simple route to dissociatively stable mono-imidazole ligated ferroheme centers that may be of use for Pt-free catalysis of O_2 reduction in low-temperature fuel cells. On the other hand, Fe^{II} meso-(pyrid-2-yl)porphine derivatives do not dimerize to any appreciable extent.

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Notes and references

‡ 2-MeIm and *N*-MeIm bind sterically unhindered ferroheme with comparable affinities.[2]

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