Water dispersed two-dimensional ultrathin Fe(III)-modified covalent triazine framework nanosheets: Peroxidase-like activity discovery and for colorimetric biosensing platform

Lining Su, Zhi Zhang, and Yuhao Xiong

a. College of Food and Bioengineering, Hezhou University, Hezhou 542899, P. R. China; b. Institute of Food science and Engineering Technology, Hezhou university, Hezhou 542899, P. R. China.

Email: xiongyuhao@yeah.net

Experimental

Materials and chemicals

1,4-dicyanobenzene (DCB) was purchased from J&K Chemical Technology (Beijing, China). Sarcosine was purchased from Beijing HWRK Chemical Corporation (Beijing, China). Sarcosine oxidase (15 U/mg) was supplied by Sigma-Aldrich (St. Louis, Mo, USA). Anhydrous zinc chloride (ZnCl₂), NaF (99.93%), and ochratoxin A (OTA) were purchased from Aladdin Reagents Corporation (Shanghai, China). 3,3′,5,5′-Tetramethylbenzidine (TMB) was purchased from TCI (Shanghai, China). The oligonucleotide was supplied by Sangon Biotech Co., Ltd. (Shanghai, China). The sequence of OTA and the FAM-modified aptamer were GAT CGG GTG TGG GTG GCG TAA AGG GAG CAT CGG ACA and 5-TCA ACA TCA GTC TGA TAA GCT AGG GAT CTC-Cy5-3′, respectively. Other analytical grade reagents were obtained from Sinopharm (Shanghai, China). Ultrapure water (18.2 M·cm) was prepared with an Ulupure purification system (Sichuan, China) and was used to prepare all aqueous solutions.

Synthesis of 2D CTF

The CTF material was synthesized using a microwave-enhanced high-temperature ionothermal method; the detailed synthetic process is described in our previous publication. The 2D CTF was synthesized as follow: 40.0 mg of CTF material and 4.0 mL of concentrated sulfuric acid (98%) were mixed well in a beaker and incubated for
5 min in a 110 °C oil bath. Subsequently, 2.0 mL of 30% H₂O₂ solution was added dropwise to the mixture and incubated for another 8 min. The mixture was allowed to cool down to room temperature, and 10 mL ultrapure water was added to the obtained dark yellow mixed solution. Then, the yellow dispersion was centrifuged at 8000 rpm for 10 min, and the supernatant solution was collected. The obtained supernatant was further centrifuged at 12000 rpm for 30 min. The precipitation was collected and washed with ultrapure water until the pH of the precipitant reached ~7.0. Finally, the precipitation was dispersed in ultrapure water to form a yellow homogeneous 2D CTF solution.

**Synthesis of 2D Fe-CTF**

The 2D Fe-CTF was prepared by impregnation modification. Briefly, 50 μL of 1.0 mM FeCl₃ solution was added to 1.0 mL of the homogeneous 2D CTF solution and incubated overnight at room temperature. Then, the obtained 2D Fe-CTF was washed five times by centrifugation with ultrapure water. Finally, the product was dispersed in ultrapure water.

**Characterization**

Fourier transform infrared (FT-IR) spectra were recorded with a PE Spectrum One FT-IR spectrometer (Perkin-Elmer, USA) by KBr powder-pressed pellets method. An S4800 field-emission scanning electron microscopy (FE-SEM, Hitachi, Japan) equipped with an energy dispersive spectroscopy (EDS) detector was used for elemental mapping. Transmission electron microscopy (TEM) images were gathered with an FEI Tecnai G2 F20 electron microscope (FEI, USA). Atomic force microscopy (AFM) was performed using a Nanoscope V Multimode 8 scanning probe microscope in ambient conditions (Bruker, Germany). X-ray photoelectron spectroscopy (XPS) analysis was conducted with a Thermo ESCALAB 250XI electron spectrometer (Thermo, USA). Raman spectral measurements were gathered with a DXR Raman spectrometer equipped with a standard 632.8 nm HeNe 10 mW laser and a CCD detector (Thermo Fisher, USA). Fluorometric measurements were performed using a LS-55 luminescence spectrometer (Perkin-Elmer, USA). Electrochemical measurements were carried out using a CHI660c electrochemical workstation.
(Shanghai, China). All UV-Vis absorption spectra were recorded with a UV-1700 spectrophotometer (Shimadzu, China).

**Investigation of peroxidase-like activity**

The peroxidase-like activity of the 2D Fe-CTF was investigated by the same method as described in our previous report. Briefly, solutions used for kinetic analysis with TMB as the substrate consisted of 780 μL of NaAc buffer (0.1 M, pH 4.0), 10 μL of the 2D Fe-CTF solution (0.2 mg mL⁻¹), 30 μL of 490 μM H₂O₂, and 30 μL of TMB with varying concentration (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7 mM). Similarly, solutions used for kinetic analysis of H₂O₂ as the substrate consisted of 780 μL of NaAc buffer (0.1 M, pH 4.0), 10 μL 2D Fe-CTF solution (0.2 mg mL⁻¹), 30 μL of 0.6 mM TMB, and 30 μL of H₂O₂ with varying concentration (0, 0.15, 0.30, 0.60, 1.20, 2.4, 4.90, and 9.80 mM). The Michaelis-Menten constant was calculated using Lineweaver-Burk plots of the double reciprocal of the Michaelis-Menten equation

\[
\frac{1}{V} = \frac{K_m}{V_{max}} \left( \frac{1}{[C]} + \frac{1}{K_m} \right),
\]

where \( V \) is the initial velocity, \( V_{max} \) represents the maximum reaction velocity, \([C]\) corresponds to the concentration on the substrates, and \( K_m \) is the Michaelis constant.

**MTT assay**

In vitro cytotoxicity was conducted as follows: HepG2 cells were first seeded in a 96 well plate and maintained in the culture medium for 24 h at 37 °C and subsequently exposed to fresh medium containing a homogeneous solution of the 2D Fe-CTF or Cu-CTF in DMSO and incubated for 20 h. After being washed with the culture medium, 120 μL aliquots of the new culture medium containing an MTT solution (10 μL, 5 mg/mL) were added to each well. After incubation for 4 h, the supernatant was removed and 200 μL aliquots of DMSO were added to each well and shaken for another 10 min. Then the resulting absorbance was quantified (\( \lambda = 490 \) nm) with an enzyme-labelling instrument (iMark, Bio-Rad, USA). All measurements were performed in triplicate, and the cell viability values were calculated according to the following formula: cell viability (%) = (absorbance of experimental group)/(absorbance of blank control group) \( \times 100\% \).

**Critical coagulation concentration (CCC) assays**
50 μL of the 2D Fe-CTF solution (1.0 mg/mL) was added into 450 μL of standard salt solutions with varying concentration values and mixed well. After incubating at room temperature for 3 h, the mixed samples were centrifuged at 2000 rpm for 2.0 min. Then the supernatant was taken and the absorbance was measured.

**Fluorescent quenching assays**

A certain amount of FAM-modified was added to different concentrations of the 2D Fe-CTF or 2D CTF (0, 20, 30, 40, 50 μg/mL) buffer (50 mM Tris-HCl, pH 7.5) solutions. Fluorescence measurements were performed after incubation for 20 min.

**Sarcosine detection**

Sarcosine detection was carried out as follows: First, 20 μL of 50 U sarcosine oxidase PBS buffer (0.2 M, pH 8.0) and 350 μL of sarcosine at various concentrations were mixed well and incubated at 38 °C for 30 min. 350 μL of acetate buffer (50 mM, pH 4.0), 20 μL of TMB (2.0 mM, ethanol solution), and 10 μL of 2D Fe-CTF solution (50 μg/mL) were subsequently added to the mixture and incubated at room temperature for 30 min. Finally, the absorbance of the mixed solution was measured at 652 nm.

**OTA detection**

Briefly, 100 μL of OTA solutions with various concentration values were mixed with 20 μL of 10 mM aptamer solution and subsequently incubated at room temperature for 60 min. Next, 10 μL of the 2D Fe-CTF solution (1.0 mg/mL) was added to the mixture and incubated at room temperature for 30 min. Then, NaCl solution was added to the aforementioned mixed solution until the final concentration reached 0.3 M. After incubating at room temperature for 2 h, centrifugation was performed at 2000 rpm for 2.0 min. Then, 20 μL of the supernatant was taken and added to 480 μL of acetate buffer (50 mM, pH 4.0), which contained 20 μL of TMB (2.0 mM, ethanol solution) and 20 μL of H₂O₂ solution (5.0 mM). After incubating at room temperature for 15 min, the absorbance of the mixed solution was measured at 652 nm.

**Fluoride ion detection**

20 μL of 2D Fe-CTF solution (50 μg/mL) was added to 400 μL F⁻ solutions with various concentration values. After incubating at room temperature for 100 min, 20 μL of TMB (10 mM, ethanol solution) and 30 μL of H₂O₂ solution (9.8 mM) were added
to the aforementioned mixed solution. After incubating at room temperature for 35 min, the absorbance of the mixed solution was measured at 652 nm.

Fig.S1 (a) Tyndall effect of 2D Fe-CTF in water, (b) photographic image of aqueous dispersion of 2D Fe-CTF, (c) photographic image of aqueous dispersion of bulk CTF, (d) Tyndall effect of 2D Fe-CTF in water after standing for 30 days, (e) photographic image of aqueous dispersion of 2D Fe-CTF after standing for 30 days and (f) photographic image of aqueous dispersion of bulk CTF after standing for 30 days.
Fig. S2 UV-vis spectrum of 2D Fe-CTF solution.

Fig. S3 FT-IR spectra of bulk CTF, 2D CTF and 2D Fe-CTF.
Fig. S4 TEM images of 2D Fe-CTF.

Fig. S5 AFM images and height profile of 2D Fe-CTF.
Fig. S6 DLS graph of 2D CTF and 2D Fe-CTF.

Fig. S7 (a) EDS elemental mappings of 2D Fe-CTF and (b) element content in a random selected area.
Fig. S8 Raman spectra of 2D CTF and 2D Fe-CTF.

Fig. S9 Absorbance changes at 652 nm in different reaction systems.
Fig.S10 (a) Hydroxyl radicals (·OH) induced the conversion of non-fluorescent terephthalic acid to highly fluorescent 2-hydroxy terephthalic acid, (b) Emission spectra of terephthalic acid in different catalytic system.

Fig.S11 The catalytic kinetic 3D spectra of 2D Fe-CTF.

Fig.S12 Arrhenius plot for the reaction velocity versus temperature of 2D Fe-CTF/TMB/H₂O₂ reaction system.
Fig. S13 Current-time responses of the 2D CTF and 2D Fe-CTF modified electrode to H$_2$O$_2$ successively added.

Fig. S14 Effect of 2D Fe-CTF and Cu-CTF on HepG2 cell viability.
Fig. S15 (a) Schematic illustration of 2D Fe-CTF as fluorescent DNA quenching substrate, (b) fluorescence spectra of 2D Fe-CTF as quenching substrate and (c) fluorescence spectra of 2D CTF as quenching substrate.

Fig. S16 Normalized concentrations of 2D Fe-CTF against (a) NaCl, (b) CaCl₂, and (c) AlCl₃ concentrations. (d) Double logarithmic plot of the critical coagulation concentrations against the ionic valency.
### Table S1 Comparison of the kinetic parameters of CCTF and other nanomaterials

<table>
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<th>Catalyst</th>
<th>Substance</th>
<th>$K_m$/mM</th>
<th>$V_{max}/10^{-8}$ MS$^{-1}$</th>
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<td></td>
<td>H$_2$O$_2$</td>
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<td>CuO-g-C$_3$N$_4$</td>
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<td>0.1</td>
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<tr>
<td></td>
<td>H$_2$O$_2$</td>
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<td>-</td>
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<tr>
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<td></td>
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<td>Apt–Au NPs/BiOCl</td>
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<tr>
<td>hybrids</td>
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<td>H$_2$O$_2$</td>
<td>0.17</td>
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**Results and discussion**
The excellent catalytic efficiency of 2D Fe-CTF could be originating from the following reasons: First and most importantly, the exfoliation of bulk CTF materials into 2D Fe-CTF with extended lateral dimensions and nanometer thickness are not only increased the surface area and charge carrier mobility, but also expected to implement fast kinetics and effectively lower mass-transfer barriers and sequential catalytic reactions\textsuperscript{15-17}. Second, after anchoring the iron ions, the 2D Fe-CTF possess many highly accessible active sites on their surface, moreover, the chelation of Fe\textsuperscript{3+} in the 2D Fe-CTF acts as bridge-like structure to link electron accepted and donated between the substrate (TMB or H\textsubscript{2}O\textsubscript{2}) and the 2D Fe-CTF, more effective charge separation in 2D CTF facilitates electron transfer from substrate to 2D Fe-CTF, which could be significant for the catalytic\textsuperscript{18, 19}; Third, due to the \(\pi-\pi\) interaction between the benzene rings in TMB and 2D Fe-CTF, the unique 2D nanosheets structure provided higher affinity for TMB. And enabling of the rapid diffusion of H\textsubscript{2}O\textsubscript{2} molecules in the surface which provided more active sites for accelerating the decomposition of H\textsubscript{2}O\textsubscript{2} to \(\cdot\text{OH}\), because of the presence of Fe(III) on the surface of 2D Fe-CTF can initiate reactions which produce the \(\cdot\text{OH}\) radical by the Fenton reaction, the newly formed Fe\textsuperscript{3+} from Fe\textsuperscript{2+} oxidized by H\textsubscript{2}O\textsubscript{2} is immediately converted back to Fe\textsuperscript{2+} \textit{via} accepting electrons from 2D CTF:\textsuperscript{20}

\[
\begin{align*}
\text{Fe(III)} & \text{ H}_2\text{O}_2 \rightarrow \text{Fe( )} + \cdot\text{OOH} + H^+; \\
\text{Fe(II)} & \text{ H}_2\text{O}_2 \rightarrow \text{Fe( )} + \cdot\text{OH} + OH^-.
\end{align*}
\]

References: