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Development of a novel enzyme-CdS nanoparticle hybrid system for the oxidation of NADH to NAD$^+$

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ABSTRACT

Inorganic semiconductor CdS nanoparticles (NPs) incorporating NADH dehydrogenase (diaphorase: DA) were successfully synthesized using a water-in-oil microemulsion method. The X-ray diffraction pattern of the enzyme-CdS NPs was assigned to a cubic structure CdS phase. The catalytic effect of enzyme-CdS NPs hybrid systems on the oxidation of dihydronicotinamide adenine dinucleotide (NADH) to nicotinamid adenine dinucleotide (NAD$^+$) was investigated. Enzyme-CdS NPs incorporating DA and ferrocene-modified polyethylenimine (Fc-PEI) (1:0.2, w/w) exhibited a significantly increased rate of NADH oxidation under illumination compared with pure CdS NPs. The redox mediator Fc-PEI acted to transfer electrons between the valence band of the CdS NPs and the flavin-bound enzyme during the oxidation reaction. Switchable oxidation of NADH in a colloidal solution of the enzyme-CdS NPs was induced with respect to the dark/light cycle. The catalytic properties of the present CdS NPs activated with an enzyme towards NADH oxidation might have future importance in biochemistry and medical applications.

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1. Introduction

Recently, new functional electrochemical entities have been developed by combining the electronic, optical, and catalytic properties of metallic or semiconductor nanoparticles (NPs) with the recognition and catalytic properties of biomolecules [1,2]. In particular, cadmium sulfide (CdS) is a widely used semiconductor material (in photovoltaic cells, diodes, thin-film transistors) with the advantages of a narrow band gap and photoelectrochemical activity in the visible range. The use of nanosized CdS represents a possible route to increase the efficiency of most photocatalytic processes owing to their very high surface area-to-volume ratio.

Biomolecule-CdS NPs hybrid systems allow the development of new biosensors [3,4], photocatalysts for CO_2 reduction [5], and fluorescence labels for biological imaging [6]. In such systems, the electrons and holes respectively generated in the conduction and valence bands of the semiconductor can be used by the enzyme to reduce and oxidize the substrate on the surfaces of the semiconductor NPs. A catalytic behavior of metallic NPs towards the biologically important conversion of dihydronicotinamide adenine dinucleotide (NADH) to nicotinamid adenine dinucleotide (NAD$^+$) was previously found by El-Sayed et al. [7]. The electrochemical oxidation of NADH to enzymatically active NAD$^+$ has attracted much interest because more than 300 enzymes have been identified as NADH-dependent dehydrogenases [8]. Hence, semiconductor NPs represent a possible route to enhance electron/hole transfer efficiency, leading to the oxidation of NADH to NAD$^+$ under irradiation with light. If a NADH dehydrogenase was incorporated into semiconductor NPs, the photoinduced oxidation of NADH to NAD$^+$ will be more performed by the enzyme immobilized in the inorganic semiconductor NPs than it on the surface of the NPs. In the present investigation, CdS NPs incorporating NADH dehydrogenase [diaphorase (EC1.6.5.2), DA], which is a ubiquitous class of flavin-bound enzymes catalyzing the oxidation of reduced NAD$^+$, were successfully synthesized using water-in-oil microemulsions (inverse micelles). Herein, we report on the catalytic effect of enzyme-CdS NPs hybrid systems on the oxidation of NADH to NAD$^+$. The catalytic properties of CdS NPs activated with such enzymes may have great future importance in biochemistry and medical applications.

2. Experimental

CdS NPs in inverse micelles were prepared by a method similar to those previously reported [9]. Reverse micelles were prepared by adding 1.0 mL water to 60 cm^3 heptane containing 4.2 g sodium di(2-ethylhexyl) sulfosuccinate. To prepare unmodified CdS NPs, aqueous solutions of 1.0 M $Cd(ClO_4)_2$ (0.235 mL) and 1.0 M Na_2S

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(0.165 mL) were added to 35- and 25-mL aliquots (A and B solutions) of the prepared reverse micelle solution, respectively. To prepare CdS NPs incorporating DA from *Clostridium kluyveri* (Wako Co., Ltd, Osaka, Japan), aqueous solutions of 1.0 M $Cd(ClO_4)_2$ mixed with 1.0 mg mL^{-1} DA and/or redox mediator solution (0.235 mL) and 1.0 M Na_2S (0.165 mL) were respectively added to the A and B solutions of the prepared reverse micelles. Polyethylenimine modified with 8 mol % ferrocene (Fc-PEI) was used as a redox mediator in this study. The polyion mediator was synthesized by a previously reported procedure [10]. After being stirred individually for 1 h, the A and B solutions were mixed together and sonicated for another 1 h, which resulted in the formation of CdS NPs in the reverse micelles. A yellow solid was obtained by vacuum evaporation of the solvent from the mixture. After subsequent dissolution of the solid in 50 mL of toluene, 1.0 M 2-(dimethylamino)ethanethiol (DMAET) was added to modify the CdS surface, and then the solution was stirred for 24 h. The modified CdS NPs were precipitated by mixing the solution with ethanol, after which the NPs were washed successively with methanol several times and finally dispersed in pure water (1.0 mg mL^{-1}).

Next, the DMAET-modified enzyme-CdS NPs (1.0 mg mL^{-1}) were mixed with 5 mM NADH in a 50 mM phosphate buffer solution (pH 8.0) and stirred under steady-state illumination for 60 min at room temperature (25 ± 2 °C). The light beam from a 150 W halogen lamp (CS-15; Cabin Industrial Co., Ltd., Tokyo, Japan) was used to illuminate the colloidal CdS NPs solution. The catalytic activity of the enzyme-CdS NPs was evaluated by monitoring the extent of NADH oxidation at 340 nm using a Hitachi spectrometer (U-1800). Quantitative measurement of NADH oxidation was also carried out using a NAD/NADH assay kit (BioVision, Inc., Palo Alto, CA, USA).

Multilayer films of anionic polystyrene sulfonate (PSS) and cationic DMAET-modified CdS NPs (or enzyme-CdS NPs) were fabricated on ITO electrodes using a layer-by-layer deposition technique [11,12]. The prepared film is hereafter denoted as $(PSS/CdS\ NPs)_n$, where n is the number of accumulated PSS and CdS NPs layers on the electrode. AFM measurements of the deposited enzyme-CdS NPs were performed with a SPM-9600 (Shimadzu Co., Ltd., Kyoto, Japan). The UV–vis absorption spectra of the immobilized CdS NPs were also recorded using the U-1800 spectrometer. The X-ray powder diffraction (XRD) pattern of the CdS NPs was measured using an X-ray diffractometer (MiniFlex; Rigaku Co. Ltd., Tokyo, Japan) with Cu-Kα rays generated at 30 kV and 15 mA.

3. Results and discussion

Fig. 1 shows an XRD pattern of the enzyme-CdS NPs synthesized using the water-in-oil microemulsion. The enzyme-CdS NPs were successfully prepared by mixing the DA- and Fc-PEI-containing (1:0.2, w/w) A solution with the B solution, as mentioned above. Onda et al. studied complex formation between cationic proteins and polyanions, and observed that the enzyme activities of protein-polyion complexes immobilized on solid substrates were much higher than that of the native protein on the same substrate [13]. They attempted to form the same complex consisting of cationic DA and anionic Fc-PEI as that used for our CdS NPs. The XRD pattern of the prepared CdS NPs agreed very closely with that of pure cubic-phase CdS (JCPDS41-1049). In Fig. 1, the peaks at 26.4, 43.5, and 52.0° were assignable to the (111), (220), and (311) reflections of the cubic CdS phase, respectively [14]. Fig. 2 shows a typical dynamic mode AFM image of the enzyme-CdS NPs deposited onto an ITO electrode by electrostatic forces. The average particle diameter was estimated to be 4 nm by measuring the heights of the imaged particles. The particles were closely packed on the electrode. Fig. 3 shows UV–vis absorption spectra obtained during the assembly of the $(PSS/enzyme\text{-}CdS\ NPs)_{1-5}$ film on an ITO electrode. A regular increase in absorption was observed after deposition of every bilayer. The absorption spectrum of the bilayer film exhibited an exciton peak at around 390 nm that coincided with that of the as-prepared CdS NPs in aqueous solution.

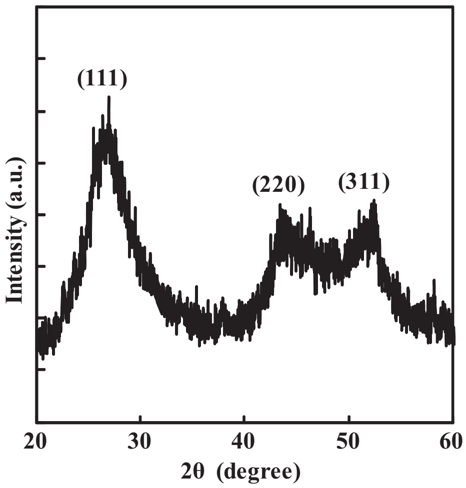

Fig. 1. XRD pattern of enzyme-CdS NPs synthesized using a water-in-oil microemulsion. The enzyme-CdS NPs were prepared by mixing solutions A, which contained DA and Fc-PEI (1:0.2, w/w), and B as described in the experimental procedure.

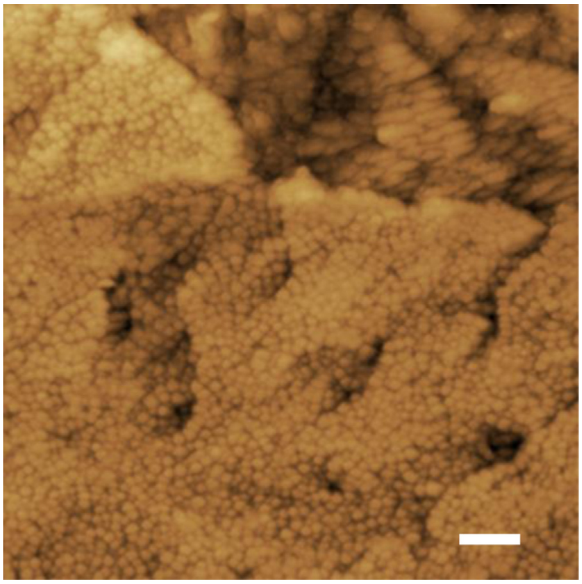

Fig. 2. Typical dynamic mode AFM image of the enzyme-CdS NPs deposited onto the ITO electrode. Scale bar is 100 nm.

Evaluation of the catalytic activity of the hybrid enzyme-CdS NPs systems for the oxidation of NADH to NAD^+ was carried out under visible illumination. Fig. 4 (A) shows that the oxidation of NADH proceeded in the colloidal solution of CdS NPs. This study for the first time demonstrated that the photoinduced oxidation of NADH was carried out by the CdS NPs. Moreover, the rate of oxidation of NADH at 60 min for the enzyme-CdS NPs in which DA and Fc-PEI (1:0.2, w/w) were incorporated was about 3-fold higher than that of the pure CdS NPs. When the enzyme-CdS NPs were prepared in absence of the redox mediator, the catalytic effect became weaker and the oxidation rate was approximately the same as that of the pure semiconductor NPs. Additionally, the rate of oxidation of NADH for the CdS NPs in which Fc-PEI was incorporated without DA was found to be similar to that of the

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