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Photo-electro-chemical properties of TiO_2 mediated by the enzyme glucose oxidase

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Abstract

Electrochemical measurements show that the enzyme glucose oxidase (GO) is adsorbed on the surface of TiO_2 without apparently changing the flat band potential of the semiconductor, indicating that it does not cause a change of the energy of conduction band electrons. On the other hand, it is observed that GO markedly increases the efficiency of the two electron reduction of O_2 to H_2O_2 which is accumulated in the solution phase.

ESR spin trapping investigations indicate that GO favors the formation of $OH^{\bullet}$ radicals, due to either the inhibition of charge recombination processes or to H_2O_2 reduction by conduction band electrons. Accordingly, photo-oxidation of different alcohols to the corresponding radical species is also enhanced in the presence of GO.

The photo-oxidation of 1,2-propandiol on TiO_2/GO is regioselective in that (i) partial oxidation to hydroxyacetone is observed and (ii) no mineralization (full combustion to CO_2) of the substrate occurs. These facts are of particular interest in the field of studies concerning the design of new photocatalytic systems with enhanced activity and controllable oxidative power.

Keywords: Radicals; TiO_2; Glucose oxidase

1. Introduction

Studies focusing on designing multicomponent systems based on the use of semiconductors have attracted quite a bit of interest in view of the development of efficient and selective heterogeneous photocatalysts [1–4]. These investigations have provided new impetus to current studies on the use of semiconductors as catalysts for pollution abatement, and gave new contributions to research on highly selective processes that are carried out under mild and environment-friendly conditions. This field of research, known as Green Chemistry, represents nowadays an important branch of environmental sciences [1].

Surface modification with small metal particles is a common, well established strategy for designing photocatalysts with improved characteristics [5–8]. Less attention has been apparently devoted to molecular derivatization of the semiconductors [9–13].

Investigations on photocatalysis have been focused mainly on TiO_2 as the photoactive semiconductor, since it fulfils important requirements such as stability, low cost and environment tolerance. Excitation of TiO_2 with light ($\lambda < 400$ nm) leads to charge separation according to Equation (1): electrons are promoted to the conduction band (e_{cb}^-) and positive holes are left in the valence band (h_{vb}^+). The importance of O_2 reduction in determining the photocatalytic activity of TiO_2 has been highlighted by numerous authors [14–17]. Capture of conduction band electrons by O_2 increases the lifetime of the holes in the valence band, thus favoring the oxidation of organic substrates, which can be possibly adsorbed on the semiconductor surface. In addition, it is generally accepted that the role of O_2 is not just that of scavenging the photogenerated e_{cb}^-. Its reduction products ($O_2^{\bullet}$ and H_2O_2)

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also take part effectively in the overall oxidation process [18–20].

Prior work by our group has been devoted to preparing and characterising composite systems in which natural enzymes [21,22] or their synthetic models [1,9,13], adsorbed or chemically linked to the semiconductor surface, enhance the efficiency of the electron transfer from photoexcited TiO_2 to O_2. In particular, we have recently shown that this process can be positively affected by the presence of catalytic amounts of glucose oxidase (GO), EC 1.1.3.4, from *Aspergillus niger* (GO) [22]. This is a flavoprotein (MW = 150 kDa) that catalyses the reduction of O_2 to H_2O_2 with the simultaneous oxidation of β-D-glucose to D-glucono-1,5-lactone [23]. GO has attracted great interest due to its robustness and stability. Surface immobilized GO, by adsorption or chemical linkage, is extensively used as a glucose biosensor; it has also been entrapped in mesoporous TiO_2, to develop a glucose biosensor based on the electrochemical detection of H_2O_2 [24,25]. GO is also employed for exploring the reactivity of redox enzymes at interfaces [26].

In our previous investigations about the effect of GO on the photocatalytic properties of TiO_2, we proposed that this enzyme is able to mediate the electron transfer process from irradiated TiO_2 to O_2 according to Equation (2) [22]. These experiments were carried out in the presence of isopropyl alcohol as h_{vb}^+ scavenger:

$$TiO_2 + h\nu \rightarrow e_{cb}^- + h_{vb}^+ \quad (1)$$

$$2e_{cb}^- + O_2 + H^+ \xrightarrow{GO} H_2O_2 \quad (2)$$

TiO_2 electrodes can be conveniently employed for investigating separately the reduction and the oxidation processes that occur simultaneously during the photocatalytic experiments, as a consequence of the primary charge separation (Equation (1)). Herein, cyclic voltammetry experiments are carried out in order to gain insights into the effect of GO on the reduction of O_2 by the conduction band electrons of TiO_2 (e_{cb}^-). We also present the results of an ESR-spin trapping investigation for detecting the short lived radical species, which are the primary oxidation products. Finally, the photocatalytic activity of the composite system TiO_2/GO is assessed in the oxidation of 1,2-propandiol in order to evaluate its ability to photocatalyze regio-selective oxidations of alcoholic functionalities.

2. Experimental

2.1. Materials

Glucose oxidase from *Aspergillus niger*, catalase free and flavin adenine dinucleotide (FAD) were purchased from Sigma. TiO_2 was the commercial Degussa P-25. All the other products were from Sigma–Aldrich, unless otherwise specified.

2.2. Electrochemical apparatus

Titanium dioxide electrodes were prepared by spreading, on a Titanium foil, a paste obtained mixing TiO_2 (Degussa P-25, 3 g), bi-distilled water (6 mL), acetylacetone (200 μL) and Triton X-100 (200 μL) with subsequent calcination at 450 °C for 30 min [27]. Electrodes had an area of 1.3 cm^2 and a resistance, measured by impedance at high frequency, of 70–80 Ω.

Cyclic voltammetry curves were performed with a EG&G potentiostat using EG&G software. A Pt sheet was used as a counter electrode and saturated calomel (SCE) as the reference. Mott–Schottky plots were obtained from cyclic voltammetry using the relationship $C = i/\nu$, where ν is the scan rate. Flat band potentials were also evaluated from plots I_{ph}^2 versus potential, with the photocurrent I_{ph} obtained from chrono-potentiometry measurements. The electrolyte was 1M $NaClO_4$ (pH 6.8–7.0).

2.3. ESR spin trapping experiments

The ESR spin trapping experiments were performed with a Bruker 220 SE spectrometer, at a microwave frequency of 9.4 GHz. *N-tert*-butyl α-phenyl nitrone (pbn) was used as spin trap, because it does not absorb light at $\lambda > 350$ nm and has a good solubility in water. Aqueous suspensions of TiO_2 containing pbn (0.05 M), GO (1 μM) and alcohol (0.1 M when required), were put into a flat quartz cell and directly irradiated into the ESR cavity at $\lambda > 350$ with a Hg medium pressure lamp. No ESR signals were observed in the dark and during irradiation of solutions of pbn/alcohol/GO in the absence of TiO_2. The spectrometer has been calibrated with α,α′-diphenyl pycryl hydrazyl (dpph); for kinetic measurements the *g* factor value was 2.0007.

2.4. Photocatalytic apparatus

TiO_2 (3 g/L) was suspended in an aqueous solution (3 mL) containing 1,2-propandiol (0.005 M). When required, GO (1 μM) was added to the solution. The sample was put in a pyrex reactor, magnetically stirred and kept in the dark for 3 h in order to reach the adsorption equilibrium on the surface of TiO_2. The amount of adsorbed enzyme was calculated by measuring the protein concentration in the solution using the Bradford Reagent (Sigma). Then, the reactor was placed in a thermostated cell holder (308 K) and irradiated with a Hg medium pressure lamp Helios Italquartz (15 mW/cm^2) using a cut off filter (Coherent-Ealing, 26-4671) which leaves only wavelengths higher than 350 nm to reach the sample (emission lines of the light source are at 365, 405, 435, 546 and 577 nm), under a saturated atmosphere of O_2. During the photochemical experiments the pH value was 5.5–6.0. Blank experiments showed that no reaction occurred in the dark or irradiating the enzyme in the absence of TiO_2.

2.5. Determination of the oxidation products

The formation of oxidation products was evaluated by GC analysis (HP 6890), using a HP-5 (Crosslinked 5% PH Me Siloxane, 30 m, 0.32 mm of internal diameter of the column, 0.25 μm of stationary phase film thickness) capillary column and a FID detector. Quantitative analysis has been carried out with calibration curves obtained from authentic samples. After the photocatalytic experiment, products that remained eventually absorbed on the irradiated film were extracted with water (2× 3 mL) and GC analysis was carried out on those portions.

2.6. Glucose oxidase activity assay

The production of H_2O_2 in the dark was measured by the FOX1 method [28] and by following the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) [29] in an aqueous solution containing phosphate buffer 50 mM, pH 6, glucose 100 mM and aliquots of the solution (10–50 μL) extracted from the cuvette that contained the TiO_2 before and at the end of irradiation. One unit of enzymatic activity (U) corresponds to the production of 1 μmol/min H_2O_2 in the dark assay. The specific activity (U/mg) of GO was calculated on the basis of the enzyme quantity (mg) extracted from the irradiated cuvette (minus the fraction adsorbed on the TiO_2 layer) and used in the dark assay.

An analogous procedure has been followed for evaluating the effect of adsorption on the enzymatic activity of GO. In this case, we have compared the production of H_2O_2 in the following two systems: (i) GO (1 μM) and glucose (100 mM) in water and (ii) GO (1 μM), glucose (100 mM) in aqueous powder dispersion of TiO_2 (1 mg/mL).

2.7. Preparation of apo-glucose oxidase

Apo-GO was obtained by eluting GO (2.5 mL, 11.4 mg, previously unfolded in urea 8 M) on a PD10 column (Sephadex G-25 M, Pharmacia) with NaCl 50 mM. UV–vis spectra of the collected fractions were recorded to confirm the extrusion of FAD from the protein. The presence of the apo-protein in a particular fraction was evidenced by measuring its concentration using the Bradford Reagent (Sigma).

3. Results and discussion

3.1. Conduction band processes

This section presents the results of an electrochemical investigation on TiO_2 electrodes in the dark, in the absence and in the presence of GO. The focus is on O_2 reduction since this is generally the fundamental process that consumes conduction band electron in photo-catalytic systems, including the one we discuss in the next section.

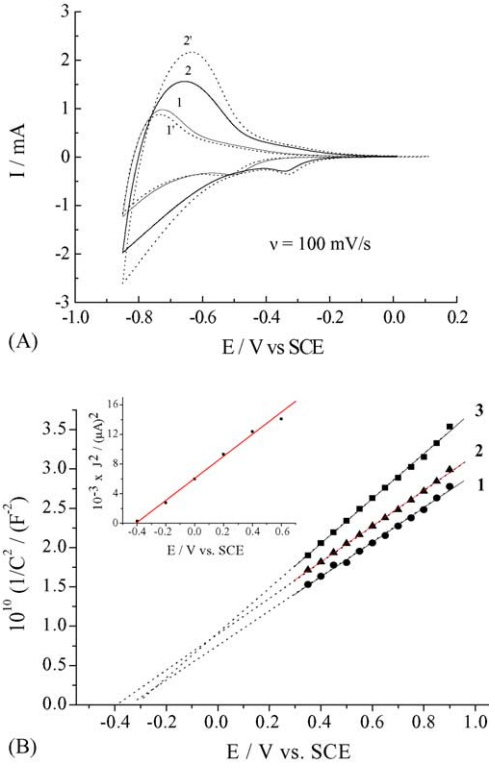


Fig. 1. (A) Cyclic voltammetry curves for a TiO_2 electrode in 0.1 M $NaClO_4$ deaerated solutions in the absence (1–1′) and in the presence (2–2′) of GO (1 μM). Scan rate: 100 mV/s. Curves 1′ and 2′ refer to cyclic voltammetry profiles after total 15 scans. (B) Mott–Schottky plots for a TiO_2 electrode in 0.1 M $NaClO_4$ with added GO (1 μM) or FAD (8 mM) from cyclic voltammetry data. Inset, evaluation of V_{fb} from plots of I^2_{ph} vs. E.

Fig. 1A shows cyclic voltammetry curves for TiO_2 in the dark, in the absence (1 and 1′) and in the presence of GO (2 and 2′). The electrodes we employed have an appreciable resistance, however, we do not aim at quantitative calculations. The curves are qualitatively similar but the stable voltammograms shows that the charge increases conspicuously and reproducibly in the presence of GO, indicating surface modification by the enzyme.

The features observed in this potential region can be attributed to adsorption–desorption processes involving hydrogen (Equation (3)):

$$H^+ + e^- \rightarrow H_{ads} \quad (3)$$

Since experiments have been done in deaerated unbuffered $NaClO_4$ solutions because the presence of, for example, phosphate buffer can interfere with the adsorption of the protein or/and of the flavin adenine dinucleotide (FAD) cofactor [30,31], one possibility is that hydrogen ions are made available by changes in the pH near the surface. On the one side, however, these possible pH changes should affect the voltammetry in opposite directions for the two cases without and with GO. On the other side, it is noteworthy that an increase in the voltammetry charge for Ti/TiO_2 electrodes has been reported [32] for non-aqueous solvents in the presence of trace water and

attributed to an enhanced contribution of a reaction of type (3). For the specific case of adsorbed proteins, Jackson et al. [33] earlier reported a similar behaviour as that seen in Fig. 1A (curves 2 and 2′) for adsorbed proteins, and ascribed it to the effect of protonated amino groups acting as a source of hydrogen.

In addition to the above results, important complementary information can be obtained from Mott–Schottky plots for TiO_2 in the absence and in the presence of GO and FAD. The data of Fig. 1B have been constructed from voltammetry experiments carried out in a potential interval (0.2–1.0 V) where faradaic processes of electron transfer do not take place and, consequently, the electrode works as a pure capacitor. Capacity can be calculated knowing that $C = i/\nu$, where i is the current in mA and ν is the scan speed in mV/s [34], and it is seen that both GO and FAD cause but a slight decrease of the capacity (by a factor <1.5). Data obtained by this procedure are confirmed, for bare TiO_2, by plots of the square of the photocurrent versus potential plots shown in the inset of Fig. 1B. In addition, analogous experiments carried out at pH 13, gave a value of V_{fb} of -0.78 V and, according with the known shift of 0.59 V/pH unit, one obtains a V_{fb} of -0.4 V at pH 6.5, in keeping with the conditions of Fig. 1B of the present work.

The value of the flat band potential derived by extrapolation from the plots of Fig. 1B is essentially the same within experimental error in the absence or in the presence of GO or FAD, suggesting that interactions between the adsorbed species and the TiO_2 surface are not strong.

Different authors have addressed the issue of protein interaction with surfaces, including TiO_2 and rather vast literature exists on this topic [35–38]. For a discussion on the nature of adsorption in the particular case of GO one should note, in particular, that the enzyme bears a slight negative charged at pH 5.5 [39,40]. At this pH, an electrostatic interaction with a positive excess charge of TiO_2 is possible (the PZC for Degussa P-25 lies in the region of 6.1–6.3). In our experimental conditions, however, the pH value is close to neutrality and we do not expect strong electrostatic interactions of the protein with the surface. An additional possibility is represented by the binding of carboxylate residues to Ti^{4+} sites [41,42]. This phenomenon may be one of the reasons for the slight decrease in the capacitance due to GO. Similar interactions of negatively charged phosphate groups of FAD with Ti^{4+} sites possibly explain the observed decrease in capacitance caused by the free cofactor. All of these mentioned interactions should be either not strong, or limited by the number of interacting groups, to explain the relative lack of an effect on the flat band potential.

The presence and role of negatively charged carboxylate groups is apparently in contrast to the presence of protons that we invoked above to explain the voltammetry behaviour of TiO_2 in the presence of GO (Fig. 1A). However, literature work reports that patches of positive and negative charge [43] can exist within the enzyme, and the presence of these

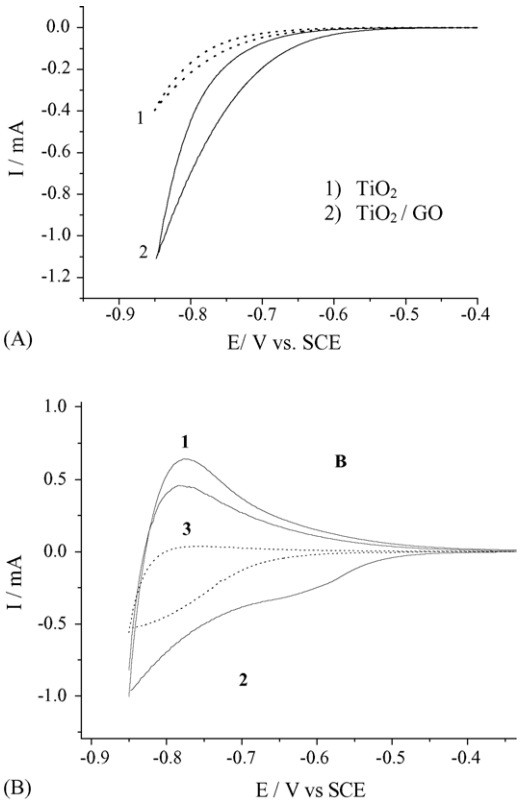


Fig. 2. (A) Cyclic voltammetry curves for a TiO_2 electrode in O_2 saturated 0.1 M $NaClO_4$ solutions in the absence (1) and in the presence (2) of GO (1 μM). Scan rate: 20 mV/s. (B) Cyclic voltammetry curves for a TiO_2 electrode in 0.1 M $NaClO_4$. (1) with GO (1 μM) in deaerated solution; (2) same as for curve (1) but after reduction of O_2; (3) without GO after reduction of O_2. Scan rate: 100 mV/s.

different sites can provide an explanation of the electrochemical data reported in the present work.

Typical cyclic voltammetry curves for the reduction of O_2 with TiO_2 electrodes in the absence and in the presence of GO are shown in Fig. 2A. Two main effects demonstrate that the electrocatalytic activity of TiO_2/GO in the reduction of O_2 is significantly higher than that of TiO_2: (i) GO causes a significant increase of the cathodic current; (ii) an anodic shift > 100 mV is also observed in the presence of the enzyme. The voltammetry curve identified as (2) in Fig. 2B is obtained at a TiO_2 electrode following O_2 reduction experiments and re-deaeration. In the presence of GO, we observe that a new cathodic peak appears which can be ascribed to the accumulation of H_2O_2 in the solution bulk, as verified from measurements with purposely added H_2O_2 [44].

Since the cofactor flavin adenine dinucleotide is the relevant redox center in GO, we carried out some measurements aiming to assess, in particular, its possible involvement in the O_2 reduction process. The ability of FAD to undergo direct electron transfer at a number of electrode surfaces, including TiO_2, is well documented [30,31]. To our knowledge, only one recent article describes an enhanced O_2 reduction at FAD modified [45]. However, in that

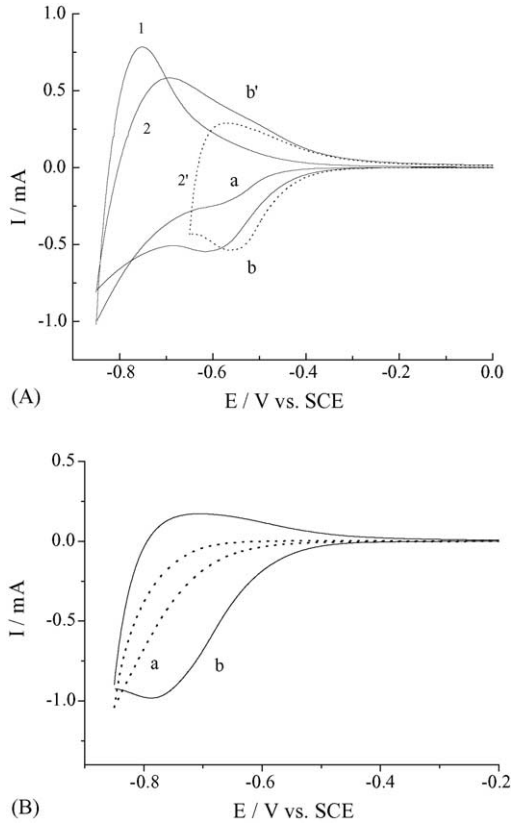


Fig. 3. (A) Cyclic voltammetry curves for a TiO_2 electrode in deaerated 0.1 M $NaClO_4$ solutions in the absence (1) and in the presence (2) of FAD (8 mM). 2′ same as for curve 2 with less negative potential limit. Scan rate: 100 mV/s. (B) Cyclic voltammetry curves for a TiO_2 electrode in O_2 saturated 0.1 M $NaClO_4$ solution: (a) in the absence and (b) in the presence of FAD (8 mM). Scan rate: 100 mV/s.

investigation FAD is electro-polymerized to form a film on a glassy carbon electrode, and effects of this polymerization are not discussed by the authors of the cited paper.

Fig. 3A shows that under our experimental conditions, the cyclic voltammetry curves for FAD under anaerobic conditions (2 and 2′) are characterized by the presence of anodic and cathodic peaks (b and b′), with the latter better defined at approximately −0.55 V. From experiments at different sweep rates (ν) we found that the current for the cathodic peak (b) depends linearly on ν thus indicating that the redox processes entails that FAD is adsorbed. This fact is in agreement with studies by Kubota et al. [31] on the adsorption of FAD and FMN on carbon fibers supported TiO_2 particles. In their detailed investigation, these authors proposed a bidentate side-on interaction of the cofactor with the oxide involving the oxygens of the phosphate groups.

Effects of FAD on the electrocatalytic efficiency of TiO_2, for O_2 reduction are considerably less pronounced than those observed with the enzyme itself (Fig. 3B) and, accordingly, no evidence of H_2O_2 accumulation, as in the case of TiO_2/GO has been found in the presence of FAD alone. Likewise, the enzyme deprived of FAD (apo-GO) was unable to improve the electrocatalytic efficiency of the semiconductor. This allows us to exclude that the GO mediated reduction of O_2 is due to some non-catalytic effect of the peptidic moiety of the protein which evidently has an important role in the mediated reduction of O_2.

To summarize results described above, we infer that both the FAD cofactor and the proteic moiety of the enzyme play a specific role in the electrocatalytic activity of the TiO_2/GO: the redox activity of the first is strongly affected by the second.

Direct electron transfer between semiconductors and adsorbed enzymes has been already observed [46]. This was always in the case of small redox proteins having an accessible prosthetic center. Since the FAD cofactor of GO is deeply buried inside the insulating structure of the protein its direct interaction with the surface of TiO_2 particle is unlike, unless structural deformations of the protein and partial exposition of the cofactor occur as a consequence of the adsorption on the semiconductor surface. Already in the dark we expected that soft proteins, such as GO, undergo significant structural deformations at hydrophilic surface/water interfaces [47]. Moreover, direct electron transfer between GO and a gold electrode surface has been observed as a consequence of its adsorption induced deformations [39,40]. On the other side, GO has been reported to maintain its enzymatic activity following electrochemistry at a platinum electrode [40]. Enzymatic activity measurements performed on our composite system confirm that adsorption on TiO_2 causes significant conformational deformations of GO, observing that it does not remain fully active once immobilized. In particular, the specific activity of GO towards its natural substrate glucose was found to decrease of about 15% in the presence of TiO_2 powder in the dark.

3.2. Alcohols oxidation

The ability of GO to mediate O_2 reduction at TiO_2 electrodes has been demonstrated in the previous paragraph. This is a key process under photocatalytic conditions since the reaction rates of electron and hole consumption must be the same at steady-state in order to maintain the electroneutrality of the TiO_2 particles, and efficient capture of the photogenerated charges (Equation (1)) by electron donors and/or electron acceptors is required in order to avoid recombination. It is also understood that information on the fate of photogenerated positive holes is important in the elucidation of the mechanism of a photocatalytic reaction.

Since pathways involving holes generally entails the formation of radicals through single electron oxidation processes, the ESR-spin trapping technique turns out to be a particularly potent tool of investigation because it allows the detection of short lived radical species [48–50]. Typical experiments were carried out irradiating powder dispersions of TiO_2 in water containing the spin trap α-phenyl *N-tert*-butyl nitrone (pbn, 0.05 M) and GO (1 μM, when required). The formation of radical species ($R^{\bullet}$) could be monitored by

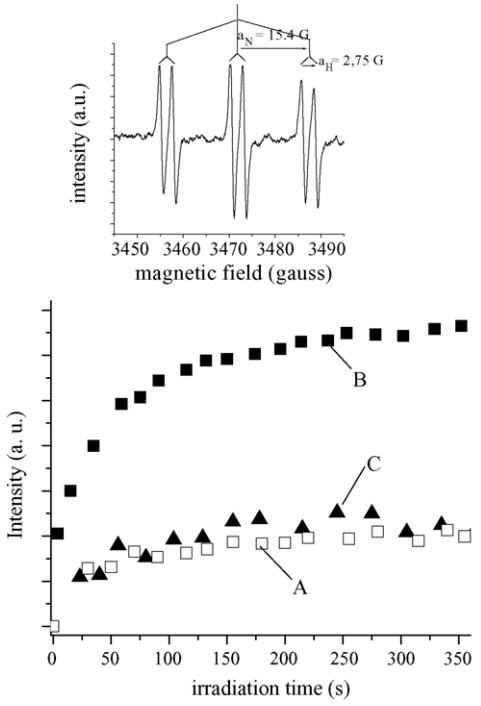
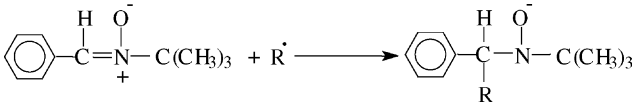


Fig. 4. ESR signal intensity of the adduct pbn-OH• as a function of time at a fixed field position obtained irradiating ($\lambda > 360$ nm): (A) TiO_2 suspension in water containing pbn (0.05 M). (B) As (A) but in the presence of GO (1 μM). (C) As (A) but in the presence of apo-GO (1 μM). Inset: ESR spin trapping spectrum of the paramagnetic adduct pbn-OH. a_N and a_H are the hyperfine splitting constants of the paramagnetic adduct due to the interaction of the unpaired electron with N and H nuclei, respectively, which have no null nuclear spin.

detecting the more stable paramagnetic nitroxides, which are formed according to Equation (4).



(4)

We obtained a spectrum which consists of a triplet of doublets (hyperfine splitting constants: $a_N = 15.4$ G, $a_H = 2.75$ G; Fig. 4, inset). A number of literature data indicate that this spectrum can be ascribed to the paramagnetic adduct pbn-OH• [33]. In particular, the values of the coupling constants fit very well into the equation $a_H = 0.604a_N - 6.53$ given by Janzen et al. for the pbn-OH• adduct in various reaction environments [51]. The trapping of OH• radicals by nitrones formed upon irradiation of TiO_2 has been already investigated in previous works [52–55].

The growth of the ESR signal of the adduct between the primary radical species and pbn as a function of time can be followed at a fixed magnetic field. In previous papers, we demonstrated that the rate of formation of this adduct can be considered proportional to the oxidation rate of the substrate by photoexcited TiO_2 [49,52]. In fact, the presence of a large excess of spin trap warrants a constant percentage of trapping, although the true radical concentration may be higher than the spin concentration derived from the intensity of the signal.

A comparison between curves A and B of Fig. 4 indicates that the formation rate of the adduct pbn-OH• increases significantly in the presence of GO. Curve C shows that the enzyme deprived of FAD does not affect the formation rate of the paramagnetic adduct. A plausible explanation of these results is that the previously discussed ability of GO to improve kinetics of O_2 reduction may inhibit charge recombination processes in photoexcited TiO_2 particles, thus favoring the subsequent oxidation of OH^- to OH• by the positive holes. However, another possible source of OH• radicals is the well known reaction between e_{cb}^- and H_2O_2 accumulated in solution during the TiO_2-assisted reduction of O_2, as pointed out by the electrochemical measurements.

The results so far discussed provide evidence that the catalytic activity of GO enhances the amount of OH• radicals photogenerated by TiO_2. Therefore, we expected that the presence of the enzyme may also affect the photocatalytic properties of this semiconductor towards organic substrates that can be oxidized by these reactive intermediates. We have previously found that GO has a marked positive effect on the oxidation of 2-propanol [22]. Herein, this study is extended to other alcoholic substrates.

Typical ESR spin trapping experiments were performed irradiating powder dispersions of TiO_2 in water solutions containing pbn (0.05 M), an alcohol (0.1 M) and GO when required (1 μM). Fig. 5 reports the growth of the ESR signals of the adducts between alcoxy radicals and pbn for methanol (Fig. 5A) and ethanol (Fig. 5B) both in the absence and in the presence of GO. For comparison we report as Fig. 5C the results obtained with 2-propanol already published in Ref. [22]. We observe that in all cases the formation rate of the adducts between pbn and the alkoxy radicals increases significantly in the presence of GO, thus indicating that in any case the enzyme has a positive effect on the oxidation rate of the alcoholic substrate to the corresponding radical species.

The presence of GO is also expected to affect the yields of final oxidation products of the alcoholic substrates. We have already verified that this enzyme has a positive effect on the oxidation of 2-propanol to acetone [22]. The results presented in the following on the oxidation of 1,2-propandiol, a simple molecule bearing two alcoholic functionalities with different reactivity, turned out to be particularly interesting for the elucidation of the photocatalytic properties of the TiO_2/GO composite system.

In a typical experiment TiO_2 (3 mg/mL) was suspended in a water solution containing the diol (0.005 M), GO or apo-GO (1 μM, when required) and irradiated ($\lambda > 360$ nm) for 180 min. The stability of GO was tested at the end of the photocatalytic experiments assaying its specific activity in the presence of glucose in aliquots of the solution extracted from the reaction vessel. This activity was found to decrease

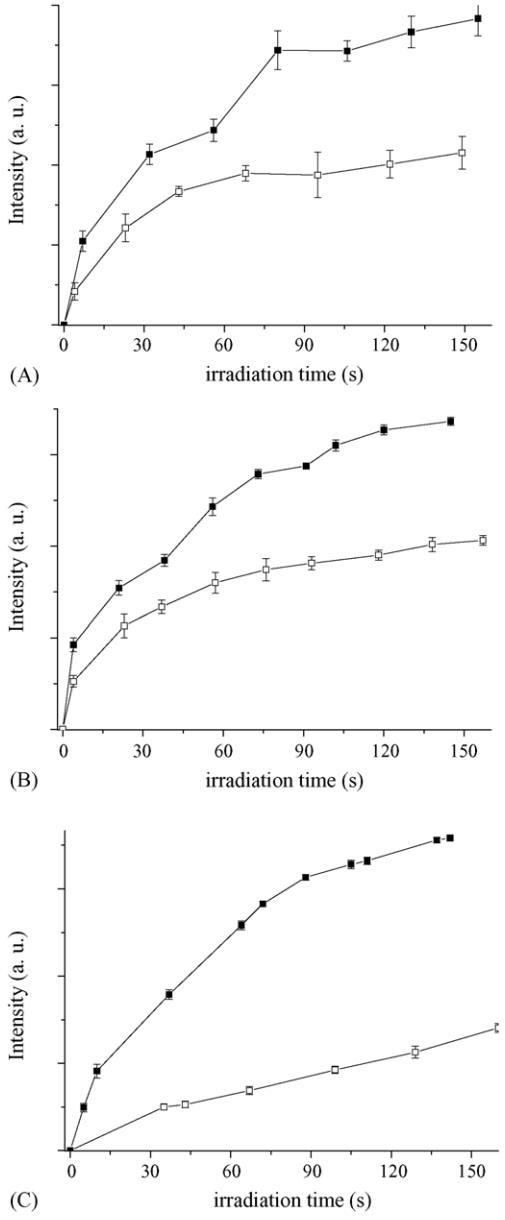


Fig. 5. ESR signal intensity of the adducts pbn-RO$^{\bullet}$ as a function of time at a fixed field position obtained irradiating ($\lambda > 360$ nm) TiO_2 suspensions in water containing pbn (0.05 M), alcohol (0.1 M) and GO (when required, 1 μM, full squares). (A) Methanol; (B) ethanol; (C) 2-propanol (already published in Ref. [22]). Reported values are the mean of three repeated experiments.

from 105 to 35 U/mg during 180 min irradiation, indicating that the enzyme underwent a slow inactivation.

Table 1 shows the results of the GC analysis of the irradiated samples. It can be clearly seen that, in agreement with the ESR spin trapping experiments, the TiO_2/GO composite system is more efficient than TiO_2 alone (see first column). Last row of Table 1 indicates that GO plays its catalytic role only when it is in its native form. This finding is in keeping with the results discussed thus far.

Complete mineralization of most organic compounds to carbon dioxide and water is typical of illuminated TiO_2, on account of the high oxidizing power of this semiconductor. The results reported in Table 1 show that a second significant effect of the enzyme is that of preventing this mineralization process, favoring, on the contrary, accumulation of partially oxidized intermediates (Scheme 1). In fact, hydroxyacetone and pyruvic acid are formed with a mass balance of 100% with the composite system, while these products were only 30% of diol disappeared with TiO_2 alone. An intermediate value of 62.5% is obtained with the TiO_2/apo-GO system.

Concerning the distribution of oxidation intermediates, it is evident from an inspection of Table 1 that TiO_2 and TiO_2/GO present conspicuous differences. In fact, while the formation of pyruvic acid is favored with TiO_2, leading to a ratio between hydroxyacetone and pyruvic acid of 0.45, in the composite system a reverse selectivity is found with a ratio of 2.7. Again, an almost intermediate value of 1.1 is obtained with the TiO_2/apo-GO system.

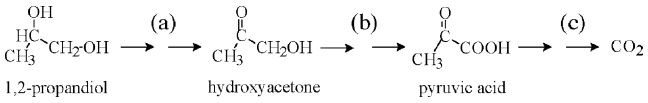
The observed influence of GO on efficiency and selectivity of the photocatalytic process may be ascribed, at least in part, to its ability to favor the formation of OH$^{\bullet}$ radicals. These species are able to initiate the oxidation of the secondary alcoholic group of the substrate considering that the possible rearrangement of the initially produced alcoxy radical leads to a stable, tertiary hydroxyalkyl radical. Accordingly, photoexcitation of TiO_2/GO leads to the formation of hydroxyacetone in larger amounts than TiO_2 alone and TiO_2/apoGO (Scheme 1a).

Another possible effect of GO on the selectivity of the photocatalytic process is its ability to control surface reactions, modifying adsorption–desorption equilibria of reagents and reaction intermediates. In fact, the protein may inhibit the hydroxyacetone adsorption, which is expected to

Table 1
1,2-Propandiol conversion and product distribution with photoexcited[a] TiO_2, TiO_2/GO and TiO_2/apo-GO

Photocatalytic system	Diol transformed[b] (%)	Mass balance[c] (%)	Product distribution (mM)[d]	
			Hydroxyacetone	Pyruvic Acid
TiO_2	48	30.4	0.23	0.50
TiO_2/GO (1 μM)	63	100	2.30	0.85
TiO_2/apo-GO (1 μM)	40	62.5	0.65	0.60

[a] TiO_2 (3 mg/mL) were immersed in a 3 mL water solution containing 1,2-propandiol (5×10^{-3} M), GO or apo-GO (1 μM, when required) and 180 min irradiated with a Hg medium pressure lamp (15 mW/cm^2, $\lambda > 360$ nm) in the presence of 760 Torr of O_2.
[b] Percentage of disappeared 1,2-propandiol.
[c] Ratio (%) between the sum of hydroxyacetone and pyruvic acid concentrations and the concentration of disappeared 1,2-propandiol.
[d] Each reported value is the mean of three repeated experiments.



Scheme 1.

be the necessary condition for inducing the oxidation of its primary alcoholic group to give pyruvic acid (Scheme 1b) [56]. Of course, the detachment of partially oxidized intermediates from the surface of the illuminated semiconductor may also avoid their subsequent mineralization to carbon dioxide and water (Scheme 1c).

4. Conclusions

Electrochemical measurements show that the enzyme GO interacts with the surface of TiO_2 and significantly improves the two electron reduction of O_2 to H_2O_2. Further electrochemical reactions involving H_2O_2 are apparently inhibited since it is found to accumulate in solution. A different oxidation pathway can be envisaged where oxidation reactions involving H_2O_2 in solution take place without a direct involvement of the semiconductor holes, which are the main responsible of mineralization processes. Again from the point of view of reaction mechanism, investigations by ESR spin trapping show that OH and alkoxy-radicals formation is enhanced in the presence of GO.

Under steady state illumination the TiO_2/GO composite system features an important change in the reactivity of 1,2-propandiol compared to TiO_2 used alone, in that: (i) formation of intermediate oxidation products with no mineralization of the substrate is observed under our laboratory experimental conditions; (ii) the photo-oxidation process becomes markedly regioselective.

The ability of the enzyme to tune the high oxidation power of the semiconductor is an important characteristic of the composite system investigated in this work, especially when the interest is focused on synthesis rather than detoxification processes.

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