

# Hydrogen Evolution from Neutral Water under Aerobic Conditions Catalyzed by Cobalt Microperoxidase-11

Jesse G. Kleingardner, Banu Kandemir, and Kara L. Bren\*

Department of Chemistry, University of Rochester, Rochester New York 14627-0216, United States

**S** Supporting Information

**ABSTRACT:** A molecular electrocatalyst is reported that reduces protons to hydrogen (H<sub>2</sub>) in neutral water under aerobic conditions. The biomolecular catalyst is made from cobalt substitution of microperoxidase-11, a water-soluble heme-undecapeptide derived from the protein horse cytochrome *c*. In aqueous solution at pH 7.0, the catalyst operates with near quantitative Faradaic efficiency, a turnover frequency  $\sim 6.7 \text{ s}^{-1}$  measured over 10 min at an overpotential of 852 mV, and a turnover number of  $2.5 \times 10^4$ . Catalyst activity has low sensitivity to oxygen. The results show promise as a hydrogenase functional mimic derived from a biomolecule.

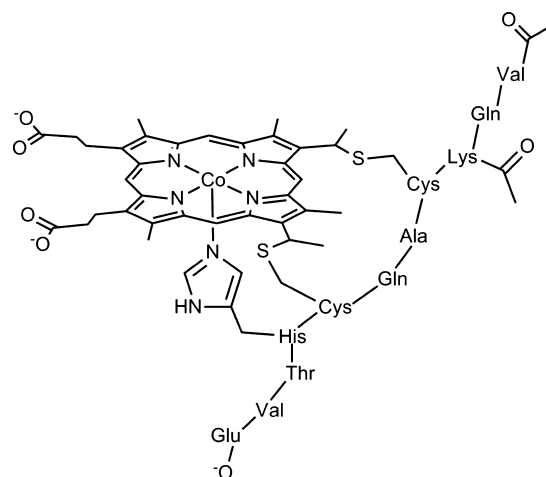
Molecular hydrogen is an energy-dense, portable fuel that has garnered significant interest in the search for alternatives to fossil fuels.<sup>1</sup> In order to be sustainable, hydrogen must be produced from noncarbon sources using catalysts based on earth-abundant elements.<sup>2</sup> Nature's hydrogenase enzymes evolve H<sub>2</sub> from water with high efficiency and activity but have a low density of active sites and are relatively unstable, with oxygen sensitivity limiting practical use of many hydrogenases.<sup>3,4</sup> Successful alternatives are inorganic heterogeneous catalysts such as nickel phosphide nanoparticles, molybdenum and tungsten sulfides, and mixed metal solids.<sup>5–8</sup>

There also is significant interest in the development of molecular functional hydrogenase mimics, as they are more amenable to detailed manipulation and study.<sup>9–11</sup> Herein, we report a water-soluble cobalt-porphyrin-peptide, cobalt-microperoxidase-11, that efficiently catalyzes hydrogen evolution from water at neutral pH under aerobic conditions.

Cobalt complexes have shown promise as molecular hydrogen-evolution electrocatalysts<sup>9–12</sup> at overpotentials as low as 40 mV<sup>13</sup> and with turnover frequencies (TOFs) as high as 600 s<sup>-1</sup> per mole of catalyst.<sup>14</sup> Turnover numbers (TONs) as high as 9000 have been reported for photocatalytic H<sub>2</sub> evolution by molecular cobalt catalysts.<sup>9,15</sup> Despite these successes, common pitfalls are dependence on organic solvents and, for systems that function in aqueous solution, the requirement of acidic conditions. Only a handful of examples exist of molecular catalysts that efficiently evolve hydrogen from water near neutral pH using inexpensive, earth-abundant elements (Table S1 in Supporting Information [SI]).<sup>16–22</sup> Oxygen-tolerant hydrogenase-functional mimics are even more rare.<sup>14,22</sup>

Among the cobalt H<sub>2</sub>-evolution catalysts, cobalt macrocyclic complexes have shown significant promise, although these

catalysts generally require organic solvent or acidic solution for function.<sup>13,17,18,23–26</sup> Inspired by these successes, we prepared the cobalt derivative of the water-soluble heme peptide known as microperoxidase-11 (MP11).<sup>27</sup> This porphyrin-peptide ligand offers significant advantages as it is water-soluble and presents five coordination sites to the metal, leaving a sixth open for interaction with substrate. The chelating ligand prevents dissociation of the axial histidine. Furthermore, this ligand is biosynthetic, biodegradable, and avoids the use of any toxic or rare elements. MP11 is obtained by pepsin digestion of horse cytochrome *c* and contains a heme covalently linked to an 11-residue peptide that confers water solubility to the heme.<sup>27</sup> After acetylation of the free amines to prevent intermolecular ligation to the open coordination site, the MP11 iron was removed via reductive demetalation.<sup>28</sup> Separation of remaining Fe(III)-containing MP11 from the demetalated derivative was accomplished via binding to histidine-immobilized Sepharose (HIS) resin<sup>29</sup> at pH 7.5 (Figure S1 in SI). Insertion of cobalt was achieved by heating with Co(II) acetate, forming bis-acetylated cobalt microperoxidase-11 (CoMP11-Ac, Figure 1). As prepared, in the presence of oxygen, the Co(III) derivative is formed. MALDI mass spectrometry confirmed the isolation of CoMP11-Ac (Figure S2 in SI). The absorption spectrum is consistent with a Co(III) porphyrin complex (Figure S3 in SI), and addition of dithionite reversibly produces a Co(II)



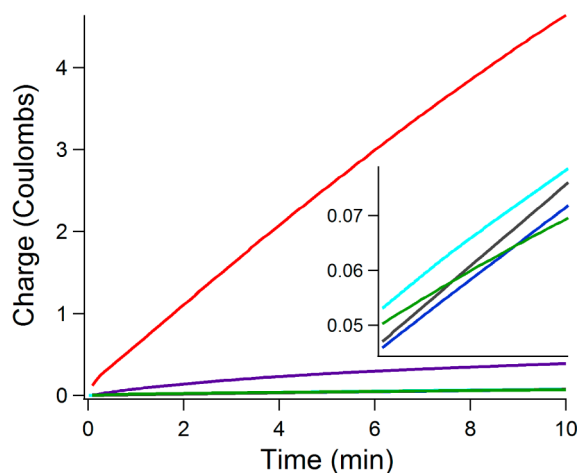
**Figure 1.** Acetylated cobalt microperoxidase-11 (CoMP11-Ac).

**Received:** July 4, 2013

**Published:** December 18, 2013

porphyrin species (Figure S4 in SI).<sup>30</sup> Details of preparation and characterization are provided in the SI.

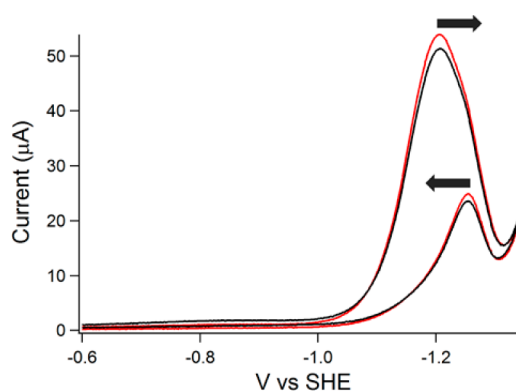
CoMP11-Ac was tested for electrocatalytic H<sub>2</sub>-evolution activity in neutral water (2 M KP<sub>i</sub>, pH 7.0) using 10-min constant potential electrolysis (CPE) experiments with a mercury pool working electrode. An increase in current as a function of catalyst concentration was observed (Figure S5 in SI) for electrolysis at -1.5 V vs Ag/AgCl (1 M KCl), and H<sub>2</sub> was detected by gas chromatography (GC) of the headspace after 10 min. The current enhancement was not seen using the Zn derivative of MP-11 (ZnMP11-Ac), even with the addition of CoCl<sub>2</sub>, nor was it observed in the presence of the MP11-Ac ligand alone (Figure 2).



**Figure 2.** Charge versus time for 10-min CPE experiments carried out under an N<sub>2</sub> atmosphere with an applied potential of -1.5 V vs Ag/AgCl (1 M KCl) in 2 M KP<sub>i</sub>, pH 7. CoMP11-Ac (1.0 μM, red) is compared with buffer (green), ZnMP11-NAc (10 μM, blue), metal-free MP11-Ac ligand (10 μM, cyan), and a combination of ZnMP11-NAc and CoCl<sub>2</sub> (10 μM each, gray). The data for background, demetalated MP11-Ac, ZnMP11-NAc, and ZnMP11-NAc/CoCl<sub>2</sub> overlap substantially and are shown in the inset with an expanded vertical scale. In purple is the current after rinsing the mercury pool with fresh buffer subsequent to electrolysis of CoMP11-Ac.

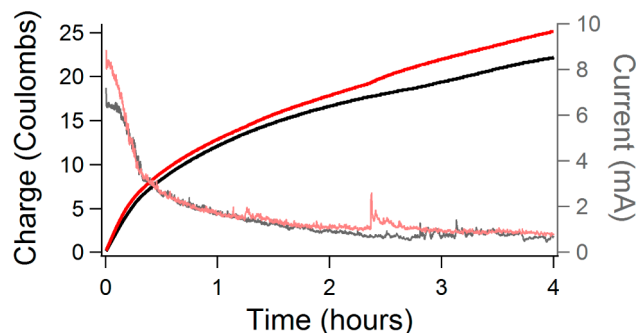
Cyclic voltammetry (CV) was performed in aqueous solutions using a hanging mercury drop electrode. For CoMP11-Ac, no Faradaic current was observed until a cathodic catalytic wave that peaked at approximately -1.20 V vs SHE (Figure 3). The peak current was found to be strongly dependent on buffer capacity (Figure S6 in SI) and pH (Figure S7–S8 in SI), while peak potential is pH independent (Figure S9 in SI). The peak current density is linearly dependent on CoMP11-Ac concentration (0.1 to 2 μM; Figures S10–S12 in SI). The shape of the catalytic wave, however, suggests degradation of catalyst during the experiment.<sup>31</sup> Neither ZnMP11-Ac, with or without CoCl<sub>2</sub>, nor demetalated MP11-Ac produces the same large decrease in the overpotential for catalytic H<sub>2</sub> evolution below that of the mercury electrode (Figure S13 in SI), indicating that a cobalt porphyrin species is responsible for the observed catalytic activity. A reversible Co(III)/Co(II) transition ( $E_m = -334$  mV vs SCE) is seen only at slow scan rates (2.5 mV/s) under acidic conditions (Figure S14 in SI).

CPE experiments were run under an N<sub>2</sub> atmosphere to determine Faradaic efficiency and to estimate TOF. After 10 min of electrolysis at -1.5 V vs Ag/AgCl (1 M KCl) in 2 M



**Figure 3.** Cyclic voltammograms of 1 μM CoMP11-Ac collected in 25 mM KP<sub>i</sub>, 1 M KNO<sub>3</sub>, pH 7. The voltammograms are shown after purging the electrochemical cell with nitrogen (red) or air (black), showing negligible difference in the catalytic peak in the presence or absence of oxygen.

KP<sub>i</sub>, pH 7, using 1.0 μM catalyst, 20 μmol of H<sub>2</sub> were evolved, as measured by GC, and 4.2 coulombs of charge were passed (after background subtraction), yielding a Faradaic efficiency of 95 ± 3%. A TOF of 6.7 mol H<sub>2</sub> s<sup>-1</sup> M<sup>-1</sup> of CoMP11-Ac was determined from the 10-min electrolysis experiments using 1.0 μM CoMP11-Ac. This TOF compares favorably with those reported for molecular H<sub>2</sub>-evolution catalysts in water (Table S1 in SI). This TOF is reported for only the first 10 min of electrolysis, because after ~15 min, activity falls off appreciably (Figure 4), and solution bleaching is observed, consistent with



**Figure 4.** CPE carried out with 1.0 μM CoMP11-Ac at -1.5 V vs Ag/AgCl (1 M KCl) with a mercury pool electrode in 2 M KP<sub>i</sub>, pH 7.0. The experiment was carried out in both an argon atmosphere (red) and in air (black). The total accumulated charge is shown as bold lines (left axis), and the current is shown as faded lines (right axis).

porphyrin degradation. It also must be noted that the TOF measured by this method will depend both on the ratio between the solution volume and surface area of the electrode and on the rate of physical mixing. Furthermore, the TOF was calculated from the total moles of catalyst in the electrolysis cell rather than the amount at the electrode surface. Adsorption of catalyst onto the electrode may increase TOF due to an increase in effective concentration at the electrode surface relative to the bulk concentration.

A test for adsorption was performed in which the mercury pool electrode was rinsed with 2 M KP<sub>i</sub>, pH 7.0, after a 10-min CPE experiment with 1 μM catalyst. The rinsed mercury was subjected again to 10 min of CPE in fresh buffer, showing only a small current enhancement relative to background (Figure 2), at a level that is ~3.5% of that obtained with 1 μM catalyst in

solution. Furthermore, hydrogen obtained (measured by GC) is less than 2% of that produced before the rinse. These results indicate a small amount of adsorption of the catalyst or a degradation product onto the mercury electrode. However, turnover of the mercury surface complicates assessment of adsorption by this method. Another indication of the absence of significant adsorption of active catalyst is that the current did not increase over multiple CV sweeps.<sup>32</sup> Additionally, the catalysis measured using a mercury electrode indicates that the activity is not due to metal particle formation.<sup>33</sup>

Cobalt porphyrins have previously been demonstrated to act as electrocatalysts for the production of hydrogen from both organic acids<sup>34</sup> and water<sup>24</sup> with high Faradaic efficiencies. A TOF was not reported for the water-soluble cobalt porphyrin catalysts, but the reported data indicate 216 nmol of H<sub>2</sub> produced per second with 2 mM catalyst in a 0.1 M TFA solution.<sup>24</sup> In contrast, CoMP11-Ac produces H<sub>2</sub> at a comparable rate at micromolar catalyst concentrations and at neutral pH. The considerable rate enhancement for H<sub>2</sub> production by CoMP11-Ac could be due to the relatively strongly donating axial imidazole ligand provided by the covalently linked peptide (Figure 1).<sup>35</sup> In cobaloxime H<sub>2</sub>-evolution catalysts, stronger axial ligands have been shown to increase the catalytic rate without increasing the overpotential.<sup>36</sup>

A valuable characteristic of a robust and useful H<sub>2</sub>-evolution catalyst is oxygen tolerance. Many known catalysts are inactive and/or degraded in the presence of oxygen, including most hydrogenase enzymes. Recent exceptions are an immobilized cobalt corrole that was found to evolve H<sub>2</sub> from 0.5 M H<sub>2</sub>SO<sub>4</sub> in an oxygen-containing atmosphere<sup>14</sup> and a cobalt diglyoxime catalyst with a pyridyl-4-hydrophosphonate axial ligand.<sup>22</sup> We noticed that neither the catalytic wave of CoMP11-Ac measured by CV (Figure 2) nor the CPE results (Figure 4) are sensitive to the presence of oxygen. Measurement of Faradaic efficiency in the presence of air over a 10-min CPE experiment yielded a value of (100 ± 4)%, the same within error as the value under nitrogen, indicating that CoMP11-Ac has the unusual property of reducing protons in the presence of oxygen. To measure Faradaic efficiency of CoMP11-Ac in the presence and absence of oxygen over longer experiments, 4-h CPE experiments at -1.5 V vs Ag/AgCl (1 M KCl) were performed on 25 nM CoMP11-Ac; representative data are shown in Figure S15 in SI. A low catalyst concentration was required for these measurements to avoid excessive pressure buildup in the sealed cell. Faradaic efficiencies under argon and air are (98 ± 2)% and (85 ± 5)%, respectively. Because air has a small effect on Faradaic efficiency in 4-h experiments, but no effect on short experiments during which the catalyst is stable, a degradation product may be responsible for decreasing Faradaic efficiency in the presence of oxygen.

Determination of TON values for 4-h experiments was done by measuring charge passed after 4 h of CPE at -1.5 V vs Ag/AgCl (1 M KCl) on 1 μM catalyst and assuming constant Faradaic efficiencies measured over 4 h. Resulting TON values are 2.5 × 10<sup>4</sup> and 1.9 × 10<sup>4</sup> under argon and air, respectively (Table S1; see SI for more details). The TON in the presence of oxygen is comparable to the highest TONs reported for non-noble-metal molecular H<sub>2</sub>-evolution electrocatalysts in neutral aqueous solution measured in inert atmospheres (Table S1 in SI).<sup>11</sup> However, it must be noted that the catalysts reported by Karunadasa et al. and Sun et al. displayed no signs of degradation after 6.1 × 10<sup>5</sup> and 5.5 × 10<sup>4</sup> turnovers,

respectively, at which point the buffer capacity of the solution was overwhelmed.<sup>17,20</sup>

The catalytic overpotential was characterized by short CPE experiments in the presence of 1.0 μM CoMP11-Ac at increasingly negative potentials. The total charge passed at the end of 1 min of electrolysis is plotted as a function of applied potential in Figure S16 in SI. The onset of catalysis occurred at an overpotential of approximately 600 mV, while the maximum current was obtained at an overpotential of approximately 900 mV (Figure S17 in SI). The overpotential is high, yet comparable to other non-noble-metal H<sub>2</sub>-evolution catalysts functioning in water at neutral pH (Table S1 in SI).<sup>16,17</sup> Nevertheless, the favorable catalytic rate, oxygen tolerance, and biosynthetic nature of CoMP11-Ac provide an excellent foundation for future optimization.

In conclusion, CoMP11-Ac is an efficient molecular catalyst for H<sub>2</sub> evolution from neutral water in the presence of oxygen. With the goal of lowering the overpotential and increasing stability and catalytic rate, more detailed studies and preparation of derivatives are underway. The presence of the peptide, aside from conferring water solubility to the catalyst, provides a means by which to functionalize or modify the catalyst by altering the sequence of the peptide or through chemical modification, and represents a general route for conferring water solubility to metalloporphyrin catalysts.<sup>37–39</sup> Finally, microperoxidases have previously been utilized in biofuel cells<sup>40</sup> and as sensors,<sup>41,42</sup> demonstrating amenability toward immobilization on electrode devices.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Detailed experimental procedures, discussion of buffer capacity dependence of results, table comparing related catalysts, characterization data for CoMP11-Ac, additional electrochemical data and plots. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

bren@chem.rochester.edu

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

We thank Bill McNamara, Will Eckenhoff, and Zhiji Han in the Richard Eisenberg Laboratory at the University of Rochester who provided assistance with catalyst characterization. This work is supported by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, Office of Science, U.S. Department of Energy, Grant No. DE-FG02-09ER16121.

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