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Electrochemistry | *Very Important Paper* |

VIP Catalase-Modified Carbon Electrodes: Persuading Oxygen To Accept Four Electrons Rather Than Two

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Abstract: We successfully exploited the natural highly efficient activity of an enzyme (catalase) together with carbon electrodes to produce a hybrid electrode for oxygen reduction, very appropriate for energy transformation. Carbon electrodes, in principle, are cheap but poor oxygen reduction materials, because only two-electron reduction of oxygen occurs at low potentials, whereas four-electron reduction is key for energy-transformation technology. With the immobilization of catalase on the surface, the hydrogen peroxide produced electrochemically is decomposed back to oxygen by the enzyme; the enzyme natural activity on the surface regenerates oxygen, which is further reduced by the carbon electrode with no direct electron transfer between the enzyme and the electrode. Near full four-electron reduction of oxygen is realised on a carbon electrode, which is modified with ease by a commercially available enzyme. The value of such enzyme-modified electrode for energy-transformation devices is evident.

The electroreduction of oxygen lies at the heart of modern energy-transformation technology, notably batteries and fuel cells.[1] To realise maximum energy output the four electron, four-proton reduction to water is essential [Eq. (1)]:

$$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O \qquad (1)$$

Otherwise, if the process stops at the two electrons, two-proton stage with the formation of hydrogen peroxide, a very significant energy-loss results, as well as deleterious effects on fuel-cell components. Accordingly, there is an intense research activity searching for cheap and effective electrode materials for the full four-electron process for which platinum is highly effective, but prohibitively expensive for many applications.[2]

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Amongst potentially attractive electrode materials, carbon stands out as being attractively cheap but its use typically leads to the formation of H_2O_2; only at high overpotentials, water is formed.[2,3] Carbon electrodes are thus not viable for O_2-reduction technology for most applications. However, if a suitable surface modification of carbon became possible to allow the four-electron process at low overpotential, this could completely transform the landscape of electrochemical materials for this application. This paper reports exactly this modification. Specifically, we use a carbon electrode to reduce oxygen into H_2O_2 and a catalase enzyme immobilized on the electrode surface to bring about the decomposition $2\,H_2O_2 \rightarrow 2\,H_2O_2 + O_2$ in a chemical rather than an electrochemical fashion.

Among the enzymes investigated electrochemically, catalase outstands due to its role as an antioxidant and its ability to decompose hydrogen peroxide to water and oxygen at fast turnover frequency.[4] Although surface-immobilized catalase shows direct electron-transfer behaviour with some electrodes,[5–13] on others, no electrochemical response is seen (for a recent review, see [14]).[7,11]

Herein, we show that catalase adsorbed on carbon electrode retains its natural redox activity, an observation that likely exploits the distant location of the buried iron, which prohibits direct electron transfer between catalase and a carbon electrode. Thus, the surface-immobilized enzyme may operate by its natural pathway of a cyclic Fe^{III}/Fe^{IV} reaction by H_2O_2 reduction and oxidation. It has been shown previously that other enzymes, such as horse radish peroxidase,[15] laccase[16,17] and bilirubin oxidase,[18] can facilitate oxygen or hydrogen peroxide reduction upon surface immobilization by using direct electron transfer or mediated electron transfer, with a lower reduction potential compared with the bare electrode. Herein, we show that up to four electron reduction of oxygen can be achieved without the need to modify the electrode to facilitate direct electrotransfer, nor is a mediator required, because the oxygen product of the enzymatic reaction is further used in a catalytic process of electrochemical oxygen reduction.

Figure 1 shows the reduction of O_2-saturated solution of 50 mM phosphate buffer and 135 mM KCl (phosphate buffered saline (PBS)), at an unmodified polished carbon fibre microelectrode ($r = 4.93$ μm, BASi Inc, Stareton, Warks, UK). The voltammogram has a half-wave potential of −0.8 V (vs. saturated calomel electrode (SCE)), and the steady-state current magnitude[19] is consistent with the known solubility of oxygen[20] ($[O_2] = 1.24$ mM), its diffusion coefficient $D_{O_2} = 1.76 \times 10^{-9}\ m^2\,s^{-1}$[21] and the transfer of two electrons as shown in Equation (2) (see calculation in the Supporting Information):

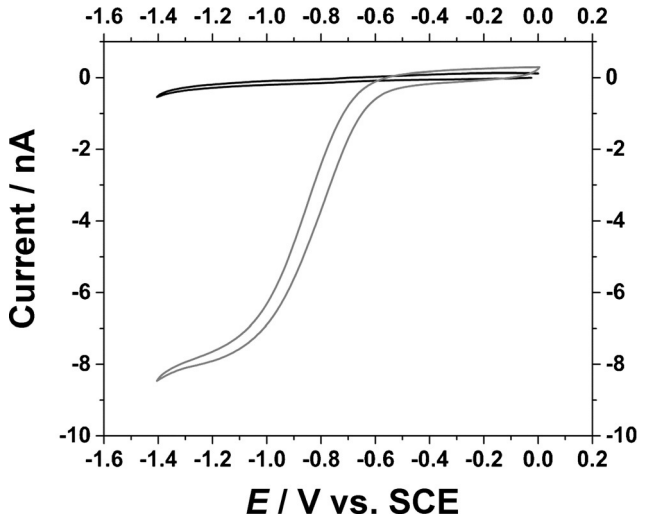


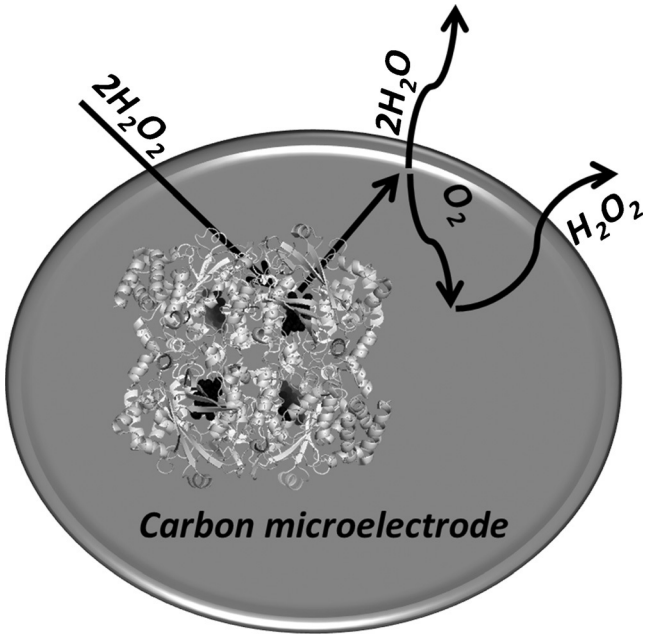
Figure 1. Cyclic voltammograms of a carbon microelectrode transferred in 1.24 mM solution of H_2O_2 free of oxygen (15 min bubbling with N_2) and 1.24 mM solution of O_2 (saturated) in 50 mM phosphate buffer and 135 mM KCl.

$$O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O_2 \qquad (2)$$

Figure 1 (upper curve) shows a potential sweep over the same range, but with a solution containing 1.24 mM H_2O_2 (after bubbling thoroughly with N_2). No reductive electrochemistry of H_2O_2 is discernible.

Next, we examined the response arising from a carbon microelectrode modified with immobilized catalase enzyme. First, a polished microelectrode was exposed to a solution of 180 μM catalase (as received from Sigma) for various times (between 5 s to 1 h). Then, the modified microelectrode was washed with water and transferred into a 10 mM H_2O_2 solution.

As can be seen from Figure 2a, the catalase-modified microelectrode gave voltammograms corresponding to oxygen reduction (for comparison, see Figure S1 in the Supporting Information), even though the solution is initially oxygen free. The limiting current increased with an increasing exposure time to the catalase solution and saturated above approximately ten minutes of pre-exposure to the catalase solution. The limiting current reflects an oxygen-reduction wave, and it implies that the enzyme immobilized on the electrode surface is capable of locally decomposing the H_2O_2 with the formation of O_2, which can then be detected electrochemically. This, in turn, is a clear indication that the enzyme is stable and active on the carbon



Scheme 1. Schematic illustration of the natural catalytic activity of catalase, followed by further reduction of the oxygen product by a suitably reductive microelectrode.

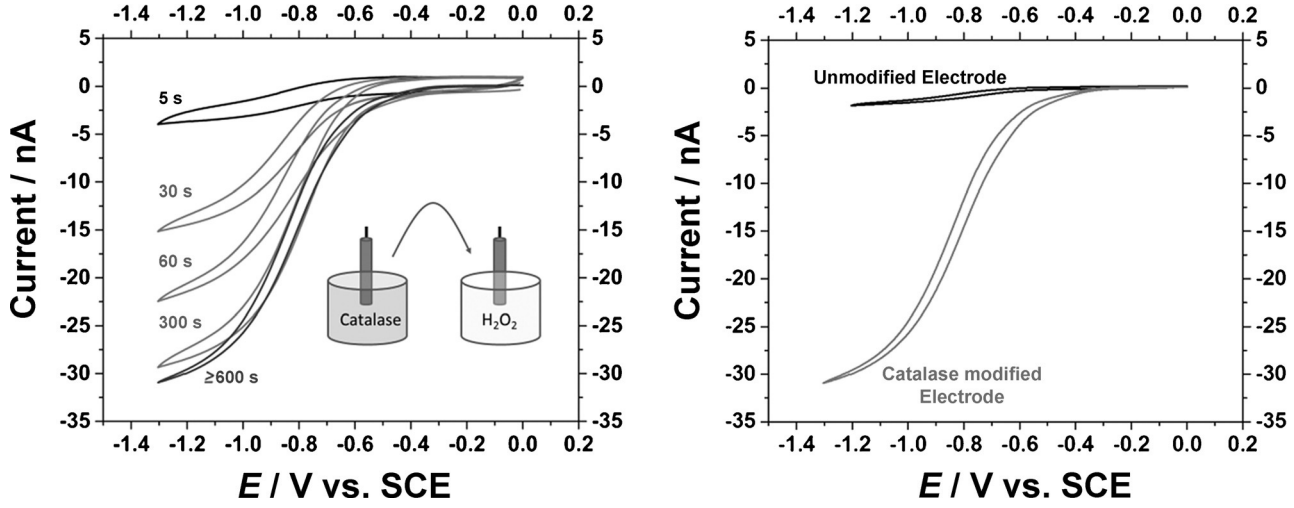


Figure 2. (a) Voltammograms of a modified carbon microelectrode transferred into 10 mM H_2O_2 + 50 mM PBS solution free of oxygen (15 min bubbling with N_2). Modification was achieved by exposing the electrode to 180 μM catalase solution prior to the transfer. Different exposure times are designated. (b) Voltammograms of a modified and unmodified carbon micro electrode exposed to 10 mM H_2O_2 in 50 mM PBS solution free of oxygen.

surface during the electrochemical measurements. The enzymatic reaction followed by the electrochemical oxygen reduction is illustrated in Scheme 1.

We next modified the surface of the carbon electrodes by immersing it in a solution of 180 μM catalase for ten minutes, when maximum response was achieved. The electrode was then washed with water and transferred to a clean aqueous oxygen-free solution of PBS buffer containing variable amounts of H_2O_2[1], but with no enzyme in the bulk solution. The voltammograms were recorded and shown in Figure 3.

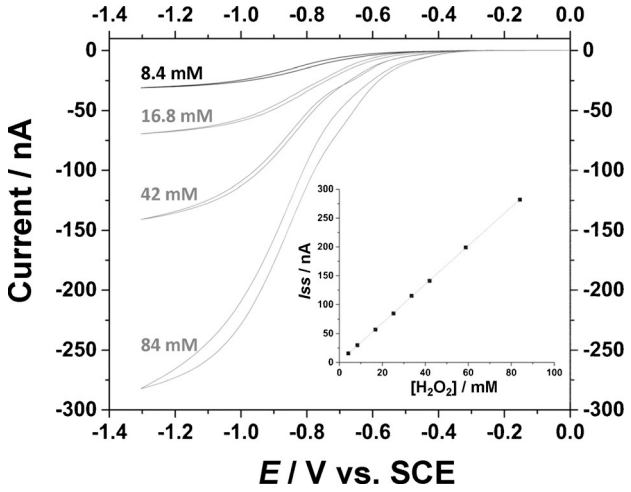


Figure 3. Voltammograms of a carbon electrode modified with catalase at different concentrations of H_2O_2 in PBS solution with oxygen free solutions (15 min bubbling with N_2). The concentrations of H_2O_2 were 4.2–84 mM. Inset shows the linear correlation of the limiting current (I_{ss}) as a function of H_2O_2 concentration. From the slope, the value of H_2O_2 diffusion coefficient is derived.

The magnitude of the steady-state-limiting currents (I_{ss}) are consistent with the diffusion-controlled reduction of oxygen assuming that all of the H_2O_2 that can diffuse to and reach the electrode surface undergoes decomposition, thus, the electrode current is expressed as shown in Equation (3)[19]:

$$I_{ss} = 4nF\frac{[C]}{2} \quad (3)$$

in which F is the Faraday constant, D is the diffusion coefficient and equals to $1.71\times10^{-9}\ m^2\,s^{-1}$ for hydrogen peroxide,[22] $[C]$ is the hydrogen peroxide concentration, r is the microelectrode radius ($r = 4.93$ μm) and $n = 2$, as was discussed above for the reduction of oxygen. The factor of a (1/2) reflects the stoichiometry of the O_2 formation from H_2O_2.

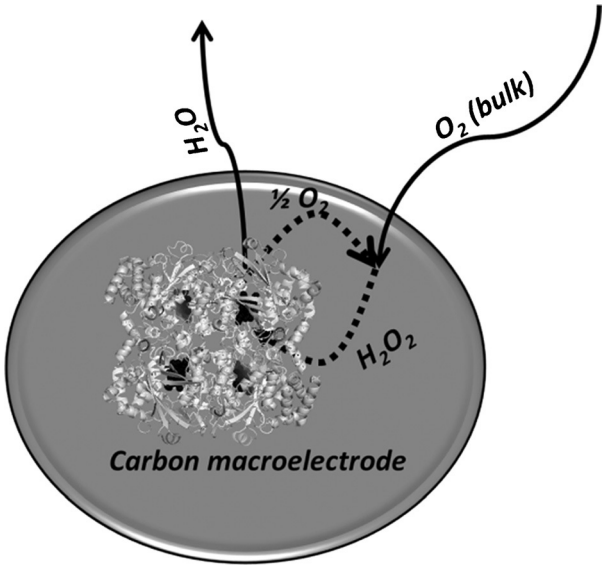
Studies of the recorded steady-state current of the catalase-modified electrode as a function of H_2O_2 concentration allowed an estimate of the H_2O_2 diffusion coefficient. From the slope of the limiting current as a function of H_2O_2 concentration, a value of $D_{H_2O_2} = 1.73\pm0.06\times10^{-9}\ m^2\,s^{-1}$ of was calculated, which is in agreement with previously reported values (Figure 3, inset). This confirms that catalase, when absorbed on a carbon surface, retains its catalytic activity with respect to the decomposition of H_2O_2. This is consistent with other reports in the literature.[7,13] According to the observed steady-state current, all of the produced oxygen is reduced by the carbon microelectrode to hydrogen peroxide ($n = 2$, Scheme 1). It is important to note that at high surface concentration of catalase, the reaction is limited by H_2O_2 diffusion, and not by conventional Michaelis–Menten kinetics.

After demonstrating the coupling between catalase enzymatic activity and carbon electrochemical reactivity towards the enzyme's product, we have examined the application of four-electron reduction of oxygen on a carbon electrode. For this, we have used a glassy carbon (GC) macroelectrode instead of a carbon microelectrode. The reason for using a macroelectrode is due to the much slower mass transfer of electroactive species to and products/intermediates away from the electrode during the electrochemical reaction.[23] Thus, the hydrogen peroxide product of the two-electron reduction of oxygen can be further decomposed by the catalase, leading to an autocatalytic reaction rather than being lost by diffusion into solution. Because catalase decomposes two hydrogen peroxide molecules into single oxygen, the autocatalytic reaction should produce a maximum amplification of: $2\sum_{n=1}^{n=\infty}1/2^n = 2$, leading to a four (2×2) electron-reduction of oxygen. This is further illustrated in Scheme 2.

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \text{ electrochemical} \quad (4)$$

$$2H_2O_2 \rightarrow H_2O_2 + O_2 \text{ enzymatic} \quad (5)$$

Figure 4a compares the experimental and simulated responses of a bare GC and a catalase-modified GC in an



Scheme 2. Schematic illustration of the autocatalytic reaction occurring at the catalase/carbon macroelectrode interface according to Equations (4) and (5).

[1] *The concentration of H_2O_2 was determined by using an extinction coefficient of 43.6 M cm at λ = 240 nm. Absorption was measured after bubbling H_2O_2 for 20 minutes in a PBS solution, similar to the conditions applied to the electrochemical measurements.*

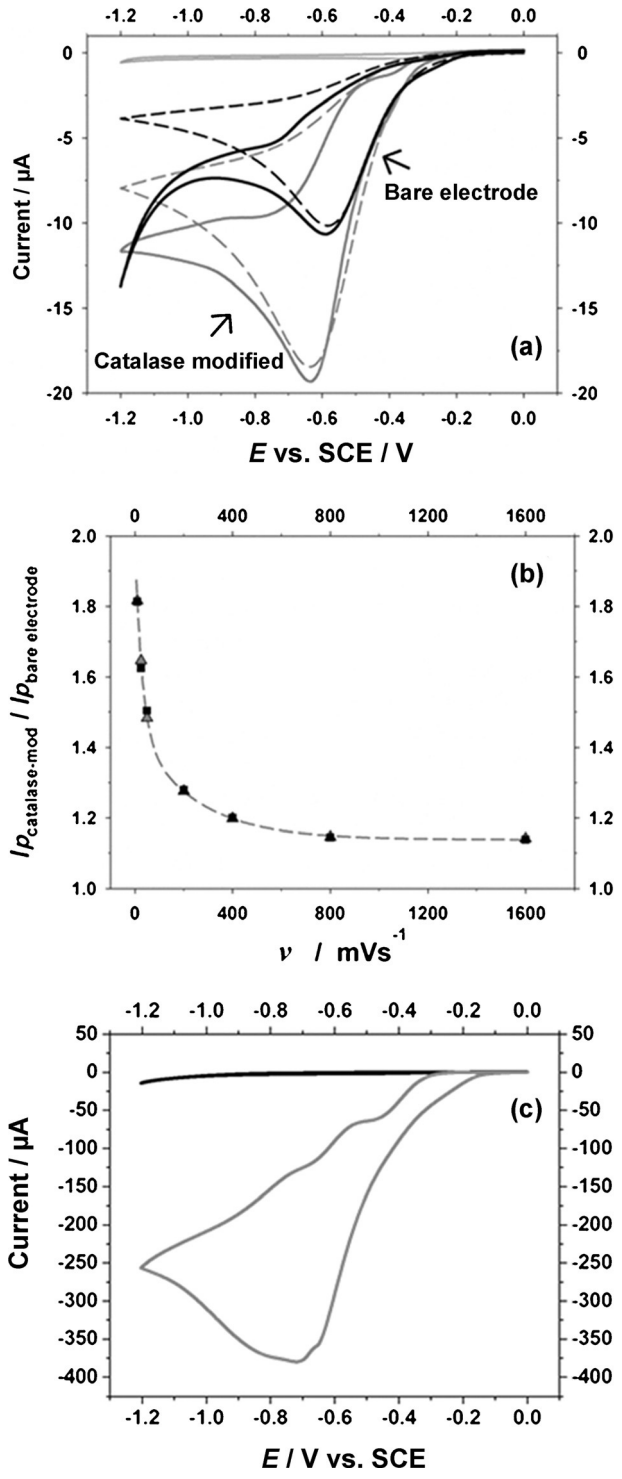


Figure 4. (a) Experimental (solid line) and simulated (dashed line) voltammograms of bare and catalase modified GC macroelectrode in saturated O_2 and PBS solution. Scan rate of 10 mV s^{-1}. (b) Experimental (squares) and simulated (triangles and dashed line) peak current ratio of catalase modified GC with respect to a bare GC electrode at different scan rates in saturated oxygen solution. (c) Voltammograms of bare and catalase-modified GC macroelectrode in 20 mM H_2O_2 PBS solution. Scan rate of 50 mVs^{-1}.

oxygen-saturated solution, recorded at scan rate of 10 mVs^{-1} (for the experimental and simulated responses at different scan rates, see Figure S2 in the Supporting Information). It is evident that the peak current potential (I_p) was nearly doubled (ratio 1.8). The results correspond to an autocatalytic process, which results in an almost four-electron reduction of oxygen at potentials similar to the conventional two-electron reduction of oxygen on a carbon electrode. The efficiency of the autocatalytic process decreases with increasing scan rates, as can be seen from Figure 4b. This observation reflects the interplay between the natural enzymatic reaction occurring on the interface and the electrochemical oxygen reduction occurring on the electrode surface with no direct electron transfer between the enzyme and the electrode.

Quantitative analysis of the autocatalytic process at the catalase-modified macroelectrode was carried out on the bases of the reaction mechanism illustrated in Scheme 2 for the electrochemical oxygen reduction in neutral media with heterogeneous decomposition of hydrogen peroxide (more details are given in the Supporting Information):[24]

$$O_2 + e^- \xrightarrow{k_0,\ \alpha,\ E_f^0} O_2^{\bullet -} \ (\text{rds}) \tag{6}$$

$$O_2^{\bullet -} + H_2O + e^- \rightarrow HO_2^- + OH^- \ (\text{fully driven}) \tag{7}$$

$$HO_2^{\bullet} + H^+ \rightarrow H_2O_2 \ (pK_a = 11.6) \tag{8}$$

$$H_2O_2 + \xrightarrow{\text{catalase}} H_2O_2 + 1/2O_2 \tag{9}$$

in which k_0, α and E_f^0 are the standard rate constant, transfer coefficient and formal potential, respectively, of the first electron transfer. The kinetic parameters of the first step were determined from the voltammograms at bare GC, in which step 4 does not take place. Next, the surface decomposition kinetics was studied from the fitting of the ratio between the peak currents at the catalase-modified and bare GC electrodes (Figure 4b). A Michaelis–Menten mechanism accounting for the surface confinement of the enzyme was considered:

$$S + E^* \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} SE^* \xrightarrow{k_2} P + E^* \tag{10}$$

in which E^* and SE^* species that are surface bound, and the rate constants are assumed to be potential independent. Under the steady-state approximation for the surface coverage of the enzyme–substrate complex (Γ_{SE}/mol cm^{-1}), the following expression for the rate (v_{cat}) of the heterogeneous enzyme reaction is obtained:

$$\upsilon_{cat} = k_2\Gamma_{SE} = \frac{k_2 c_s^0 \Gamma_{E,tot}}{k_M + c_s^0} \tag{11}$$

in which $K_M = \frac{k_{-1}+k_2}{k_1}$, $\Gamma_{E,tot}$ is the surface coverage of catalase and c_s^0 is the surface concentration of the substrate (i.e., hydrogen peroxide), which is subject to mass transport by diffusion. Making use of the above-mentioned formalism and the numerical method described in the Supporting Information, a very satisfactory description of the variation of the peak ratio with scan rate was achieved (Figure 4b) with $\frac{K_2\Gamma_{E,tot}}{K_M} = 2.5\times10^{-2}$, and K_M is in the order of approximately 100 μM.

The four-electron reduction of oxygen was further illustrated when the modified electrode was introduced to a solution of hydrogen peroxide. As can be seen in Figure 4c (upper curve), the GC macroelectrode is inert to hydrogen peroxide reduction, whereas the modified GC is active. This scenario is analogues to the results shown in Figure 2b for a carbon microelectrode. However, the peak current produced by the modified GC macroelectrode (red curve) corresponds to a four-electron reduction of oxygen (see calculation in the Supporting Information), consistent with the slow mass transport on a macroelectrode, thus allowing the autocatalytic reaction to take place (unlike in the case of a microelectrode).

In conclusion, we have shown that catalase immobilized on a carbon electrode maintains its natural functionality. The deeply buried heme of the catalase does not allow direct electron transfer with the glassy carbon electrode. Rather, the natural activity of the enzyme is retained and hence can be exploited, and the oxygen product is sensed directly by the carbon microelectrode. The rate-limiting step of this reaction is the hydrogen peroxide diffusion to the electrode, as shown from the catalase-modified microelectrode response to solution containing different hydrogen peroxide concentration. In the case of a macroelectrode, nearly four-electron reduction of oxygen at a catalase-modified glassy carbon was observed at slow scan rates. This energetically effective process is realized with a simple and quick modification of the carbon electrode with catalase. Moreover, the four-electron reduction occurs at low potentials, in which the conventional two-electron reduction at a bare glassy electrode occurs. This simple technique can be efficiently applied to electrode materials, in which hydrogen peroxide reduction occurs at higher overpotentials than oxygen reduction, such as carbon electrodes, and bring about up to twice energy output at the potential corresponding to oxygen reduction at the electrode.

Experimental Section

Sodium chloride, potassium phosphate monobasic (> 99%, KH_2PO_4) and potassium phosphate dibasic (≥ 98%, K_2HPO_4) were supplied by Sigma. All solutions were made with ultrapure water from Millipore with resistivity not less than 18.2 MΩ cm at 298 K. For the electrochemical measurements, a 4.93 μm carbon microelectrode or a 1.33 mm radius glassy carbon macroelectrode were used as a working electrode. The electrodes were polished with micropolish alumina (Buehler) of decreasing particle size (3.0, 0.1 and 0.01 μm). All solution were made at pH 7, achievable through the use of appropriate $K_2HPO_4/K_2H_2PO_4$ phosphate buffer and confirmed by using a Hannah pH 213 pH meter. For experiments, in which the absence of oxygen was required, solutions were deoxygenated by using oxygen-free nitrogen (N_2, BOC, Guildford, UK) for at least 20 min. For experiments performed in O_2-saturated solutions, solutions were purged with O_2 (O_2, BOC, Guildford, UK) for 30 min prior to the measurements, in the same way as N_2. Experiments were conducted at 295 ± 2 K within a Faraday cage by using a saturated calomel reference electrode (SCE) and a graphite rod counterelectrode. An Autolab PGSTAT 12 was used for all experiments. The simulations of the voltammograms were done in a similar fashion to the ones reported earlier.[24] A full explanation of the simulation method is given in the Supporting Information.

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Keywords: electrochemistry · catalase · enzymes · hydrogen peroxide · oxygen reduction

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