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Fluorescein as a visible-light-induced oxidase mimic for signal-amplified colorimetric assay of carboxylesterase by enzymatic cascade reaction

Li Liu,[a,b]Chaoqun Sun,[a] Juan Yang,[a] Ying Shi[a], Yijuan Long[a], and Huzhi Zheng*[a]

Abstract: We found that fluorescein possessed high visible-light-induced oxidase mimetic activity and could transform colorless 3,3′,5,5′-tetramethylbenzidine (TMB) into blue oxidized TMB (oxTMB) without unstable and destructive H_2O_2 under visible light illumination. Instead, fluorescein uses oxygen as a mild and green electron acceptor, and its activity can be easily controlled by the switching "on/off" of visible light. In addition, the visible-light-induced catalytic mechanism was elucidated in detail and, as the main reactive species h^+ and $O_2^{\bullet-}$ accounted for TMB oxidation. Based on the fact that fluorescein diacetate (FDA) possessed no activity and generated active fluorescein *in situ* in the presence of carboxylesterase (CaE), a signal-amplified sensing platform through cascade reaction for CaE detection was constructed. Our proposed sensing system displayed excellent analytical performance for the detection of CaE in a wide linear range from 0.040 to 20 U/L with a low detection limit of 0.013 U/L. This work not only changes the conventional concept that fluorescein is generally considered to be photocatalytic inert, but also provides a novel sensing strategy by tailoring the enzyme mimetic activity of fluorescein derivatives with analyte.

Introduction

Recently, considering the intrinsic drawbacks of natural enzymes (*e.g.* horseradish peroxidase,HRP), such as high cost in large scale production, poor stability of catalytic activities against denaturation, digestion or the variations of environmental conditions,[1] researchers have therefore endeavoured to develop artificial enzymes such as nanozymes[2], metal-organic frameworks[3] and small molecule-based mimic peroxidises[4], as viable alternatives. Although these artificial peroxidases exhibit some advantages over HRP, for example, low in cost, easy to prepare, high stability and so on,[1-4] most of them need destructive and unstable H_2O_2 as an oxidant to obtain high catalytic activity, which may become obstacle to further applications. More significantly, owing to lack of simple, green and cost-effective means to modulate the catalytic activity of nanozymes, it is highly desired to exploit new class of enzyme mimics that their activities can be easily modulated without the aid of H_2O_2.

In response to demand, Wang et al.[5-9] have exploited several nanomaterials to mimick oxidase that make use of visible light as a simple and rapid mean to tune the oxidase-mimicking activity, which ignites the exploration of photocatalytic enzyme mimics. Unfortunately, most previous pioneering works focus on nanozymes whose preparation involve organic solvents or surfactants as toxic sources, rigorous conditions (e.g. a high reaction temperature, high pressure) or complex synthesis procedures, which may hinder in the large-scale production and widespread practical applications. Thus, it is imperative to develop new types of photoinduced oxidase mimics possessing new properties and potentials.

Fluorescein has been exploited in various areas for instance ophthalmology, bioimaging and biosensing for their versatile fluorescent properties.[10] Nevertheless, the applications of fluorescein are mainly concentrated on their fluorescent properties[11] and enhancer effect on the chemiluminescence[12]. To the best of our knowledge, there has been not any study devoted to the photocatalytic property as mimetic oxidase so far.

In this paper, excitingly, the novel visible-light-induced oxidase-mimicking activity of fluorescein was unprecedentedly found. Upon visible light irradiation, fluorescein can catalyze the oxidation of typical enzyme catalytic chromogenic substrates such as 3,3',5,5'-tetramethylbenzidine (TMB), o-phenylenediamine (OPD) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) leading to the typical color changes, and its function was the same as natural oxidase. What is more, fluorescein exhibits a higher affinity to TMB in comparison with HRP.

On the basis of fluorescein diacetate (FDA) without oxidase-like activity and fluorescein, *in situ* generated by carboxylesterase (CaE) catalyzing FDA hydrolysis, possessing excellent photocatalytic capability to transform colorless TMB into blue oxidized TMB (oxTMB), a signal amplification sensing strategy through cascade reaction for CaE detection was elaborated (illustrated in Scheme 1). The limit of detection reached to 0.013 U/L and the practicability of the sensing platform was also validated in biological samples. In the view of low cost, commercial availability FDA and robust, excellent photocatalytic fluorescein, our proposed protocol is simple, cheap, and easy to perform. To the best of our knowledge, for the first time we discovered that organic small molecules possess intrinsic visible-light-induced oxidase-mimicking activity and this is the first instance of colorimetric strategy for CaE detection. Meanwhile, this work also offers a new insight into the application of fluorescein derivatives to develop colorimetric biosensors based on the analyte tuned visible-light-induced oxidase-mimicking activity.

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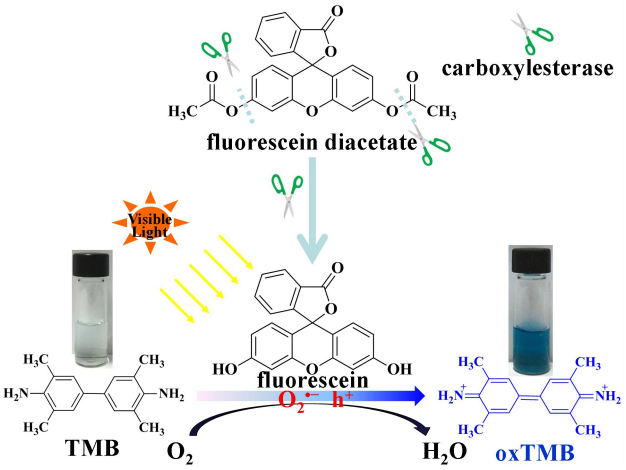


Scheme 1 The proposed principle for CaE detection based on cascade reaction.

Results and Discussion

Visible-light-induced oxidase-mimicking activity of fluorescein

Firstly, the visible-light-induced oxidase-mimicking activity of fluorescein was evaluated by the catalysis of a typical oxidase chromogenic substrate TMB. In the presence of visible light irradiation, fluorescein could catalyze the oxidation of TMB and produce a deep blue color, with maximum absorbance at 370nm and 652 nm (Figure 1A, red line). In contrast, neither the mixture of fluorescein and TMB without visible light irradiation nor TMB alone irradiated by visible light without fluorescein exhibited any color variation (Figure 1A, black line and blue line). Apparently, these results above confirmed that fluorescein possessed visible-light-induced oxidase-mimicking activity.

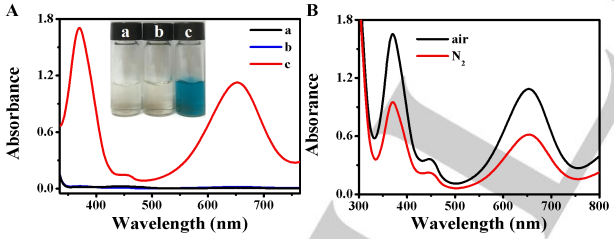


Figure 1. (A) UV-vis absorbance spectra of different reaction systems: (a) 0.50 mM TMB (black line) under visible light irradiation, (b) 1.0 μM fluorescein + 0.50 mM TMB without visible light irradiation (blue line) and (c) 1.0 μM fluorescein + 0.50 mM TMB under visible light irradiation (red line) in HAc-NaAc buffer (0.10 M, pH 4.0). The insert shows the corresponding optical photographs. (B) The absorbance spectra of TMB oxidation catalyzed by illuminated fluorescein conducted in air saturated solution (black line) and nitrogen saturated solution (red line).

Oxidase can utilize O_2 as oxidant to catalyze an oxidation-reduction reaction. As showed in Figure 1B, the catalytic activity of fluorescein was inhibited in the N_2-saturated reaction system, further validating its oxidase-mimicking property. Different from the characteristic that peroxidase always requires the help of high concentration H_2O_2 to achieve high catalytic activity, [13] fluorescein employs greener dioxygen as oxidant to replace destructive H_2O_2 and the photocatalytic activity can be easily tuned by the on/off state of the light.

To further verify the visible light induced oxidase-mimicking activity of fluorescein, we carried out the experiments using other enzymatic substrates, *i.e.*, OPD and ABTS to replace TMB, producing the typical yellow or green color with characteristic absorption peak at 448nm or 417 nm (Figure S1), respectively, which indicated that the oxidase-mimicking activity of fluorescein was not only restricted to TMB.

Influence factors on the visible-light-induced oxidase-mimicking activity of fluorescein

The relative catalytic activity of fluorescein was, like natural enzymes, dependent on pH and substrate concentration. The optimal pH and the substrate (TMB) concentration were 4.0 and 0.50 mM, respectively (Figure S2A and B). As shown in Figure S3C, the absorbance increased depending on the added amount of fluorescein, suggesting that the catalysis was catalyst-concentration-dependent. Figure S2D showed a good linear relationship between absorbance and fluorescein concentration over the range from 1.0 nM to 1.0 μM.

Catalytic mechanism of fluorescein as artificial oxidase

In order to elucidate the reactive species generated in the catalytic reaction induced by visible light, different quenchers that can scavenge the relevant reactive species including hydroxyl radicals (•OH), singlet oxygen ($^1O_2$), superoxide anions ($O_2^{\bullet-}$) and photo-generated holes (h^+) were added to the catalytic system. As illustrated in Figure S3, almost no change in the absorbance of the oxTMB can be observed after adding •OH scavenger isopropanol (IPA) or tert-butanol (TBA)[8]. It indicated that •OH could not be generated in the catalytic reaction. Catalase, an efficient enzyme for decomposing of H_2O_2 to H_2O and O_2,[8] could not affect the photocatalytic activity, indicating that H_2O_2 was not produced by illuminated fluorescein. On the contrary, the catalytic activity of fluorescein is apparently suppressed in the presence of h^+ scavenger like ethylenediaminetetraacetic acid (EDTA)[8],ammonium oxalate (AO)[14] or KI[8], indicating that h^+ is one of the main oxidative species. N_2 was purged in the reaction system to perform TMB oxidation under an oxygen free atmosphere. The catalytic activity of fluorescein decreased obviously under the oxygen free condition, indicating dissolved oxygen in solution plays an important role in the photostimulated catalytic oxidation of TMB by fluorescein (Figure 1B and S3A). As illustrated in Figure S3A, the absorbance didn't decrease obviously in the presence of NaN_3, which was utilized to scavenge $^1O_2$ [15]. It indicated that $^1O_2$ was not mainly reactive oxygen species (ROS) in the catalytic reaction. In addition, the absorbance dramatically decreased after adding superoxide dismutase (SOD), which was utilized to effectively scavenge $O_2^{\bullet-}$,[16] showing that $O_2^{\bullet-}$ radicals may be also main reactive specie during the catalytic process. Because TMB, OPD or ABTS reacts with p-benzoquinone (BQ), a typical $O_2^{\bullet-}$ scavenger,[17,18] methyl orange (MO) in place of these substrates is usually used to evaluate the catalytic

performance of photocatalysts[19]. The visible-light-induced MO oxidative degradation catalyzed by fluorescein was considerably suppressed after the introduction of BQ (Figure S3B), suggesting that $O_2^{\bullet-}$ plays a key role in this catalytic system. As the above results demonstrated that h^+ and $O_2^{\bullet-}$ as dominant reactive species could be responsible for the TMB oxidation catalyzed by fluorescein under visible light illumination.

Electron spin resonance (ESR) spectroscopy accepted as powerful and direct detection method of ROS was used to further confirm the ROS involved in the oxidation, unequivocally with DMPO as spin traps for $O_2^{\bullet-}$ [20]. ESR signal cannot be observed from the reaction between fluorescein and DMPO without visible light irradiation (Figure 2). Due to concomitant exposure of DMPO and fluorescein to visible light for 5 min, $O_2^{\bullet-}$ was generated, as verified by a strongly characteristic peak of the typical DMPO-$O_2^{\bullet-}$ adduct with an intensity ratio of 1:1:1:1[20](Figure 2), demonstrating that fluorescein could catalyze O_2 to generate $O_2^{\bullet-}$ radicals, similar to the case of oxidase[21]. The intensity of these ESR signals enhanced when visible light irradiation time increased to 10 min (Figure 2). All experimental results mentioned above provide a direct evidence to support that fluorescein catalytically generates $O_2^{\bullet-}$ under visible light illumination and the quantity of $O_2^{\bullet-}$ generated depends on the irradiation time.

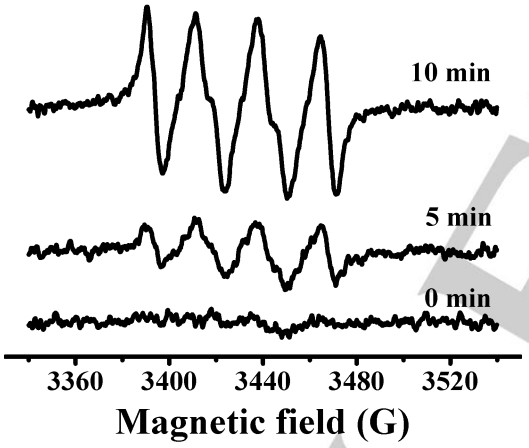
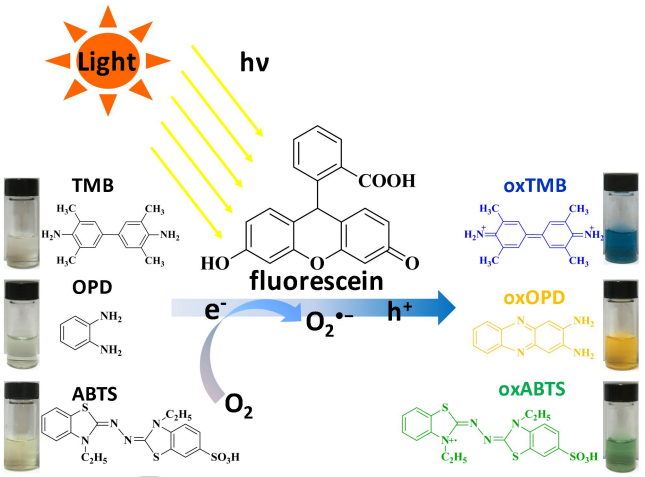


Figure 2. Time-dependent changes in the ESR spectra of 10 μM fluorescein with 50 mM spin trap DMPO after being photoirradiated with visible light for 0, 5 or 10 min.

Fluorescein has high molar extinction coefficient (ε_{490} = 8.8 × 10^4 cm^{-1} M^{-1}) and absorbs light in the visible regime.[22] When fluorescein is irradiated by visible light, it absorbs photons and results in the corresponding generation of electrons in the lowest unoccupiedmolecular orbital (LUMO) and leaving h^+ in the highest occupied molecular orbital (HOMO) to react with TMB directly. On the other hand, the photogenerated electrons on LUMO of fluorescein might be trapped by dissolved oxygen (as an electron acceptor), forming $O_2^{\bullet-}$ radicals. To validate the photoinduced charge transfer of fluorescein, the experiment of photoelectrochemistry was conducted. The rapid rise of the photocurrent of fluorescein upon illumination with a reproducible response to on/off cycles (Figure S4) is indicative of the effective charge generation, separation, and transfer process. On the basis of the above results, the schematic illustration of catalytic oxidation of TMB by photoactivated fluorescein is shown in Scheme 2.



Scheme 2 Proposed mechanism for visible light stimulated oxidase-mimicking activity of fluorescein

The oxidation and reduction potential of fluorescein is 0.87 and -1.18 V *vs.* saturated Ag/AgCl, respectively,[23] thus HOMO and LUMO level was calculated to be 1.07 and -0.98 V versus standard hydrogen electrode (NHE). Because the HOMO level of fluorescein was more positive than that of TMB (rang from 0.22 to 0.70 V *vs.* NHE), [24,25] the photogenerated h^+ can oxidize TMB directly. In addition, the LUMO level of fluorescein was more negative than that of oxygen (-0.046 V *vs.* NHE).[26] Therefore, dissolved oxygen can accept the excited electrons of fluorescein to generate $O_2^{\bullet-}$. All these results provided direct evidence supporting that fluorescein acted as a functional mimic of oxidase.

Steady-state kinetic analysis

Next, the visible-light-stimulated oxidase-mimicking activity of fluorescein was investigated according to steady-state kinetic analysis. As shown in Figure 3, typical Michaelis-Menten curves were observed and a series of the steady-state kinetic parameters (K_m: Michaelis-Menten constant; V_{max}: maximum initial velocity; K_{cat}: catalytic constant, where [*E*] is fluorescein concentration; and K_{cat}/K_m: catalytic efficiency) were calculated using the Lineweaver-Burk equation. The K_m is an indicator of the binding affinity between enzymes and substrates, and a high K_m value represents a weak affinity whereas a low value suggests a high affinity.[27] As summarized in Table 1, the K_m value of fluorescein as peroxidase mimic was 8.3 fold larger than that of it as oxidase mimic, indicating that fluorescein as visible light induced artificial oxidase has a higher affinity to TMB than that of it as artificial peroxidase. Quite impressively, the K_m value of fluorescein as mimic oxidase, was much lower than that of HRP (K_m = 0.434 mM^{-1})[13], indicating that it had a higher affinity to TMB than that of HRP. Additional, the maximum initial velocity (16 fold), catalytic constant (1.6×10^3 fold) and catalytic

efficiency (1.3×10^4 fold) of the visible-light-induced oxidase mimetic fluorescein was higher than that of peroxidase mimetic fluorescein, demonstrating that fluorescein has high catalytic activity under visible light irradiation.

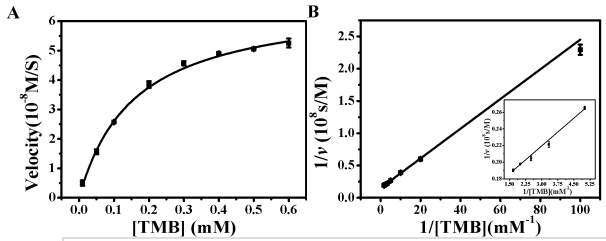


Figure 3. Steady-state kinetic assay (A) and double reciprocal plots (B) of fluorescein as oxidase mimic under visible light illumination using TMB as the substrate. The velocity (v) of the reaction was measured using 1.0 μM fluorescein and TMB concentration was varied in HAc-NaAc buffer (0.10 M) at pH 4.0. Error bars represent the standard deviation of three repeated measurements.

Table 1. Comparison of the K_m, V_{max}, K_{cat} and K_{cat}/K_m of fluorescein as oxidase mimic, fluorescein as peroxidase mimic.

Catalyst	Concentration[μM]	K_m[mM]	V_{max}[10^{-8} M·s^{-1}]	K_{cat}[10^{-2} s^{-1}]	K_{cat}/K_m[M^{-1}·s^{-1}]	Reference
Fluorescein as oxidasemimic	1.0	0.158±0.009	6.717±0.117	6.717	0.425	This work
Fluorescein as peroxidase mimic	100	1.31±0.129	0.418±0.011	4.18×10^{-3}	3.19×10^{-5}	[4]

The working principle of CaE sensing

We found the functional group changes of fluorescein may significantly impact on its catalytic performance. After introducing carboxylic ester groups into xanthene ring of fluorescein skeleton, the visible light-induced catalytic activity is lost (Figure S5A) due to the lack of light absorption in the visible region (Figure S5B). In virtue of these attractive features, it is easy and flexible to take advantages of functional groups-mediated photocatalytic activity to rationally design some facile, label-free colorimetric platforms. Based on this scenario, CaE, an enzyme specific for cleavage of the carboxylic ester bond, and its substrate FDA were employed as proof-of-concept to *in situ* regulate the catalytic capability of fluorescein derivatives. Typical routes to detect CaE have been established by virtue of fluorescein-release to induce the recovery of fluorescence signal.[28-34] In our work, we proposed an enzymatic cascade reaction colorimetric strategy for CaE detection using FDA as an analyte-active probe, which could initiate the fluorescein-catalyzed chromogenic reaction of TMB to act as an amplifier. Thus, a high sensitivity towards CaE could be anticipated.

As illustrated in Scheme 1, CaE catalytically hydrolyzes inactive FDA to yield active fluorescein and then the *in situ* produced fluorescein under visible light irradiation catalyzescolorless TMB oxidation to form blue oxTMB, which allows visual or spectroscopic detection. In order to examine the feasibility of the designed enzymatic cascade reaction for CaE sensing, the absorption spectra of the mixture of TMB and FDA in the absence and presence of CaE under visible light irradiation were investigated. Just as expected, the solutions without CaE show almost colorless solution and no obvious absorption (inset and curve “a”, Figure 4). Upon addition of CaE, the color of TMB changes from colorless to blue, which is reflected by absorption spectra (inset and curve “b”, Figure 4). On account of the fact that the linear relationship between absorbance and fluorescein concentration (Figure S2D) and the content of fluorescein controlled by CaE concentration, the resulting absorbance changes at 652 nm relates directly to CaE concentration (Figure 5). The above-mentioned results verified the feasibility of the sensing strategy. Notably, both visible light irradiation and *in situ* produced fluorescein through enzymatic cascade reaction are very vital to endow fluorescein with enhanced catalytic efficiency, thus our proposed sensing platform can exhibit powerful signal amplification performance.

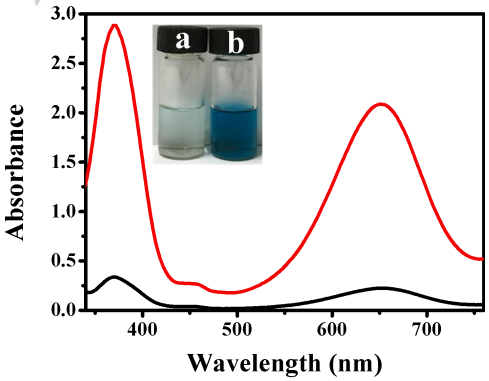


Figure 4. Absorption spectra of TMB-FDA systems in the absence (a) and presence (b) of 10 U/L CaE after 10 min visiblelight irradiation. Reaction condition: 0.50 mM TMB, 5.0 μM FDA, 0.10 MHAc-NaAc buffer (pH 4.0). Inset showed the color change of the corresponding samples.

Optimization of the sensing conditions.

In order to obtain the optimal performance of the CaE sensing system, the effect of pH and FDA concentration was investigated. As depicted in Figure S6A, absorbance first enhanced accompanied by the increase of pH from 1.0 to 4.0, and got a maximum at pH 4.0, then decreased with further increasing pH value. So, pH 4.0 was selected as the optimal pH value for subsequent experiments. As displayed in Figure S6B, absorbance showed a rising tendency and achieved a plateau once the FDA concentration was higher than 5.0 μM. Therefore,

the optimal concentration of FDA was 5.0 μM for CaE detection. While for convenience, the subsequent experiments were conducted at room temperature (25 °C).

Analytical performance of CaE detection

As can be seen from Figure 5, along withthe augment of CaE concentration the absorbance increases. As expected, the blue color deepened as the CaE concentration increased, which could be observed directly by the naked eye (inset in Figure S5B,). Figure S7 depicted the corresponding calibration plot and indicated that absorbance is linear with the logarithm of CaE concentration. The linear equation is A = 0.7801 lg[CaE] + 1.4595 (R^2 = 0.9971) in the range from 0.040 to 20 U/L with a low detection limit (LOD) of 0.013 U/L (3σ, n=11). The relative standard deviation (RSD) was 3.0% for detecting 10 U/L CaE (n =11), implying the good reproducibility of the assay. As shown in Table S1, the proposed method exhibits wider linear range and lower LOD than that of the existing optical methods.[28-35] The high sensitivity can be attributed to the enzymatic cascade reaction through *in situ* generation fluorescein as signal amplifier.

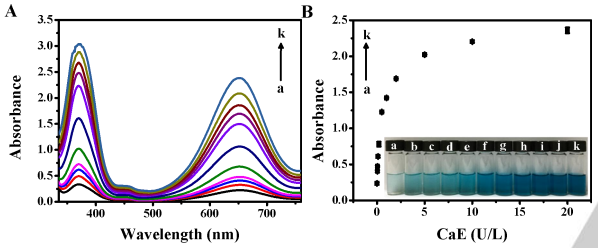


Figure 5. (A) Absorption spectra of the sensing system in the presence of CaE with different concentrations. (B) CaE concentration-dependent absorbance response, the insert shows the corresponding optical photographs. a→k: 0, 0.04, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 U/L. Error bars represent the standard deviation of three repeated measurements.

Next, the selectivity of the method was evaluated and presented in Figure S8. The results from interference assays demonstrated that various endogenous molecules in serum including other enzymes (trypsin, lysozyme and ChOx), serum albumin (BSA and HSA), glucose, common amino acids (Glu, Arg, Tyr, Lys, Gly, Ser, Try, Gln, Cys), GSH and metal ions (K^+, Zn^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+} and Al^{3+}) showed negligible effect on the CaE sensing system, indicative of satisfactory selectivity to CaE (Figure S8A,). The results presented in Figure S8B indicated that the method could provide good ability to resist interference from common metal ions and biological species. The aforementioned findings clearly indicated that our newly constructed approach is reliable, sensitive and suitable for CaE determination in complex real samples.

To validate the suitability and reliability of the sensing platform in biological samples, CaE activity in FBS was measured using standard addition method. As shown in Table S2, the recovery for all the samples ranged from 95.4-108.2%, and RSD ranged from 2.6-3.8%, which revealed the practicability and feasibility of the method in complex biological samples.

Conclusions

In summary, fluorescein possesses intrinsic visible-light-included oxidase-mimicking activity and exhibits higher affinity to TMB than HRP. In addition, we elucidated the photocatalytic mechanism in detail and found h^+ and $O_2^{\bullet-}$ were the main reactive species account for TMB oxidation. Fluorescein uses oxygen as a mild and green electron acceptor (not relies on destructive H_2O_2), and the high catalytic activity can be attain fast and simply only through 10 min visible light irradiation. Significantly, its activity is easily switched on by visible light and tuned by introduction of groups. As found, induction of carboxylic ester groups caused the loss of activity. On basis of this novel property of fluorescein, we have successfully constructed a facile, economical, fast and green colorimetric sensing strategy through enzymatic cascade reaction as a signal amplification technique for highly sensitive and selective CaE detection. With the benefit of the versatility in varieties and functionalities of fluorescein derivatives, as well as the schemes of functional groups-mediated photocatalytic activity, such integration will be promising in future sensor developments and the application of fluorescein as enzyme mimics. We also develop a simple strategy for biosensing by tailoring the enzymcatic activity with analyte, which might guide the design of other artificial enzymes for different biological applications.

Experimental Section

Reagents and chemicals

Fluorescein (F, Reagent Ph. Eur. grade), 3,3′,5,5′-tetramethylbenzidine (TMB, 99%), methyl orange (MO, ACS reagent, 85 %), sodium azide (NaN_3,99.5%), carboxylesterase from porcine liver (CaE, EC 3.1.1.1, 18 units/mg), bovine serum albumin (BSA,98%),human serum albumin (HSA,96%),cholesterol oxidase from cellulomonas species (ChOx, EC 1.1.3.6, 31 units/mg), L-glutamic acid (Glu, TLC grade, 99%),lysozyme from chicken egg white (E.3.2.1.17, 63628 units/mg) were purchased from Sigma-Aldrich. Fluorescein diacetate (FDA, HPLC grade, 98.0%) were purchased from TCI Chemical Industry (Tokyo, Japan). Trypsin (EC 3.4.21.4, 213 units/mg) was purchased from Worthington Biochemical Corporation (Lakewood, USA). Sodium acetate anhydrous (99.99% metals basis), 2-methoxyethanol (99.7%, GC), L-tryptophan (Try, 99%), L-glutamine (Gln, 99%), L-serine (Ser, 99%), catalase (CAT, 35000 units/mg), p-benzoquinone (BQ, 99%), potassium iodide (99%), ammonium oxalate monohydrate (AO, 99.8%), ethylenediaminetetraacetic acid (EDTA, 99.5%), *tert*-butyl alcohol (TBA, 99.5%) and ispropyl alcohol (IPA, 99.5%) were obtained from Aladdin Reagent Co., Ltd. (Shanghai, China). L-glycine (99%) was from Alfa Aesar. Tris (99.9%), reduced glutathione (GSH, 99%) L-cysteine (Cys, 99%), L-lysine (Lys, 99%), L-tyrosine (Tyr, 99%) and L-arginine (Arg, 99%) were from Genview (USA). Superoxide dismutase from bovine erythrocytes (SOD, 2500 uints/mg), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS, reagent grade) and o-phenylenediamine (OPD, reagent grade) were purchased from Sangon Co., Ltd. (Shanghai, China). Acetate (HPLC grade, ≥99.9%), hydrochloric acid (HCl, Guaranteed reagent) and H_2O_2 (30%) was obtained from Macklin biochemical Co. Ltd. (Shanghai, China). Fetal bovine serum (FBS) was purchased from Gibco (Australia). All other reagents were of analytical grade and were used directly without further

purification.Ultrapure water (18.2 M) was prepared with a Milli-Q system (Millipore, USA) and used throughout the experiments.

Visible-light-induced oxidase-mimicking activity of fluorescein

The visible-light-induced oxidase-mimicking activity of fluorescein was monitored by the catalytic oxidation of TMB, ABTS and OPD. In a typical experiment, 200 μL of fluorescein (10.0 μM) and 200 μL of chromogenic substrate (5.0 mM TMB, 5.0 mM OPD or 50 mM ABTS) were added into 1.0 mL acetate buffer solution (0.20 M, pH 4.0) and the mixture was diluted with ultrapure water to a volume of 2.0 mL. The reaction solution was irradiated for 10 min with a 150 W halogen tungsten lamp (CTTH-150W, Crown Tech. Inc, USA) equipped with a long-pass cutoff filter (λ ≥ 400nm) to provide visible light at room temperature and then the absorbance at 652 nm was recorded using a UV-2450 UV-Vis spectrophotometer (Shimadzu, Japan). The light intensity of the light source was fixed at 213.4 mW/cm^2 and measured using a radiant power meter (91150V, Newport Corporation, Stratford, CT USA).

Kinetic assay

The steady kinetic assays were performed by monitoring the absorbance change at 652 nm with a 1 min interval. 5.0 mL of 0.10 M acetate buffer (pH 4.0) containing 1.0 μM fluorescein and varied TMB concentrations was illuminated by visible light for 1 min, and then the absorbance was recorded. The Lineweaver-Burk plot: $1/v = K_m/(V_{max}[S]) + 1/V_{max}$, was used to calculate kinetic parameters, where v is the initial velocity, V_{max} is the maximal reaction velocity, K_m is the Michaelis constant and [S] corresponds to TMB concentration.

Electron spin resonance (ESR)

10 μM fluorescein and 50 mM spin trap 5,5-dimethyl-1-pyrroline N-oxide (DMPO) in anhydrous methanol solution were photoirradiated with visible light for 0, 5 or 10 min and then the prepared samples were transferred to a quartz capillary tube and placed in the ESR cavity. The ESR spectra were recorded on a JES FA200 ESR spectrometer (JEOL, Japan). A 500 W high pressure mercury lamp equipped with an ultraviolet cutoff filter (λ ≥ 420nm) provided visible light at room temperature.

Photocurrent Measurements

The photocurrent measurements were performed with a photocurrent work-station (Lvium, Nethrlands). A conventional three-electrode system was used for photocurrent measurement, in which a platinum wire was served as the counter electrode, a saturated Ag/AgCl was served as the reference electrode and a bare or fluorescein modified glassy carbon electrode with 4 mm diameter was served as the working electrode, respectively. All the photocurrent measurements were performed at a constant potential of 0 V (*vs* saturated Ag/AgCl). A 0.10 M acetate buffer solution (pH 4.0) was used as the supporting electrolyte for photocurrent measurements.

CaE detection

The CaE activity assay was based on its enzymatic hydrolysis of FDA to generate fluorescein for catalyzing the oxidation of TMB under visible illumination. In a typical experiment, 200 μL of CaE was incubated with 200 μL of FDA (50 μM dissolved in 2-methoxyethanol) for 30 min at 37 °C in pH 8.0 Tris-HCl buffer solutions (200μL, 10mM). Following that, 200 μL of 5.0 mM TMB, 1.0 mL NaAc buffer solution (0.2 M, pH 4.0) and 200 μL ultrapure water were added in the above solution and illuminated for 10 min under visible-light (λ ≥ 400 nm). Finally, the absorbance of the oxTMB at 652 nm was measured by a UV-Vis spectrophotometer.

Determination of CaE in FBS

Firstly, 20 μL FBS (2%) was spiked with different concentration of CaE solution and diluted to 500 μL using Tris-HCl buffer (pH 8.0, 1 mM). Next, 200 μL of 50 μM FDA solution was added into as-prepared samples of FBS containing various concentration of CaE. After incubation at 37 °C for 30 min, 1.0 mL NaAc buffer (0.2mM, pH 4.0) was added and the above mixture diluted to 1.8 mL using ultrapure water. Finally, 200 μL of TMB stock solution (5.0 mM) were added. The absorbance at 652 nm was recorded after visible light illuminating for 10 min.

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Keywords:colorimetric method• visible-light-induced oxidase mimic• fluorescein• carboxylesterase• cascade reaction

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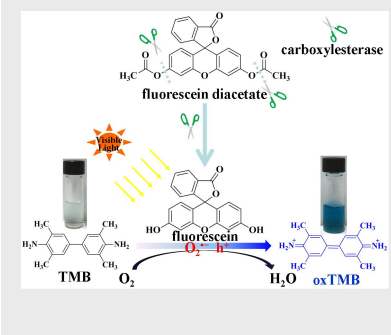

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Fluorescein as a visible-light-induced oxidase mimic for signal-amplified colorimetric assay of carboxylesterase by enzymatic cascade reaction

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