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A Single Iron Porphyrin Shows pH Dependent Switch between "Push" and "Pull" Effects in Electrochemical Oxygen Reduction

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ABSTRACT: The "push–pull" effects associated with heme enzymes manifest themselves through highly evolved distal amino acid environments and axial ligands to the heme. These conserved residues enhance their reactivities by orders of magnitude relative to small molecules that mimic the primary coordination. An instance of a mononuclear iron porphyrin with covalently attached pendent phenanthroline groups is reported which exhibit reactivity indicating a pH dependent "push" to "pull" transition in the same molecule. The pendant phenanthroline residues provide proton transfer pathways into the iron site, ensuring selective $4e^-/4H^+$ reduction of O₂ to water. The protonation of these residues at lower pH mimics the pull effect of peroxidases, and a coordination of an axial hydroxide ligand at high pH



emulates the push effect of P450 monooxygenases. Both effects enhance the rate of O_2 reduction by orders of magnitude over its value at neutral pH while maintaining exclusive selectivity for $4e^-/4H^+$ oxygen reduction reaction.

■ INTRODUCTION

Heme-based metalloenzymes are ubiquitous in nature, and these enzymes catalyze biologically important chemical transformations.^{1–10} Heme proteins like hemoglobin (Hb) and myoglobin (Mb) bind O_2 reversibly and acts as O_2 storage and transport proteins.^{3,11-15} In all aerobic organisms, O_2 is reduced to H_2O by cytochrome c oxidases (CcO).^{16–23} Cytochrome P450 utilizes O2 and catalyzes hydroxylation, epoxidation, and oxo atom transfer of a wide variety to substrates^{9,10,24-30} and is involved in catabolism and hormone biosynthesis,³¹ while peroxidases (e.g. horseradish peroxidase, cytochrome c peroxidase) catalyze the oxidation of organic compounds using H_2O_2 as an oxidant $^{4,32-35}$ and are involved in immune response. Their diverse reactivity is attributed to the different heme-binding axial ligand and distal residues.^{4,36-38} Peroxidases, for example, have highly conserved histidine and arginine residues in their distal pocket along with an axial histidine ligand (Figure 1A),³⁹ while cytochrome P450 uses thiolate ligation originating from a cysteine residue and highly conserved threonine residue in the distal pocket (Figure 1B).⁴⁰ A common step within the catalytic cycle of these oxidases and monooxygenases is the O-O bond heterolysis of a low spin ferric hydroperoxo intermediate species (Fe^{III}-OOH, "compound 0")⁴¹ to get a high valent reactive metal-oxo intermediate called "compound I", which is best described as Fe^{IV}=O (ferryl) species bound to a one-electron oxidized porphyrin cation radical (formally two-electron oxidized relative to the resting ferric state).^{9,10,21,24-27,29,31,32,42} The thiolate side chain of the cysteine residue coordinates to the Fe atom of the heme cofactor in cytochrome P450.44 This



Figure 1. Active site structures of (A) peroxidase (PDB ID: 1ATJ),³⁹ (B) Cytochrome P450 (PDB ID: 1AKD).⁴⁰ Color codes: carbon \rightarrow off white, nitrogen \rightarrow blue, oxygen \rightarrow red, iron \rightarrow brown and sulfur \rightarrow yellow. Hydrogen atoms are omitted in the crystal structures for clarity.

ends up in an anionic compound 0 species in cytochrome P450, which is basic, and the protonation at the distal oxygen atom of the bound hydroperoxide species occurs via a prearranged proton transfer pathway involving the threonine residue.⁵⁰ The electron density on the thiolate (or any other anionic trans axial ligand) is crucial for the enhancement of the

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Figure 2. (A) Cobalt(II) hangman porphyrin, (B) FeL2, (C) Fe-MARG, (D) α_4 -FeFC₄ (Fc: Ferrocene), and (E) Fe-bisPhen (this paper).





"The reagents are indicated above the arrows, and the spectroscopic data of the intermediates are included in the Supporting Information.

 pK_a of the bound hydroperoxide, and the phenomenon is wellknown as the "push" effect. ^{4,29,38,44,51-54} Alternatively, imidazole functional group of histidine coordinates with heme in HRP to produce a neutral compound 0.The histidine and protonated arginine residue in the distal site exert a "pull" effect, 55,56 which facilitate the heterolytic O–O bond cleavage of the Fe^{III}-OOH (compound 0).^{48,57–60}The H-bonding interactions between active site ligands and the second sphere residues play a vital role in regulating the functions of these heme metalloenzymes. Recent investigations with synthetic pubs.acs.org/IC

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Figure 3. (A) Molecular structure of Zn-bisPhen (3b). C (gray), N (blue), O (red), Zn (violet). H atoms and solvent molecules are omitted for clarity. Two coordinated water molecules (W_1 and W_2) in the crystal are indicated by black circular rings. (B) The water channel (red balls) in HRP (PDB ID: 1SCH)⁷⁸ with the key arginine residue is shown, and hydrogen bonds are indicated by yellow lines. (C) Schematic representation of the pull effect (left) in peroxidase and push effect (right) in cytochrome P450 enzymes.

iron porphyrins have revealed that the strong H-bonding interaction to the distal oxygen atom from the protonated distal residues polarizes the O–O bond, pulling its O–O σ^* down in energy. This allows better backbonding into the O–O σ^* from the low spin ferric center weakening the O–O bond for further cleavage.^{61,62} The presence of basic residues in the distal site and nature of axial ligation play a vital role in regulating the pull and push mechanism, respectively.^{4,51}

The spatial control of the hydrogen bonding and proton delivery to the proper site is key for the generation of the high valent compound I intermediate from compound 0. In this spirit, the effect of H-bonding during oxygen reduction reaction (ORR) has been explored in artificial synthetic analogues. For example, a series of cobalt(II) hangman porphyrins having pendant carboxylic acid residues in the distal superstructure (Figure 2A) are found to assist the selective reduction of O₂ to H₂O in acidic water medium.⁶³ Iron porphyrins with tetratriazole residues attached to the redox active ferrocene groups (α_4 -FeFc₄, Figure 2D) can selectively reduce O₂ to H₂O in aqueous media across a wide pH range.^{64,65} The hydrogen bonding interaction favors O₂ binding and reduction from the side with these hydrogen bonding interactions.^{64,66–68} Recently, a series of mononuclear iron porphyrins with distal basic residues ([FeL2, Figure 2B]⁶⁹ and [Fe-MARG, Figure 2C]⁷⁰), physisorbed on edge-plane graphite (EPG) electrode, have been shown to selectively reduce O_2 to H_2O at pH 7. The protonated pendant basic residues in these iron porphyrins stabilize the Fe^{III}-OOH species by strong H-bonding interactions and facilitate the O-

O bond cleavage via a pull effect, promoting peroxidase activity as well as selective $4e^{-}/4H^{+}$ reduction in ORR.^{62,69–71}

In this manuscript, we report a mononuclear iron porphyrin complex containing two phenanthroline moieties above the porphyrin plane (Figure 2E). The two distal residues in HRP is the inspiration behind the installation of these phenanthroline residues and its performance as an ORR electrocatalyst is investigated. The ORR data reveal that the complex selectively reduces O_2 to water over a wide range of pH when adsorbed on EPG electrode. The complex exhibits push effect similar to P450 due to the axial hydroxide ligation to the metal at higher pH. At lower pH, the protonated phenanthroline residues exert pull effect like peroxidases. The rate of O_2 reduction can be tuned up by several orders of magnitude by taking advantage of the pH dependent "push—pull" effect in a single mononuclear iron porphyrin.

RESULTS

Synthesis and Characterization. The α, α -atropisomer of 5,10-di(*ortho*-aminophenyl)-15,20-diphenylporphyrin (DAPP, Scheme 1, [2]) is utilized as starting material for the synthesis. It has been synthesized and purified as reported previously.⁷² The ¹H NMR of the ligand (Supporting Information, spectral data 6[i]) indicate that it is isolated as the pure α, α -atropisomer of 5,10-di(*ortho*-aminophenyl)-15,20-diphenylporphyrin. The metal free ligand (bisPhen, Scheme 1,[3]) is synthesized by condensation between DAPP (Scheme 1, [2]) and 1,10-phenanthroline-2-carbonyl chloride⁷³ (Scheme 1,



Figure 4. (A) CV (under anaerobic conditions) for Fe(III/II) couple of Fe-bisPhen catalyst at 100 mV/s scan rate at pH 7 phosphate buffer, using 100 mM KPF₆ as the supporting electrolyte and using Ag/AgCl (satd. KCl) reference and Pt wire as counter electrode on edge plane graphite surface. (B) RRDE data of Fe-bisPhen immobilized on EPG at pH 7 phosphate buffer, using 100 mM KPF₆ as the supporting electrolyte, at 10 mV/s scan rate at 300 rpm rotation rate using a Pt counter electrode and Ag/AgCl (satd. KCl) as reference electrode. (C) LSV of Fe-bisPhen physisorbed on EPG in air saturated pH 7 phosphate buffer, using 100 mM KPF₆ as the supporting electrolyte, at a scan rate of 100 mV/s with multiple rotations using Ag/AgCl (satd. KCl) reference and Pt wire as counter electrode.(D) K–L plot of Fe-bisPhen catalyst (red bold line) at a potential of –400 mV is given. and the theoretical plots for 2e⁻ (green dashed line) and 4e⁻ processes (black dashed line) are indicated in the figure.

[1e]). Finally, the free ligand is metalated with iron (ferrous bromide) or zinc acetate using standard protocols.^{74,75} All the synthetic protocols for the steps depicted above (Scheme 1) are described in details in the experimental section. These free ligands and their Fe (Fe-bisPhen, Scheme 1, [3a]) and Zn (Zn-bisPhen, Scheme 1, [3b]) complexes are characterized using ESI-MS, ¹H NMR, UV-visible absorption spectroscopy, cyclic voltammetry, and single crystal X-ray diffraction techniques (Supporting Information, Figures S1-S3 and spectral data 6[i-vi]). Single crystals of Zn-bisPhen (dark purple color) are obtained by diffusion of diethyl ether in a chloroform solution of the complex. The solid-state structure of the Zn-metalated complex is obtained using single crystal Xray diffraction. The complex Zn-bisPhen (Scheme 1, [3b]) crystallizes with a $P\overline{1}$ space group (Figure 3A). The central Zn atom is in a square pyramidal geometry with an axial water ligand. The Zn–N distances are (2.06 ± 0.01) Å, which are in the range observed for other Zn(II) porphyrinates.^{76,77} The metal (Zn) coordinated water molecule (W_1) is directly hydrogen bonded to one of the nitrogen atom of the pendant phenanthroline groups, and the second water molecule (W_2) forms hydrogen bond with the other nitrogen atom of the phenanthroline moiety and the metal(Zn) coordinated water molecule (W_1) (Figure 3A). The N(phen)—O(W₂) and N(phen)— $O(W_1)$ distances are ~2.83 and ~2.73 Å, indicating strong hydrogen bonding with the water molecules. Thus, the phenanthroline residues help to establish a water channel into the active site, which is a unique attribute of this iron porphyrin complex and emulates the architecture of HRP (Figure 3B). All the parameters of the crystal structure are tabulated in the Supporting Information (Table S1).

Heterogeneous Oxygen Reduction Reaction in Aqueous Medium. The Fe-bisPhen (Schemes 1 and 3a) complex was physisorbed on the edge-plane graphite (EPG) electrode, and their electrochemical response was investigated under heterogeneous conditions at neutral pH 7 buffer. In dry degassed pH 7 buffer, Fe-bisPhen shows electrochemically reversible $Fe^{III/II}$ redox process at -222 mV vs Ag/AgCl (satd. KCl) (Figure 4A). By integrating this redox wave, the surface coverage of Fe-bisPhen is found to be $(8.21 \pm 0.15) \times 10^{-11}$ mol/cm^2 The Fe-bisPhen complex shows electrocatalytic O₂ reduction in air saturated pH 7 phosphate buffer solution, which is further examined using a rotating disk electrochemistry (RDE) technique. Linear sweep voltammetry (LSV) of the catalyst at different rotation rates shows a substrate diffusion limited electrocatalytic O₂ reduction current (Figure 4C) with an onset potential that overlays the reduction process of Fe^{III} to Fe^{II} (satd. KCl) (Figure 4A). The LSV current



Figure 5. (A) Overlay of CV (under anaerobic conditions) for Fe(III/II) couple at 100 mV/s on edge plane graphite surface in pH 5, 6, 7, 8, 9, 10, and 11 phosphate buffers, using 100 mM KPF₆ as the supporting electrolyte and using Ag/AgCl (satd. KCl) reference and Pt wire as counter electrode. Anaerobic CV data for additional pH values are shown in the Supporting Information (Figure S4). (B) The plot of Fe^{III/II} potential (green dots) with pH and its simulation (blue line) with $pK_{a1} = 5.5$, $pK_{a2} = 8.0$, and $E_m = -0.215$ mV.

increases with increasing rotation rate of the electrode. The kinetics of O₂ reduction was determined with RDE experiment using Koutecky-Levich (K-L) analysis⁷⁹ following the equation below:

$$i^{-1} = i_{\rm K}(E)^{-1} + i_{\rm L}^{-1} \tag{1}$$

where $i_{\rm K}$ is the kinetic current expressed as

$$i_{\rm K}(E) = nFA[O_2]k_{\rm cat}\Gamma_{\rm catalyst}$$
⁽²⁾

and $i_{\rm L}$ is the levich current and expressed as

$$i_{\rm L} = 0.62 {\rm nFA}[{\rm O}_2] (D_{\rm O2})^{2/3} \omega^{1/2} \nu^{-1/6}$$
(3)

where n is the number of electrons transferred to the substrate, F is the Faraday constant, A is the macroscopic area of the disc (0.096 cm^2) , $[O_2]$ is the concentration of O_2 in an air saturated buffer (0.21 mM) at 25 °C, k_{cat} is the second-order rate constant of catalytic O_2 reduction, $\Gamma_{catalyst}$ is the catalyst surface coverage in mol/cm², D_{O2} is the diffusion coefficient of O_2 $(1.96 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1})$ at 25 °C, ω is the angular velocity of the disc, and v is the kinematic viscosity of the solution (0.009 cm^2 s^{-1}) at 25 °C.⁸⁰

Plot of the inverse of the catalytic current (I^{-1}) at multiple rotation rates across several potentials in the mass transfer region with the inverse square root of the angular rotation rate $(\omega^{-1/2})$ is linear. The second-order rate constant of electrocatalytic O2 reduction can be calculated from K-L plot using eq 2. The k_{cat} values obtained using the intercept on the EPG electrode surface for the Fe-bisPhen complex is (1.85 ± 0.02) \times 10⁵ (Figure 4D, red line). The slope obtained from the experimental data is almost identical to that predicted for a 4e⁻ process (Figure 4D, black line) and very different from that predicted for a 2e⁻ process (Figure 4D, green line). The selectivity for O₂ reduction is better quantified specifically by monitoring the partially reduced oxygen species (PROS) formation via rotating ring disc electrochemistry (RRDE).^{79,81,82} In an RRDE experiment, any PROS, like O_2^- and H_2O_2 , produced at the working electrode reaches at the Pt ring encircling the working electrode due to the hydrodynamic force created by rotation of the electrode. The Pt ring is held at a fixed potential (0.7 V at pH 7) such that it oxidizes the PROS back to O2 and generates an oxidative current. The ratio of ring to disk current is a direct measure of the extent of PROS generated in situ and can be used to measure product selectivity, e.g., the Fe-bisPhen on EPG is found to produce (5.01 ± 0.15) % PROS in pH 7 phosphate buffer, suggesting a 95% selectivity for 4e⁻/4H⁺ ORR (Figure 4B), consistent with the RDE results (Figure 4C).

pH Dependence ORR. The Fe-bisPhen complex is physisorbed on edge-plane graphite (EPG) electrode and analyzed under aqueous conditions at various pH from 4 to 11. In the presence of 1 atm N₂, Fe-bisPhen displays electrochemically reversible $Fe^{III/II}$ redox process at all these values (Figure 5A and Figure S4). The pH dependence shows cathodic shift of ~140 mV from pH 6 to 9. The pH dependence is consistent with a Fe^{III} $-OH + e^- + H^+ \rightarrow Fe^{II} -$ OH₂ proton coupled electron transfer (PCET) transition of the Fe-bisPhen complex (Figure 5B, blue line).

In the corresponding aerated buffer solutions, the complex shows electrocatalytic O2 reduction. LSV of the catalyst indicates that mass transfer limited catalytic O2 reduction current is achieved at all those negative potentials. Both RDE and RRDE experiments (Figure 6 and Figure 7) are performed for the determination of kinetics and selectivity of ORR at different pH's. Across the entire pH range evaluated, this catalyst shows >95% selectivity for the complete four electron reduction of oxygen to water on EPG electrode in aqueous buffer medium (Table 1), as indicated by the slope of the I^{-1} vs $\omega^{-1/2}$. The rates obtained at these values for selective 4e^{-/} 4H⁺ ORR show interesting variations. The ORR rate constant increases from 1.85 \pm 0.02 \times 10⁵ M⁻¹ s⁻¹ at pH 7 to 123 \pm 11 \times 10⁵ M⁻¹ s⁻¹, i.e. ~100 times higher, at pH 9 (Table 1). Below pH 6, the rate again increases up to \sim 40 times to \sim 60 \times $10^5 \text{ M}^{-1} \text{ s}^{-1}$ (Table 1) by lowering the pH down to 4. Such Ushaped rate profile across this pH range (Figure 8), which is absent in iron porphyrins with triazole-based hydrogen bonding distal pocket, indicates two different pH dependent mechanisms at play and responsible for enhancing the rate of ORR.⁶⁵

DISCUSSIONS

During the past several years, investigation of heterogeneous electrochemical ORR catalyzed by iron porphyrin using in situ surface enhanced resonance Raman spectroscopy coupled to rotating disc electrochemistry has been used to investigate the mechanism of electrochemical ORR catalyzed by different



Figure 6. LSV data of Fe-bisPhen catalyst physisorbed on EPG surface at a scan rate of 100 mV/s with multiple rotations in pH 5, 6, 8, 9, 10, and 11 phosphate buffers using 100 mM KPF₆ as the supporting electrolyte and Ag/AgCl (satd. KCl) reference and Pt wire as counter electrode. (inset) K–L plot of the Fe-bisPhen catalyst (red bold line). The theoretical plots for 2e⁻ (green dashed line) and 4e⁻ (black dashed line) processes are indicated in the inset. The extended K–L plot of the Fe-bisPhen catalyst is also provided in the Supporting Information (Figure S6). The RDE data for additional values are shown in Supporting Information (Figure S5).

synthetic heme and heme/Cu systems (Table 2).^{83–87} Under heterogeneous aqueous conditions, irrespective of nature of axial ligands (H₂O in the absence of a covalently attached axial ligand) and distal metal, this six coordinate (6C) low spin (LS) Fe^{III}-OOH species gets accumulated during catalysis, indicating that the rate of O–O bond cleavage of the above species is the rate-determining step for ORR catalyzed by iron porphyrins (Table 2).^{86,87} The selectivity of ORR is determined from the site of protonation of an intermediate 6C LS Fe^{III}-OOH species formed during oxygen reduction.⁸⁸ If protonation occurs at the proximal oxygen, then H₂O₂ is released (2e⁻/2H⁺ reduction product) (Scheme 2, pathway 2). On the other hand, protonation at the distal oxygen facilitates O–O bond cleavage, and H_2O is produced $(4e^-/4H^+ O_2 reduction product)$ (Scheme 2, pathway 1).^{56,89} These results all suggest that the 6C LS Fe^{III}-OOH species, which have been observed in almost all cases investigated, is important in controlling not only the selectivity but also the overall rate of reaction under these reaction conditions (Scheme 2).^{69,83,88,90–92} Introduction of H-bonding residues in the distal pockets of mononuclear iron porphyrin could stabilize this Fe^{III}-OOH species and direct selective protonation of the distal oxygen of a Fe^{III}-OOH species favoring facile and selective $4e^-/4H^+$ oxygen reduction.⁶⁹ These conclusions were



Figure 7. RRDE data of Fe-bisPhen catalyst deposited on the EPG surface at a scan rate of 10 mV/s with 300 rpm rotation in pH 5, 6, 8, 9, 10, and 11 phosphate buffers using 100 mM KPF₆ as the supporting electrolyte and Ag/AgCl (satd. KCl) reference and Pt wire as counter electrode. The RRDE data for additional values are shown in Supporting Information (Figure S7).

vetted by investigating the kinetics of O–O bond heterolysis in organic solvents as well where a covalently attached pendent distal amine/guanidine residues (Figure 2B, C) could enhance the rate of heterolytic O–O bond cleavage by several orders of magnitude relative to a control sample without pendant groups.⁶² The pendant phenanthroline used here results in a water channel which is poised to translocate the proton to the bound axial -OOH ligand of a putative Fe^{III}-OOH intermediate from bulk water. This is evident from the hydrogen bonded water network observed crystallographically in the Zn-bisPhen-

 OH_2 complex (Figure 3A) between the pendant phenanthroline and the bound axial ligand.

The rate of $4e^{-}/4H^{+}$ electrochemical ORR by Fe-bisPhen at pH 7 is $(1.85 \pm 0.02) \times 10^{5} \text{ M}^{-1} \text{ s}^{-1}$, which matches those reported for mononuclear iron porphyrins without push or pull effects exerted by anionic trans axial ligands and pendant proton transfer residues, respectively.^{64,65} Experimentally, the ORR rate increases by 2 orders of magnitude as the pH of the buffer solution is increased from 7 to 11 (Figure 8, blue points). At a higher pH the trans axial H₂O ligand of a putative solvent bound H₂OFe^{III}-OOH species is likely to be

Table 1. Electrochemical ORR Data of Fe-bisPhen at Different pH Values

pН	$k_{\rm cat} \ ({\rm M}^{-1}{\rm s}^{-1}) \times 10^{-5}$	PROS (%)
4	(59.8 ± 0.7)	(2.52 ± 0.3)
4.67	(65.9 ± 1.2)	(0.39 ± 0.4)
5	(27.4 ± 0.4)	(2.09 ± 0.25)
6	(6.09 ± 0.09)	(3.86 ± 0.2)
7	(1.85 ± 0.02)	(5.01 ± 0.15)
8	(29.0 ± 0.6)	(3.03 ± 0.1)
9	(123 ± 11.0)	(1.43 ± 0.2)
10	(121 ± 10.0)	(0.03 ± 0.1)
11	(169 ± 15.0)	(0.03 ± 0.05)



Figure 8. Plot of log of second order rate constant or ORR vs pH. The red line indicates the fit to the experimental data (blue diamonds) using two pK_a values of 5.6 \pm 0.1 and 7.9 \pm 0.1.

Table 2. Fe–O and O–O Vibrations Identified in Situ Using SERRS-RDE during ORR Catalyzed by Different Systems^{83–87}

	system	Fe O (cm ⁻¹)	$O O (cm^{-1})$
FeTPP		634	830
PhO trans axial l	igand	587	830
Fe/Cu Mb* (CcO biosynthetic models)		ND	801
Fe/Cu	⁶ L-Fe(OH ₂)Cu	ND	819
synthetic model	⁶ L-Fe(Im)Cu (Im- Imidazole)	545	847

deprotonated to a ⁻OH trans axial ligand. The pK_a of this change is estimated to be 7.9 ± 0.1 from the data (Figure 8, red line). Similar 100-fold enhancement in ORR rate was also observed for thiolate bound iron porphyrins.⁹³ Due to its greater push effect, a hydroxide ligand can increase the pK_a of Fe^{III}-OOH species and making the O–O bond cleavage faster to release water akin to a thiolate axial ligand. To validate such a possibility, an imidazole ligand (neutral; stronger σ donor than H₂O) and dissolved Cl⁻ axial ligand (π anionic ligand; weaker than OH⁻) are used to investigate the ORR at pH 7 (Figure S8 and S9). The ORR data show that, while the selectivity of ORR remains the same for both these axial ligands, the rate of ORR increased from (1.85 ± 0.02) × 10⁵ M⁻¹ s⁻¹ with H₂O to (1.71 ± 0.07) × 10⁶ M⁻¹ s⁻¹ with

imidazole and (1.75 \pm 0.09) \times $10^{6}~M^{-1}~s^{-1}$ with Cl^- as axial ligands (Table 3). Imidazole and Cl⁻, both being better axial ligands to a Fe^{III}-OOH species relative to H₂O, affect an order of magnitude faster ORR relative to H_2O . Note that the determination of rates using K-L analysis can only be performed for complexes that are sufficiently stable, which requires them to be selective for $4e^{-}/4H^{+}$ ORR across a wide range of pH values. Thus, simpler porphyrins like FeTPP⁹⁴ cannot be included in this comparison. However, the tetraferrocene functionalized FeTPP (α_4 -FeFc₄, Figure 2D) is selective for $4e^{-}/4H^{+}$ ORR in this pH range, and its rates have been reported earlier.⁶⁴ A similar rise in ORR rate is also observed (Figure 8, purple dots) in α_4 -FeFc₄, where the pK_a for the change is different due to the fact that the hydrogen bonding group in α_4 -FeFc₄ is a triazole instead of a phenantroline in Fe-bisPhen. This observation adds credence to the proposal that a trans OH⁻ ligand, produced at higher values, is responsible for acceleration of ORR rates by a factor of 100.

When the pH of the solution is lowered from 7 to 4, the ORR rate increases from $(1.85 \pm 0.02) \times 10^5$ to $\sim 60 \times 10^5$ M^{-1} s⁻¹, i.e., a 40-fold increase (Table 1, Figure 8). A plot of the ORR rate with pH (Figure 8) indicates a pK_a of (5.6 ± 0.1) for this enhancement of rate at low pH, which matches very well the pK_a of phenanthroline (~5) in water.⁹⁵ The α_4 -FeFc₄ complex which bears distal triazole residues having $pK_a < 2$ does not show any rate enhancement (Figure 8, purple) at this pH. Thus, protonation of the pendant phenanthroline residues enhance the rate of ORR by 40-fold. A phenanthroline strapped porphyrin had also proposed rate enhancement of ORR due to phenantroline protonation in organic solvents.⁶⁸ Similar enhancement of the ORR rate was observed for iron porphyrins with pendant pyridine/amines which, on protonation at neutral pH values, were found to be efficient in accelerating the rate of ORR by orders of magnitude.^{62,69,70}

In summary, installation of two phenanthroline groups in a mononuclear iron porphyrin enables it to reduce O_2 selectively to H_2O over a wide range of pH values. At low values, the phenanthrolines are protonated and behave like the protonated histidine of HRP, exerting a pull effect (Scheme 3) and resulting in a 40-fold increase in ORR rate. At high pH values, the trans axial water gets deprotonated to a hydroxide, which exerts a push effect (Scheme 3) similar to cysteine in cytP450, resulting in 100-fold increase in ORR rate. The pyridyl groups (a) directly hydrogen bond to axial ligands on the metal center which (b) results in a water channel. Both features allow direct delivery of protons from bulk solvent to the iron center, imparting selectivity for $4e^-/4H^+$ ORR while the pH dependent push-pull effect tunes the rates favorably over 2 orders of magnitude.

EXPERIMENTAL DETAILS

Materials and Methods. All reagents used are of the highest grade commercially available. 1,10-Phenanthroline, hydrogen peroxide solution 30% (w/w) in H₂O, benzoyl chloride, anhydrous ferrous bromide (FeBr₂), and 2,4,6-collidine were purchased from Sigma-Aldrich. All solvents, zinc acetate [Zn (OAc)₂·2H₂O], thionyl chloride, triethylamine, and Na₂SO₄ were purchased from Merck and used without further purification. Unless otherwise mentioned, solvents were distilled, dried, and deoxygenated pursuant to established protocols for use in the glovebox. Preparation and handling of air-sensitive materials were carried out under an inert atmosphere in the glovebox. Unless otherwise mentioned, all reactions were performed at room temperature, and column chromatography

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Scheme 2. Schematic Representation of the Factors Determining the $2e^{-}/2H^{+}$ vs $4e^{-}/4H^{+}$ Selectivity in O₂ Reduction by Iron Porphyrin Complexes⁴



"The shaded oval ring represents the porphyrin ligand; red and blue color denote the proximal and distal oxygen atoms of a bound hydroperoxide ligand, respectively.

Table 3. Rate of ORR at pH 7 $(k_{cat}, M^{-1}s^{-1})$						
Fe-bisPhen	Fe-bisPhen Cl ⁻	Fe-bisPhen Imz	$lpha_4$ -FeFc $_4$			
$(1.85 \pm 0.02) \times 10^5$	$(1.75 \pm 0.09) \times 10^{6}$	$(1.71 \pm 0.07) \times 10^{6}$	$(1.20 \pm 0.20) \times 10^{5}$			





"The shaded oval ring represents the porphyrin ligand. Two phenyl rings are omitted in the left and right structures for clarity.

was performed on silica gel (60-120 mesh) with neutral alumina. Elemental analyses were performed on a PerkinElmer 2400 series II CHN analyzer. Electrospray ionization (ESI) mass spectra were recorded with a Waters QTOF Micro YA263 instrument. The absorption spectra are measured in the SHIMADZU spectrograph (UV-2100). NMR tubes were purchased from Wilmad-Lab Glass. Room temperature ¹H NMR spectra were collected on a Bruker DPX-500 spectrometer. X-ray single crystal data were collected at 120 and 298 K using radiation on a Bruker SMART APEX diffractometer equipped with CCD area detector using graphite monochromatic Mo $K\alpha$ (λ = 0.71073 Å) radiation. The APEX II software package was used for data collection, data reduction, and structure solution refinement. The hydrogen atoms were refined isotropically, and their locations were determined from a difference Fourier map. Structure solving was done using the direct method and refined in a routine manner.

Electrochemical Measurements (Heterogeneous). *Cyclic Voltammetry.* The cyclic voltammograms (CV) are recorded on a CH Instrument bipotentiostat model 700E. A glassy carbon electrode (2 mm diameter) was used as a working electrode. A Pt wire was used as a counter electrode. The measurements were made against an Ag/

AgCl (saturated KCl) aqueous reference electrode with scan rates varying from 50 to 500 mV/s.

Electrode Modification. Physiabsorption on EPG. A 50 μ L portion of catalyst from a 1 mM solution of the respective catalysts in chloroform (CHCl₃) was deposited on a freshly cleaned EPG electrode mounted on a RDE setup. After evaporation of the solvent, the surface was rinsed with CHCl₃, sonicated in ethanol, and thoroughly dried with N₂ gas. Finally, the modified electrodes were washed with milli-Q water before using them for electrochemical experiments.

Coverage Calculation. The coverage of a catalyst on an electrode is estimated by the integrated area under the corresponding oxidation/reduction currents of the respective species obtained from their voltammogram under N₂. The experiments were repeated three times and average values with standard deviation has been reported. The value can be obtained from the equation: $\Gamma = Q/nFA$ [where Q = integrated area under the reduction/oxidation current in coulomb, n = no of electron involved in the process (n = 1), F =96 500 C, and A = microscopic area of the disk (0.096 cm²)]

Rotating Disc Voltammetry. The RDE measurements were performed on a CHI 700E bipotentiostat with a Pine Instruments modulated speed rotor fitted with an E6 series Change-disc tip. The pubs.acs.org/IC

Article

Scheme 4. Synthetic Scheme of the Fe-bisPhen Imz



graphite surface was cleaned by polishing it uniformly on a Silicon carbide grinding paper. The complex was physisorbed on the disc as described above. The RDE experiments were carried out by measuring LSV at 50 mV/s scan rate at different rotation rates using Ag/AgCl (saturated KCl) reference and Pt counter electrodes.

Rotating Ring Disk Electrochemistry: Partially Reduced Oxygen Species Detection and Calculation. The platinum ring was polished using alumina powder (grit sizes: 1 μ , 0.3 μ , and 0.05 μ) and electrochemically cleaned. The EPG disc with the catalyst is inserted in the RRDE assembly which is then mounted on the rotor and immersed into a cylindrical glass cell equipped with Ag/AgCl reference (saturated KCl) and Pt counter electrodes. In this technique, the potential of the disk is swept from positive to negative, and when O_2 is reduced, any H_2O_2 , i.e., a 2e⁻ reduction product of O_2 produced in the working disk electrode is radially diffused to the encircling Pt ring, which is held at a constant potential of 0.7 V and oxidizes the H_2O_2 back to O_2 . The ratio of the $2e^{-}/2H^{+}$ current (corrected for collection efficiency) at the ring and the catalytic current at the disk is expressed as PROS and it provides an in situ measure of the $2e^{-}/2H^{+}$ reduction side reaction. The collection efficiency (CE) of the RRDE setup is measured in a 2 mM K₃Fe(CN)₆ and 0.1 M KNO₃ solution at 10 mV/s scan rate and 300 rpm rotation speed. A $17 \pm 1\%$ CE is generally recorded during these experiments. The potential at which the ring is held during the collection experiments at pH 7 for detecting H2O2 is 0.7 V. The PROS are generally determined at potentials where the Pt ring current is at the maximum.

Electrochemical Measurements (Homogeneous). Cyclic voltammetry measurement of homogeneous, nonaqueous solution containing 1 mM Fe-bisPhen was collected on a CH Instruments (CHI) model 710D potentiostat using a three-electrode configuration. Glassy carbon (3 mm, CHI), platinum wire, and silver wire electrodes were used as the working, auxiliary, and reference electrodes, respectively. Ferrocene was used as internal standard. $[Bu_4N][ClO_4]$ (100 mM) was used as supporting electrolyte.

Synthesis. 1,10-Phenanthroline-2-carboxylic Acid. 1,10-Phenanthroline-2-carboxylic acid (**1e**, Scheme 1) was prepared following the previous reported procedure starting from 1,10-phenanthroline (**1a**, Scheme 1)³ after characterization of the product obtained in every step with ESI-MS and ¹H NMR spectroscopy.

 α, α -5, 10-Di(ortho-aminophenyl)-15, 20-diphenylporphyrin (DAPP). The α, α -atropisomer of 5,10-di(ortho-aminophenyl)-15,20diphenylporphyrin (2, Scheme 1) was synthesized and separated accordingly following a previously reported procedure.⁷² The α, α isomers that have their 2-aminophenyl groups above the same face of the porphyrin macrocycle can be separated in a column. They are more polar than the α, β -isomers which have their 2-aminophenyl groups above the two opposite faces of the porphyrin. Again, the 5,10isomers which have their 2-aminophenyl groups on two adjacent *meso*-aryl substituents are slightly more polar than the 5,15-isomers. So, the pure α, α -atropisomer of 5,10-di(ortho-aminophenyl)-15,20diphenylporphyrin is isolated by using column chromatography using silica gel (100–200 mesh size) and characterized by ¹H NMR spectroscopy, where the peak integration indicated only a single atropisomer in the product. ¹H NMR (400 MHz, CDCl3, 25 °C): δ , ppm = 8.96–8.93 (m, 8H), 8.27–8.25 (m, 3H), 7.91 (t, 2H), 7.82–7.78 (m, 5H), 7.59 (t, 3H), 7.21–7.18 (m, 3H), 7.02 (d, 2H), 3.50 (s, 4H), –2.60 (s, 2H).

Bisphenanthroline Substituted α , α -5,10-Di(ortho-aminophenyl)-15,20-diphenylporphyrin (bisPhen). At first 1,10-phenanthroline-2-carboxylic acid (673 mg,3 mmol) was refluxed with 30 mL of SOCl₂ solvent for 1 h under inert atmosphere. Then, SOCl₂ was evaporated, and solid light-yellow mass was obtained. The dry solid DAPP (644.78 mg,1 mmol) was added to that yellow mass under inert condition. The system was put under vacuum for 15 min to make it free from oxygen and moisture. After that, 35 mL of dry DCM was added followed by 10 mL of dry THF. The resulting mixture was neutralized with dry triethylamine (1.08 mL, 7.8 mmol) under ice cold conditions and stirred for 4 days. Then, the final mixture was evaporated in a rotary evaporator and worked up with a dichloromethane-water mixture and was dried over anhydrous Na2SO4 and evaporated to obtain solid product. The compound was further purified by column chromatography using neutral alumina with DCM-MeOH (5%) mixture as the eluent, and purple colored compound was isolated (bisPhen, 3, Scheme 1).Yield: (898.61 mg, 85%); ¹H NMR (400 MHz, CDCl3, 25 °C): δ, ppm = 10.55 (s, 2H), 9.18 (s, 2H), 8.90 (d, 2H), 8.66 (d, 2H), 8.59 (d, 2H), 8.52 (s, 2H), 8.21 (d, 3H), 8.04 (d, 5H), 7.94 (t, 3H), 7.67-7.57 (m, 13H), 6.79 (d, 2H), 6.57 (t, 4H), 5.30 (s, 3H), 4.96 (s,2H), 3.65 (d,1H), -2.70 (s, 2H). UV-vis (CH₂Cl₂): $\lambda_{max} = 423$ nm (Soret), 516 nm, 551 nm, 591 nm, 650 nm (Q bands). ESI-MS (positive ion mode in ACN): m/z (%) =1057.3721(100) [bisPhen]H

Bisphenanthroline Substituted α, α -1,2-Diaminotetraphenyl Iron Porphyrin (Fe-bisPhen). The bisPhen ligand (50 mg, 0.0473 mmol) was dissolved in dry degassed THF, and about 30 μ L of collidine was added to it and stirred for 5 min. Then, FeBr₂ (40.8 mg, 0.189 mmol) was added to the solution, and it was stirred overnight. After the completion of the reaction, the solvent was removed, and the reaction mixture was worked up with a dichloromethane-water mixture after treatment with dil. HCl (4 M, 30 mL) to remove excess FeBr₂. The organic layer was dried with Na2SO4 and evaporated through a rotary evaporator. The reddish-brown colored solid was isolated (FebisPhen, 3a; Scheme 1). Yield: (47.23 mg, 90%); Elemental analysis calcd (%) for [Fe-bisPhen] ($C_{70}H_{42}FeN_{10}O_2Br$): C 75.68, H 3.81, N 12.61; Found: C 71.31, H 3.71, N 11.71; ¹H NMR (500 MHz, CDCl₃, 25 °C): δ , ppm = 77.43, 77.31 (pyrollic protons), 11–14 (meta hydrogens of phenyl rings of the porphyrin); UV-vis (CH_2Cl_2) : $\lambda_{max} = 416$ nm (Soret), 511 nm, 585 nm, 685 nm (Q bands); ESI-MS (positive ion mode in ACN): m/z (%) = 1110.2841 (100) $[Fe^{III}-bisPhen]^+$

Bisphenanthroline Substituted α, α -1,2-Diaminotetraphenyl Zinc Porphyrin (Zn-bisPhen). To a solution of the bisPhen ligand (100 mg, 0.095 mmol) in 25 mL of THF was added Zn(OAc)₂·2H₂O (58 mg, 0.316 mmol), and the reaction was stirred for 6 h. The solvent was evaporated and extracted with DCM. After work up, the organic layer was collected and dried over anhydrous Na₂SO₄. Finally, purple colored solid compound (Zn-bisPhen; **3b**, Scheme 1) was isolated. Yield: (95.39 mg, 90%). ¹H NMR (400 MHz, CDCl3, 25 °C): δ , ppm = 9.75 (s, 2H), 9.06 (s, 2H), 8.91 (d, 2H), 8.80 (s,2H), 8.75 (d, 2H), 8.54 (d, 2H), 8.27 (d, 2H), 8.08 (s, 2H), 7.98 (d, 3H), 7.89 (t, 2H), 7.76 (d, 2H), 7.68–7.61(m, 7H), 7.53(d, 4H), 6.96(d, 2H), 6.84(d, 2H), 6.71 (d, 2H), 6.03 (q,2H), 5.41 (d, 2H).UV–vis (CH₂Cl₂): λ_{max} = 428 nm (Soret), 558 and 598 nm (Q bands).

Imidazole Bound Fe-bisPhen (Fe-bisPhen Imz). For preparation of 1 mL of a 2 mM stock solution, we added 2.22 mg of Fe-bisPhen in 1 mL of DCM in a glass tube. Separately, we had 3.40 mg of imidazole in 250 μ L of DCM. Then, the two solutions were mixed in the glass tube, and the resulting solution was precipitated to isolate solid product by adding excess nonpolar solvent such as hexane to the mixture and drying under vacuum. The imidazole bound Fe-bisPhen (3c, Scheme 4) was characterized by absorption spectroscopy. Yield: (2.14 mg, 91%). UV–vis (CH₂Cl₂): $\lambda_{max} = 423$ nm (Soret), 556 nm (Q-band).

ASSOCIATED CONTENT

③ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.inorgchem.0c02408.

UV-visible spectroscopy data, homogeneous cyclic voltammetry data, heterogeneous electrochemical data, crystallographic data, and spectral data (PDF)

Accession Codes

CCDC 2023938 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

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