

Combinatorial Biomimetics. Optimization of a Composition of Copper(II) Poly-L-Histidine Complex as an Electrocatalyst for O₂ Reduction by Scanning Electrochemical Microscopy

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We report a simple combinatorial approach of preparing mimics for enzymes with rapid screening as electrocatalysts for oxygen reduction by scanning electrochemical microscopy (SECM).¹ Poly-L-histidine (poly-his) was used as a matrix and ligand to complex Cu²⁺ to mimic the active sites of oxygen reductases. An array of Cu²⁺-poly-his spots with different compositions was deposited on a glassy carbon (GC) substrate by the dispensing technique described previously,² and their electrocatalytic activity for oxygen reduction was evaluated by SECM.

Electrodes modified with polymer-based films³ containing charge carriers (e.g., redox mediators attached to a polymer backbone) and enzymes (e.g., laccase) dispersed into a matrix permeable to reactants and electrolyte show excellent activity for oxygen reduction.⁴ Enzyme-modified electrodes have been extensively explored,^{5–7} and enzymes are used in many chemical processes. However, although they show outstanding activity and high specificity and selectivity, they have the drawback that they must work at near physiological conditions and are often expensive and unstable during extended usage. These limitations have led to extensive efforts to synthesize enzyme biomimetics, largely based on synthesizing compounds with a center that is like the three-dimensional catalytic active site. In most enzymes, the spatial arrangement of amino acids and the prosthetic group encompassing the active site determines the specificity of the enzyme for a reaction. The active sites of redox enzymes often contain metal ions, such as Cu²⁺, Fe²⁺, and Co²⁺, and there are numerous reports of the synthesis of artificial catalysts, such as for oxygen reduction.⁸ The active site of laccase contains four Cu ions coordinated with histidine and other amino acids.^{9,10} An alternative to the complex synthesis that often characterizes biomimetic chemistry is a rather simple, pseudo-evolutionary approach based on the preparation of small test samples of different composition by an automated dispensing technique and the rapid screening of candidate materials, such as by SECM. We use copper ion complexes with poly-his as a proof-of-the-concept test system.

To gain a better understanding of the Cu-poly-his complexes, we also studied the electrochemical (EC) behavior of the Cu²⁺-poly-his complex in both solution and as the complex-coated thin film on GC electrodes in aqueous solutions at various pHs. No previous investigations reported electrocatalytic properties of Cu²⁺-poly-his for oxygen reduction, although a number of studies discussed the structures and binding stability of copper poly-his complexes.^{11–13} Solution phase electrochemical studies, discussed in more detail in the Supporting Information, were carried out for mixtures of Cu²⁺ and poly-his in deaerated 0.5 M Na₂SO₄ at pH 5.8, with or without O₂ at a GC electrode. Cyclic voltammograms (CVs) (Figure S1, see Supporting Information) show the O₂ reduction peak at ~ -0.30 V (all potentials vs Ag/AgCl). In 1 mM Cu²⁺ solution, CV showed the reduction of Cu²⁺ to Cu⁰ with a cathodic peak potential at -0.1 V with stripping of the Cu⁰ on

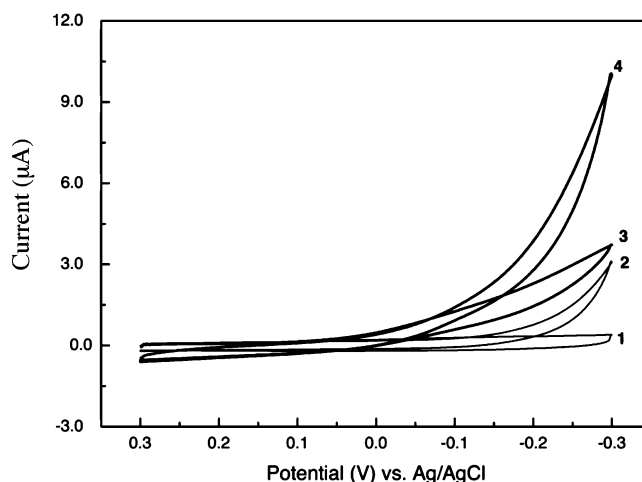


Figure 1. Cyclic voltammograms of Cu²⁺-poly-his-modified GC electrode in 0.2 M PBS (pH 7) compared with bare GC electrode in the absence or presence of O₂. The condition of modified films: 8 mM histidine residues, 2 mM Cu²⁺ at pH 5. Potential scan rate, $\nu = 50$ mV/s. Curve 1: bare GC, no O₂. Curve 2: bare GC, with O₂. Curve 3: modified GC, no O₂. Curve 4: modified GC, with O₂.

the reverse scan and with an anodic peak potential of 0.07 V. In the presence of poly-his at pH 4.5 (Figure S2, see Supporting Information), the magnitude of the Cu²⁺ reduction current decreased. When the concentration of histidine residues was 10 times that of Cu²⁺, the stripping reoxidation peak disappeared in the reverse scan, and a solution phase oxidation peak was observed at 0.41 V. In an O₂-saturated solution, the oxygen reduction commenced at -0.05 V in the solution containing Cu²⁺-poly-his complex; this is slightly more positive than that in the absence of complex (~ -0.13 V).

The electrocatalysis of O₂ reduction at Cu²⁺-poly-his-modified GC electrodes¹⁴ compared with bare GC electrodes is shown in Figure 1. Curves 3 and 4 show the CVs at a GC electrode coated with Cu²⁺-poly-his in PBS solution (pH 7) in the absence or presence of O₂, respectively. The reduction of Cu²⁺-poly-his coated on GC electrodes started at ~0.06 V in the absence of O₂. O₂ reduction on Cu²⁺-poly-his-modified GC electrodes (curve 4) started at ~ -0.07 V, which was more positive than that on the bare electrode (~ -0.13 V, curve 2). At a bias of -0.3 V, the current in the presence of O₂ at a Cu²⁺-poly-his-modified GC electrode was 2.3-fold higher than that at bare GC electrode. At a current density of ~40 $\mu\text{A}/\text{cm}^2$, the O₂ reduction on the Cu²⁺-poly-his-modified GC electrode occurred at a potential ~0.13 V more positive than that on the bare electrode (compare curves 2 and 4 in Figure 1). These results suggest an electrocatalytic process for oxygen reduction at Cu²⁺-poly-his complex-modified GC electrodes.

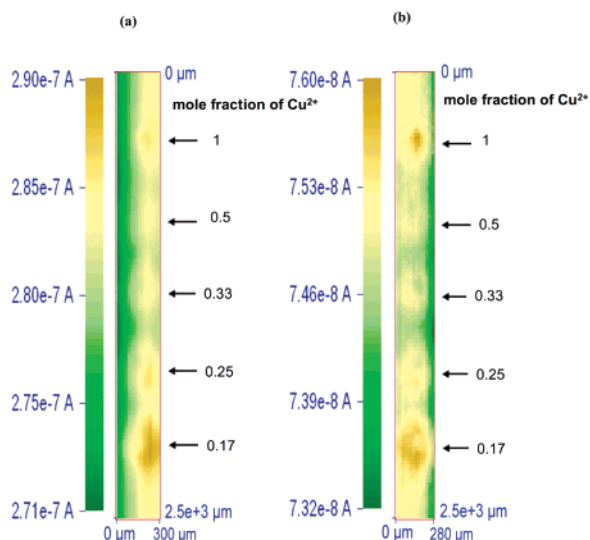


Figure 2. SECM images of Cu^{2+} -poly-his arrays containing spots with a different mole fraction of Cu^{2+} at pH 7 phosphate buffer, $i_T = 73$ nA (i_T : tip current), (a) $E_S = -0.3$ V vs Ag/AgCl; (b) $E_S = -0.2$ V vs Ag/AgCl. Tip raster rate = $100 \mu\text{m/s}$.

Arrays of Cu^{2+} -poly-his complex spots of various compositions were deposited on a GC plate ($10 \times 10 \times 1$ mm, Alfa) by using a piezo-based microarrayer.¹⁵ The tip generation–substrate collection mode of SECM was used to image activity of the arrays^{15,16} because of its high sensitivity (pA) and relative insensitivity to changes of the tip–substrate distance.¹⁶ SECM images of the activity for oxygen reduction of Cu^{2+} -poly-his spots were obtained in 0.2 M phosphate buffer at pH 7 (Figure 2). A $25 \mu\text{m}$ diameter Pt tip positioned $40 \mu\text{m}$ away from the GC surface was scanned in the xy plane (parallel to spot rows) at $100 \mu\text{m/s}$, while O_2 was electrogenerated from H_2O at a constant tip current. The substrate array potential (E_S) was held at -0.2 and -0.3 V. The substrate current (i_S), measured as a function of the tip position to produce the SECM image, was higher when the O_2 -generating tip scanned over a more active spot. Thus, the magnitude of the electrocatalytic activity of any of the spots is reflected in the magnitude of i_S .

For the arrays shown in Figure 2, O_2 electroreduction was seen for mole fractions of Cu^{2+} above 0.17. Spots containing poly-his alone washed away before imaging, however, the Cu^{2+} -poly-his spots adhered well enough for several imaging scans. The spots containing only Cu^{2+} also catalyzed oxygen reduction. This suggests that Cu^0 was formed during the heat treatment for evaporation of glycerol. For the arrays studied, the highest efficiency and stability of the complex for oxygen reduction reaction was found at a Cu^{2+} mole fraction in the range of 0.17–0.35, suggesting that the stoichiometry of the complex and the geometrical arrangement of Cu^{2+} in the poly-his may play a role in the electrocatalytic process. However, these complexes still show poor activity. The collection

efficiency of the complexes for oxygen is still low; only less than 20% of oxygen produced by the tip was reduced on an active spot. More than 20 different arrays were prepared and scanned, and 85% of these showed catalytic activity (see Supporting Information.)

While the results here represent proof of principle, the performance of the best material is still inferior to that of laccase. However, these represent only preliminary studies, and different polypeptides and their mixtures and different preparation conditions will be investigated.

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Supporting Information Available: Additional figures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (14) Detailed procedure for the preparation of the Cu-poly-his-modified glassy carbon electrodes in Supporting Information.
- (15) Experimental details in Supporting Information. Briefly, two different solutions containing 10 mM poly-L-histidine and 10 mM $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, respectively, were first prepared; 5% v/v glycerol was added to each solution to avoid premature evaporation of the spots. The dispenser was filled with poly-L-histidine solution ($3 \mu\text{L}$), which was dispensed in a programmed number of drops at each site. Each composition was prepared repeatedly to test the reproducibility. Thus, the arrays contained 5 rows and 5 columns of spots. After dispensing the poly-L-histidine spots, the dispenser was emptied, thoroughly rinsed first with ethanol and then with Milli-Q water, and refilled with $3 \mu\text{L}$ of Cu^{2+} solution. The platter positioned the dispenser exactly over the previously prepared poly-L-histidine spots, and a number of drops of Cu^{2+} solution dispensed with the total sum of drops were always equal to 12. The spots of the array were then agitated for 5 min in a Vortex Genie 2 agitator (Fisher). The array was heated in the oven at 100°C overnight to dry the spots. Spots were 150 – $300 \mu\text{m}$ diameter and spaced $400 \mu\text{m}$ apart. The area of the 5×5 array was $2.5 \text{ mm} \times 2.5 \text{ mm}$, and 50 min was required to scan the complete array at a tip raster rate of $100 \mu\text{m/s}$.
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