TEMPO as a Promising Electrocatalyst for the Electrochemical Oxidation of Hydrogen Peroxide in Bioelectronic Applications

Sofiene Abdellaoui,[=] Krysti L. Knoche,[=,*] Koun Lim, David P. Hickey,[*] and Shelley D. Minteer[**,z]

Departments of Chemistry and Materials Science and Engineering, University of Utah, Salt Lake City, Utah 84112, USA

A number of bioelectronic applications work with oxidase enzymes and many of them can operate with small molecule or polymer redox mediators. However, for some oxidases, there are no known redox mediators able to mediate electron transfer. Therefore, electron transfer must occur through peroxide production and oxidation at the electrode surface. Organic redox catalysts such as oxoammonium cations, are able to oxidize H_2O_2 to form nitroxyl radicals, which can be electro-oxidized and regenerate the oxoammonium cation form. In this study, we investigate the ability to use TEMPO as a platform for the electrocatalytic oxidation of H_2O_2 at different pHs. The results have shown that TEMPO can be used to monitor H_2O_2 in broad pH range (≥ 4) at 530 mV (vs SCE). Combining TEMPO with cholesterol oxidase, we have shown the possibility to monitor the cholesterol oxidation with a linear range between 20 μM and 2.5 mM with a sensitivity of 54.86 mA cm^{-2} M^{-1}. Furthermore, we have studied the electrocatalytic oxidation of oxalate by oxalate oxidase for biofuel cell applications. These combined results demonstrate TEMPO as a promising electrocatalyst applied for the development of electrochemical biosensors or enzymatic biofuel cells.

Hydrogen peroxide (H_2O_2) is an enzymatic product of oxygen (O_2) reduction, which can be catalyzed by an exhaustive list of oxidase enzymes including glucose oxidase, alcohol oxidase, lactate oxidase, urate oxidase, cholesterol oxidase, D-amino acid oxidase, glutamate oxidase, lysine oxidase, and oxalate oxidase. Hydrogen peroxide is the smallest and simplest peroxide and is of great interest in multiple fields as a disinfectant, as a propellant in the aerospace industry, and as a biomarker for biological decomposition in the food industry.[1] It also applied in the defense system of some insects,[2] the immune system,[3] and regulation of cellular processes.[4] Development of enzymatic biosensors presents a significant utility for the detection and quantification of the large number of oxidase substrates for fundamental studies as well as diagnostic and industry applications. Different techniques for the detection of oxidase substrates that have been described in the literature include spectrophotometry,[5] fluorimetry,[6] chemiluminescence,[7] and fluorescence,[8] but most of them are costly and time consuming. Hydrogen peroxide can be oxidized electrochemically, and thus electrochemical techniques have also been used as detection methods. Electrochemistry is commonly described as a simpler, cheaper, faster, and more sensitive detection technique for the development of oxidase substrate biosensors and enzymatic biofuel cells.[9,10]

The direct electrochemical oxidation and reduction of H_2O_2 require high overpotentials (>+0.65 V for oxidation and >−1.7 V for reduction versus NHE). These high potentials limit analytical applications involving the oxidation or reduction of H_2O_2 in complex media, because media electrolysis causes interference and can foul the electrode surface. In order to decrease the required overpotential and increase both specificity and sensitivity, it is necessary to utilize redox catalysts or redox mediators capable of reacting with H_2O_2. Prussian blue and other metal hexacyanoferrates have been used extensively to detect hydrogen peroxide, because they are capable of oxidizing H_2O_2 at a low potential (−50 mV vs. Ag/AgCl).

However, these transition metal complexes or materials lack operational stability in neutral and alkaline solutions.[1] Hemic proteins, such as horseradish peroxidase, catalase, cytochrome c, hemoglobin, microperoxidase, and myoglobin, are capable of direct electron transfer with an electrode and thus have been used to construct biosensors.[11,12] Despite the high selectivity obtained with the use of these types of enzymes, the optimization of the direct electron transfer between the enzymatic active sites and the electrode surface remains a challenge and their stabilities are still limited. Carbon nanotubes, metals, and metal oxides have also been described as possible electrocatalysts for the electrochemical oxidation of H_2O_2, but they remain non-selective, often toxic, and costly.

Organic redox catalysts, such as TEMPO, have previously been shown to catalytically oxidize hydrogen peroxide.[36] In these reports, TEMPO is electrochemically oxidized from the stable nitroxyl radical to an oxoammonium cation and subsequently acts as the recipient of two consecutive single electron transfers from hydrogen peroxide to generate two molecules of the nitroxyl radical form of TEMPO, one molecule of O_2, and two protons as shown below (Scheme 1).[36] The oxoammonium ion of TEMPO, formed from the electro-oxidation of the nitroxyl radical, has typically been shown to oxidize primary alcohols and aldehydes to form an hydroxylamine, which can undergo a second oxidation to regenerate the nitroxyl radical. However, it is also able to react with hydrogen peroxide via single electron transfer as described above. We sought to utilize conditions for which TEMPO could be used to selectively react with hydrogen peroxide without catalytically reacting with alcohol functional groups in solution. In this way, the high reactivity of TEMPO with hydrogen peroxide could be combined with the selectivity afforded by an oxidase enzyme to construct a highly sensitive and highly selective biosensor. In this work, we demonstrate the application of electrocatalytic oxidation of hydrogen peroxide by TEMPO in two different enzymatic contexts: the first is an enzymatic biosensor for the detection of cholesterol using cholesterol oxidase and the second is an enzymatic biofuel cell utilizing oxalate as a fuel source with oxalate oxidase. It is important to note that oxalate is not a common fuel for biofuel cells, but an intermediate in a number of enzymatic cascades.[33,34]

[=]These authors contributed equally to this work.
[*]Electrochemical Society Student Member.
[**]Electrochemical Society Fellow.
[z]E-mail: minteer@chem.utah.edu
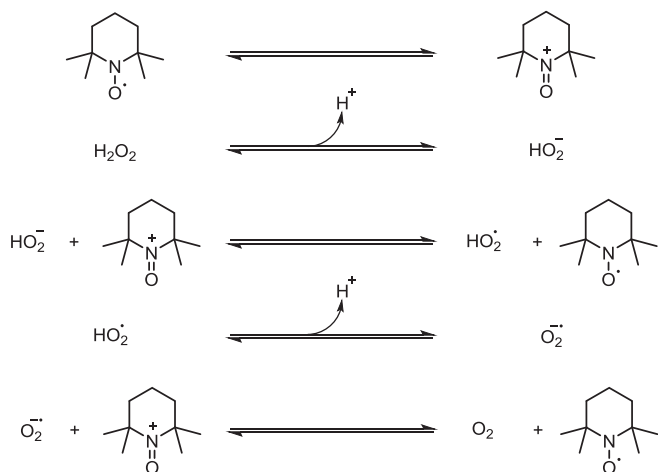
Scheme 1. Equilibrium of single electron between oxoammonium ion and H_2O_2.

Experimental

Materials.— All reagents were obtained from commercial sources and used without further purification. TEMPO (free radical, 2,2,6,6-tetramethylpiperidine 1-oxyl), Triton X-100, and sodium oxalate were purchased from Sigma-Aldrich. Sodium phosphate dibasic and monobasic, H_2O_2 solution at 30%, and citric acid were purchased from Fisher scientific. Cholesterol was purchased from Alfa Aesar. Cholesterol oxidase (ChOx) from *Streptomyces lividans* (38.7 U/mg; E.C. number 1.1.3.6) was purchased from Sekisui Enzyme. Oxalate oxidase (OxOx) from barley (45 U/ml, E.C. 1.2.3.4) was provided as a gift from Amano.

Electrocatalytic oxidation of H_2O_2 by TEMPO.— The electrocatalytic oxidation of H_2O_2 by TEMPO was characterized in solution by cyclic voltammetry (CV) using a conventional three-electrode set up with a saturated calomel (SCE) as the reference electrode, platinum mesh as the counter electrode, and a glassy carbon electrode (GCE) (3 mm diameter), purchased from CH Instruments, as the working electrode. The CVs were carried out at 10 mV s^{-1} with the CH Instrument 611e potentiostat in a solution of 5 mM TEMPO in the presence and absence of 50 mM H_2O_2 in 200 mM phosphate buffer at pH 7.4 and 25°C. A control was carried out in the same buffer with only 50 mM H_2O_2. Potential axes are plotted in the convention where positive potential is on the left and the oxidation current is plotted as negative. The pH range was also performed by CV with 5 mM TEMPO and 50 mM H_2O_2 in 200 mM citrate-phosphate buffer from pH 3 to 8. Finally, the concentration range of H_2O_2 (0 to 100 mM) was carried out with 5 mM TEMPO in 200 mM phosphate buffer at pH 7.4 and 5.2.

Electrochemical measurements with cholesterol oxidase and oxalate oxidase.— The electrochemical measurements with TEMPO and oxidases were also performed by CV at 5 mV/s with 5 mM TEMPO in 200 mM phosphate buffer at pH 7.4 for ChOx and pH 5.2 for OxOx. Before each electrochemical measurement with ChOx, 8 U of enzyme in 5 ml were incubated 3 min at 25°C with different concentrations of cholesterol (0 to 2.5 mM) in 1% of Triton X-100. Before electrochemical measurements with OxOx, 4 U of enzyme were incubated for 10 min with a concentration range of oxalate (0 to 50 mM) at 25°C.

Biofuel cell measurements.— An enzymatic biofuel cell was tested using an H cell where the cathode was Toray paper (geometric area 1 cm^2) coated with laccase, anthracene modified multi-walled carbon nanotubes, and tetrabutylammonium bromide modified Nafion and immersed in an O_2 saturated 200 mM phosphate buffer at pH 5.2.[37]

The anode was an unmodified Toray paper electrode (geometric area 1 cm^2) immersed in a solution of OxOx, 100 mM oxalate, and 5 mM TEMPO in an O_2 saturated 200 mM phosphate buffer at pH 5.2. The separator was a Nafion 212 membrane. Before the addition of TEMPO and electrochemical measurements, 16 U of enzyme and 100 mM oxalate (or 0 mM oxalate) were incubated 30 minutes. Linear polarization was performed at 1 mV/s from the open circuit potential to 0.01 V with triplicate biofuel cells.

Results and Discussion

In order to verify the ability of TEMPO to mediate the electrooxidation of H_2O_2, comparative cyclic voltammograms were performed on 5 mM TEMPO in phosphate buffer solution at pH 7.4 in the absence and presence of H_2O_2 (Figure 1). A characteristic, reversible redox signal of TEMPO is observed where the electrooxidation peak current of the hydroxylamine form occurs at 530 mV (vs. SCE). A catalytic signal with a current density of 2.32 mA cm^{-2} at 530 mV (vs. SCE) is generated when TEMPO is in the presence of 10 mM H_2O_2. The same figure also shows that there is no discernible oxidation signal in the voltammogram at the same concentration of H_2O_2 in the absence of TEMPO.

The electrocatalytic efficiency of TEMPO is dependent on the pKa of its hydroxylamine form, which needs to be deprotonated and oxidized to regenerate the nitroxyl radical.[13] Figure 2 shows the effect of pH on the electrocatalytic oxidation of H_2O_2 by TEMPO. A low activity is observed at acidic pH, because below the pKa of hydroxylamine, the coupled deprotonation/ oxidation is no longer facilitated.[14] However, the oxidation signal remains significant above pH 4, demonstrating that TEMPO could work with H_2O_2-producing oxidases that operate in acidic pH ranges.

Oxidases are frequently used in the development of biosensors and biofuel cells. While oxidases can be found that operate in a broad range of pHs, biosensors are commonly designed with oxidases that work at neutral (physiological) and acidic pH. In order to investigate the use of TEMPO to monitor H_2O_2 at both acidic and physiological pH, a series of CVs was performed on a concentration range of H_2O_2 at both pH 7.4 and 5.2. Figure 3 shows the calibration curves obtained from H_2O_2 oxidation signals by TEMPO at 530 mV (vs. SCE) at the two pH values. The calibration curves were fitted to traditional Michaelis-Menten kinetic parameters to give a calculated j_{max} (maximum current density) of 3.73 ± 0.12 and 0.24 ± 0.02 mA cm^{-2} and K_M (Michaelis-Menten constant) of 7.08 ± 0.85 and 5.36 ± 1.75 mM for pH 7.4 and
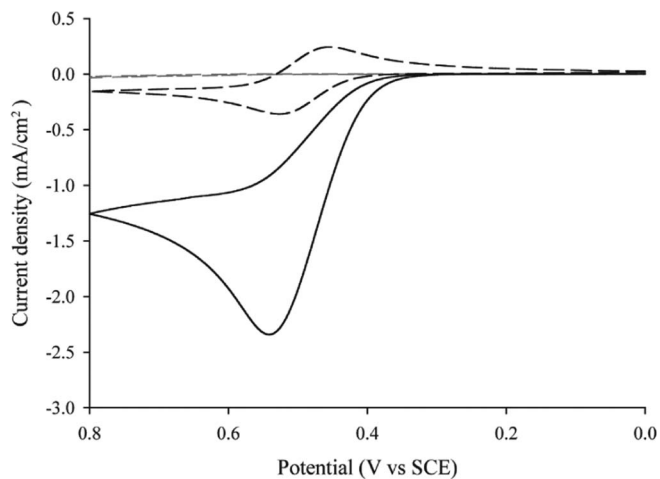

Figure 1. Representative CVs of 5 mM TEMPO in the absence (black dashed line) and presence (black solid line) of 10 mM H_2O_2. The dashed gray line is a CV carried out in the presence of 10 mM H_2O_2, but without TEMPO. All experiments were performed with a glassy carbon working electrode (3 mm diameter), platinum mesh counter electrode, and saturated calomel reference electrode (SCE) in 200 mM phosphate buffer (pH 7.4) at 10 mV/s and 25°C.
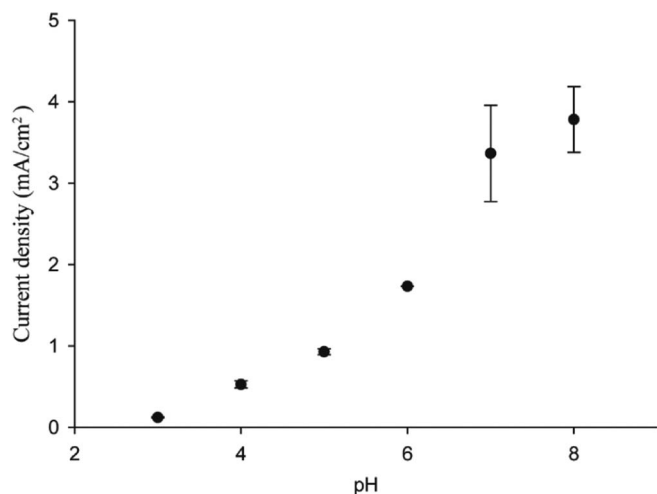
Figure 2. pH profile of the electrocatalytic oxidation of 50 mM H_2O_2 by 5 mM TEMPO at 530 mV (vs. SCE). Experiments were performed with a 3 mm glassy carbon working electrode and platinum mesh counter electrode in 200 mM citrate phosphate buffer at 10 mV/s at 25°C.

Figure 4. CVs of 5 mM TEMPO with cholesterol oxidase (ChOx) in the absence (black dashed line) and presence (black solid line) of 2.5 mM cholesterol. The gray dashed line is a CV carried out in the presence of 5 mM TEMPO and 2.5 mM cholesterol, but without ChOx. Inset: current density at 530 mV (vs. SCE) versus concentration range of cholesterol. All experiments were performed with a glassy carbon working electrode (3 mm diameter), platinum mesh counter electrode, and SCE reference electrode in 200 mM phosphate buffer (pH 7.4) and 1% Triton X-100 at 5 mV/s and 25°C.

5.2, respectively. These results demonstrate that not only is TEMPO a stable catalyst, but it is able to monitor H_2O_2 in a broad pH spectrum including acidic and physiological pH.

After demonstrating the ability to electro-oxidize H_2O_2 with TEMPO, we have investigated the ability of TEMPO to mediate electro-oxidation of H_2O_2 produced by oxidases. In this study, two enzymes that operate in different pH ranges were chosen as models. Commercial cholesterol oxidase (ChOx) from *Streptomyces lividans* operates in a neutral pH range and oxalate oxidase (OxO) from barley operates in an acidic pH range.

Cholesterol oxidase is a flavoenzyme able to catalyze the oxidation of cholesterol to cholest-4-en-3-one using oxygen as an electron acceptor to form H_2O_2 (Eq. 1).

$$Cholesterol + O_2 \rightarrow Cholest\text{-}4\text{-}en\text{-}3\text{-}one + H_2O_2 \qquad [1]$$
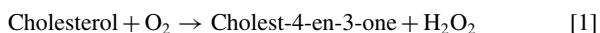
This enzyme has been used extensively in the development of cholesterol biosensors for the medical and food industries. Different electrode designs have been studied, including mediated electron transfer
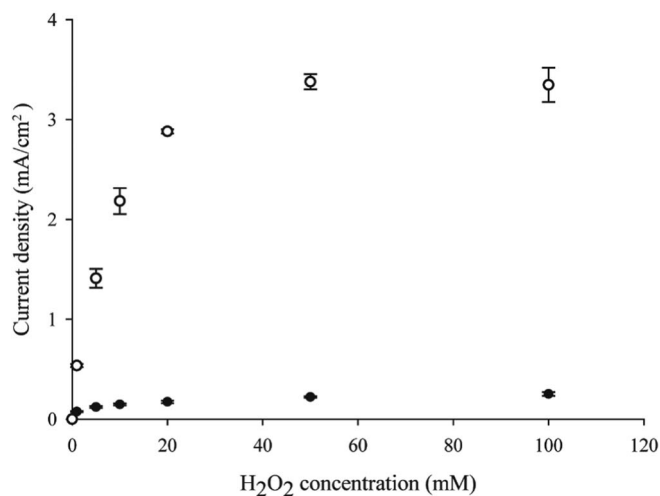

Figure 3. Calibration curve of 5 mM TEMPO in presence of different concentration of H_2O_2 at pH 7.4 (empty circle) and 5.2 (filled circle). All experiments were performed with a glassy carbon working electrode (3 mm diameter), platinum mesh counter electrode, and saturated calomel reference electrode (SCE) in 200 mM phosphate buffer (pH 7.4 and pH 5.2) at 10 mV/s and 25°C.
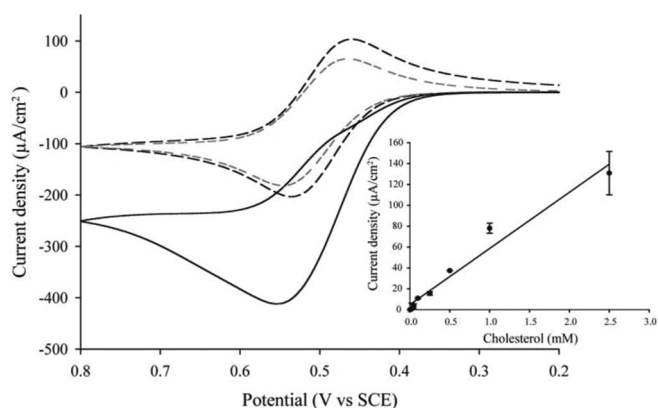
directly with the FAD cofactor buried in the enzyme[15] and H_2O_2 monitoring by horseradish peroxidase[16] or by metals[17] and modified nanotubes.[18] CVs were performed on TEMPO and ChOx at pH 7.4 in order to examine the use of TEMPO to monitor the enzymatic cholesterol oxidation (Figure 4). In Figure 4, a representative cyclic voltammogram obtained for TEMPO, ChOx, and 2.5 mM cholesterol exhibits a significant electrocatalytic signal of 400 $\mu A\ cm^{-2}$ at 530 mV. No electrocatalytic signal is observed in the absence of cholesterol when ChOx is still present nor in the absence of ChOx when cholesterol is still present. A calibration curve for cholesterol, shown in the inset of the Figure 4, indicates a linear response range from 20 μM to 2.5 mM cholesterol with a sensitivity of 53.9 ± 4.3 mA $cm^{-2}\ M^{-1}$. Compared to recent cholesterol biosensors described in the literature (Table I), these values are competitive in terms of having high sensitivity and a broad linear range. Considering that these results were obtained with a non-optimized system working at physiological pH, we can suggest that TEMPO has great potential for use in a real cholesterol biosensor.

Oxalate oxidase belongs to the cupin superfamily characterized by conserved motifs and a β-barrel domain fold.[19] Its redox center contains a mononuclear manganese ion coordinated by amino acids and it is able to catalyze the oxidation of oxalate to CO_2 and the reduction of oxygen to H_2O_2 (Eq. 2).

$$Oxalate + O_2 \rightarrow 2CO_2 + H_2O_2 \qquad [2]$$
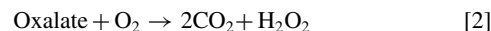
Some studies have used OxOx to develop oxalate biosensors.[27–30] It is important to note that there are no known redox mediators for oxalate oxidase, so all oxalate oxidase biosensors have had to operate via the detection of the hydrogen peroxide enzymatic product. Designs include electrochemical detection of oxalate using edge plane and basal plane pyrolytic graphite electrodes[28] where there was a high overpotential and electrochemical detection of H_2O_2 using multi-walled carbon nanotube-gold nanoparticle composites,[28] carboxylated multi-walled carbon nanotubes in a polyaniline composite film,[30] and chromium hexacyanoferrate[27] as catalysts.

Here, cyclic voltammetry was used to examine the use of TEMPO with oxalate and OxOx in phosphate buffer at pH 5.2 (Figure 5). A representative CV obtained for OxOx, 5 mM TEMPO, and 50 mM oxalate exhibits a significant catalytic signal of 340 $\mu A\ cm^{-2}$ at 530 mV when compared with the CV of TEMPO and OxOx in the absence of oxalate (250 $\mu A\ cm^{-2}$ at 530 mV). No electrocatalytic behavior is observed for TEMPO and oxalate in the absence of OxOx either (220 $\mu A\ cm^{-2}$ at 530 mV). The current densities at 530 mV for concentrations of oxalate between 2 and 50 mM in the presence of

Table I. Comparison of recent cholesterol biosensors.

Electrode design	LOD (μM)	Linear range (mM)	Sensitivity (mA cm^{-2} M^{-1})	Reference
ChOx/Ppy/PB/GCE	0.6	0.02–0.1	0.55	20
IL/ChOx/PB/GCE	4.4	0.01–0.4	400 mA/M[1]	21
ChOx/PTZ/SPE	2.3	0.015–0.15	33.1	16
ChOx/PEDOT/PMB/GCE	1.6	0.01–0.22	79	22
ChOx/NiFe2O4-CuO-FeO-chitosan nanocomposite	0.0313	0.13–12.95	16.54	23
ChOx/MUA/AuNPs/dithiol/AuE	34.6	0.04–0.22	45.96	24
ChOx/AuPt/chitosan/IL/GCE	10	0.05–6.2	90.7	25
ChOX/HRP/AuNPS/PDDA/MWCNTs/GCE	2.2	0.01–1.05	18.6	17
ChOx/PANI/PVP/Graphene	1	0.05–10	34.77	26
ChOx/TEMPO/GCE	20	0.02–2.5	54.86	Present work

Abbreviation: ChOx, cholesterol oxidase; Ppy, polypyrrole; PB, Prussian blue; GCE, glassy carbon electrode; IL, ionic liquid; PTZ, phenothiazine; SPE, screen-printed electrode; PEDOT, poly(3,4-ethylenedioxythiophene); PMB, poly (methylene blue); MUA, 11-mercaptoundecanoic acid; AuNPs, gold nanoparticles; AuE, gold electrode; AuPt, gold platinum electrodeposition; HRP, horseradish peroxidase; PDDA, poly-(diallyldimethyl-ammonium chloride); MWCNTs, multi-walled carbon nanotubes; PANI, polyaniline; PVP, poly(vinylpyrrolidone).

TEMPO and OxOx are shown in the Figure 5 inset. Although linearity may only exist from 2 to 10 mM, the signal increases with concentration of oxalate up to 50 mM. Other oxalate biosensors only measure in the μM range.[27] TEMPO, therefore, has possible applications in an oxalate biosensor where it enhances the H_2O_2 signal. Fitting the curve to traditional Michaelis-Menten kinetic parameters gives a calculated K_M of 3.96 ± 0.56 mM.

TEMPO also has implications for enzymatic biofuel cells. Researchers sometimes add an enzyme such as catalase to an oxidase cathode (commonly glucose oxidase) to consume the H_2O_2 produced by the oxidase because peroxide contamination has a negative effect on the long term stability of the enzyme.[31,32] TEMPO would help make this strategy even more effective. TEMPO could also be used to facilitate consumption of H_2O_2 at an oxalate oxidase bioanode, similar to the glucose oxidase bioanode described above. Or, it could be used for two purposes in an oxalate/H_2O_2 enzymatic biofuel cell, where oxalate is oxidized at the OxOx bioanode or the OxOx product H_2O_2 becomes the biocathode (catalase or peroxidase) substrate.[35]

Here, an oxalate/O_2 enzymatic biofuel cell was tested, where TEMPO, oxalate, and OxOx were in solution with a Toray paper electrode for the anode and the cathode consisted of laccase. Figure 6 shows representative power density curves for a biofuel cell with 100 mM oxalate and a biofuel cell with no oxalate present. When oxalate is present, there is an average maximum power density of 4.19 ± 0.85 μW cm^{-2} and an average maximum current density of 74.4 ± 11.9 μA cm^{-2} compared with an average maximum power density of 1.56 ± 0.43 μW cm^{-2} and an average maximum current density of 27.2 ± 0.8 μA cm^{-2} when there is no oxalate present.

Conclusions

This study has shown that TEMPO can be used to selectively electro-oxidize H_2O_2 at 530 mV (vs SCE) in a broad range of pHs (≥4). Thereby, TEMPO has been combined with cholesterol oxidase to monitor cholesterol oxidation. Compared to recent electrochemical cholesterol biosensors, a competitive linear range and sensitivity was obtained. Furthermore, the electro-oxidation of H_2O_2 produced from the oxalate oxidation with oxalate oxidase has also been studied. These results allow us to present a nitroxyl radical as a promising electro-catalyst applied for the development of electrochemical biosensors or enzymatic biofuel cells. Future work will evaluate other nitroxyl radicals such as ABNO (9-Azabicyclo[3.3.1]nonane N-oxyl) with lower redox potential to study their catalytic activity toward H_2O_2 in order to decrease the oxidation potential for higher selectivity. Bioelectrodes containing immobilized TEMPO and oxidases could be designed to
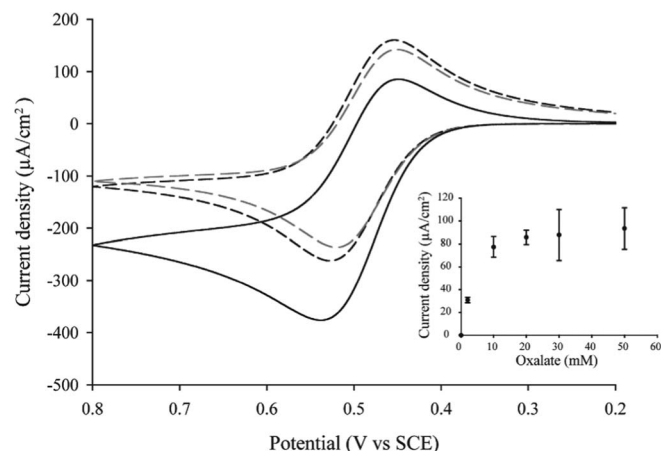

Figure 5. Representative CVs of 5 mM TEMPO with oxalate oxidase (OxOx) in the absence (black dashed line) and presence (black solid line) of 50 mM oxalate. The gray dashed line is a CV carried out in the presence of 5 mM TEMPO and 50 mM oxalate, but without OxOx. Inset: current density at 530 mV (vs. SCE) versus concentration range of oxalate. All experiments were performed with a glassy carbon working electrode (3 mm diameter), platinum mesh counter electrode, and SCE reference electrode in 200 mM phosphate buffer (pH 5.2) at 5 mV/s and 25°C.
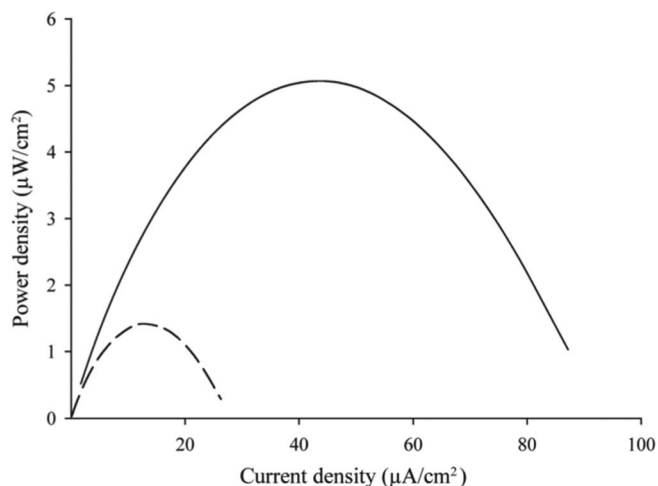

Figure 6. Representative power density curves for a biofuel cell with oxalate oxidase and TEMPO anode and laccase cathode in pH 5.2 phosphate buffer. The solid line is when the anode contains 100 mM oxalate and the dashed line is in the absence of oxalate.

improve the current signal and to facilitate the development of biosensors or biofuel cells.

References

1. W. Chen, S. Cai, Q.-Q. Ren, W. Wen, and Y.-D. Zhao, *The Analyst*, **137**(1), 49 (2012).
2. A. C. McIntosh and A. Prongidis, in *Design and Nature V: Comparing Design in Nature with Science and Engineering*, C. Brebbia and A. Carpi, eds., Vol. 138, p. 265–272 (2010).
3. P. Niethammer, C. Grabher, A. T. Look, and T. J. Mitchison, *Nature (London)*, **459**(7249), 996 (2009).
4. S. Neill, R. Desikan, and J. Hancock, *Current Opinion in Plant Biology*, **5**(5), 388 (2002).
5. R. F. P. Nogueira, M. C. Oliveira, and W. C. Paterlini, *Talanta*, **66**(1), 86 (2005).
6. J. H. Lee, I. N. Tang, and J. B. Weinstein-Lloyd, *Analytical Chemistry*, **62**(21), 2381 (1990).
7. S. Hanaoka, J.-M. Lin, and M. Yamada, *Analytica Chimica Acta*, **426**(1), 57 (2001).
8. A. Gomes, E. Fernandes, and J. L. F. C. Lima, *Journal of Biochemical and Biophysical Methods*, **65**(2–3), 45 (2005).
9. D. W. Kimmel, G. LeBlanc, M. E. Meschievitz, and D. E. Cliffel, *Analytical Chemistry*, **84**(2), 685 (2012).
10. N. J. Ronkainen, H. B. Halsall, and W. R. Heineman, *Chemical Society Reviews*, **39**(5), 1747 (2010).
11. A. K. M. Kafi, G. Wu, and A. Chen, *Biosensors and Bioelectronics*, **24**(4), 566 (2008).
12. L. Gorton, A. Lindgren, T. Larsson, F. D. Munteanu, T. Ruzgas, and I. Gazaryan, *Analytica Chimica Acta*, **400**(1–3), 91 (1999).
13. J. Kulys and R. Vidziunaite, *Journal of Molecular Catalysis B: Enzymatic*, **37**(1–6), 79 (2005).
14. D. P. Hickey, M. S. McCammant, F. Giroud, M. S. Sigman, and S. D. Minteer, *Journal of the American Chemical Society*, **136**(45), 15917 (2014).
15. A. N. Sekretaryova, V. Beni, M. Eriksson, A. A. Karyakin, A. P. F. Turner, and M. Y. Vagin, *Analytical Chemistry*, **86**(19), 9540 (2014).
16. M. Eguílaz, R. Villalonga, P. Yáñez-Sedeño, and J. M. Pingarrón, *Analytical Chemistry*, **83**(20), 7807 (2011).
17. R. Khan, A. Kaushik, P. R. Solanki, A. A. Ansari, M. K. Pandey, and B. D. Malhotra, *Analytica Chimica Acta*, **616**(2), 207 (2008).
18. G. Li, J. M. Liao, G. Q. Hu, N. Z. Ma, and P. J. Wu, *Biosensors and Bioelectronics*, **20**(10), 2140 (2005).
19. M. M. Whittaker and J. W. Whittaker, *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry*, **7**(1-2), 136 (2002).
20. J.-P. Li and H.-N. Gu, *Journal of the Chinese Chemical Society*, **53**(3), 575 (2006).
21. X. Liu, Z. Nan, Y. Qiu, L. Zheng, and X. Lu, *Electrochimica Acta*, **90**, 203 (2013).
22. S. Kakhki, M. M. Barsan, E. Shams, and C. M. A. Brett, *Analytical Methods*, **5**(5), 1199 (2013).
23. J. Singh, M. Srivastava, P. Kalita, and B. D. Malhotra, *Process Biochemistry*, **47**(12), 2189 (2012).
24. U. Saxena, M. Chakraborty, and P. Goswami, *Biosensors and Bioelectronics*, **26**(6), 3037 (2011).
25. A. Safavi and F. Farjami, *Biosensors and Bioelectronics*, **26**(5), 2547 (2011).
26. N. Ruecha, R. Rangkupan, N. Rodthongkum, and O. Chailapakul, *Biosensors and Bioelectronics*, **52**, 13 (2014).
27. S. Yadav, R. Devi, S. Kumari, S. Yadav, and C. S. Pundir, *Journal of Biotechnology*, **151**(2), 212 (2011).
28. B. Sljukic, R. Baron, and R. G. Compton, *Electroanalysis*, **19**(9), 918 (2007).
29. C. S. Pundir and M. Sharma, *J. Sci. Ind. Res. India*, **69**, 489 (2010).
30. C. S. Pundir, N. Chauhan, N. Rajneesh, M. Verma, and M. Ravi, *Sensors and Actuators B: Chemical*, **155**(2), 796 (2011).
31. A. Zebda, C. Gondran, A. Le Goff, M. Holzinger, P. Cinquin, and S. Cosnier, *Nature Communications*, **2**, 370-Article No.: 370 (2011).
32. C. Agnes, M. Holzinger, A. Le Goff, B. Reuillard, K. Elouarzaki, S. Tingry, and S. Cosnier, *Energy & Environmental Science*, **7**(6), 1884 (2014).
33. S. Xu and S. D. Minteer, *ACS Catalysis*, **2**(1), 91 (2012).
34. R. L. Arechederra and S. D. Minteer, *Fuel Cells*, **9**(1), 63 (2009).
35. A. Pizzariello, M. Stred'ansky, and S. Miertuš, *Bioelectrochemistry*, **56**(1-2), 99 (2002).
36. S. Goldstein, G. Merenyi, A. Russo, and A. Samuni, *Journal of the American Chemical Society*, **125**(3), 789 (2003).
37. M. T. Meredith, M. Minson, D. Hickey, K. Artyushkova, D. T. Glatzhofer, and S. D. Minteer, *ACS Catalysis*, **1**(12), 1683 (2011).