Hybrid Bilayer Membrane: A Platform To Study the Role of Proton Flux on the Efficiency of Oxygen Reduction by a Molecular Electrocatalyst

Ali Hosseini,* Christopher J. Barile, Anando Devadoss,[†] Todd A. Eberspacher, Richard A. Decreau, and James P. Collman

Department of Chemistry, Stanford University, Stanford, California 94305, United States

S *Supporting Information*

ABSTRACT: In this report, we present a novel platform to study proton-coupled electron transfer (PCET) by controlling the proton flux using an electrode-supported hybrid bilayer membrane (HBM). Oxygen reduction by an iron porphyrin was used as a model PCET reaction. The proton flux was controlled by incorporating an aliphatic proton carrier, decanoic acid, into the lipid layer of the HBM. Using this system, we observed a different catalytic behavior than obtained by simply changing the pH of the solution in the absence of an HBM.

Proton-coupled electron transfer (PCET) reactions are central to several biological and artificial energy conversion processes.[1,2] The four-electron reduction of O_2 to water is one of the most significant reactions that involves PCET. O_2 reduction by molecular metal complexes is important in understanding biological systems such as cytochrome *c* oxidase (CcO)[3] and in the construction of efficient cathodes for fuel cells.[4] Traditionally, the pH of the bulk solution is varied in order to study the mechanism of PCET reactions.[2,5,6] The accompanying shift in the thermodynamic potential of the redox center gives limited information about the role of the proton flux in PCET processes. More recently, Nocera and co-workers synthesized hangman porphyrins in which a distal acid—base group is positioned near the redox center in order to control the proton transfer.[7,8] However, the nature of the proton flux and its effect on the reactivity of a catalytic center remains largely unknown.

In this work, we constructed a catalyst-embedded hybrid bilayer membrane (HBM) to study the effects of proton flux on O_2 reduction (Figure 1A). This methodology enables control of the proton flux to study PCET reactions independent of the pH of the bulk solution.

In an HBM system, a monolayer of lipid molecules is anchored to a self-assembled monolayer (SAM) of alkanethiols that are covalently attached to a gold electrode. The polar head groups of the lipids are oriented outward toward the aqueous solution and the hydrophobic tails inward to the hydrophobic SAM.[9–14] It has been established that the nature of the underlying SAM dictates the rate of electron transfer to the appended redox species.[15,16] Previous work has shown that rapid electron transfer to the catalytic site is necessary to minimize partial reduction of O_2 (i.e., formation of superoxide and/or hydrogen peroxide).[17,19]
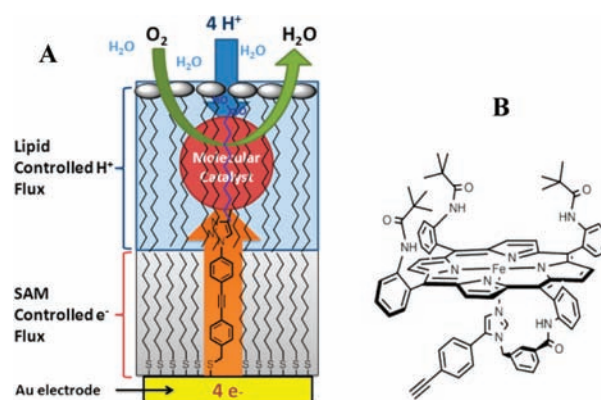

Figure 1. (A) Schematic diagram representing the HBM system. (B) Fe porphyrin catalyst used in this study.

In this study, the composition of the lipid monolayer was altered by using aliphatic proton carriers in order to control the proton flux to the catalyst embedded in the HBM. O_2 reduction by an iron porphyrin (Figure 1B) was used as a model catalyst to study the effects of proton flux on PCET reactions. To eliminate electron transfer as the rate-limiting step, we covalently immobilized the Fe porphyrin onto a SAM containing azide-terminated conjugated thiols and a decanethiol diluent[18] using the Cu(I)-catalyzed click reaction.[19]

The electrocatalytic reduction of O_2 with the exposed Fe porphyrin-appended SAM (no monolayer of lipid) has been well-studied.[17,19] In this case, the current increased until it was limited by diffusion of O_2 from the bulk solution to the Fe porphyrin (Figure 2 gray). When the Fe porphyrin was embedded in an HBM,[20] the O_2 reduction current was much lower and peaked at a more negative potential (Figure 2 black). Protons do not diffuse readily through lipid bilayers, and in nature, proton transport is tightly regulated by various channels and mediators.[21] Therefore, it was expected that proton migration through the lipid layer of the HBM would be slow. This was supported by the independence of the midpoint potential of the Fe(II)/Fe(III) couple on the pH of the solution in the HBM system (Figure S5 in the Supporting Information), which is in contrast to the 59 mV per decade shift observed for an exposed catalyst.[22] Since the four-electron reduction of O_2 is coupled to four protons, it might be predicted that the catalytic current
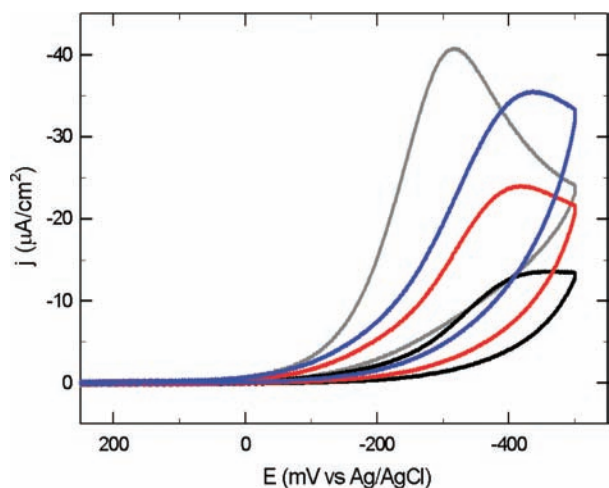
Figure 2. Catalytic current due to O_2 reduction by an immobilized Fe porphyrin at 25 mV/s in pH 7 phosphate buffer with 0.1 M KCl: exposed SAM (gray), HBM (black), HBM with 0.5 equiv of decanoic acid (red), and HBM with 1 equiv of decanoic acid (blue).

Figure 3. Average number of electrons consumed per O_2 molecule reduced for an exposed SAM (red square) and in the HBM systems containing decanoic acid (black triangles).

would be limited by the rate of proton transfer. In this case, there would be either no reaction or a slow and steady current rise at a high overpotential resembling that for a slow-electron-transfer régime.[17,19] However, since we observed a current that peaked, the catalysis was limited by O_2 diffusion[23] and not by the proton flux. This suggests that in the aprotic environment created by the HBM, O_2 undergoes one-electron reduction to superoxide.

We accelerated the proton transport from the lipid−solution interface to the catalytic site by incorporating exogenous decanoic acid into the vesicles prior to the formation of the HBM (Figure S1). It has been demonstrated that protons can be transported across a lipid bilayer by incorporating either an aliphatic acid or an amine into the membrane. In their resting state, these amphiphilic proton carriers orient themselves with their polar head groups at the lipid−water interface.[27] "Flip-flop" diffusion results in proton transfer across the membrane in the presence of a driving force such as a pH gradient.[28,29]

The molar ratio of decanoic acid to 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) inside the lipid layer of the HBM was used as an independent variable to control the rate of proton flux to the catalyst. Incorporating 0.5 equiv of decanoic acid into the HBM system enhanced the O_2 reduction while maintaining a hydrophobic environment (Figure 2 red). In this case, the catalyst generated a current higher than that for the HBM-embedded Fe porphyrin without decanoic acid but still lower than that for the catalyst on an exposed SAM. Increasing the decanoic acid concentration in the lipid layer to 1 equiv relative to DMPC resulted in even more rapid proton transport to the active site, which was reflected in a higher current (Figure 2 blue).

Figure 2 illustrates that at each decanoic acid concentration, the catalytic current increased until it was limited by O_2 diffusion. We observed no change in the potential at which the current peaked in the HBM systems (Figure 2 black, red, blue; Figure S4). Since this thermodynamic potential was constant, the effective pH at the catalytic site did not change with the concentration of decanoic acid incorporated into the lipid layer. We hypothesize that the increased catalytic current reflects a greater proton flux across the lipid layer of the HBM.
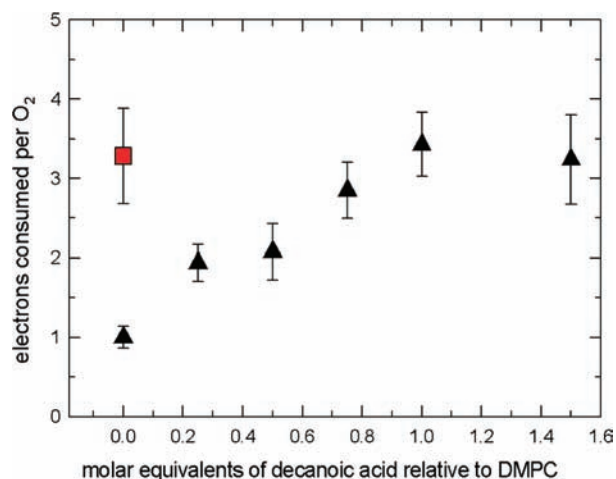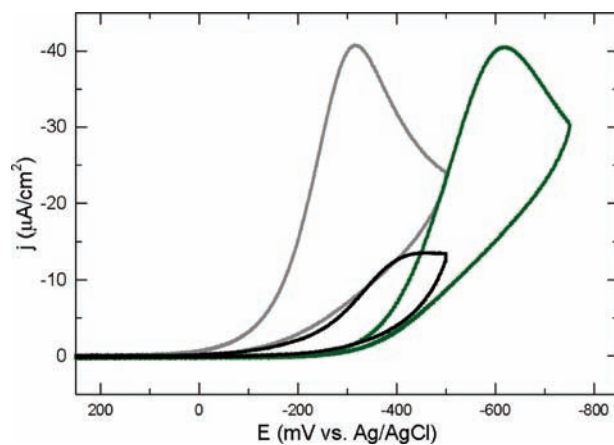

Figure 4. Catalytic current due to O_2 reduction by an immobilized Fe porphyrin at 25 mV/s in phosphate buffer with 0.1 M KCl on an exposed SAM at pH 7 (gray) or pH 13 (green) and inside an HBM at pH 7 (black).

Since the total concentration of O_2 in the bulk solution is constant, the total charge under the catalytic current is proportional to the average number of electrons consumed per O_2 molecule. The formation of an HBM creates an aprotic hydrophobic environment around the catalyst, and it is known that in aprotic solvents, O_2 undergoes one-electron reduction to superoxide.[30] We propose that in the absence of decanoic acid, O_2 is reduced by a single electron to give superoxide in the HBM system. This is supported by a comparison of the integrated current under the catalytic wave of the Fe porphyrin on the exposed SAM with that of the Fe porphyrin embedded in the HBM without decanoic acid. This analysis gives the average number of electrons consumed per O_2 molecule. The ratio of these two values is ∼3.4, which is consistent with the results of previous H_2O_2 collection experiments using interdigitated array microelectrodes for the exposed Fe porphyrin (80% four-electron and 20% two-electron),[18] in which the average number of electrons per O_2 molecule reduced was determined to be ∼3.6.

As the proton flux was accelerated by incorporating additional decanoic acid into the HBM, the average number of electrons consumed per O_2 molecule increased. Figure 3 illustrates that the number of electrons, as determined by the ratio of integrated current, changed from 1 to 3.4 as the concentration of decanoic acid increased from 0 to 1 equiv relative to DMPC, with no further change observed at 1.5 equiv. This suggests that as the amount of decanoic acid in the lipid is increased, the reduction of O_2 changes from one-electron to two-electron to mostly four-electron. Alternatively, the ratio of four-electron to one-electron reduction simply increases.

We compared the control of the proton flux in the HBM system to the results of varying the concentration of protons in solution by changing the pH of the buffer system. O_2 reduction with an exposed Fe porphyrin-appended SAM at pH ~13 resulted in a catalytic current density similar to that observed at pH 7, with the expected thermodynamic shift in the potential at which the current peaked (Figure 4).

These results illustrate that the lipid layer of the HBM removes all protic sources (H_2O and H_3O^+) from the lipid–SAM interface. The incorporation of decanoic acid into the HBM accelerates the kinetics of proton transfer during O_2 reduction, whereas the pH of the bulk solution influences the thermodynamics of the reaction.

In conclusion, we have demonstrated a relatively simple convergent approach for constructing an electrode-supported HBM that can be used for the direct study of PCET reactions under controlled proton flux. This approach provides additional insight into the role of protons and proton flux in PCET reactions that cannot be achieved by simply changing the pH of the bulk solution.

■ ASSOCIATED CONTENT

ⓢ **Supporting Information.** Experimental procedures and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
alih2@stanford.edu

Present Addresses
†Hitachi Chemical Research Center, Inc., Irvine, CA 92617.

■ ACKNOWLEDGMENT

■ REFERENCES

(1) Costentin, C.; Robert, M.; Saveant, J. M. *Acc. Chem. Res.* **2010**, *43*, 1019–1029.

(2) Huynh, M. H. V.; Meyer, T. J. *Chem. Rev.* **2007**, *107*, 5004–5064.

(3) Ferguson-Miller, S.; Babcock, G. T. *Chem. Rev.* **1996**, *96* 2889–2907.

(4) Gewirth, A. A.; Thorum, M. S. *Inorg. Chem.* **2010**, *49*, 3557–3566.

(5) Madhiri, N.; Finklea, H. O. *Langmuir* **2006**, *22*, 10643–10651.

(6) Abhayawardhana, A. D.; Sutherland, T. C. *J. Phys. Chem. C* **2009**, *113*, 4915–4924.

(7) Rosenthal, J.; Nocera, D. G. *Acc. Chem. Res.* **2007**, *40*, 543–553.

(8) Chng, L. L.; Chang, C. J.; Nocera, D. G. *Org. Lett.* **2003**, *5*, 2421–2424.

(9) Plant, A. L. *Langmuir* **1993**, *9*, 2764–2767.

(10) Plant, A. L.; Brighamburke, M.; Petrella, E. C.; Oshannessy, D. J. *Anal. Biochem.* **1995**, *226*, 342–348.

(11) Plant, A. L. *Langmuir* **1999**, *15*, 5128–5135.

(12) Twardowski, M.; Nuzzo, R. G. *Langmuir* **2003**, *19*, 9781–9791.

(13) Twardowski, M.; Nuzzo, R. G. *Langmuir* **2004**, *20*, 175–180.

(14) Hosseini, A.; Collman, J. P.; Devadoss, A.; Williams, G. Y.; Barile, C. J.; Eberspacher, T. A. *Langmuir* **2010**, *26*, 17674–17678.

(15) Devaraj, N. K.; Decreau, R. A.; Ebina, W.; Collman, J. P.; Chidsey, C. E. D. *J. Phys. Chem. B* **2006**, *110*, 15955–15962.

(16) Chidsey, C. E. D *Science* **1991**, *251*, 919–922.

(17) Collman, J. P.; Devaraj, N. K.; Decreau, R. A.; Yang, Y.; Yan, Y. L.; Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D. *Science* **2007**, *315*, 1565–1568.

(18) The rate of electron transfer from the gold electrode to the appended porphyrin through this aromatic linker has been estimated to be greater than 5×10^4 s^{-1}.[15]

(19) Collman, J. P.; Decreau, R. A.; Lin, H. W.; Hosseini, A.; Yang, Y.; Dey, A.; Eberspacher, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 7320–7323.

(20) The formation of a full monolayer of lipid on the Fe porphyrin-appended SAM was confirmed by the shift in the midpoint potential of the Fe(II)/Fe(III) couple from −93 mV for the open SAM to −37 mV for the HBM (Figure S3). The observed change in the interfacial capacitance (6 to 4 μF/cm^2) of the electrode after exposure to the lipid vesicle solution was also consistent with the formation of a full HBM layer.

(21) Jain, M. K. *Introduction to Biological Membranes*, 2nd ed.; Wiley: New York, 1988.

(22) Boulatov, R.; Collman, J. P.; Shiryaeva, I. M.; Sunderland, C. J. *J. Am. Chem. Soc.* **2002**, *124*, 11923–11935.

(23) It should be noted that O_2 has a partition coefficient of >2 in DMPC relative to water at 25 °C[24] and can readily permeate through the hybrid membrane. In addition, the Fe porphyrin molecule resides close to the water–HBM interface (~1 nm), and diffusion of O_2 from the aqueous phase to the catalyst should be extremely rapid. The diffusion coefficient of O_2 at 25 °C in a DMPC bilyaer is 1.8×10^{-5} cm^2/s,[25] which is comparable to that of water (1.9×10^{-5} cm^2/s).[26] The time required for O_2 to diffuse through 1 nm in the HBM is on the order of 1 ns. The experimental time scales are in the range of 1 s.

(24) Subczynski, W. K.; Hyde, J. S. *Biophys. J.* **1983**, *41*, 283–286.

(25) Windrem, D. A.; Plachy, W. Z. *Biochim. Biophys. Acta* **1980**, *600*, 655–665.

(26) Chang, P.; Wilke, C. R. *J. Phys. Chem.* **1955**, *59*, 592–600.

(27) Srivastava, A.; Singh, S.; Krishnamoorthy, G. *J. Phys. Chem.* **1995**, *99*, 11302–11305.

(28) Schönfeld, P.; Schild, L.; Kunz, W. *Biochim. Biophys. Acta* **1989**, *977*, 266–272.

(29) Kornberg, R. D.; McConnell, H. M. *Biochemistry* **1971**, *10* 1111–1120.

(30) Maricle, D. L.; Hodgson, W. G. *Anal. Chem.* **1965**, *37*, 1562–1565.