Bionic radical generation and antioxidant capacity sensing with photocatalytic graphene oxide–titanium dioxide composites under visible light

Weiguang Ma, Dongxue Han, Nan Zhang, Fenghua Li, Tongsun Wu, Xiandui Dong and Li Niu

OH radicals as reactive oxygen species in an organism were applied to assay antioxidant capacity since the obtained results present high biological relevance. As a proper photocatalyst, titanium dioxide was employed to generate OH radicals under ultraviolet light irradiation. However, ultraviolet light can damage molecular probe (DNA or protein) during the detection of antioxidant capacity, which interferes with the results. In this article, a novel composite graphene oxide–titanium dioxide (GO–TiO2) nanocomposite was synthesized, which can generate numerous OH radicals just under visible light irradiation. In addition, a novel electrochemical antioxidant capacity sensor was designed with GO–TiO2 composites as source of OH radicals and DNA as a molecular probe. Antioxidants were measured by using the suppression of the decline of reduction current of methylene blue used as an intercalating agent for DNA after irradiation and OH-mediated DNA damage. Using gallic acid (GA) as a model antioxidant species, the detection of GA at levels as low as 0.85 mg L\(^{-1}\) was possible. The antioxidant capacity of other antioxidants was also assayed. Finally, the novel sensor was applied to the determination of antioxidant capacity in tea.

Introduction

Reactive oxygen species (ROS) represent an important class of radical species generated in biological systems, and usually include superoxide, \(\text{H}_2\text{O}_2\) and OH radicals.\(^7\) High concentration of ROS can result in unrestricted oxidation of DNA, protein and membrane lipids, which in turn leads to oxidative destruction of the cell and can cause serious diseases such as cancer, cardiovascular disease, diabetes mellitus, neurodegenerative disease and aging.\(^8\) Fortunately, antioxidants (like glutathione, lipid acid, uric acid and flavonoids, etc.) can effectively scavenge ROS to protect organism; so to assess the antioxidant capacity is important in science and practice in the interests of human health. At present, on the basis of the chemical reaction involved, major antioxidant capacity assay can be roughly divided into two categories: (a) single electron transfer reaction based assay, such as copper reduction assay,\(^9,10\) ferric reducing antioxidant power,\(^7\) Folin–Ciocalteu (F–C),\(^9\) 2,2-diphenyl-1-

\(\text{DPPH}\) assay\(^10\) and Trolox equivalent antioxidant capacity assay.\(^11\) (b) Hydrogen atom transfer reaction based assay, like the oxygen radical absorbance capacity,\(^12,13\) total radical-trapping antioxidant parameter,\(^14\) \(\beta\)-carotene bleaching by \(\text{ROO}^+\) and low-density lipoprotein oxidation.\(^15\) Among these methods, varieties of free radicals were chosen. As stable free radicals, 2,2-diphenyl-1-picyrlyhydrazyl (DPPH), 2,2’-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and galvinoxyl have been widely used to investigate the antioxidant capacity in food and drug industry. OH radicals as a universal radical in organisms and which possess high reaction activity can be used to evaluate antioxidant capacity in vitro. The results obtained present high biological relevance and as a result have generated more and more interest from researchers. At present, there are mainly two ways to produce OH radicals,\(^17\) (a) the Fenton reaction, reduced transition metal ions, such as Fe(II), Cu(I) or Cr(II) react with \(\text{H}_2\text{O}_2\) through a one-electron redox reaction producing \(\text{OH}\) radicals and hydroxide anion. (b) Photocatalysis at catalyst surface, for example, TiO2 can absorb special light and react with \(\text{H}_2\text{O}\), generating plenty of \(\text{OH}\) radicals. Compared to the Fenton reaction, photocatalysis is convenient for its controllability and preferred by many researchers.\(^18,19\)

Since the first landmark paper was published on TiO2 photocatalytic water splitting by Fujishima and Honda,\(^20\) more and more researchers have paid attention to this field, and many works have been published.\(^21,22\) TiO2 has excellent photocatalytic
performance such as easy availability, long-term stability, and nontoxicity. However, the wide band gap of TiO2 (~3.2 eV, anatase) only allows it to absorb ultraviolet light (~387 nm) which limits utilization of solar light.21 Meanwhile, molecular probes (such as protein or DNA) can be damaged by ultraviolet light24 which can interfere with the result to a certain extent in the detection of antioxidant capacity. Therefore, it is of significant importance to develop a TiO2-based photocatalyst which can generate plenty of OH radicals under visible-light irradiation.

Graphene as two-dimensional macro-molecular sheet of carbon atoms with a honeycomb structure has excellent electronic conductivity and mechanical properties.25 It was used to modify TiO2, and then extend the light harvesting range. These hybrid materials have been used in degradation of environmental pollutants, solar cells, water-splitting and in antibacterial applications,23,24 etc. As is known, it is very difficult to dissolve graphene in water and other solvents,27 which seriously affects the qualities of graphene and TiO2 composites including aggregation of graphene and inhomogeneous TiO2 nanoparticles. Results of Zhang et al.28 showed that good contact between graphene and TiO2 can greatly improve the photocatalytic efficiency. GO possesses similar properties to graphene. What is more, it can easily dissolve in water. So, in this paper GO and TiCl3 were applied as precursors to synthesize uniform GO–TiO2 hybrid materials. The as-prepared GO–TiO2 hybrid materials show photocatalytic efficiency under visible-light irradiation. For the first time, GO–TiO2 composites have been employed as a source of OH radicals and DNA as a molecular probe to design a novel electrochemical antioxidant capacity sensor. Meanwhile, the antioxidant capacity of tea in five different brands was detected successfully.

Experimental

Preparation of GO–TiO2

GO was obtained from natural graphite powder (320 meshes, Shanghai Chemical) by a modified Hummers method.29 The as-prepared GO was diluted by deionized water, and then used to prepare the GO–TiO2 composites. GO–TiO2 composites were synthesized by using the self-assembly method reported by Chao et al.29 with some modification. Typically, GO and sodium dodecylsulfate (SDS, 0.05 mol L−1) were mixed together, and then 50 mL TiCl3 (0.12 mol L−1, Alfa) was added with modest stirring for one hour. Subsequently 10 mL of Na2SO4 (0.6 M) and 5 mL of H2O2 (1 wt%) were added, and the obtained mixed solution stirred for 16 hours at 90 °C. Then separated precipitates were washed with water and ethanol three times, dried at 70 °C, and calcined in air at 400 °C with a heating rate of 5 K min−1 for 2 h. Finally, the obtained composites were treated with a cleaning process involving three cycles of centrifugation/washing/re-dispersion in water and dried at 70 °C in air. The control experiments using multi-walled nanotube (MWNT) or deionized water were performed as described above. The amount of GO (0.5 mg mL−1), MWNT (2.4 mg mL−1), deionized water and SDS in different composites are listed in Table 1.

Characterization

X-ray diffraction (XRD) patterns of the samples were carried out in the range of 15–80° (2θ) using a D/MAX 2500V/PC X-ray diffractometer (Cu Kα radiation, λ = 0.15406 nm), operated at 40 kV and 30 mA. X-ray photoelectron spectroscopy (XPS) was recorded with an ESCALAB-MKI250 photoelectron spectrometer with Al Kα X-ray radiation as the X-ray source for excitation. Transmission electron microscope (TEM) and high-resolution transmission electron microscope operating (HRTEM) images were obtained with a Tecnai G2 high-resolution transmission electron microscope operating at 200 kV. The UV-visible diffused reflectance spectra (DRS) were performed on the dry-pressed disk samples using a Hitachi U-3900 spectrophotometer equipped with an integrating sphere assembly, using BaSO4 as the reference sample. Fluorescence emission spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer with an excitation wavelength of 315 nm.

Fluorescence measurements

The samples were prepared by the following method: 0.5 mL of 100 g L−1 different photocatalytic materials in absolute ethanol was placed in Petri dish with a glass rod. The as prepared Petri dishes were dried in air and then calcined at 300 °C for 2 hours. Subsequently, the Petri dishes were poured into 8 mL of 0.5 mmol L−1 terephthalic acid (TA, Alfa) and illuminated at 420 nm for 15 min. NaOH was added to the system to maintain an alkaline environment (pH = 10), in which TA has a preferable solubility. The light source was homemade 9 W LED bulbs. The light was 15 cm overhead the as-prepared Petri dishes. After irradiation, the solution was immediately transferred into a quartz cell for fluorescence measurements. The control experiments were also conducted under the same conditions as the above statement except for not adding the photocatalytic materials.

Agarose gel electrophoresis analysis

The samples were prepared similar to fluorescence measurements. Instead of TA, 0.1 mg mL−1 DNA solution which contained 30 mmol L−1 GA or not was cast into Petri dishes and then illuminated at 420 nm for 20 min or 30 min. 5 μL illuminated DNA and DNA marker were analyzed by gel electrophoresis in 1.5% agarose gel. The gel was stained by 1 μg mL−1 ethidium bromide, visualized under UV light, and photographed using a JS-680B video system. The control experiment was also illuminated 30 min without photocatalysis.

DNA damage and detection

First, 10 μL of 2 mg mL−1 GOB–TiO2 composites and 10 μL of 0.6 mg mL−1 DNA solution (salmon tests ds-DNA sodium salt, Sigma) were cast on to the glassy carbon electrode (d = 3 mm), respectively and dried at room temperature. This as-prepared electrode was denoted as DNA modified electrode. Then 20 μL deionized water was cast on the DNA modified electrode and illuminated at 420 nm. The light was 8 cm overhead the DNA modified electrode. After illumination, the electrode was
washed with deionized water and immersed into 2 \( \text{mol L}^{-1} \) methylene blue (MB, Shanghai Chemical) solution with stirring for 10 min at open circuit. The square wave voltammetry (SWV, increment potential \( \Delta E = 4 \text{mV} \), step amplitude \( \Delta E' = 25 \text{mV} \) and frequency \( f = 10 \text{Hz} \)) was recorded immediately on electrochemical station (CHI, 660A) in a three electrodes system. The as-prepared electrode was used as working electrode, and a platinum wire and Ag/AgCl (3 \( \text{mol L}^{-1} \) KCl) were used as auxiliary and reference electrode, respectively.

**Antioxidant capacity detection**

Five brands of tea were bought from the local supermarket. 0.5 g tea was added into 50 mL boiling deionized water, and then it was filtered by normal funnel after 30 min extraction. The filtrate was diluted with deionized water and used to detect antioxidant capacity. Just as the detection of damaged DNA, the process of antioxidant capacity detection was very similar; except that the modified electrodes were finally cast by a solution that contains antioxidants (tea diluted solution, GA solution, ascorbic acid AA, glutathione GSH, Trolox, or resveratrol RES). The time of irradiation was 30 min.

DPPH radical scavenging activity was reported by Brand-Williams et al. with a little modification. 5 mg DPPH (Alfa) was dissolved in 125 mL absolute ethanol. 0.2 mL various of tea...
A diluted solution or Trolox solution was added in 0.8 mL DPPH solution and reacted for 15 min without light. The reacted solution could be immediately detected by a UV-visible spectrophotometer. The absorbance was recorded at 514 nm.

F–C method was referenced by Ainsworth and Gillespie. 

100 μL of various tea diluted solution or GA solution was added to 2 mL microtubes; then, 200 μL of F–C reagent (10%, v/v, Sigma) was introduced with a thorough vortex; at last, 800 μL of Na₂CO₃ (0.7 mol L⁻¹) was added to the above solution and the mixture was incubated at 20 °C for 2 hours. The reacted solution was detected by UV-visible spectrophotometer. The absorbance was recorded at 756 nm.

Results and discussion
Characterization of GO–TiO₂ composites
The XRD patterns of the as-prepared GOB–TiO₂, MWNT–TiO₂ and TiO₂ exhibit similar characters as shown in Fig. 1. The
Fig. 10 Efficiency of hydroxyl radical scavenging of several antioxidant compounds.

Table 2 Detection of antioxidant capacity by three different methods

<table>
<thead>
<tr>
<th>DNA sensor, GA (mg g⁻¹)</th>
<th>F–C method, GA (mg g⁻¹)</th>
<th>DPPH method, Trolox (mg g⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>B1 47.45</td>
<td>60.29</td>
<td>141.20</td>
</tr>
<tr>
<td>B2 66.61</td>
<td>62.94</td>
<td>201.10</td>
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<tr>
<td>B3 41.86</td>
<td>78.40</td>
<td>191.50</td>
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<tr>
<td>B4 24.34</td>
<td>18.55</td>
<td>49.90</td>
</tr>
<tr>
<td>B5 38.39</td>
<td>39.82</td>
<td>86.30</td>
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peaks at 2θ values of 25.3, 37.8, 48.0, 53.9, 55.1, 62.7, 68.8, 70.3, 75.0° can be indexed to (101), (004), (200), (105), (211), (204), (116), (220), and (215) crystal planes of anatase TiO₂, respectively. Yet, three more diffraction peaks at 27.4, 36.1 and 41.2° are only observed in TiO₂ without GO or MWNT, which correspond to (110), (101) and (111) crystal planes of rutile TiO₂. So we assume that GO and MWNT can affect the crystal form of TiO₂. Notably, we cannot observe the diffraction peak of GO (not shown) at 22.14° in the GO–TiO₂ composites. The reason for this might be that it is shielded by the main peak of anatase TiO₂ at 25.4°.²³

The chemical state of elements in GOB–TiO₂ and GO has been analyzed by the XPS, and the results are shown in Fig. 2. The peaks of C 1s at 284.6 eV and 286.6 eV correspond to the C–C bond and C–O bond.⁴⁴ Compared to GO, the peak of GOB–TiO₂ at 286.6 eV decreased, caused by the C–O bonds being partly transformed into carbonate species in the process of heating at 400 °C. At the same time, another strong peak is observed at 288.6 eV in GOB–TiO₂, which confirms the existence of carbonate species.²⁵ Meanwhile, as shown in Fig. 2c, the peaks of Ti 2p located at 458.5 eV and 464.3 eV are assigned to Ti 2p₁/₂ and Ti 2p₃/₂, respectively, which is in good agreement with the binding energy values of Ti⁴⁺ in pure anatase.¹⁷ Moreover, the results correspond with that of XRD.

Fig. 3 shows the TEM images of as-prepared GOB–TiO₂, MWNT–TiO₂ and GO. The wrinkles on the plane of GO³⁸ can be clearly observed on Fig. 3a. GO with plenty of carboxylic acid, hydroxyl, and epoxide groups can easily adsorb Ti³⁺ and then form dispersed TiO₂ nanoparticles, which can be evidenced by the TEM of Fig. 3b and c. The size of TiO₂ nanoparticles is about 10 nm. However, TiO₂ nanoparticles were found to be aggregated on MWNT (Fig. 3d). As shown in Fig. 3e, HETEM images clearly illustrated the crystalline nature of TiO₂. FFT was used to calculate the spacing of the TiO₂ layer in its crystalline lattice, and the result showed that crystal lattice fringes observed in Fig. 3f originate from anatase TiO₂,¹⁷ which is also agreed with XRD.

UV-vis diffuse reflectance spectra (DRS) of GOB–TiO₂, MWNT–TiO₂ and TiO₂ have been recorded in Fig. 4a. As can be clearly seen, the addition of GO or MWNT induces the increased light absorption intensity in both of the UV and visible light regions, when compared to the bare TiO₂. The width of band gap was a key role to determine light absorption. A plot of the transformed Kubelka–Munk function as a function of energy of light is shown in Fig. 4b, by which the roughly estimated band gaps are 2.53, 2.69 and 3.02 eV corresponding to GOB–TiO₂, MWNT–TiO₂ and TiO₂, respectively. Analogous band gap narrowing of TiO₂ was found in the case of TiO₂–CNT and TiO₂–graphene composite materials.²⁶,²⁸ Due to band gap narrowing of TiO₂, it can effectively improve the catalytic efficiency under the visible light regions.

Generation of OH radicals

When TiO₂ is irradiated by special light, the electron can jump from valence band to conduction band, leaving a hole behind. Then, adsorbed water or hydroxide ions are trapped by holes to produce OH radicals. Subsequently, electrons are trapped by the reaction with adsorbed O₂ to produce superoxide radical, which then forms more OH radicals.²⁷ TA can react with OH radicals, producing 2-hydroxynaphthalene acid, which can emit visible fluorescence with the excitation of 315 nm, and used for measurement for OH radicals. As shown in Fig. 5a, with the increased content of GO in the GO-TiO₂ composites, fluorescence intensity is increased and then decreased. These experimental results imply that optimum GO quantity exists and that GO can stop the contact between TA and TiO₂, resulting in decreased catalytic efficiency.²⁸ Therefore, GOB–TiO₂ composites were selected and used to interact with DNA and sense antioxidant capacity. Compared to TiO₂ and MWNT–TiO₂ (shown Fig. 5b), GOB–TiO₂ presented stronger fluorescence intensity, which indicated that more OH radicals can be generated. It is estimated there are two possible reasons: (a) by dispersed TiO₂ on GO or MWNT, the band gap of TiO₂ is narrowed (Fig. 4b), which can effectively improve the catalytic efficiency under the visible light regions and lead to the production of more OH radicals. (b) The catalysis effect can be affected by interfacial contact between TiO₂ and carbonaceous nanomaterials. Since the dispersibility of TiO₂ on the GO is more homogeneous than that on MWNT as proved by TEM (Fig. 3b–d), the photo-generated electron can easily transport from TiO₂ to GO. Therefore, the recombination is decline between electron and hole which make TiO₂–GO show high catalysis effect.

DNA damage and detection

OH radical as a member of ROS in organisms can seriously damage DNA and lead to diseases when present in high concentrations. Therefore, DNA as a molecular probe was used...
to assay antioxidant capacity,⁵³–⁵⁵ to nearly reflect real conditions within an organism. In order to detect DNA damage with GOB–TiO₂ as the source of OH radicals, agarose gel electrophoresis was performed. As shown in Fig. 6, after illumination for 30 min, the length of DNA was found to be less than 1000 base pairs (bp) in majority with the photocatalysis GOB–TiO₂ (lane c), which was shorter than that of control experiment (lane e length more than 2000 bp). The result demonstrated that DNA was seriously damaged by OH radicals generated from GOB–TiO₂ and broken into short fragments. Due to addition of GA (lane d), an amount of DNA was found to keep the length over 2500 bp dramatically. The result revealed that antioxidant could efficiently protect DNA from being damaged.

Due to the high sensitivity and convenience electrochemical methods were used to quantify DNA damage.⁴²–⁴⁶ The guanine base, one of the four bases in DNA, can be easily oxidized since it has low oxidative potential with respect to the three other bases. Fig. 7 displays the oxide peak of guanine base at 0.92 V. With the increase of radiation time, the oxide peak of guanine base decreased. It has been demonstrated that DNA can be seriously damaged by OH radicals generated on GO–TiO₂ under the 420 nm irradiation. Hence, antioxidant capacity could be detected using GO–TiO₂ as OH radicals’ source.

**Determination of antioxidant capacity**

It has been reported that DNA or guanine base as a molecular probe was used to assay antioxidant capacity.⁴⁷ However, due to high oxidation potential excess substance could interfere in the detection. In order to solve this problem, MB was applied to assay antioxidant capacity since it has low electrochemical redox potential and high sensitivity toward ds-DNA structure changes.¹⁸–²⁰ As shown in Fig. 8, after irradiation for 30 min, the reduction current of MB was sharply decreased (Fig. 8, red). With increase in irradiation time, the reduction current of MB decreased (not shown). After addition of GA, the decline of reduction current was suppressed (Fig. 8, blue). It was demonstrated that the novel antioxidant was able to assay antioxidant capacity.

It is well known that GA possesses strong antioxidant capacity,⁶⁹ so we first investigated the antioxidant capacity of GA with the novel sensor. As shown in Fig. 9, the reductive current of MB recovered after adding the GA. The greater the concentration of GA, the higher the peak current of MB. In the insert (Fig. 9), the reduction current of MB was plotted against the concentration of GA, a linear range from 10 to 100 mg L⁻¹ of GA was found. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the equations; LOD = 3S/K and LOQ = 10S/K, respectively, where S is the standard deviation of a blank and K is the slope of the calibration line.⁷ The LOD and LOQ for GA were found to be 0.85 mg L⁻¹ and 2.82 mg L⁻¹. The RSD was 3.2% (n = 6) at 20 mg L⁻¹. The method showed excellent linearity (R² = 0.991) over a relatively broad concentration range of GA with respect to the guanine-biosensor (0.10–0.50 mg L⁻¹).⁴¹

For the sensing method, the possible interfering substances were detected. The results showed that 1000-fold glucose, fructose, malic acid, glycine and 100-fold cysteine did not interfere with the detection of GA at 20 mg L⁻¹.

In order to compare the efficiency of OH radical scavenging, several antioxidants (AA, GSH, TROLOX, GA, and RES) were tested at a concentration of 20 mg L⁻¹ under the same experimental conditions. The results are shown in Fig. 10. The efficiency was expressed as the percentage of the reduction current according to the following expression: %efficiency = Ia/Ib × 100, where Ia is the reduction current of MB measured after DNA damage in the presence of the antioxidant compound, Ib is the reduction current of MB without damage. It was found that AA presented the highest antioxidant efficiency (52.0%). A similar result was observed by Barroso et al.⁷⁰ The lowest values were GSH and TROLOX, 17.0 and 20.0%, respectively. GA and RES showed a similar antioxidant efficiency of 27.5 and 30.1%, respectively.

**Determination of the antioxidant capacity of tea**

Tea contains phenols and acids which have strong antioxidant capacity.⁷¹ The antioxidant capacity of five different brands tea was investigated with the as-prepared DNA sensor. In Table 2, the results are expressed as GA equivalents (milligram of GA per gram of tea). As expected, all brands tea showed remarkable antioxidant capacity. Green tea (B1, B2, and B3) presented high antioxidant capacity, 47.45, 66.61 and 41.86 mg g⁻¹, respectively. With respect to green tea, B4 and B5 showed lower antioxidant capacity. The reason for this may depend on the type and extent of fermentation and drying treatment, which may cause oxidation and condensation of phenolics.

Due to the robustness, simplicity and cost-effectiveness, F–C method is widely used to assay phenolic compounds. In general, phenolic compounds content correlates with antioxidant activity and seems to play an important role in stabilizing lipid oxidation. Therefore, this method was introduced and the results were expressed as GA equivalents. As shown in Table 2 the results with the DNA sensor agrees with the F–C method.

The DPPH radical scavenging activity was also evaluated and expressed by Trolox equivalents (milligram of Trolox per gram of tea). As expected, green tea (B1, B2, and B3) presented higher antioxidant capacity with respect to that of B4 and B5. Good agreement of DPPH method and F–C method demonstrated that this novel DNA sensor can be applied to assay antioxidant capacity.

**Conclusions**

In this article, a novel antioxidant sensor has been successfully designed via GO–TiO₂ nanoparticles. GO–TiO₂ composites with high photocatalysis effect have been synthesized and applied to generating OH radicals. The as-prepared composite materials with improved visible-light-harvesting ability, high charge-hole mobility, and low electron–hole recombination exhibited high photocatalytic performance and stability. As a source of OH radicals, it is very convenient to control the generation process of OH radicals just with visible light irradiation. OH radicals were further employed to damage DNA and antioxidants generated to protect DNA from damage, these were applied in real samples to assay antioxidant capacity in tea. Compared with other reported methods that detect antioxidant...
capacity, the present method presents three advantages: (a) OH radicals are chosen, which provide better biocompatibility to oxidative destruction than synthetic radicals in organisms; (b) in contrast to the Fenton reaction, it is convenient to control generation of OH radicals with visible light irradiation; (c) as a molecular probe, DNA was applied to evaluate the antioxidant capacity. The obtained results are predicted to have important biological relevance with applications in the food and medical industry.

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