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Water-Soluble Polymer-Bound Biomimetic Analogues of Cytochrome *c* Oxidase Catalyze 4e$^-$ Reduction of O_2 to Water

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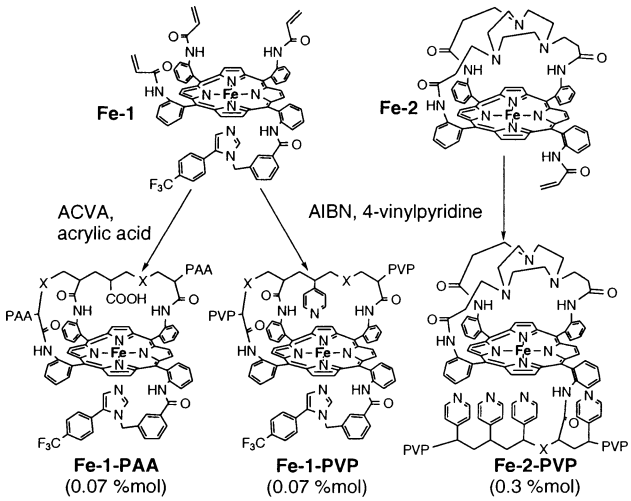
Activity toward catalytic O_2 reduction by polyacrylic acid (PAA) and polyvinylpyridine (PVP) polymers containing functional analogues of the O_2-reducing site of cytochrome *c* oxidase (CcO) has been studied in solution and on the electrode surface. Pronounced effects of the porphyrin microenvironment on the selectivity and turnover frequency of the catalysts were observed.

The design of artificial models of cytochrome *c* oxidase and the investigation of their electrocatalytical properties have attracted considerable interest.[1] Previously we synthesized model compounds of the bimetallic (heme/Cu_B) reaction site,[2,3] wherein an Fe-coordinating imidazole or pyridine and a protecting superstructure are both tethered to an Fe porphyrin. These CcO analogues catalyze 4-electron reduction of O_2 to H_2O at physiological potentials and pH.[1,4] A closer relation to the natural system, where the catalytic site is buried in a protein, could be achieved by incorporating these model compounds into polymers. The latter allow studies in aqueous solution where the monomeric species itself is insoluble or tends to aggregate[5] and also isolate catalytic sites in a less-polar microenvironment (similar to that in CcO) than is accessible to electrode-adsorbed catalysts.[1,4] Herein we report the synthesis of three artificial copolymerized CcO models (Scheme 1) and their catalytic O_2 reduction behavior.

Scheme 1. Structures of the Porphyrin Monomers and Their Copolymers[a]



[a] Average molar concentrations of Fe porphyrins in polymers is given in parentheses. Further details are provided in the Supporting Information. ACVA: 4,4′-azobis(4-cyanovaleric acid). AIBN: 2,2′-azobis(isobutylnitrile). X: polymer-backbone((−CH(COOH)CH_2−)$_n$ and (−CH(4-pyridinyl)-CH_2−)$_n$).[6]

Monomer **Fe-1** carries a coordinating imidazole to mimic the proximal imidazole of the CcO's heme a_3 but lacks the appropriate distal superstructure. **Fe-2** on the other hand is protected on one side by a triazacyclononane (TACN) cap but the other face remains open. Polymerization of these porphyrins with suitable comonomers[6] yields structures more relevant to the heme/Cu_B site, which are also water-soluble. **Fe-1** acquires a distal superstructure from the polymer matrix. The pyridine moiety of polyvinylpyridine (PVP) serves as the fifth ligand to **Fe-2**.

As synthesized, the polymers contained ferric porphyrins, which can be reduced with bisacetylacetonato-bis(1-methyl-5-carboxylimidazole)ruthenium(II), **RuIm.** Titration of **Fe-1-PAA** (PAA = polyacrylic acid) with **RuIm** leads to a shift of the Soret band from 421 to 437 nm along with the appearance of a new pronounced band at 565 nm,[6] indicative of the formation of a 5-coordinate Fe^{II} porphyrin.[7] In contrast,

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(2) Collman, J. P.; Sunderland, C. J.; Boulatov, R. *Inorg. Chem.* **2002**, *41*, 2282.
(3) Collman, J. P.; Boitrel, B.; Fu, L.; Galanter, J.; Straumanis, A.; Rapta, M. *J. Org. Chem.* **1997**, *62*, 2308.
(4) (a) Boulatov, R.; Collman, J. P.; Shiryaeva, I. M.; Sunderland *J. Am. Chem. Soc.* **2002**, *124*, 11923. (b) Shiryaeva, I. M.; Collman, J. P.; Boulatov, R.; Sunderland *Anal. Chem.* **2003**, *75*, 494.
(5) Numerous polymer-bound systems have been developed as hemoprotein mimics (E.g.: Wang, J. H. *Acc. Chem. Res.* **1970**, *3*, 90. Mathew, T.; Kuriakose S. *J. Porphyrins Phthalocyanines* **1999**, *3*, 316 and references therein) or epoxidation catalysts (E.g.: Nestler, O.; Severin K. *Org. Lett.* **2001**, *2*, 3907). Anson and Saveant studied reduction of O_2 to H_2O_2 by Co(tpp) adsorbed in Nafion using $Ru(NH_3)_6^{2+}$ as the electron mediator: Anson, F. C.; Ni, C. L.; Saveant, J. M. *J. Am. Chem. Soc.* **1985**, *107*, 3442 and references therein.
(6) See Supporting Information for further details.

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reduction of monomeric porphyrins **Fe-1** and **Fe-2** under comparable conditions invariably led to 6-coordinate Fe^{II} derivatives (λ_{max}: 430, 531 nm). Hence, the polymeric network in **Fe-1-PAA** effectively protects the open site, which remains accessible to small exogenous ligands, as evidenced by the spectral change observed when CO was bubbled into a solution of the reduced catalyst. **Fe-1-PVP** remained 6-coordinate in the ferrous state, suggesting coordination of pyridine to the distal site. However, rapid UV−vis spectral changes upon exposure of **Fe-1-PVP** to CO indicate that such coordination is labile.

Bubbling O_2 into solutions of **Fe^{II}-1-PAA** or **Fe^{II}-2-PVP** causes immediately spectral changes regenerating the band at 421 nm (the Fe^{III} species). In contrast, **Fe^{II}-1-PVP** reacted with O_2 much more slowly so that the final spectrum was not obtained until after several hours. No formation of μ-oxo dimers (λ_{max}: ~408 nm)[8] is observed, confirming that the polymer protects the porphyrins against dimerization.

Fe-1-PAA also effectively catalyzes oxidation of **RuIm** by O_2. For example, in the presence of 0.5 mol % **Fe-1-PAA** the half-life of **RuIm** in oxygenated aqueous solution dropped from 13 to 5 s. This indicates that **Fe^{II}-1-PAA** serves as a catalyst of a reaction between O_2 and **RuIm**. This setup reproduces the sequence of reactions that form the basis of spectrophotometric assays of CcO activity, wherein $Ru(NH_3)_6^{2+}$ is used as a reductant of CcO in the presence of O_2.

Catalytic O_2 reduction by the copolymers was also studied electrochemically, wherein the electrode serves the role of the external reductant, such as **RuIm** in the experiments described above, or ferrocytochrome *c* in vivo. The polymers tend to adsorb spontaneously from an aqueous solution on the electrode surface. As a result, cyclic voltammetry (CV) of aqueous solutions of the polymers under N_2 is dominated by surface-confined species. Strong adsorption of the polymers to electrode allowed us to study O_2 reduction by the surface-confined catalysts in contact with an air-saturated aqueous solution using rotating ring-disk voltammetry[11] (Figure 1). The average number of electrons per O_2 molecule (n_{av}) was determined by comparing the ring-to-disk current ratio for a bare graphite electrode and for a catalyst-modified electrode, assuming that H_2O and H_2O_2 are the only products.

All three polymers catalyze O_2 reduction beyond the H_2O_2 level.[12] The relative catalytic activity and selectivity of these three polymeric catalysts can be rationalized in terms of

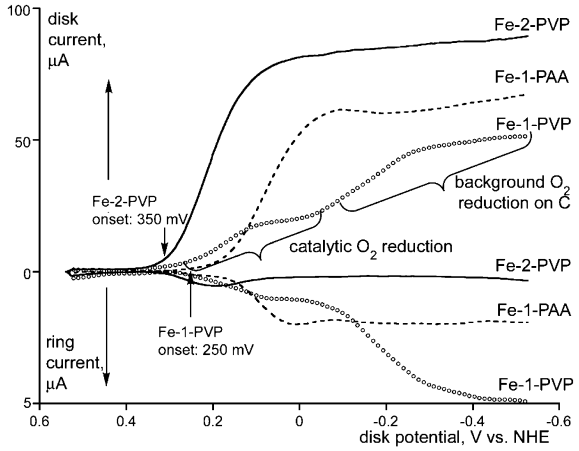

Figure 1. O_2 reduction catalyzed by biomimetic CcO analogues in polymers in an air-saturated aqueous pH = 4[9] buffer containing 0.1 M KNO_3 as the supporting electrolyte.[10] Electrode rotation rate: 200 revolutions per minute; collection efficiency: 15%. Note the different scales for the disk and ring currents.

different microenvironments around the catalytic sites. **Fe-1-PVP** is the least active catalyst ($TOF_{max} \sim 0.1\ s^{-1}$),[6] most likely because coordination of PVP's pyridine to Fe^{II} decreases the population of the catalytically active 5-coordinate Fe^{II} centers. Hence, pyridine serves as an endogenous inhibitor, resulting in catalytic behavior similar to that observed for monomers in the presence of CN^-.[13] Displacement of partially reduced oxygen species from Fe by this pyridine during catalytic turnover may account for the low catalytic selectivity of **Fe-1-PVP** ($n_{av} \sim 3.3$).

Eliminating this endogenous competitor without changing the structure of the catalytic site (catalyst **Fe-1-PAA**) results in a significant increase in the catalytic activity (Figure 1, $TOF_{max} \sim 10\ s^{-1}$).[6] Further increase both in the activity and the selectivity of O_2 reduction is observed upon introduction of a more biologically relevant and structurally more rigid distal superstructure (**Fe-2-PVP**, $TOF_{max} > 10^2\ s^{-1}$, $n_{av} = 4$, $E_{1/2} = 0.2$ V vs NHE).[6]

The 100 mV difference in the onset potentials manifested by **Fe-2-PVP** and **Fe-1-PAA** (Figure 1) correlates with the difference in the $Fe^{III/II}$ potentials observed under anaerobic conditions, which is probably due to more efficient stabilization of the Fe^{III} state by more σ-basic imidazole.[6]

An acidic group in the O_2-binding pocket of **Fe-1-PAA** may account for the different selectivities of **Fe-2-PVP** and **Fe-1-PAA**, by facilitating dissociation of a partially reduced oxygen ligand by its protonation through a proton-relay mechanism. Indeed, the amount of PRS[10] produced by **Fe-1-PAA** inversely correlates with pH.[9] In contrast, the PVP matrix in **Fe-2-PVP** may effectively lower the local proton concentration, suppressing the proton-relay mechanism of PRS release.

Here we have shown that polymeric matrixes can be used to tune predictably catalytic properties of biomimetic Fe porphyrins toward reduction of O_2 by affecting the microenvironment around the catalytic sites. We expect this

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(8) Fleischer, E. B. *J. Am. Chem. Soc.* **1971**, *93*, 3162.
(9) At pH 7 only **Fe-1-PAA** gave reproducible results; **Fe-1-PVP** and **Fe-2-PVP** could not be studied at higher pH.
(10) The disk current corresponds to the catalytic reduction of O_2 whereas the partially reduced species (PRS) (if any) produced in the catalytic cycle are detected at the ring by oxidizing them back to O_2 at a suitable potential. The bare graphite was used as a reference for $2e^-$ reduction of O_2.
(11) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 2001.
(12) Many electrode-confined simple Fe porphyrins reduce O_2 with $n > 2$ at sufficiently reducing potentials; see for example: Shigehara, K.; Anson, F. C. *J. Phys. Chem.* **1982**, *86*, 2776 and ref 1 above.
(13) Collman, J. P.; Boulatov, R.; Shiryaeva, I. M.; Sunderland, C. J. *Angew. Chem., Int. Ed.* **2002**, *41*, 4139.

approach to allow preparation of more faithful analogues of the heme/Cu site for further biomimetic studies.

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Supporting Information Available: Synthetic procedures and electrochemical and spectroscopical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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