Colorimetric and electrochemical dual-mode uric acid determination utilizing peroxidase mimics activity of CoCu bimetallic nanoclusters

Yaopeng Liu[a,b,c], Wei Zhao[d,e],Yi Gao[a,b,c], Qing Zhuo[d,e], Tingting Chu[a,b,c], Chengyu Zhou[a,b,c], Wensheng Huang[a,b,c], Yin Zheng[*a,b,c] and Yingru Li[* d,e]

*Corresponding author

E-mail address: zhengyin0617@163.com; Liyingru@outlook.com

2. Experimental section

2.1. Materials and instruments

Uric acid (UA), 3,3′,5,5′-tetramethylbenzidine (TMB), 5,5-Dimethyl-1-pyrroline N-oxide (DMPO), L-Glycine (Gly) and other amino acid were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. (Shanghai, China). Cobalt acetate tetrahydrate ($C_4H_6CoO_4·4H_2O$), Copper acetate monohydrate ($C_4H_6CuO_4·H_2O$), 1,3,5-Benzenetricarboxylic acid (BTC), terephthalic acid (TA), ethylene glycol, N, N-dimethylformamide (DMF), hydrogen peroxide (30% H_2O_2), sodium acetate (NaAc), acetic acid (HAc), potassium phosphate monobasic (KH_2PO_4) and sodium phosphate dibasic (Na_2HPO_4) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ultrapure water was used as experimental water throughout the experiment.

Ultraviolet spectra were performed by a Shimadzu UV-2550 UV-Visible spectrophotometer (Shimadzu Co., Kyoto, Japan). Electrochemical experiments were performed using a CHI-660E electrochemical workstation (Chenhua Apparatus Co., Shanghai, China). Fluorescence spectra were recorded on an RP-5301PC fluorescence spectrophotometer (Shimadzu Co., Kyoto, Japan). The scanning electron microscope (SEM) and transmission electron microscopy (TEM) images were obtained using a scanning electron microscope (Quanta FEG 250 and FEI Tecnai F20, FEI, USA). The material was confirmed using AVATAR 370 Fourier Transform Infrared Spectrometer (Thermo-Nicolet, USA). The crystal structures of the samples were characterized by X-ray diffraction (XRD-7000, Shimadzu, Japan). X-ray Photoelectron Spectroscopy (XPS) was recorded by Kratos AXIS Supra spectrometer (Ultima IV, Rigaku Corporation, Japan). N_2 sorption analysis was recorded on the AUTOSORB IQ porosimeter (Quantachrome, America).

2.2. Synthesis of Co@Cu-BNCs

Firstly, $C_4H_6CoO_4·4H_2O$ (1.0 g 4.0 mmol), $C_4H_6CuO_4·H_2O$ (1.0 g 5.0 mmol), BTC (3.0 g 14.3 mmol) were dissolved into 30 mL DMF, and then the obtained solution was transferred into 30 mL ethylene glycol to form a mixed solution. After vigorously stirring the solution for 0.5 hours, it was transferred to a high-pressure reactor. The reactor was heated to 160 °C in an oven, and held at this temperature for 10 hours. After being naturally cooled to room temperature, the Co@Cu-BNC precursor was obtained. After washing with ethanol and deionized water, drying under vacuum at 70 °C, a slightly light green powders were produced. Then the precursor was heated to 700 °C at 5 °C min^{-1} under nitrogen atmosphere in a tube furnace and maintained for 2 hours, and CoCu Bimetal Nanoclusters (Co@Cu-BNCs) was obtained.

2.3. Fabrication of the enzymatic-like Modified Electrode

The glassy carbon electrode (GCE) was polished on a polishing cloth containing 0.05 μm polishing powder (Al_2O_3) and then ultrasonicated in anhydrous ethanol and ultrapure water for 3 minutes, respectively. 5 mg Co@Cu-BNCs material in 0.5 mL DMF was ultrasonicated for 2 hours to make the

dispersive solution, and then 3 μL of the dispersion it was added to the surface of the GCE by a pipette. Finally, the decorated GCE was vacuum dried at 90 °C, named as Co@Cu-BNCs/GCE.

2.4. Peroxidase mimics activity of Co@Cu-BNCs

By examining the catalytic activity of the Co@Cu-BNCs at various temperatures (20 – 50 °C) and pH (3.0 – 8.0), the optimum reaction condition for the colorimetric system was determined. The catalytic activity was calculated based on $(A_s/A_m)\times100$, where A_s is the absorbance value of each reaction system after stabilization and A_m is the maximum absorbance value of all the reaction systems studied.

To study the peroxidase-like activity of Co@Cu-BNCs, TMB was chosen as the chromogenic substrate. Typically, 30 μL of Co@Cu-BNCs solution (1.0 mg mL^{-1}) was added into 1.97 mL of NaAc-HAc buffer solutions (0.2 M, pH=4.5) containing TMB (10 mM) and H_2O_2 (125 mM) in a colorimetric cylinder. The UV-Visible spectrum was obtained after reaction at 30 ℃ for 60 minutes.

2.5. Steady-state kinetic Study for peroxidase mimics

To determine the affinity between the Co@Cu-BNCs and H_2O_2 (or TMB), a series of substrate concentrations of H_2O_2 (or TMB) were added into NaAc-HAc buffer solutions (1.9 mL, 0.2 M, pH=4.5) containing Co@Cu-BNCs (15 μg mL^{-1}). In the TMB+H_2O_2+Co@Cu-BNCs system, steady-state kinetic experiments were implemented under the optimum reaction conditions by altering the TMB or H_2O_2 concentrations. On a UV-Visible spectrophotometer, kinetic tests were conducted in time course mode to record the change in absorbance at 652 nm. According to the Michaelis-Menten equation[1], $\nu=V_{max}[S]/(K_m+[S])$, the kinetic constants were calculated, where ν is the initial rate, [S] is the substrate concentration, V_{max} is the maximum velocity, and K_m is the Michaelis constant.

2.6. Catalytic mechanism for peroxidase mimics activity.

In order to explore the reactive oxygen species (ROS) in this system, some radical scavengers were used. For example, thiourea (·OH scavenger), NaN_3 ($^1O_2$ scavenger) and p-benzoquinone ($O_2^{\cdot-}$ scavenger) were added respectively to the TMB+H_2O_2+Co@Cu-BNCs system. The concentrations of thiourea, NaN_3 and p-benzoquinone were 0.25 mM or 0.5 mM. In addition, the determination of ·OH was based on the reaction of TA and OH. TA is capable of capturing ·OH and generating 2-hydroxy terephthalic acid with unique fluorescence around 435 nm. The concentrations of TA, H_2O_2 and nanozymes were 0.6 mM, 2.5 mM and 15 μg mL^{-1}, respectively. Then, the fluorescence spectra of the different system were evaluated (λ_{ex}=315 nm, λ_{em}=435 nm). By using the DMPO (80 mM) as spin trapping agent, the generation of the·OH radical was further confirmed by the electron spin resonance (ESR) experiment.

2.7. Dual-mode sensing platform for UA detection

For UA detection, 100 μL of UA with different concentration, 40 μL of H_2O_2 (125 mM) with various concentration, 30 μL of TMB (10 mM) and 30 μL of Co@Cu-BNCs (1.0 mg mL^{-1}) were added to NaAc-HAc buffer solutions (1.8 mL, 0.2 M, pH=4.5) in sequence. Then, the mixed solution was kept at 30 ℃ for 15 minutes and successively the UV-Visible spectrum of the mixture was recorded.

All electrochemical detections were performed in a typical three-electrode system at room temperature, using a bare GCE or Co@Cu-BNCs/GCE as working electrodes, a platinum electrode as the auxiliary electrode, and a saturated calomel electrode ($Hg/KCl/Hg_2Cl_2$) as the reference electrode. The concentration of UA in a phosphate-buffered solution (PBS, 0.1 M, pH=6.0) was determined by differential pulse voltammetry (DPV).

2.8. UA assay in human serum samples

The serum was collected from a group of volunteers from the Affiliated Hospital of Hubei Minzu University. The standard additive method was employed to show the practical applicability[2]. After diluting the serum with ultra-pure water to a final dilution of 200 times, the serum sample was added with UA standard solution at varying concentrations. Then, UA was detected with the same procedures described above.

References

1. J. Chen, F. Xu, Q. Zhang and S. Li, *Anal Chim Acta*, 2021, **1180**, 338740.

2. Z. Ma, L. Yang, Y. Wang, M. Wang, W. Qi and Z. He, *Chemical Engineering Journal*, 2021, **416**.