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Light-Driven, Quantum Dot-Mediated Regeneration of FMN To Drive Reduction of Ketoisophorone by Old Yellow Enzyme

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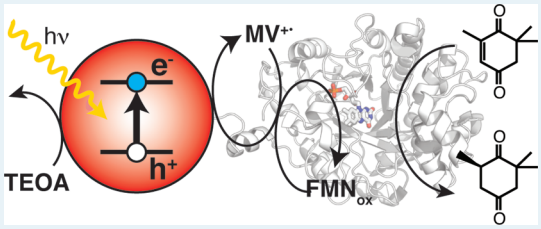
Supporting Information

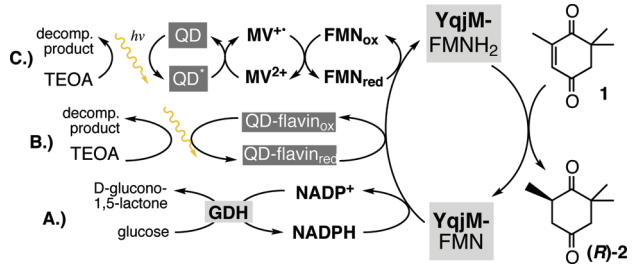
ABSTRACT: We report the full reduction of the biological cofactor FMN with visible light using CdSe quantum dots and methylviologen as an electron relay. In turn, these reducing equivalents can drive the stereospecific reduction of ketoisophorone by an old yellow enzyme homologue from *Bacillus subtilis* (YqjM). The experiments demonstrate the current capabilities and limitations of quantum dots as part of a cofactor regeneration system and pave the road for future studies aimed at new and improved in situ light-driven cofactor regeneration strategies.



KEYWORDS: *biocatalysis, quantum dots, photoreduction, redox mediator, old yellow enzyme, cofactor regeneration*

Cofactor regeneration is an integral part for a wide spectrum of biocatalytic transformations, yet it is best known in the context of $NAD(P)^+$/NAD(P)H recycling. The nicotinamide cofactors are the most frequently used two-electron donor/acceptors for biotransformations involving oxidoreductase family members (E.C. 1), an important class of enzymes in organic synthesis due to their efficiency and versatility, as well as high chemo-, stereo-, and regioselectivity.[1,2] Because economic and practical considerations favor cofactor recycling, traditional redox cofactor regeneration in these systems is accomplished by running reactions in whole cell extracts or in the presence of a second, complementary $NAD(P)^+$/NAD(P)H-dependent enzyme, such as NAD(P)H oxidase, as well as formate or glucose dehydrogenase (Scheme 1A).[3−6] Separately, electrochemical methods involving organometallic complexes of Ru(II) or Rh(III) have been employed.[7,8] An attractive yet less explored alternative to the established cofactor recycling methods is the photochemical regeneration of redox partners. The development of effective light-driven cofactor regeneration systems promises not only simpler reaction conditions but also an economical and environmentally sustainable solution. However, the recycling of $NAD(P)^+$/NAD(P)H relies on simultaneous electron and hydride transfer. This presents a challenge for photochemical cofactor regeneration because each absorbed photon can transfer only one electron in most chromophores, and stepwise reduction of the cofactor is largely prevented by the exceedingly high redox potential for the second electron-transfer step.[9] Fortunately, a number of oxidoreductases also accept flavin cofactors as alternate two-electron donors/acceptors, a fact that can be utilized for light-driven reactions because the flavin's isoalloxazine moiety tolerates two consecutive single-electron transfers. Such photochemical reduction of flavin, in turn, can be coupled to enzymatic reactions.[10−12]

Scheme 1. Enantioselective YqjM-Catalyzed Reduction of Ketoisophorone 1 to (*R*)-Levodione 2, Using Cofactor Regeneration Systems Based on (A) Glucose Dehydrogenase (GDH), (B) Light-Driven CdSe QD-FMN Reduction, and (C) Light-Driven QD-Methylviologen (MV)-Flavin Cascade



Attracted by the renewable nature of light-mediated cofactor regeneration, we have explored the use of CdSe quantum dots (QDs) as a tunable and efficient system for energy capture and electron delivery. When the size of semiconductor nanoparticles is smaller than the exciton radius (typically a few nanometers), the conduction band electron and valence band hole energy levels become quantum-confined, giving rise to size-dependent absorption and emission spectra of QDs.[13] These colloidal particles have a high absorption cross section, long excited state lifetime, and good photostability, making them ideal light absorbers in photocatalytic systems. Additional versatility and potential functional benefits arises from the possibility for surface functionalization, enabling us to tailor QDs for specific reaction environment and to utilize the nanoparticle as an immobilization matrix for the biocatalyst.[14−22]

We initially evaluated a light-driven cofactor regeneration system based on the direct coupling of the riboflavin to the QD

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surface via a bidental thiol/borate linker (Scheme 1B). Although successful assembly of the QD−flavin adduct and initial photoinduced charge separation was verified via fluorescence quenching and transient absorption measurements, the absence of accumulation of reduced cofactor suggested rapid recombination of the charge-separated state, despite the use of a sacrificial electron donor, such as triethanolamine (TEOA) (data not shown).

In contrast, complete photochemical reduction of FMN was achieved using CdSe QDs with methylviologen (MV^{2+}) as an electron relay system and TEOA as a sacrificial electron donor (Scheme 1C). We first demonstrated the photogeneration of $MV^{+\bullet}$ radical in an aqueous solution containing CdSe-QD (light absorber), MV^{2+}, and TEOA. Upon illumination of the solution with visible light (see Figure 1 caption for detailed

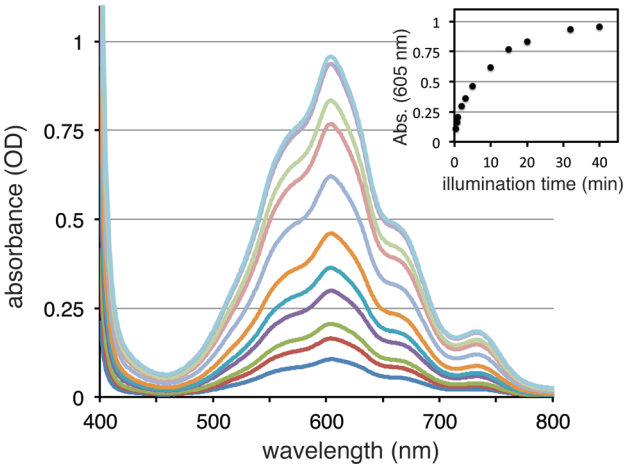

Figure 1. Generation of MV radicals upon 40-min irradiation with visible light in the presence of CdSe QDs and TEOA. The difference (after−before irradiation) spectra are shown after 0.33−40 min of illumination (from bottom to top). Inset: time-dependent accumulation of $MV^{+\bullet}$ based on absorbance at 605 nm. The 2-mL reaction mixture (10 μM CdSe, 250 μM MV^{2+}, and 200 mM TEOA) was illuminated with a xenon lamp (11.5 mW, filtered from 410 to 520 nm).

experimental conditions), $MV^{+\bullet}$ radicals were formed, as indicated by the distinct absorption peaks at 605 and 395 nm in the difference spectra shown in Figure 1. In this spectral region, MV^{2+} has negligible absorption, and the difference spectra (after−before illumination) correspond to the spectra of the photogenerated $MV^{+\bullet}$ radicals. The $MV^{+\bullet}$ concentration reached a maximum within 40 min when ~46% (~115 μM) of the initial MV^{2+} had been reduced.

The photogenerated $MV^{+\bullet}$ can be used to reduce flavin, as shown by the spectral evolution of an aqueous solution of QDs, MV^{2+}, and TEOA in the presence of FMN (Figure 2A). The spectrum of the initial solution (black) is dominated by the absorption of the QD, with a distinct exciton band at 580 nm and absorption features extending to higher energy. Upon 10 min of illumination with visible light (see Figure 2A caption), $MV^{+\bullet}$ radicals were formed (green), as previously demonstrated in Figure 1. At this stage, 105 μL of 2 mM FMN_{ox} stock solution was injected under strictly anaerobic conditions, which caused spectral changes (red spectrum) consistent with secondary electron transfer from the in situ-generated $MV^{+\bullet}$ radical to the flavin cofactor. The complete reduction of FMN took only a few seconds. The difference spectrum of the flavin

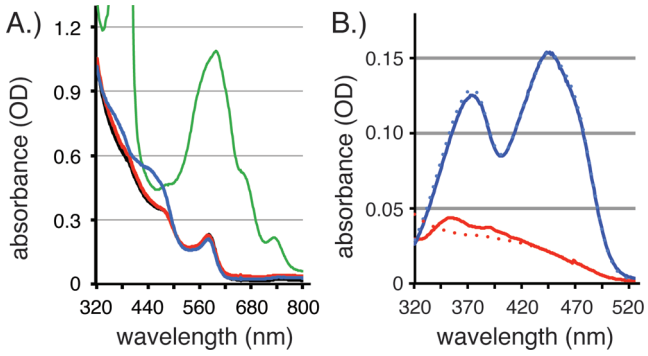

Figure 2. (A) Absorption spectra of QD-assisted and MV radical-mediated FMN reduction in four stages. Stage I (black): MV^{2+} solution (10 μM CdSe, 250 μM MV^{2+}, 200 mM TEOA) prior to illumination. Stage II (green): $MV^{+\bullet}$ formation upon illumination (xenon lamp, 30 mW of 410−520 nm light for 10 min). Stage III (red): solution after addition of FMN_{ox} (20 μM). Stage IV (blue): Oxidation of FMN after exposure to air. (B) Difference absorption spectra for FMN. Reduction of FMN (stage III−I) is shown as a solid red line, whereas reoxidation (stage IV−I) is marked by a solid blue line. Dashed lines represent the reference spectra of FMN_{red} (red) and FMN_{ox} (blue).

after reduction by $MV^{+\bullet}$ (Figure 2B, red) matches the FMN_{red} reference spectrum. The integrity of the flavin was confirmed by recovery of the FMN_{ox} spectrum upon exposure to air (Figure 2B, blue). Under the current photochemical conditions, the $MV^{2+}/MV^{+\bullet}$ couple functions as an efficient and recyclable electron relay to achieve light-driven full reduction of the FMN.

In turn, the accumulated FMN_{red} should be able to drive subsequent redox processes. We sought to demonstrate this principle by coupling the QD/MV^{2+}-based cofactor regeneration system to the activity of the old yellow enzyme homologue YqjM from *Bacillus subtilis*.[3] YqjM is a flavin-dependent ene-reductase for the reduction of α,β-unsaturated alkenes. Although the enzyme normally derives its reducing equivalents from NADPH, FMN_{red} can substitute as an electron shuttle to drive the stereoselective conversion of ketoisophorone **1** to (*R*)-levodione **2**. Upon addition of YqjM and **1** to the photogenerated FMN_{red}, the formation of (*R*)-**2** was detected with an enantiomeric excess (ee) of 64% (Figure 3A). The ee value represents a low estimate because the starting material already shows detectable amounts of both stereoisomers of **2**. Accompanying the progress of the enzymatic reaction, the oxidation of the cofactor from FMN_{red} to FMN_{ox} is also observed (Supporting Information Figure S1). Separately, we validate the contribution of YqjM in the reaction mixture by omission of (a) YqjM and (b) FMN and YqjM. In the absence of enzyme, no reaction of **1** was observed. In contrast, omission of enzyme and cofactor prompted the direct conversion of **1** by $MV^{+\bullet}$ to yield racemic product.

Next, the efficiency of the QD system was compared with two established cofactor regeneration systems: the light-driven reduction of MV^{2+} by $[Ru(bpy)_3]^{2+}$ complex, as well as the traditional coupled enzyme system involving glucose dehydrogenase (GDH).[23] Although product formation by YqjM in combination with GDH outperformed both photocatalysts by ~5-fold (Supporting Information Figure S2), the rate of product formation and final yield of (*R*)-**2** with CdSe QD and $[Ru(bpy)_3]^{2+}$ were similar, with TEOA as a sacrificial electron donor (Figure 3B). For both systems, the rates of $MV^{+\bullet}$ radical generation are comparable, and the calculated quantum yield

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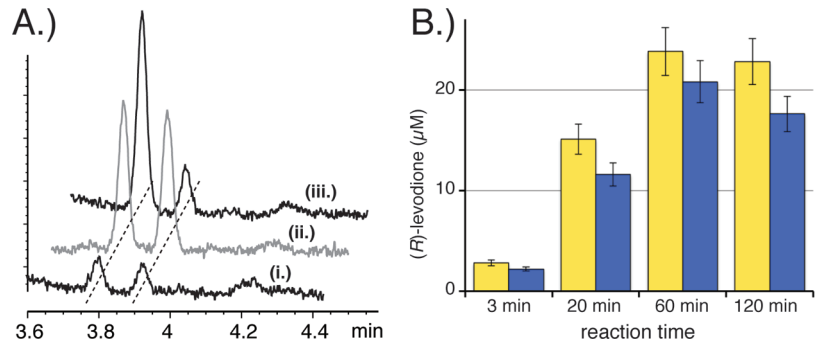

Figure 3. Light-driven formation of levodione by the CdSe QDs/MV/YqjM system. (A) GC analysis of (*R*) and (*S*)-levodione (t_R = 3.79 and 3.91 min) in (i) starting material, (ii) the nonenzymatic reduction and (iii) stereospecific reduction by YqjM. Starting material **1** not shown (t_R = 3.48 min) (B) Comparison of product formation by the CdSe QDs/MV/YqjM system (yellow) with $[Ru(bpy)_3]^{2+}$ complex (blue).

was around 5% (Supporting Information Table S1), suggesting that $MV^{+\bullet}$ radical generation is the main efficiency-limiting factor of photodriven biocatalysis in these systems. When the Ru(II) complex is used with EDTA as a sacrificial electron donor, the amount of $MV^{+\bullet}$ radical generated is almost 40% higher than with TEOA. Unfortunately, EDTA causes precipitation of QDs, making a direct comparison not possible. Notably, we observed a halt in product formation after 60 min, an effect that we attribute to YqjM inhibition by TEOA decomposition product.

To investigate the factors that limit the efficiency of $MV^{+\bullet}$ radical generation, we have carried out transient absorption studies of QD-MV^{2+} complexes. The experimental setups for the femtosecond-to-microsecond transient absorption measurement have been described previously.[24] To avoid the spectral overlap between the QD and $MV^{+\bullet}$ radical, we used a smaller QD (with 1S exciton band at 530 nm). As shown in Figure 4A,

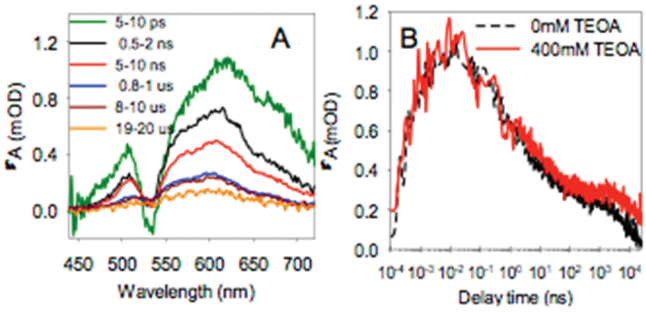

Figure 4. Transient absorption spectra and kinetics of QD-MV^{2+} solutions. (A) Transient absorption spectra in the presence of TEOA at indicated delay time after 400 nm excitation, showing the formation of $MV^{+\bullet}$ radical band centered at ~605 nm. (B) Comparison of $MV^{+\bullet}$ radical (605 nm) formation and decay kinetics in solutions with (red) and without (black) TEOA.

the transient spectrum at 10 ps after 400 nm excitation indicated formation of the $MV^{+\bullet}$ radical absorption band centered at 605 nm, consistent with ultrafast charge transfer between QDs and MV^{2+}.[25,26] The negative feature at 530 nm can be attributed to the shift of the QD 1S exciton band in the electric field caused by the charge-separated state.[24]

Separately, the formation and decay kinetics of the $MV^{+\bullet}$ radicals in solutions with and without TEOA were compared (Figure 4B). $MV^{+\bullet}$ radicals formed within 10 ps in both solutions, suggesting that the initial charge separation is efficient in both systems. In the absence of TEOA, nearly all photogenerated $MV^{+\bullet}$ radicals decayed in 20 μs, presumably through charge recombination with the holes in the QD. The process is none-single exponential and has a half-life of ~5 ns. With 400 mM of TEOA, the decay of the radical was slowed down. At 20 μs, ~15% of the radical remained. It is interesting to note that the effect of TEOA occurs mainly after 1 μs, after over 65% of the $MV^{+\bullet}$ radical has decayed by charge recombination. It suggests that TEOA is an inefficient sacrificial electron donor for the QD and the radical generation efficiency can be greatly enhanced if a more efficient sacrificial donor could be found.

In summary, we present evidence for the suitability of the CdSe QD system to reduce important biological electron carriers such as FMN. In the presence of MV^{2+} as the initial electron acceptor and mediator, as well as TEOA as sacrificial electron donor, $MV^{+\bullet}$ radicals can be generated by visible illumination with ~5% quantum yield. The photogenerated $MV^{+\bullet}$ radicals can subsequently be used to reduce FMN, which in turn can drive the stereospecific reduction of **1** by the YqjM. Transient absorption study of the system suggests that the process of hole-filling in the QD by TEOA is the main limiting factor in achieving higher yields of reduced FMN from visible light and quantum dots. Both the identification of more efficient sacrificial electron donors for QDs and tailoring of nanostructures to slow down charge recombination and to enhance hole removal are possible ways to improve the efficiency. The improvement of these kinds of cofactor regeneration systems could provide significant benefit to industrial processes, offering a greener technology and lower manufacture cost of many important molecules.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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