Photo-manipulation of activity of enzymes bound to inorganic nanomaterials

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Abstract

The present review introduces various enzymatic reaction systems employing an external light source (visible or UV light) to initiate enzymatic reaction and/or adjust its reaction rate, that is, photo-manipulation of enzymatic activity. In general, a photon-receptor such as semiconductor with a suitable band gap size is needed to interact with enzymes because enzyme molecules are typically not photosensitive. When irradiated, photocarriers (electrons or holes) in the photon-receptors are transferred from the photo-receptors to enzymes for excitation, and then enzymatic reaction breaks out. Hence, switching of enzymatic activity is achievable by turning on/off the light source. The fact that photocarriers are triggers, indicates that redox enzymes that can be stimulated by negative electrons or positive holes are well-matched for the proposed systems. In addition, it has been reported that light-induced-environmental changes (pH, temperature) around enzymes are also useful for strict control of enzymatic activity.

Keywords: Enzyme; Photoirradiation; Photoswitching; Bioluminescence; Semiconductor nanoparticles

1. Introduction

Since enzymatic reactions have excellent substrate selectivity and a large turnover number (k_{cat}) even at moderate conditions, enzymes extracted from living bodies have been widely employed in a variety of fields including food and chemical industries. In the case of application of enzymes *in vitro*, strict control of their activity (physicochemical stability, catalytic performance) is demanded to produce valuable chemicals efficiently. Various techniques to adjust enzymatic activities have been proposed up to date. Most simple examples are hybridization with solid supports, that is, immobilized enzymes to consolidate fragile biomolecules [1] and fixation of enzyme on a metal electrode especially for biosensing [2,3]. On the other hand, photo-manipulation of enzymatic activity has recently attracted much interest especially at scientific application of enzymes because use of focused or pulsed light succeeds in spatial and time-resolved control.

Even though the photo-manipulation has several advantages aforementioned, there are few enzymes responding to photon energy directly except for fluorescent proteins. To break through the situation, hybridization of photo-active materials with enzymes has been studied extensively in past decades, and then many researchers have reported photon-induced enzymatic activity control using a variety of photo-active materials. Such photo-manipulation of enzymatic activity is believed to be a critical tool in the fields of biochemical engineering (industrial application of biocatalysts) and biochemistry (particularly for bioimaging). In conventional studies, hybridization of an enzyme with a support has been carried out only to improve physical (thermal) and chemical stability [4], in other words, the support for enzymes may behave only as a stabilizer. By contrast, it should be noted that hybridization with a photo-active material realizes to append various intrinsic functions (optical, magnetic, electrical, etc.) to enzymes.

Hence, the present review briefly mentions principle and mechanism of photon-induced activity modulation of enzymes immobilized on photo-active materials. Especially, we focus inorganic nanomaterials such as nanoparticles, nanodots and nanosheets as photo-accepted materials as schematically illustrated in Fig. 1.

2. Photoswitching of redox enzymes on semiconductor nanomaterials

Inorganic semiconductor nanomaterials can absorb photons with various energy levels (wavelengths) depending on their band gap energy. Therefore, if energy received by

semiconductor is transferred to enzymes, enzymatic activity will be activated according to wavelength, intensity and beam size of incident light. To do the energy transfer effectively, it would be significant to chemically or physically adsorb on semiconductor nanomaterials.

Within our knowledge, it seems that Niemeyer and coworkers firstly demonstrated possibility of such strategy for peroxidases [5]. They succeeded in light-triggered catalytic activity control of peroxidase adsorbed on quantum dots (QDs). Since the QDs (CdS) were capped with negatively charged mercaptoacetic acid, horseradish peroxidases (HRP) with a slight positive charge could be electrostatically bound to surface of QDs (QD-HRP) at neutral pH. It is known that HRP can oxidize a variety of organic substrate in the presence of H_2O_2. UV light (λ = 366 nm) irradiation to the QD-HRP induced oxidation of a substrate (Amplex Red) without H_2O_2 in contrast that only QDs or HRP hardly caused the enzymatic reaction. Needless to say, the reaction did not proceed in the dark. Therefore, the enzymatic activity was easily switched on/off by turning on/off the UV light. The authors explained that reactive oxygen species (ROS) such as hydroxyl and/or superoxide anion radicals were evolved on surface of QDs under the UV light irradiation, where hydroxyl radicals would be produced by reaction of H_2O with holes in the valence band of CdS and electrons in the conduction band would reduce dissolved oxygen gas to form superoxide anion radicals. Then the ROS excited (oxidized) HRP followed by the enzymatic oxidation of Amplex Red. Furthermore, the strategy could be extended to other peroxygenases (Cyp152A1) or proteins having peroxidase like activity (myoglobin). On the other hand, King et al. recently discovered that light irradiation to nitrogenase MoFe protein hybridized with CdS nanorods brought about N_2 reduction to NH_3 at moderate conditions [6], although conventional and industrial synthesis of NH_3 from N_2 and H_2 is acquired at high temperature and pressure (recognized as the Harber-Bosch process). Different from the first case in Ref. [5], the reduction was triggered by migration of electrons generated in the conduction band of irradiated CdS to the nitrogenase MoFe proteins.

In summary, it was proven that irradiation to semiconductor nanocrystals generated photocarriers (holes in the valence band and electrons in the conduction band), and then the photocarriers excite redox enzymes followed by enzymatic reactions of appropriate substrates at ambient conditions as illustrated in Fig. 1. Among numerous enzymes, redox enzymes must be well matched to semiconductors that generate photocarriers useful for

excitation of enzyme by charge transfer.

Since utilization of CdS as a receptor of photon energy should be avoided due to toxicity of Cd, our group has claimed that semiconducting layered oxides composed of nontoxic elements are useful as host nanomaterials to manipulate enzymatic activity [7]. In this study, as a result of mixing of exfoliated Fe-doped layered titanate (FTO) nanosheets and HRP (Fig. 2), periodical crystal structure of FTO intercalated with HRP (HRP-FTO) was formed via an electrostatic interaction between negatively charged FTO and positive HRP at an appropriate pH (< 5) [8]. Besides layered titanates, such hybridization biomolecules have been reported for other inorganic nanosheets including zirconium phosphates and layered double hydroxides [9,10]. It was verified that the hybridization process did not deteriorate enzymatic activity of HRP. As FTO has a wide band gap corresponding to UV light, near UV (365 nm) was irradiated to a reaction mixture of HRP-FTO and a substrate (Amplex Ultrared). As a result, the UV light irradiation took place enzymatic oxidation of the substrate and photoswitching was accomplished by turning on/off the light (Fig. 3). Mechanism of fundamental photon-induced enzymatic reaction of HRP-FTO seems to be identical to that of HRP-adsorbed QDs aforementioned. Briefly, The resting HRP in the interlayer space of FTO is transformed into activated state (Compound I) as a result of oxidation with holes generated in the valence band of the irradiated FTO. Subsequently, Compound I leads to the enzymatic conversion of Amplex ultrared accompanied by two-electron reduction to regenerate the resting state. FTO would not only provide photocarriers to trigger the enzymatic reaction, but also protect HRP from photodenaturation [11].

Aforementioned techniques have utilized UV light as a trigger to initiate an enzymatic reaction cycle. However, UV light frequently induces denaturation of biomolecules due to its high photon energy. Therefore, visible light with moderate energy appears to be suitable for photo-manipulation of enzymatic activity. Kang et al. have used carbon quantum dots (CQDs) as a photo-absorber [12]. Besides CQDs are clear of worry about toxicity different from CdS, their physicochemical durability, aqueous solubility and attractive multicolor photoluminescence may be valuable in the biological fields [13]. In Ref. [12], lipases catalyzing hydrolysis of lipids were adsorbed on surface of CQDs. The adsorption of lipases with low solubility in an aqueous phase to CQDs might proceed through hydrophobic interaction between them, although the authors only explained that the hybridization was due to "non-covalent bonds". Enzymatic activity was investigated

for hydrolysis of olive oil as a fatty acid. As compared with free lipase, binding to CQDs improved the activity about 10% under visible light irradiation from a xenon lamp, in contrast that the activity in the dark was dropped by 30% as a result of the hybridization. According to determination of kinetic parameters, it was concluded that the preferred influence of visible light irradiation was based on enhanced affinity to hydrophobic substrate and structural change of lipases favorable to disintegrate olive oil. As a related research, Reisner et al. employed CQDs with positive (CQDs(+)) or negative surface charges (CQDs(-)) as photo-absorbers and investigated of photosensitizing effect of CQDs on enzymatic activity of reductases (hydrogenase (H2ase) and fumarate reductase (FccA)) under visible light illumination [14]. Needless to say, electron transfer from irradiated CQDs to the reductases is a trigger for starting a catalytic cycle. In both case, CQDs(+) behaved as prominent electron donors rather than CQDs(-). At both enzymes, the surface-exposed electron entry sites are surrounded by negatively charged residues, and hence the sites attract CQDs(+) strongly as compared with CQDs(-), resulting in more efficient direct electron transfer from CQDs to enzymes.

We have also devoted much effort to develop an enzymatic reaction system driven by visible light irradiation. n-Type iron oxide (α-Fe_2O_3, hematite) and heavily Fe-doped titanate with narrow band gaps, which can be excited by visible region, was used to achieve the aim [15,16]. In the former case, a thin film of α-Fe_2O_3 on a Pt plate was prepared by a photoelectroless deposition technique in a Fe^{2+} salt solution and subsequent heat treatment to crystallize the as-deposited film as thermodynamically stable α-Fe_2O_3. The fabricated thin film was immersed in a solution containing peroxidase at an appropriate pH, then the peroxidase was adsorbed on the surface of α-Fe_2O_3 via electrostatic interaction. To study possibility of visible-light-driven enzymatic reaction (Fig. 4), three kinds of LED light sources with different wavelength, i.e., blue (474 nm), green (529 nm) and red (636 nm), were utilized, where the α-Fe_2O_3 could be excited by blue and green emissions but not by red emission according to a photoabsorption spectrum. As anticipated, irradiations of blue and green lights could cause the photo-induced enzymatic reactions as shown in Fig. 5. In contrast, red light did not remain any evidence of the reaction. Interestingly, a reaction rate under green light was faster than that under blue light, although the former had lower photon energy. Together with the photo-induced enzymatic reaction, the blue light might cause reduction of Fe^{3+} to Fe^{2+} by excited electrons and then the Fe^{2+} would dissolve into a reactant solution. As a result,

the photo-enzymatic reaction rate would be slowed down. Hence, it was revealed that there is optimum wavelength of incident light depending on semiconductor employed to maximize efficiency of the photo-induced enzymatic reaction.

Table 1 summarizes literatures with respect to photo-enzymatic reactions using semiconducting nanomaterials. As well as the aforementioned studies, other host semiconducting nanomaterials have been also adapted to photo-manipulation of enzymatic activity. For example, TiO_2 nanoparticles [17] or $BiVO_4$ [18] loaded with noble metals to apply a photocatalytic reaction system (artificial photosynthesis) and semiconducting polymer dots for photodynamic therapy and imaging of cancer cells [19] were proposed to date.

3. Diversity of photo-manipulation of enzymatic activity

As denoted in the former section, several research groups have accomplished the photo-manipulation of enzymatic activity by interaction with semiconductor nanomaterials behaving as photon receptors. Recently, diverse strategies to manipulate enzymatic activity with photon energy have been reported.

Simon et al. claimed that activity of HRP bound to gold nanoparticles (HRP-AuNP) could be controlled by laser light irradiation [20]. Since gold shows low specific heat capacity and high thermal conductivity among various transition metals and alloys, laser irradiation (λ = 532 nm, corresponding to a wavelength of surface plasmon resonance of AuNP) to a reactant mixture including the HRP-AuNP increased local temperature around the HRP-AuNP due to photoabsorpion by AuNP. Consequently, the HRP connected to AuNP was deactivated then a reaction rate was reduced, because enzymatic activity was deteriorated by heating (thermal deactivation) in the temperature range examined. Judging from the rate reduction by laser irradiation, temperature difference between bulk solution and HRP-AuNP was estimated to be about 3^{o}C at highest laser intensity. Although the temperature rising might appear to be small, change in activity of enzyme, which is extremely sensitive to environmental temperature compared with other catalysts, could be detected. The authors have also demonstrated that decrease in activity was related to output power of laser, implying that photo-manipulation of enzymatic activity based on temperature fluctuation was possible.

A unique phenomena about photo-induced environmental alteration and subsequent change in enzymatic activity has been recently reported by another group. Kohse et al.

have realized photo-switching of enzymatic activity in a reactant solution under coexistence of a pH-jump reagent [21]. The reaction was composed of mainly two steps. At first, laser light irradiation (490 nm) brought about release of H^+ from 2-nitrobenzaldehyde used as a pH-jump reagent. Then, the decrease in pH could activate acidic phosphatases, in other words, the laser light became a trigger of enzymatic reaction of the phosphatases. In conclusions, it was revealed that photoirradiation can modify local conditions surrounding enzymes in an appropriate solution, and hence their activity can be easily manipulated.

4. Influence of supports on emission properties of bioluminescence

Among various enzymes, some of them participate in a photoemission process. For instance, HRP catalyzes an oxidative emission reaction of luminol and aequorin (photoprotein) releases blue light under the presence of Ca^{2+}. This section describes effects of solid supports on emission properties of photoproteins or photoenzymes, although the effect slightly differs from the main topic of this manuscript.

Lu and co-workers have reported that intensity of luminescence of luminol catalyzed by HRP significantly increased after capsuling of HRP with polymer (polyamide) and subsequent loading of AuNP [22]. The authors consider that the result is based on metal enhanced bioluminescence (MEB) that means luminescence from luminol is coupled to surface plasmon of AuNP, where the polymer layer plays an important role to separate HRP and AuNP at a suitable distance. That is, in a short distance (< 5 nm), most luminescence is transferred to surface plasmon accompanied by non-radiation transition, while longer distance (> 20 nm) between them causes hardly MEB. On the other hand, our group also found analogous enhanced bioluminescence from luminol catalyzed by HRP in the presence of titanate nanosheets especially at diluted HRP concentrations [23]. According to dynamic light scattering, it was revealed that aggregated HRP molecules are peptized by coexistence with titanate nanosheets. Hence, the increment of bioluminescence intensity would be due to an increase in effective (apparent) concentration of HRP.

5. Conclusions

Several studies demonstrating photo-manipulation of enzymatic activity were summarized in this manuscript. Since activity of most enzymes is not related to photo-

irradiation, typically a photon-receptor such as an inorganic semiconductor is required to be hybridized with enzymes in order to construct the photo-manipulation system. Fundamental mechanism can be described as follows. The photon-receptor is excited when irradiated and then generated photocarriers are transported to enzymes. The resultant enzymes cause a catalytic reaction. Therefore, different from conventional enzymatic reaction systems, the reaction rate can be adjusted by controlling irradiation wavelength and/or intensity. In other words, photo-switching of enzymatic activity is achieved. As photo-irradiation can easily focus on a specific area desired, it appears that spatially resolved enzymatic reaction will occur. Moreover, it is well-known that loading of noble metal nanomaterials (especially nanoparticles) to semiconductors stimulates charge (electron-hole pair) separation in the field of photocatalysis, and hence the loading may be also effective in enhancement of efficacy of photoenzymatic reactions. However, the goal is not achieved at present. In near future, extensive researches for many kinds of enzymes and nanomaterials [24] are required to prove usefulness of the proposed technique.

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Table 1 Various photon-manipulation systems of enzymatic activity.

Enzyme	**Photon receptor***	**Band gap energy / eV**	**Wavelength of incident light / nm or source**	**Substrate**	**Enzymatic reaction**	**Ref.**
HRP	CdS NDs	2.4 (bulk)	366	Amplex red	Oxidation	[5]
Nitrogenase	CdS NRs	2.4 (bulk)	405	N_2	Reduction	[6]
HRP	Fe-doped titanate NSs	3.1	365	Amplex ultra red	Oxidation	[7]
Lipase	C NDs	-	Xe lamp	Olive oil	Hydrolysis	[12]
Hydrogenase,	C NDs	-	AM 1.5G	H^+	Reduction	[14]
Fumarate reductase				Fumarate		
HRP	α-Fe_2O_3 thin film	2.2	474, 529	Amplex ultra red	Oxidation	[15]
	Fe-doped nanometric titanate NSs	2.8 (10 mol% Fe-doped titanate)				[16]

*NDs: nanodots, NRs: nanorods, NSs: nanosheets

Figures

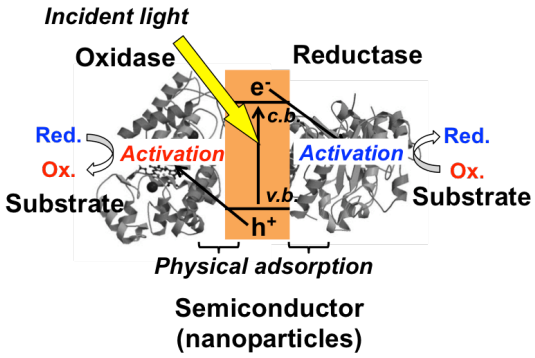

Fig. 1: Schematic illustration describing photo-manipulation mechanism of activity of redox enzymes (oxidase or reductase) bound to semiconductor. Typically, the immobilization of enzyme is carried out through electrostatic interaction (physical adsorption) between semiconductor and enzyme. Electrons in the conduction band (c.b) and holes in valence band (v.b) generated by irradiation from an external light source are migrated to reductase and oxidase, then cause reduction and oxidation of substrates, respectively.

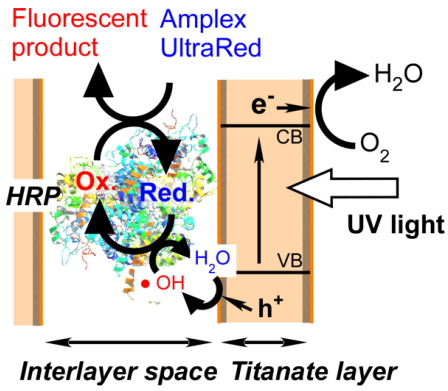

Fig. 2: Schematic model of UV light-induced enzymatic reaction of HRP intercalated into titanate layers. Reprinted with permission from Kamada, et al., Chem. Mater., 23, 2968. Copyright (2011) American Chemical Society.

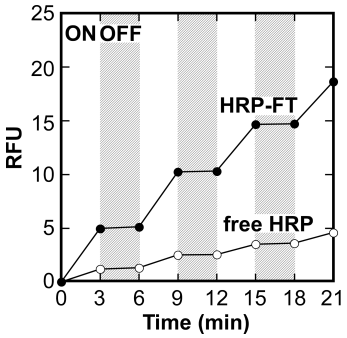

Fig. 3: Photo-switching behavior (data) of HRP existing in interlayer space of Fe-doped layered titanate (HRP-FT). Relative fluorescent unit (RFU) related to product concentration of enzymatic reaction increases only under UV-light irradiation (ON). Reprinted with permission from Kamada, et al., Chem. Mater., 23, 2968. Copyright (2011) American Chemical Society.

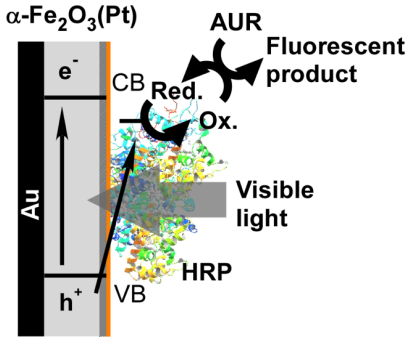

Fig. 4: Schematic illustration of photo-induced enzymatic reaction by HRP adsorbed on Pt-doped α-Fe_2O_3 thin film. Amplex Ultrared (AUR) is catalytically oxidized to a fluorescent product by the HRP bound to the film under visible light illumination. Reprinted with permission from Kamada, et al., J. Phys. Chem. C, 116, 20694. Copyright (2012) American Chemical Society.

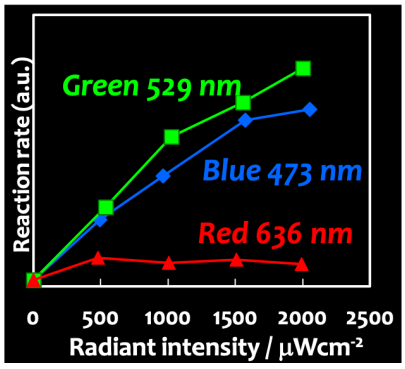

Fig. 5: Effects of radiant intensity on photo-induced enzymatic reaction rate of HRP-adsorbed hematite thin films under irradiation of colored light. Reprinted with permission from Kamada, et al., J. Phys. Chem. C, 116, 20694. Copyright (2012) American Chemical Society.