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Electrochemical sensing devices using ATCUN-Cu(II) complexes as electrocatalysts for water oxidation

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ABSTRACT

This work demonstrated that electrochemical sensing devices can be fabricated by electrocatalytic water oxidation. First, we suggested that the Cu(II) complexes with amino terminal Cu(II)- and Ni(II)-binding (ATCUN) peptides showed good electrocatalytic ability toward water oxidation. In contrast to the previously reported electrocatalysts for water oxidation, the ATCUN-Cu(II) electrocatalysts can operate at neural pH with lower oxidation potential. Furthermore, the ATCUN-Cu(II) metallopeptides can be easily modified onto the surface of nanomaterials to produce nanocatalysts for water oxidation. To demonstrate the analytical performances of the ATCUN-Cu(II) electrocatalysts as electrochemical signal labels, DNA analysis was performed in a sandwich option using gold nanoparticles (AuNPs) as the carriers. The method detected DNA down to 0.1 pM. This is the first time that H_2O molecule was employed as the electrocatalytic substrate in electrochemical sensing devices.

1. Introduction

To improve the detection sensitivity of electrochemical sensors, considerable attention has been devoted to the integration of recognition elements with electronic elements for the preparation of detection/signal probes [1,2]. Enzymatic signal amplification with horseradish peroxidase (HRP), alkaline phosphatase (ALP), or glucose oxidase (GOx) is one of the most commonly used strategies for electrochemical sensing [3]. In particular, electrochemical devices with multiple signal amplification steps, such as enzyme-loaded nanomaterials and enzymatic reaction plus redox cycling, can greatly improve the sensitivity [1–3]. Versus natural enzymes, mimetic oxidoreductases for electrochemical sensing offer easy production, low cost, and high stability [3]. Typical examples include metal-dependent DNAzymes, metal nanocatalysts, and mimetic molecules loaded onto metal-organic frameworks (MOFs) [4–11]. However, the practical applications of both enzymes and mimetic catalysts still remain limited in electrochemical sensing devices because of their costly and complicated preparation processes, cross-talk interference, and/or requirements for deoxygenation to exclude interference from dissolved oxygen [12,13]. Moreover, the catalytic amplification strategies usually require the addition of extra substrates (e.g., glucose and hydrogen peroxide) and favor the use of redox mediators to facilitate electron transfer. Water is the reaction medium for most of electrochemical sensors. However, to the best of our knowledge, there is no report of electrochemical sensing device with H_2O molecule as the electrocatalytic substrate.

For electrocatalytic water oxidation, metal complexes and noble and earth-abundant metals have attracted significant interest as catalysts. In particular, copper-based water oxidation catalysts are attractive because of the high abundance and low cost of copper, including copper-bipy complex, copper-carbonate complex and copper-triglycylglycine [14–23]. These catalysts exhibit high current density and good stability for water oxidation. However, the catalysts require high pH for Cu(II) binding and/or operate at high oxidation potential for electrocatalytic oxidation of water. This limits their practical utility for developing electrochemical sensing devices because the high oxidation potential will bring high background current and a high pH environment will hamper the probe/target interactions. Thus, for developing electrochemical sensing devices with H_2O molecule as the substrate, the imperative

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work is to find a water oxidation electrocatalyst that can operate at low oxidation potential and neutral pH.

Amino terminal Cu(II)- and Ni(II)-binding (ATCUN) motif is present in a protein or peptide, which coordinates to Cu(II) with picomolar affinity [24–27]. The motif has a structural characterization comprising of a free NH_2-terminus, a histidine residue in the third position from the N-terminus, and two intervening peptide nitrogens (denoted as NH_2-X-X-His sequence). In the present work, we studied the redox properties of different ATCUN-Cu(II) metallopeptides and found that they showed similar attributes as the Cu(II)-based electrocatalysts toward water oxidation [14–21]. However, in contrast to the previously reported electrocatalysts, the ATCUN-Cu(II) metallopeptides exhibit high stability in neutral pH environment and can operate at low oxidation potential. Moreover, the metallopeptides can be readily attached to the surface of nanomaterials. This affords an efficient signal transduction platform for electrochemical sensing devices. To demonstrate the analytical performance of the mimetic catalysts for electrochemical sensing, DNA analysis was performed in sandwich mode with gold nanoparticles (AuNPs) as the carriers to detection probes and ATCUN-Cu(II) metallopeptides.

2. Experimental

2.1. Chemicals and reagents

All peptides were synthesized and purified by ChinaPeptides Co., Ltd. (Shanghai, China). The DNA strands were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). Their sequences are presented in Supplementary Material (Table S1). Tris(carboxyethyl)phosphine (TCEP), tris-(hydroxymethyl)aminomethane hydrochloride (Tris–HCl), 6-mercapto-1-hexanol (MCH), KH_2PO_4 and K_2HPO_4 were purchased from Sigma-Aldrich (Shanghai, China). Other reagents were of analytical grade and obtained from the Aladdin Reagent Company (Shanghai, China). All solutions were prepared with deionized water treated with a Milli-Q purification system.

2.2. Voltammetric characterization of peptide-Cu(II) complexes

The cyclic voltammograms (CVs) of peptide-Cu(II) complexes were collected using a CHI 660E electrochemical workstation (CH instrument, Shanghai, China) in 0.2 M phosphate-buffered saline solution (PBS, pH 7.4). The reference, counter and working electrodes are a Ag/AgCl, a platinum foil and a glassy carbon electrode, respectively. The glassy carbon electrode was polished with 0.05 μm alumina powder on wet cloth and then sonicated in 50% ethanol for 30 s. Unless otherwise specified, the concentration ratio of peptide to Cu(II) kept at 1.5:1 to avoid interference from free Cu(II).

2.3. Catalytic oxidation of water by $KMnO_4$

0.5 mL of 1 mM $KMnO_4$ solution in PBS was mixed with 0.5 mL of 20 μM free Cu(II), peptide or ATCUN-Cu(II) (1:1). The mixed solution was irradiated with 100 W lamp for 30 min. The color change was observed by naked eyes. The photographic pictures were taken by camera on a mobile phone.

2.4. Preparation of AuNPs/DNA/DCH-Cu(II)

The 13 nm AuNPs were prepared with the trisodium citrate reduction method. The total AuNPs concentration was determined to be 11.2 nM with a molar absorptivity of $2.7 \times 10^8\ M^{-1}\ cm^{-1}$ at 520 nm. The DNA and peptide molecules were attached onto the AuNPs surface through a ligand-exchange reaction between thiolated DNA as well as peptide and citrate-stabilized AuNPs. To evaluate the immobilization ability of AuNPs for DCH peptide, 0.5 mL of the prepared AuNPs were mixed with 0.5 mL of 10 μM DCH solution in the presence of 100 μM TCEP for 12 h and then centrifuged at 13,000 r/min for 5 min. The free peptide in the supernatant solution was measured by mass spectrometry. The average number of peptide per gold nanoparticle was calculated to be 782 ± 37. The preparation of DNA/DCH-modified AuNPs was performed by mixing 0.5 mL of the prepared AuNPs suspension with 0.5 mL of 5 mM PBS (pH 7.4) containing a given concentration of detection probe DNA and 500 μM TCEP for 30 min. This step was followed by adding 0.5 mL of 10 μM DCH peptide solution to the mixture for 12 h incubation. The resultant suspension was centrifuged and washed twice with the PBS buffer to remove excess DNA and peptide. We also optimized the concentration ratio of detection probe DNA to DCH used for the nanocatalysts preparation. The current was initially intensified by increasing the DNA/DCH concentration and then decreased beyond 1:250. Thus, the DNA/DCH concentration ratio kept at 1:250 for the preparation of the nanocatalysts. For the preparation of AuNPs/DNA/DCH-Cu(II) nanocatalysts, 1.5 mL of PBS containing 3 μM Cu(II) was added to disperse the DNA/DCH-modified AuNPs suspension. The resultant AuNPs/DNA/DCH-Cu(II) nanocatalysts were characterized by a Cary 60 spectrophotometer and a FEI Tecnai G2 T20 transmission electron microscope (TEM) (Hillsboro, OR, USA). Before use, the nanocatalysts was diluted to 2 nM (equivalent AuNPs) with the PBS buffer.

2.5. DNA detection

The DNA self-assembly monolayers (SAMs)-covered electrode with medium surface density was prepared following the reported protocol [28]. Briefly, the gold electrode was polished with 0.05 μm alumina, cleaned with piranha solution (H_2O_2/H_2SO_4 (v/v) = 1/2) for 5 min and rinsed with water. Then, the clean electrode was immersed in 10 mM Tris–HCl buffer (pH 7.4) containing 2 μM capture probe DNA, 1 mM EDTA, 10 mM TCEP and 0.1 M NaCl for 1 h. To block the unreacted gold surface and facilitate the hybridization, the DNA SAMs-covered electrode was soaked with 1 mM MCH for 2 h. For DNA analysis, 25 μL of PBS buffer containing a given concentration of target DNA and 0.1 M NaCl was mixed with 25 μL of the prepared AuNPs/DNA/DCH-Cu(II) suspension for 10 min. Then, the sensing electrode was incubated with the mixed solution for 1 h. After hybridization, the electrode was washed with water and then placed in 0.2 M PBS (pH 7.4) for voltammetric measurement.

3. Results and discussion

The Cu(II) center in the reported Cu(II)-N_4 water oxidation electrocatalysts adopts a square-planar or square-based structure with the four N donors of ligands including triglycylglycine (Fig. 1A) [16–18,21]. Note that Cu(II) is also coordinated to ATCUN peptide in a square plannar configuration with a 1:1 binding mode [25]. For this view, we investigated the redox properties of different ATCUN-Cu(II) metallopeptides, including neutral as well as positively or negatively charged tripeptides (GGH, KGH, RTH, DAH), tetrapeptides (GGHG, KGHG, RTHD, DAHF), and C-terminally aminated tripeptides (GGH-NH_2, KGH-NH_2) (Figs. 1B–D and S1–S5). Representative voltammetric responses of Cu(II) complexes with GGH, GGHG, and GGH-NH_2 are shown in Fig. 1B. For GGH-Cu(II), an irreversible CV wave with an oxidation potential of 0.83 V was observed at a scan rate of 100 mV/s (curve 1 in Fig. 1B) or higher (Fig. 1C). The irreversible CV wave has a similar characteristic to the Cu(II)-based electrocatalysts toward water oxidation [14–21,29–31]. This is indicative of a catalytic process in which the Cu(II) is generated