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2D transition metal carbide MXene as a robust biosensing platform for enzyme immobilization and ultrasensitive detection of phenol

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ABSTRACT

MXene-Ti_3C_2, as a new class of two-dimensional (2D) transition metal carbides (or nitrides), has been synthesized by exfoliating pristine Ti_3AlC_2 phases with hydrofluoric acid. The SEM and XRD images show that the resultant MXene possesses a graphene-like 2D nanostructure. and the surface of MXene has been partially terminated with -OH, thus providing a favorable microenvironment for enzyme immobilization and retaining their bioactivity and stability. Considering the unique metallic conductivity, biocompatibility and good dispersion in aqueous phase, the as-prepared MXene was explored as a new matrix to immobilize tyrosinase (a model enzyme) for fabricating a mediator-free biosensor for ultrasensitive and rapid detection of phenol. The varying electrochemical measurements were used to investigate the electrochemical performance of MXene-based tyrosinase biosensors. The results revealed that the direct electron transfer between tyrosinase and electrode could be easily achieved via a surface-controlled electrochemical process. The fabricated MXene-based tyrosinase biosensors exhibited good analytical performance over a wide linear range from 0.05 to 15.5 μmol L^{-1}, with a low detection limit of 12 nmol L^{-1} and a sensitivity of 414.4 mA M^{-1}. The proposed biosensing approach also demonstrated good repeatability, reproducibility, long-term stability and high recovery for phenol detection in real water samples. With those excellent performances, MXene with graphene-like structure is proved to be a robust and versatile electrochemical biosensing platform for enzyme-based biosensors and biocatalysis, and has wide potential applications in biomedical detection and environmental analysis.

1. Introduction

With the growing interest in low dimensional nanomaterials, two-dimensional (2D) transition metal carbides or nitrides called MXenes have attracted extensive attention due to their unique morphology and properties. The 2D layered MXenes are synthesized by selectively removing "A" layers from bulk $M_{n+1}AX_n$ phases (where M is an early transition metal, A is an A-group mostly from groups 13 and 14 of a periodic table, and X is C and/or N, n = 1–3) (Barsoum, 2000), resulting in the chemical formula of $M_{n+1}X_n$. Because the metallic nature of the M-A bond is weaker than the M-X bond, the etching procedure can be successfully achieved (Meshkian et al., 2015).

Ti_3AlC_2 is one of the 70-plus group of ternary carbides and nitrides ($M_{n+1}AX_n$), and the MXene-Ti_3C_2 can be obtained by exfoliating Ti_3AlC_2 with hydrofluoric acid (Naguib et al., 2011). Due to the unsaturated surface with unpaired electrons, the surfaces of MXene-Ti_3C_2 are easily terminated with various functional groups (e.g., -O, -OH or/and -F group) during the etching procedure without changing the metallic conductivity (Khazaei et al., 2013; Zhang and Dong, 2017). The formation of strong bonds between the Ti and the attached groups make the surface-functionalized Ti_3C_2 more stable. The mechanical flexibility of O-functionalized Ti_3C_2 improves a lot because of the significant charge transfer from the inner Ti-C bonds to the outer Ti-O surface (Fu et al., 2016; Guo et al., 2015). When the surface of MXene-Ti_3C_2 adsorbs the -OH group, the interlayer coupling in the OH-terminated Ti_3C_2 is stronger than that in the F- or O-terminated one attributing to the formation of hydrogen bonds between the layers in the former (Khazaei et al., 2017). With the intriguing physical and chemical properties, such as graphene-like structure, large electrochemically active surface, metallic conductivity, high stability, excellent

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mechanical properties, especially good dispersion in aqueous solution (Eames and Islam, 2014; Naguib et al., 2011, 2014), MXene has been widely used in metal (Li, Na, K, Ca) ion batteries (Dong et al., 2017; Er et al., 2014; Mashtalir et al., 2013), supercapacitors (Lukatskaya et al., 2013; Zha et al., 2016; Zhang and Dong, 2017), fuel cells (Xie et al., 2013), absorbents (Mashtalir et al., 2014; Peng et al., 2014), electronic devices (Liu et al., 2015; Lorencova et al., 2017; Rakhi et al., 2016; Wang et al., 2014, 2015).

Enzyme biosensors, as alternative analytical tools, have been extensively used for on-site monitoring environmental pollutants for the advantages of simplified operation, cost-efficient, fast response, inexpensive instrument and minimal requirement for sample pretreatment. The biosensing materials play an important role for the development of enzyme biosensor (Wu et al., 2012a). To date, carbon-based nanomaterials, such as graphene, carbon nanotube and mesoporous carbon, have been widely used as biosensing materials for enzyme immobilization and biosensor fabrication, due to their large specific surface areas, good biocompatibility and excellent electrical conductivity. However, these nanocarbons always have hydrophobic nature, which is difficult to disperse uniformly in water phase. As is well known, the uniform dispersion of nanomaterials in water solution is crucial to advance their applications in enzyme-based biosensors. In order to alter the hydrophobic surface, these nanocarbons were functionalized with hydroxyl or carboxylic groups (Malig et al., 2012; Si and Samulski, 2008; Xu et al., 2008) and amphiphilic polymers or surfactants (Lotya et al., 2009). Although, the solubility of the nanomaterials were improved, it is inevitable to decrease their electrical conductivity and introduce other materials to the biosensing platform. In our previous study, room temperature ionic liquids (RTILs) were used to alter the solubility of graphene (Lu et al., 2014) and mesoporous carbon (Wu et al., 2012b). The hydrophobic nanomaterials become water-soluble and remain the excellent electrical conductivity, but the high-purity RTILs are very expensive and the preparation process of RTIL-carbon nanocomposite is highly complicated yet time-consuming. Therefore, MXene with hydrophylic surface and excellent metallic conductivity could be a good candidate as an excellent immobilization matrix for fabricating electrochemical biosensors. Up to date, MXene-Ti_3C_2 either in pristine form or combined with TiO_2 nanoparticles has been used to immobilize Hemoglobin for the detection of H_2O_2 or $NaNO_2$ (Liu et al., 2015; Wang et al., 2014, 2015), displaying good performances. To the best of our knowledge, the studies about MXenes for enzyme-based biosensors are very limited (Rakhi et al., 2016). Considering the unique structure and excellent properties of MXenes, more systematic studies about MXenes for enzyme immobilization and biosensor fabrication should be carried out.

In this study, MXene-Ti_3C_2 has been synthesized by exfoliating pristine Ti_3AlC_2 phases with hydrofluoric acid. The resultant MXene possesses a graphene-like 2D nanostructure and the conductivity are comparable with those of multilayer graphenes (Naguib et al., 2014). More importantly, the surfaces of MXene have been partially terminated with -OH, which could provide an aqueous-like biocompatible microenvironment for the immobilized enzyme molecules and retaining their bioactivity and stability (Das and Prabhu, 1990; Lu et al., 2006). Considering the unique metallic conductivity, biocompatibility and good dispersion in aqueous phase, the as-prepared MXene-Ti_3C_2 was explored as a matrix to immobilize tyrosinase (Tyr, a model enzyme) for fabricating a mediator-free biosensor. The resulting MXene-Ti_3C_2 based biosensor exhibited excellent analytical performances with high sensitivity, fast response and low detection limit for determination of phenol. The proposed biosensing method also demonstrated good repeatability, reproducibility, long-term stability and high recovery for phenol detection in water samples. The MXene-Ti_3C_2 with graphene-like structure is evidenced to be a robust electrochemical biosensing platform for enzyme-based biosensors, and has wide potential applications in biomedical detection and environmental analysis.

2. Materials and methods

2.1. Materials

Chitosan (Chi, from shrimp shells, ≥ 75% deacetylated) and tyrosinase (from mushroom, > 1000 units mg^{-1}) were purchased from Sigma (USA). Phenol was purchased from J&K Chemical Ltd. (Beijing, China). 50 mmol L^{-1} phosphate buffer solution (PBS, pH 6.0) were prepared by mixing standard solutions of Na_2HPO_4 and NaH_2PO_4. Unless otherwise stated, PBS (50 mmol L^{-1}, pH 6.0) was deoxygenated and used as the electrolyte in electrochemical experiments. Milli-Q water (18 MΩ cm) was used throughout all experiments.

2.2. Apparatus and methods

Scanning electron microscopy (SEM) images were obtained with a field emission scanning electron microscopy JSM-7800F (JEOL, Japan). The powder X-ray diffraction (XRD) patterns were obtained on a X′pert Pro X-ray diffractometer (PANalytical, Holland) with Cu $K_{\alpha 1}$ radiation (λ = 1.5406 Å) over the 2θ range of 5 − 80°. The scan step-width was set to 0.033° and the scan rate was 0.1° s^{-1} at room temperature. Fourier transform infrared (FTIR) spectra were obtained by using a Spectrum GX apparatus (Perkin-Elmer Company, USA).

Electrochemical impedance spectroscopy (EIS) measurements were carried out with a Metrohm Autolab PGSTAT 302 N Potentioatat/Galvanostat (Eco Chemie, The Netherlands) in a 1 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.5 mol L^{-1} KNO_3, with a scan frequency range from 1 × 10^4 to 1 × 10^{-1} Hz and an amplitude of 10 mV. Cyclic voltammetry (CV) and current-time (I-t) measurements were carried out on glassy carbon electrodes (GC, 3 mm diameter) by using a CHI 440B electrochemical workstation (CHI Instruments Inc., USA). The measurements were based on a three-electrode system consisting of the modified GC electrode as the working electrode, an Ag/AgCl electrode (KCl concentration of 3 mol L^{-1}) as the reference electrode, and a platinum wire as the auxiliary electrode.

2.3. Preparation of MXene

Firstly, the pristine Ti_3AlC_2 was prepared by the solid-liquid reaction (Hu et al., 2016). Briefly, the elemental powders of Ti, Al and graphite in a molar ratio of 3: 1.1: 1.88 were mixed with agate balls and then heated to 1550 °C for 2 h in a following argon atmosphere. Then, MXene-Ti_3C_2 was synthesized by exfoliating the pristine Ti_3AlC_2 phases with hydrofluoric acid (HF) (Naguib et al., 2012, 2011). Typically, 1.0 g Ti_3AlC_2 powder was slowly added to 120 mL 40 wt% hydrofluoric acid solution. The reaction mixture was stirred at 300 rpm for 72 h at 25 °C. After that, the mixed solution was centrifuged at 6000 rpm for 5 min, and the powder was collected after discarding the supernatant. Then, the resulting powder was washed with distilled water repeatedly four times. Finally, Ti_3C_2 was collected by filtering the solution using a polytetrafluoroethylene membrane (0.22 mm pore size) and dried in vacuum oven at 60 °C for 12 h.

2.4. Construction of MXene based tyrosinase biosensor

The MXene based tyrosinase biosensor was prepared by a simple casting method. Prior to modification, a GC electrode with 3 mm diameter was polished on a polishing cloth with 1.0, 0.3 and 0.05 μm alumina powder successively, and washed with Milli-Q water followed by sonicating in ethanol and Milli-Q water. Then the electrode was dried with purified nitrogen stream. With the optimization of the experimental conditions, the final compositions containing tyrosinase, MXene, and chitosan were 2.5 mg mL^{-1}, 0.4 mg mL^{-1} and 1.5 mg mL^{-1}, respectively. The preparation process of biosensor was as follows: Firstly, 10 μL tyrosinase solution (10 mg mL^{-1}, dissolved in PBS) was added into 20 μL MXene suspension (0.8 mg mL^{-1}, dispersed