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Intrinsic peroxidase-like activity and the catalytic mechanism of gold @ carbon dots nanocomposites

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The well-dispersed AuNPs@CDs nanocomposites were successfully synthesized from the reduction of chloroauric acid by carbon dots (CDs) at room temperature. The as-prepared AuNPs@CDs were characterized by transmission electron microscopy (TEM), fourier transform infrared spectroscopy (FTIR), Raman spectroscopy and X-ray photoelectron spectroscopy (XPS), and the results indicate the AuNPs@CDs nanocomposites with core-shell structure. The AuNPs@CDs are found to possess intrinsic peroxidase-like activity, and the catalytic activity is higher than that of AuNPs. The mechanism of peroxidase-like activity of AuNPs@CDs was investigated by fluorescence spectroscopy, electron spin resonance and cyclic voltammetry, and it is found that the mechanism could be ascribed to facilitate the electrontransfer between TMB and H_2O_2.

1. Introduction

Peroxidase is a class of efficient enzyme and has been widely applied in food industry, biotechnology, medical science, chemical industry and environmental science.[1] Unfortunately, natural peroxidases suffer greatly from high production cost and their instability, as they are so much sensitive to the environment. Accordingly, searching for natural peroxidase or artificial imitation peroxidase with good stability and low cost is urgently needed. Recently, Fe_3O_4 has been reported to possess intrinsic peroxidase-like activity, similar to that of natural horseradish peroxidase (HRP), which has been drawn extensive attention.[2] Subsequently, nanometal oxides (e.g. CuO, Co_3O_4 and MnO_2),[3-5] carbon nanomaterials (e.g. graphene oxide (GO)),[6] carbon dots (CDs),[7, 8] and noble metal nanoparticles or nanoclusters (e.g. AuNPs, AuNCs and AgNPs)[9-13] have been demonstrated to exhibit enzyme mimetic activity. Additionally, hybrid materials (e.g. AuNPs/citrate-functionalized graphene nanosheets[10] and Au@Pd nanoparticles-graphene hybrids[14]) have also been reported to display surprisingly high peroxidase-like activity. Comparing with HRP, nano-enzymes have good activity, high stability, simple preparation process, low cost and tunable surface/size. These properties make them possess potential in sensor to detect H_2O_2,[15] glucose,[7, 9, 16] calcium,[17] acetylcholine[18] and ascorbic acid.[19] Moreover, most of these nano-enzymes display stronger affinity between the enzyme and the substrate than that of HRP. However, some of them need complex synthesis process and time-consuming post-treatment. Thus it is still necessary to search a simple and green method to synthesize nanomaterials with peroxidase-like activity.

CDs as a new class of nanomaterial have been applied to the fields of bioimaging, sensor, catalysis, optoelectronic device, nanomedicine, etc.[20-23] As we know, CDs not only possess stable photoluminescence, fantastic biocompatibility and low cytotoxicity, but also display super conductivity, rapid electron-transfer properties. Moreover, abundant oxygen-containing functionalized groups on the surface of CDs make them much easier for forming environmentally friendly hybrid materials. These characteristic properties could be used to further enhance the catalytic activities of the original metal nanoparticles.[24-26] For example, CDs-Pt,[27] Co@CDs[28] have been reported to display higher intrinsic peroxidase-like activity than metal nanoparticles because of the synergistic effect of metal nanoparticles and CDs. Noble metal nanoparticles and the hybrid nanomaterial have been also reported as peroxidase,[9, 10] however, to the best of our knowledge, there is no detailed investigation on composites of AuNPs and CDs as peroxidase. We are interested in whether the AuNPs and CDs nanocomposites possess peroxidase-like activity, and if so, what is the mechanism of the nanocomposites as peroxidase?

In this paper, AuNPs@CDs nanocomposites were synthesized. The morphology and surface structure were characterized by TEM, FTIR, Raman spectroscopy and XPS. The peroxidase-like activity of the prepared AuNPs@CDs has been investigated by catalyzing the oxidation of TMB in the presence of H_2O_2. The mechanism of peroxidase-like activity of AuNPs@CDs was probed by fluorescence spectroscopy, electron spin resonance and cyclic voltammetry.

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2. Experimental

2.1 materials

Chloroauric acid ($HAuCl_4$. $4H_2O$; ≥ 99 wt %) was purchased from Sinopharm Chemical Reagent Co., Ltd. Urea (≥ 99 wt %), citric acid (≥ 99.5 wt %), sodium citrate (≥ 99 wt %) and disodium hydrogen phosphate (≥ 99 wt %) were purchased from Shanghai Runjie Chemical Reagent Co., Ltd. 3,3',5,5'-tetramethlbenzidine (TMB; ≥ 98 wt %), horseradish peroxidase (HRP, 160 U· mg^{-1}) and terephthalic acid (TA; ≥ 99 wt %) were supplied by Aladdin Industrial Corporation. 5,5'-dimethyl-1-pyrroline-N-oxide (DMPO; ≥ 99 wt %, chromatographic grade) and sodium polyacrylate (PAA; average Mw～5100) were purchased from Sigma-Aldrich. Hydrogen peroxide (H_2O_2; 30 wt %) was obtained from Chinasun Specialty Products. Co., Ltd. All reagents were used directly without further purification, and all aqueous solutions were prepared with ultrapure water (18.2MΩ).

2.2 Synthesis and characterization of AuNPs@CDs

Synthesis of CDs: The CDs were obtained through the previous reported method.[29] Briefly, 0.200 g of citric acid and 0.087 g of urea were dissolved in 20 mL of water and the solution was transformed into a digestion vessel, which was placed in the microwave digestion container and heated by use of 900 W power at temperature of 150 ℃ for 5 min. After the reaction was completed, the microwave digestion vessel was cooled down naturally. Then the obtained CDs solution was stored at room temperature for use.

Synthesis of AuNPs@CDs and AuNPs: The AuNPs@CDs nanocomposites were prepared by a chemical reduction route. The experimental procedure is described as follows: a certain amount of sodium polyacrylate was dissolved in water (82.5 mL), and mixed with chloroauric acid solution (2 mM, 37.5 mL) in a 250 mL round-bottom flask with a magnetic stir bar. The CDs solution (2 mg/mL, 30 mL) was added to trigger the reaction after stirring for 10 min. The pale yellow solution gradually turned pink and then red wine, and the mixture was kept stirring for 3 h at room temperature to complete the reaction. The AuNPs@CDs was collected by centrifugation at 12500 rpm for 10 min, and the deposits were washed by ultrapure water three times to remove the unreacted precursor. The purified AuNPs@CDs were dissolved in ultrapure water and the concentrations were determined by the UV-Vis spectrum.[30] The AuNPs were synthesized according to the literature.[31]

Characterizations: UV-vis absorption spectra of the samples were carried out by UV2450 spectrophotometer (Hitachi, Japan). The fluorescence spectra of the samples were carried out by FLS920 fluorescence spectrometer (Edinburgh, UK). The transmission electron microscopy (TEM) images of the CDs and AuNPs@CDs were observed by a JEOL-2010 electron microscope (JEOL, Japan) operating at 200 kV. The fourier transform infrared (FTIR) spectrum of AuNPs@CDs was measured by a NICOLET iS10 (Thermo Fisher, America) spectrometer ranging from 500 to 4000 cm^{-1}. The Raman spectra of PAA and AuNPs@CDs were performed by a Renishaw Invia Raman spectrometer (Renishaw, UK) with 514.5 nm as laser excitation. The X-ray photoelectron spectroscopy (XPS) spectra of AuNPs@CDs were performed by a ESCALAB 250 spectrometer (Thermo Fisher, America).

2.3 Peroxidase-like activity and kinetic analysis of AuNPs@CDs

The peroxidase-like activity of AuNPs@CDs was carried out by the catalytic oxidation of TMB in the presence of H_2O_2. Typically, a certain amount of TMB, H_2O_2, and the AuNPs@CDs solution were mixed with citrate–phosphate buffer (0.2 M) solution at a certain pH and temperature. The reaction was monitored by recording the absorbance at 652 nm belonged to the oxidation product of TMB using UV-vis spectrophotometer. The apparent steady-state kinetic analysis were carried out by using AuNPs@CDs (1.5 mg/L) as nano-enzyme in citrate–phosphate buffer solution (pH 3.8, 0.2 M) at various concentrations of TMB, fixed H_2O_2 concentration (300 mM) and temperature of 40 ℃. The apparent kinetic parameters were calculated using the Michaelis-Menten model: $V_0 = V_{max} \times [S]/(K_m + [S])$, where V_o is the initial velocity, V_{max} is the maximum reaction velocity, $[S]$ is the substrate concentration and K_m is the Michaelis constant.

2.4 Fluorescence measurments

TA solutions (0.5 mM) were mixed with H_2O_2 and different concentration of AuNPs@CDs in citrate–phosphate buffer solution (pH 5, 0.2 M). A series of sample solutions were exposed to UV light. The fluorescence spectrum of the solution in the region of 330 ~ 550 nm was recorded at excitation wavelength of 315 nm with a slit width being 2 nm.

2.5 Electron spin resonance experiment

All Electron spin resonance (ESR) experiments were performed on Bruker EMX A300 spectrometer at room temperature. The sample solutions were put in glass capillary tubes and the tubes were inserted into the ESR cavity. ESR parameters settings were as follows: modulation amplitude 1 G, scan range 3420-3620 G, microwave power 6.4 mW and time constant 163.84 ms for detection of spin adducts using spin trap DMPO. Tubes containing DMPO (20 mM), H_2O_2 (200 mM) and various concentrations of AuNPs@CDs (3 mg/L - 30 mg/L) in citrate–phosphate buffer solution (pH 3.8, 0.2 M) were exposed to UV light for 10 min and characterized immediately.

2.6 Electrochemical properties of AuNPs@CDs

The cyclic voltammetry and amperometric measurments were carried out by CHI 660B (Chenhua, China). The glassy carbon electrode (GCE, 3.0 mm in diameter) was polished with 0.3 mm and 0.05 mm alumina slurry and washed by ultrapure water. Then the solution (6 μL, 120 mg/L) of AuNPs@CDs was dropped on the pre-processed GCE surface and allowed to air dry at room temperture. A three-electrode system composed by a saturated calomel electrode as reference, a platinum wire as auxiliary electrode and the AuNPs@CDs modified GCE or bare GCE as working electrode was used for all electrochemical experiments. In amperometric experiments, the current-time date of modified GCE or bare GCE system were recorded upon successive addition of 20 mM H_2O_2 in the time lags of 40 s in the citrate–phosphate buffer solution (pH 3.8, 0.2 M) at applied potential of -1.4 V.The ultrapure water was bolied before use.

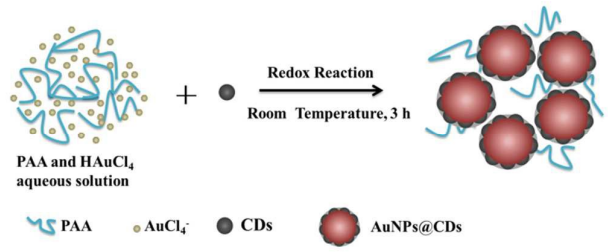
3. Results and discussion

3.1 Synthesis of AuNPs@CDs

AuNPs@CDs nanocomposites were synthesized in PAA-$HAuCl_4$ aqueous solution by a chemical reduction route at room temperature. In this process, gold nanoparticles were in-situ reduced by CDs and assembled on the surface of PAA-CDs soft template as depicted in Scheme 1. Sodium polyacrylate (PAA) was employed as soft template, and CDs acted as both stabilizer and reduction agent due to the intrinsic abilities of CDs to act as electron donors and acceptors. In the synthesis process, the CDs were used to substitute for traditional reduction agent. And the CDs possess many advantages that traditional reductant is incomparable, such as easily controlling the process, avoiding complicated post-treatment and avoiding some of environment pollution.[26, 32-36]

3.2 Characterization of AuNPs@CDs

The morphologies of the as-prepared CDs and AuNPs@CDs nanocomposites were investigated by TEM. As shown in Fig. 1A, the diameter of CDs is approximately 3.1±0.4 nm by calculating the average size of 100 nanoparticles. The TEM image of AuNPs@CDs is shown in Fig. 1B, indicating an average size of 16.3±1.7 nm. A higher magnification TEM image of the AuNPs@CDs is shown in Fig. 1C, which suggests AuNPs@CDs with core-shell nanostructure and the ultrathin carbon layer about 1～2 nm.



Scheme 1: The schematic illustration of the reaction process.

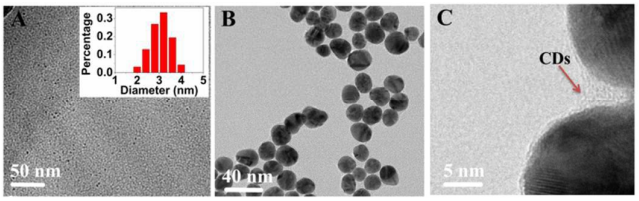


Figure 1: (A) TEM image of the as-prepared CDs. (inset: size distribution of CDs for TEM image.) (B,C). TEM images of the AuNPs@CDs with different magnifications.

The surface structure of AuNPs@CDs was investigated by FTIR. As shown in Fig. 2A, the broad peak centred at 3420 cm^{-1} is attributed to the stretching vibration of hydroxyl group, while the peak at 1647 cm^{-1} is related to the C=O stretching vibration of carboxide. These functional groups maybe come from the CDs on the AuNPs@CDs surface.

To further confirm the presence of CDs on the surface of AuNPs@CDs, the Raman spectra of PAA and AuNPS@CDs were carried out. Comparing with the Raman spectrum of PAA, there are two peaks in the Raman spectrum of AuNPs@CDs (Fig. 2B). The peak at 1570 cm^{-1} (G band) is assigned to sp^2-bond carbon atoms in a two-dimensional hexagonal lattice, and the peak at 1339 cm^{-1} (D band) represents disordered carbon.[37, 38] The results suggest that the CDs cover the surface of AuNPs@CDs, and the intensities ratio (I_D/I_G) of the peaks demonstrates that the CDs could be composed primarily of graphite structure with sp^2 hybridization.

The AuNPs@CDs nanocomposites were further characterized by XPS. As shown in Fig. 2C, the doublet $4f_{7/2}$ and $4f_{5/2}$ peaks are corresponding to the metallic state of Au (0). In addition, the peak of C 1s was observed and it can be divided into three peaks belonged to the C-C/C=C and the oxygenated functional groups (C-O and C=O), respectively (Fig. 2D).[39] These results from XPS measurements suggest the formation of AuNPs@CDs nanocomposites, which is consistent with the results from TEM, FTIR and Raman spectrum.

Furthermore, the optical properties of the AuNPs@CDs were investigated. The AuNPs@CDs solution presents wine red color (inset in Fig. 3A) and displays a strong absorption at 524 nm (Fig. 3A) belonged to the AuNPs characteristic absorption. As shown in Fig. 3B, the fluorescence intensity of AuNPs@CDs is remarkably weaker than that of CDs, which may be caused by the formation of AuNPs@CDs and the electron transfer from CDs shell to AuNPs core. This phenomenon was also observed in other metal-NPs@CDs system.[28]

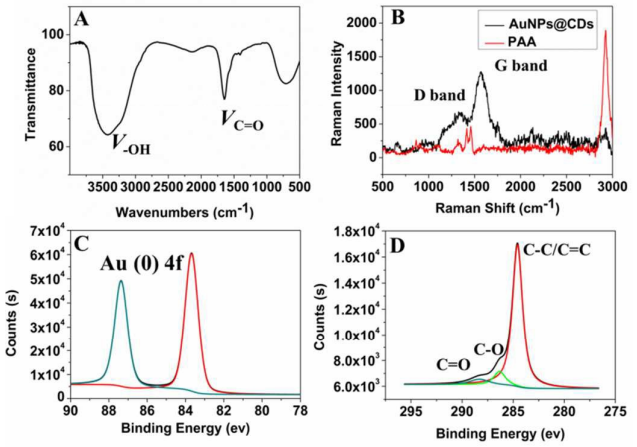


Figure 2: (A) The FTIR spectrum of the AuNPs@CDs. (B) The Raman spectra of AuNPs@CDs and PAA. The XPS spectra of Au 4f (C) and C 1s (D).

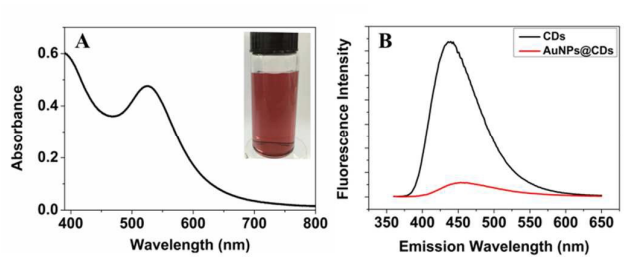


Figure 3: (A) The UV-vis spectrum of the AuNPs@CDs. The inset shows the color of the AuNPs@CDs solution. (B) The emission fluorescence spectra of CDs and AuNPs@CDs at excitation of 345 nm.

3.3 peroxidase-like activity and kinetic analysis of AuNPs@CDs

The peroxidase-like activity of AuNPs@CDs was investigated by catalyzing oxidization of TMB in the prescence of H_2O_2. A series of control experiments were carried out to investigate whether the catalytic reaction was caused by the AuNPs@CDs as shown in Fig. 4A. It is clear that the mixed solution of TMB ,H_2O_2 and AuNPs@CDs (or AuNPs) shows blue color (inset in Fig. 4A). For systems of AuNPs@CDs/TMB/H_2O_2 and AuNPs/TMB/H_2O_2, the absorbances at maximum wavelength of 652 nm were observed as shown in Fig. 4A. In comparison with AuNPs@CDs relevent systems, no absorbance was detected at 652 nm for system of the TMB/H_2O_2, PAA/TMB/H_2O_2, CDs/TMB/H_2O_2 and AuNPs@CDs/TMB. The above results show that the change of absorbance at 652 nm caused by peroxidase-like activity of AuNPs@CDs and AuNPs. The absorbance of AuNPs@CDs/TMB/H_2O_2 system is much stronger than that of AuNPs/TMB/H_2O_2 system in the same reaction time. It means that the the preoxidase-like activity of AuNPs@CDs is higer than that of AuNPs. In comparison with AuNPs, the improvement of peroxidase-like activity of the AuNPs@CDs could be attributed to the synergistic effect of AuNPs and CDs.[27, 28] In addition, catalytic oxidation rate of TMB by H_2O_2 increase with the concentration of AuNPs@CDs in the region of 1~6 mg/L (Fig. 4B).

As we know, the catalytic activity of natural enzyme is affected by the pH and temperature of the reaction condition. Thus the effect of pH and temperature on the catalytic activity of AuNPs@CDs was explored based on the relative activity A_i/A_m, where A_i was the absorbance at 652 nm at each condition and A_m was the maximum one. The plots of A_i/A_m against pH and temperature are displayed in Fig. 4C and Fig. 4D, respectively. The results suggest that the optimal experimental conditions are pH of 3.8 and temperature of 40 ℃. It's noteworthy that relative activity (59 %) of AuNPs@CDs was maintained, much higer than that of AuNPs/Cit-GNs (about 48 %)[10] and HRP (about 5 %) at 55 ℃,[28] which indicates that the AuNPs@CDs have good thermal stability.

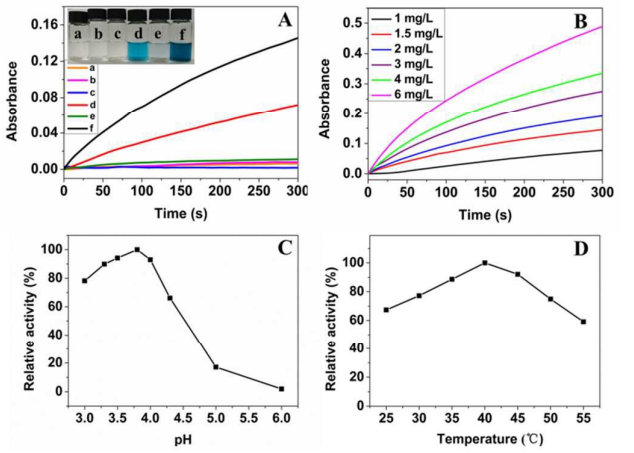


Figure 4: (A) Time-dependent absorbance changes at 652 nm of TMB in different reaction system and the color of different reaction system (inset in A): (a) TMB/H_2O_2, (b) PAA/TMB/H_2O_2 (1.13 mg/mL PAA), (c) CDs/TMB/H_2O_2 (0.4 mg/mL CDs), (d) AuNPs/TMB/H_2O_2 (1.5 mg/L AuNPs), (e) AuNPs@CDs/TMB (1.5 mg/L AuNPs@CDs) and (f) AuNPs@CDs/TMB/H_2O_2 (1.5 mg/L AuNPs@CDs). (B) Time-dependent absorbance changes at 652 nm of different concentration of the AuNPs@CDs in AuNPs@CDs/TMB/H_2O_2 system. The effect of pH (C) and temperature (D) on the catalytic activity of the AuNPs@CDs. Reaction conditions: All experiments were carried out using 1.5 mg/L AuNPs@CDs in citrate–phosphate buffer solution (pH 3.8, 0.2 M) with TMB (0.2 mM), H_2O_2 (200 mM) as substrates at temperature of 40 ℃, unless otherwise stated.

Under the optimal condition, steady-state kinetics experiments were performed to further investigate the peroxidase-like activity of AuNPs@CDs by changing one substrate concentration while fixing that of the other substrate. Typical Michaelis-Menten curve was obtained at a fixed H_2O_2 concentration (300 mM), and the maximum reaction velocity (V_m) and Michaelis costant (K_m) were calculated and shown in Table 1. As we all know, K_m is an indicator of the affinity of enzyme for the substrate, and the smaller value of K_m indicates the stronger affinity between the enzyme and the substrate. A comparison of the kinetic parameters of AuNPs@CDs with reported peroxidase is listed in Table 1. The apparent K_m value for AuNPs@CDs with TMB as substrate is 0.0587 mM, which is far lower than that of HRP (0.434 mM)[2], AuNPs (0.74 mM)[10] and Co@CDs (0.32 mM)[28]. These results demonstrate that the AuNPs@CDs display better affinity to TMB than that of AuNPs, which may result from that a suitable amount of carboxy groups on the surface of AuNPs@CDs is conducive to attracting the amino group of TMB electrostatically.[10] The Lineweaver-Burk plots at a series of fixed concentration of H_2O_2 are shown in Fig. 5B, where the slops of the lines are parallel, and the character of ping-pang mechanism is clearly indicated. This suggests that, like HRP and the other reported nano-enzyme,[2, 10, 40] the AuNPs@CDs bind and react with one substrate, and then react with the second substrate.

Table 1 Comparison of the kinetic parameters of AuNPs@CDs and the reported peroxidase. K_m is the Michaelis constant, V_{max} is the maximal reaction velocity.

catalyst	substrate	K_m[mM]	V_{max}[10^{-8} M.s^{-1}]	Ref
AuNPs@CDs	TMB	0.0587	1.86	This study
HRP	TMB	0.434	10.0	[2]
AuNPs	TMB	0.74	12.15	[10]
Co@CDs	TMB	0.32	5.45	[28]

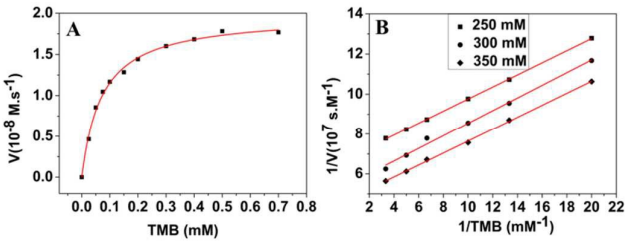


Figure 5: (A) The steady-state kinetic assay of the AuNPs@CDs nanocomposites. The concentration of H_2O_2 was 300 mM and that of TMB was varied. (B) Double reciprocal plots of the activity of the AuNPs@CDs nanocomposites with the concentration of H_2O_2 fixed at 250 mM (300 mM and 350 mM) and that of TMB was varied. Reaction conditions: 0.2 M Citrate–phosphate buffer solution (pH 3.8), temperature 40 ℃, the concentration of AuNPs@CDs was 1.5 mg/L.

3.4 Mechanism of peroxidase-like activity of AuNPs@CDs

According to previous researches, the peroxidase-like catalytic mechanism of nanoenzyme could be generally classified into three different ways: (1) the generation of hydroxyl radical (· OH), assigned to iron oxide,[41, 42] Co_3O_4/rGO nanocomposites,[43] (2) the generation of O_2, assigned to the BSA-MnO_2,[5] and (3) the electron transfer process, assigned to natural emzyme,[44] Co_3O_4 nanoparticles[4] and PVP-Ir nanocomposites.[45] In order to clarify the peroxidase-like catalytic mechanism of AuNPs@CDs, a series of experiments were carried out.

Fluorescence quenching and spin trapping technique were used to demonstrate whether the hydroxyl radical was generated during the catalytic process of AuNPs@CDs as peroxidase. In the method of fluorescence quenching, TA was used as a probe for detection of hydroxyl radical. It is believed that H_2O_2 produces hydroxyl radical under UV radiation. TA can react with · OH and converts to the highly fluorescent 2-hydroxy-terephthalic acid. The mixed solution of TA, H_2O_2 and various AuNPs@CDs concentration were irradiated at UV light. The fluorescence intensity of TA/H_2O_2/AuNPs@CDs/UV system decreases with increase of AuNPs@CDs concentration as shown in Fig. 6A. The results suggest that AuNPs@CDs can reduce the production of · OH by catalyzing the decomposition of H_2O_2 in a concentration dependent manner. It indicates that catalytic process of AuNPs@CDs as peroxidase hardly generates hydroxyl radical.

Combination of spin trapping technique and ESR spectroscopy was used to probe hydroxyl radical in the enzyme catalysis process. In this method, DMPO used as spin trap matter can react with · OH to generate the stable DMPO/· OH spin adduct, which has a typical four lines ESR spectrum with relative intensity being 1:2:2:1. ESR was performed on DMPO/H_2O_2/UV system with and without the presence of AuNPs@CDs to evaluate the catalytic mechanism by means of exploring the effect of the AuNPs@CDs on hydroxyl radical signal intensity. The DMPO/· OH adduct signal intensity is reduced after adding AuNPs@CDs as shown in Fig. 6B, which suggests that the catalytic process could not generate hydroxyl radical. The results coincide with those observed in the fluorescence measurements of TA/H_2O_2/AuNPs@CDs/UV system. These results also suggest a great potential of AuNPs@CDs involved in the antioxidant therapeutics avoiding generation of · OH which may lead damage to lipids, proteins and DNA, during the decomposition of H_2O_2.

Electrochemical measurments can be used to explore whether the catalytic mechanism of peroxidase could be ascribed to the electron transfer process.[4, 7] Cyclic voltammetry and amperometric measurments were preformed to investigate the peroxidase-like catalytic mechanism of AuNPs@CDs. Cyclic voltammograms of the AuNPs@CDs modified GCE electrode in the prescence of H_2O_2 and absence of H_2O_2 are shown in Fig. 7A. The current observed in the prescence of H_2O_2 is larger than that of the abscence of H_2O_2. At the same time, amperometric responses of bare GCE and the AuNPs@CDs modified GCE upon successive additions of H_2O_2 are displayed in Fig. 7B. Comparing with the reduction current at bare GCE, the reduction current rises sharply to reach a steady state at AuNPs@CDs modified GCE with addition of a certain amout of H_2O_2. These results from cyclic voltammetry and amperometric measurments suggest that the AuNPs@CDs nanocomposites possess the ability of electron transfer between electrode and H_2O_2 in the electrochemical process. It is thought that the AuNPs@CDs could be conducive to the electron transfer between TMB and H_2O_2 to form the oxidation product of TMB in the catalytic process by the AuNPs@CDs as nano-enzymes. Therefore, it is reasonable to conclude that the peroxidase-like activity of AuNPs@CDs may originate from the promotion of the electron transfer between TMB and H_2O_2, and the proposed mechanism is displyed in Fig. 7C.

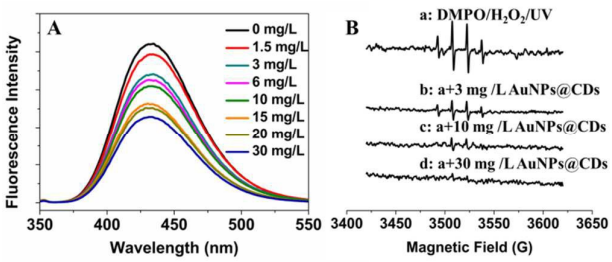


Figure 6: (A) Fluorescence emission spectra of TA in the presence of H_2O_2 and different concentration of AuNPs@CDs in 0.2 M citrate–phosphate buffer with pH 5.0. (B) The effect of the AuNPs@CDs on the formation of hydroxyl radical in the DMPO/H_2O_2/UV system. Samples were mixture of DMPO (20 mM), H_2O_2 (200 mM) and various concentrations of AuNPs@CDs in 0.2 M citrate–phosphate buffer with pH 3.8.

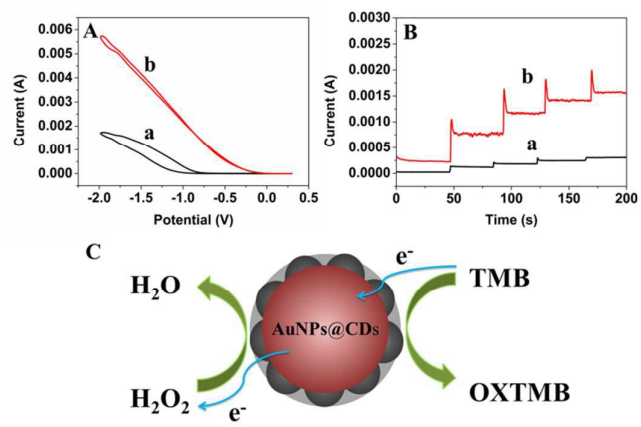


Figure 7: (A) Cyclic voltammograms of the AuNPs@CDs modified GCE electrode in 0.2 M citrate–phosphate buffer (pH 3.8) in the absence (a) and in the presence of H_2O_2 (200 mM)(b). Scan rate: 50 mV/s. (B) Amperometric response of bare GCE (a) and the AuNPs@CDs modified GCE (b) in 0.2 M citrate–phosphate buffer (pH 3.8) at applied potential of -1.4 V upon successive additions of H_2O_2 (20 mM). (C) Proposed mechanism over AuNPs@CDs as peroxidase.

4 conclusion

In summary, AuNPs@CDs nanocomposites with uniform size and high stability were synthesized using a simple and green method with CDs working as reduction agent. The peroxidase-like activity of AuNPs@CDs was extensively investigated. Kinetic studies show that the Michaelis costant (k_m) for TMB with AuNPs@CDs as peroxidase is much smaller than that of HRP and AuNPs. The peroxidase-like catalytic mechanism of AuNPs@CDs could be ascribed to that the AuNPs@CDs facilitate the electron transfer between TMB and H_2O_2. Due to their good catalytic activity and biocompatibility, AuNPs@CDs possess potential applications in fields of biotechnology and clinical diagnosis as enzymatic mimics, etc.

Acknowledgements

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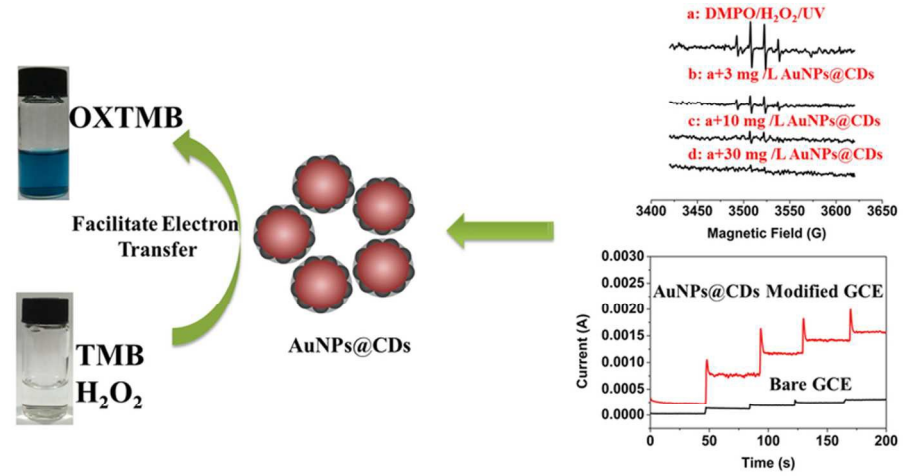

The mechanism of AuNPs@CDs as nano-enzyme catalysing the oxidation of TMB in the presence of H_2O_2

40x20mm (600 x 600 DPI)