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Research Article

A System of Photocatalysis for NAD+ Regeneration of Product of (*S*)-1-Pheylethanol by Enzymic Catalysis

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Abstract

In this study, a system of photocatalysis for NAD+ regeneration of enzymatic catalysis was constructed. The optimal conditions for the coupling reaction of photocatalysis and biocatalysis were explored. Blue light was chosen for the efficient reaction and the optimal concentration of VB_2 (vitamin B_2, riboflavin) was determined. NAD+-dependent (*R*)-1-phenylethanol dehydrogenase was used in the reaction for transforming (*R*)-1-phenylethanol to acetophenone. The byproducts of the reaction were just H_2O and O_2 by means of catalase. The coupling reaction of catalysis and photocatalysis can be used for obtaining (*S*)-1-phenylethanol through racemization of 1-phenylethanol.

Keywords: NAD+; Photocatalysis; Biocatalysis; Riboflavin; (*R*)-1-phenylethanol dehydrogenase

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1. Introduction

In recent years, increasing attention has been focused on photoredox catalysis, particularly using visible light [1].The cheap light emitting diode (LED) light sources have been used to photocatalytic reactions, the significantly deceased energy demand of LED photocatalysis make it attractive envisioning environmentally acceptable syntheses [2]. The photocatalysis have effectively applied in organic chemistry and the possible environmental impact is necessary to substantiate 'green claims' [2-5].

Biocatalysis is a green chemical process. Oxidoreductases are attractive biocatalyst for chiral selective oxidation or reduction, but most of them often require cofactor. Nicotinamide Adenine Dinucleotide (NAD+) or reduced NAD+ (NADH) is a kind of important cofactor of oxidoreductases, which serve as sources of redox equivalents, are prohibitively expensive, and therefore can't be applied stoichiometrically [6,7]. Hence, several cofactor regeneration methods that enable the substoichiometric use of the cofactors have been developed. One approach that is commonly used in industry is whole-cell catalysis which relies on the microbial cell to both express the enzyme for regenerate the cofactor [8-10]. The bi-enzymatic system and electrochemical technique for efficient cofactors regeneration are also applied in industrial biocatalytic applications [11-14]. The concept of artificial photosynthesis was used to cofactor regen-

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eration has recently been demonstrated. For example, Nam *et al.* studied visible light-driven NADH regeneration sensitized by proflavine and flavin derivatives [15,16], Lee *et al.* studied photocatalysis NAD^+ regeneration with flavin adenine dinucleotide (FAD) as a reducing power and Rauch *et al.* using flavin mononucleotide (FMN) as photosensitizer achieved NAD^+ regeneration under LED light irradiation [2,17].

Flavin derivatives can use as hydrogen acceptors for *in situ* regeneration of coenzyme [18]. Riboflavin (VB_2) is responsible for the redox activity of cofactors FMN and FAD. It exhibited high turnover numbers under photolysis condition [19]. Furthermore, VB_2 is cheaper and easily available than FMN and FAD in chemical market. VB_2 was considered as the most likely candidate to explain the "natural" photodegradation of pollutants in the environment [20].

The optically active (*S*)-1-phenylethanol is a kind of chemical chiral building block and used for synthetic intermediate in pharmaceutical and agrochemical industries. It can be produced by deracemization of racemic 1-phenylethanol catalyzed NAD^+-dependent (*R*)-1-phenylethanol dehydrogenase [21]. Therefore in this study, VB_2 is used as photocatalyst for NAD^+ regeneration in enzymatic preparation of (*S*)-1-phenylethanol.

2. Materials and methods

2.1 Materials

The chemicals were in highest grade and purchased from Sinopharm Chemical Reagent Corporation (Nanjing, China). VB_2 was obtained from Aladdin Industrial Corporation (Shanghai, China). The catalase (from ox liver, 3500U/mg) was supplied by Shanghai Titan Science Co., Ltd. (Shanghai, China) and the LED (18 W) purchased from Open Moon Co., Ltd. (Guangzhou, China).

2.2 Purification of (*R*)-1-phenylethanol dehydrogenase

The (*R*)-1-phenylethanol dehydrogenase was extracted from the strain of *Lysinibacillus* NUST506 which was obtained from the previous studies [21]. The bacterial strain was cultured in 500 mL LB medium with 0.1% (v/v) 1-phenylethanol (pH 7.0) and shaken at 37 °C with 120 rpm for 12 hours. The cells were harvested by refrigerated centrifugation at 4 °C (8,000 × g, 5 min) and washed with deionized water thrice.

All purification steps were carried out at 4 °C. The cell pellet was resuspended in deionized water and broken by ultrasonic processor for an hour [22]. The supernatant after centrifugation (10,000 × g, 10 min) was used as the crude enzyme. Ammonium sulfate was added to 50% saturation and put it at 4 °C for 2 hours. Moreover, the supernatant was collected by centrifugation (10,000 × g, 10 min), then 85% saturation of ammonium sulfate was added and incubated at 4 °C for 2 hours. After centrifugation (10,000 × g, 10 min), add a small volume deionized water to resuspend the precipitate. Then, the enzyme solution was dialyzed against deionized water. The preparative polyacrylamide gel electrophoresis (PAGE) was performed by Prep Cell Model 491 (Bio-Rad, USA) as Han described to get the (*R*)-1-phenylethanol dehydrogenase, [23]. The densities gel and separated gel were 5% (m/v) and 8% (m/v), respectively. The enzyme extract with loading buffer was loaded on the preparative PAGE and ran at 200 V for 12 hours with cooling water circulation. Proteins were continuously eluted by Na_2HPO_4-citric acid buffer (10 mM, pH 7.0) and collected by fraction collector all the time.

2.3 Assay of (*R*)-1-phenylethanol Dehydrogenase Activity

The enzymatic activity was assayed with a spectrophotometer (Hitachi U1800, Japan) by monitoring the increase or decrease in absorbance at 340 nm, which is the characteristic absorption wavelength of NADH (ε_{340} = 6.22 mM^{-1} cm^{-1}) [24-26]. To a test tube, 2.7 mL of Gly-NaOH buffer (50 mM, pH 8.0), 0.1 mL of (*R*)-1-phenylethanol (24 mM), 0.1 mL of NAD^+ (5 mM) and 0.1 mL of (*R*)-1-phenylethanol dehydrogenase solution were added. The mixture was incubated at 37 °C for 1 min in order to get initial reaction rates. In the control group, 0.1 mL of deionized water was used instead of 0.1 mL of (*R*)-1-phenylethanol dehydrogenase solution in the original reaction system. One unit of the enzyme activity was defined as the amount of (*R*)-1-phenylethanol dehydrogenase which produced 1 μmole of NADH per minute at 37 °C and pH 8.0.

2.4 Photocatalysis Reaction

The reaction was performed in a light-proof incubator. The concentration of NADH was assayed by absorbance at 340 nm. The effects of different light were performed using red, yellow, green and blue LEDs. The reaction solu-

tion consisted of 0.2 mL NADH (5 mM), 0.02 mL VB_2 (1 mM) and 3.78 mL sodium phosphate buffer (50 mM, pH 7.0) in silex glass tube. The residual of NADH was measured. The effects of pH were determined by pH range of 5 to 10. Different 50 mM buffer solutions were used to set the optimal pH value, Na_2HPO_4-Citric acid buffer was used for pH 5-6, sodium phosphate buffer for pH 7-8 and Gly-NaOH buffer for pH 9-10. The residual of NADH was determined after 10min incubation at 37 °C. The effects of VB_2 (0.01 mM to 2 mM) on photocatalysis were also detected at 37 °C. The reaction solution was consisted of 0.02 mL VB_2, 0.2 mL NADH (5 mM) and 3.78 mL phosphate buffer (50 mM, pH 8.0). The residual of NADH was determined after 30 min incubation.

2.5 Coupling of Enzymatic Catalysis and Photocatalysis

To the test tube, 0.05 mL 164 mM (*R*)-1-phenylethanol, 0.1 mL 5 mM NAD^+, 0.01 mL 20 mM VB_2, 0.01 mL catalase (3500 U/mL) and 0.5 mL (*R*)-1-phenylethanol dehydrogenase (200U/mL) were added to 2.33 mL phosphate buffer (200 mM, pH 8.0), the reaction was performed under blue light at 37 °C. An equal concentration of (*S*)-1-phenylethanol and (*R,S*)-1-phenylethanol instead of the (*R*)-1-phenylethanol was used to determine the chiral specificity. The concentrations of components during the reaction were analyzed by High performance liquid chromatography (HPLC) every 12 hours.

2.6 High Performance Liquid Chromatography

HPLC (Shimadzu, LC-10AT, Japan) was used for detecting the concentration of 1-phenylethanol and acetophenone. All reaction liquids were centrifuged at 10,000 × g for 10 min before analyzing. The samples were detected by a Shimadzu VP-ODS C18 column (250 mm × 4.6 mm) with a mobile phase of acetonitrile-ammonium acetate (25 mM ammonium acetate) (1:1, v/v) at a flow rate of 1.0 mL/min detected with Shimadzu SPD-10A detector at 210 nm in room temperature.

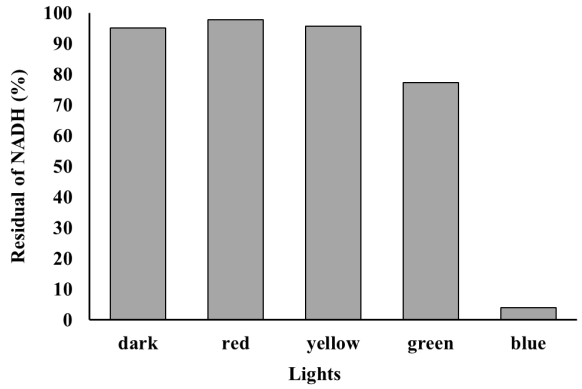

Figure 1. The decrement of NADH under different kind of LED at 37 °C.

3. Results and Discussion

3.1 Effects of Different Light and VB_2 Concentration on Photocatalysis Reaction

Different kinds of LED light for photocatalysis were tested. The conversion of NADH under different lights was showed in Figure 1. The decrement of NADH in dark, red, yellow, green and blue LED lights were 4.9%, 2.3%, 4.3%, 22.7%, and 96%, respectively. Under blue light, significantly decrement of NADH was observed. The decrement of NADH under blue light in 30 min was showed in Figure 2. The decrement of NADH was about 98% in 30 min. So, blue light was selected for next experi-

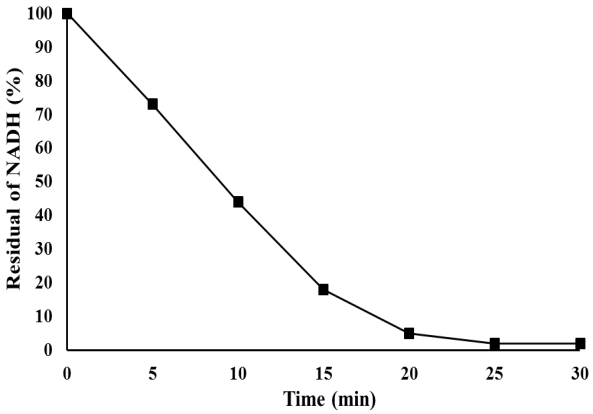

Figure 2. The residual of NADH in 30 min under blue light at 37 °C

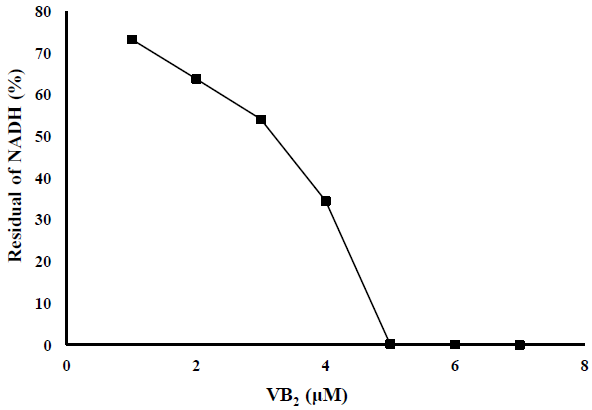

Figure 3. The conversions of NADH under different concentration of VB_2

ments for the conversion of NADH. We studied the effects of different concentration of VB_2 to photocatalysis for choice a suitable concentration of VB_2 to the reaction system. The result was showed as Figure 3. The concentration (5μM) of VB_2 was considering the proper concentration to the photocatalysis reaction system.

Figure 4 showed the conversion of NADH was gradually decreased with the increase of pH. The conversion was about 50% at pH 8.0 after incubation 10 min at 37 °C. Considering both the effect of pH on photocatalysis, the effect of pH on enzyme activity and the pH tolerance of the enzyme, pH 8.0 was selected for next coupling reaction.

3.2 Coupling Enzymatic Catalysis and Photocatalysis

In order to building an efficient regeneration system for NAD^+ from NADH, the enzymatic catalysis and photocatalysis was coupled for reduction of (*R*)-1-phenylethanol to acetophenone (Figure 5). The end-products of the reaction were acetophenone, H_2O and O_2 by means of catalase.

As showed Figure 6, (*R*)-1-phenylethanol was added to the reaction and NAD^+ regenerated by VB_2 under blue light irradiation. The (*R*)-1-phenylethanol dehydrogenase catalyzed the oxidation of (*R*)-1-phenylethanol to acetophenone and the reduction of the NAD^+ to NADH. Then, the NADH was oxidized to NAD^+ by VB_2 as hydrogen acceptor. Finally, the VB_2 was oxidized under blue light irradiation. The acetophenone was increased gradually with the decrease of (*R*)-1-phenylethanol in the reaction when (*R*)-1-phenylethanol added. After 50 hours, 2.75 mM (*R*)-1-phenylethanol was oxidized to acetophenone by coupling enzymatic catalysis and photocatalysis with the cofactor of 0.17 mM NAD^+.

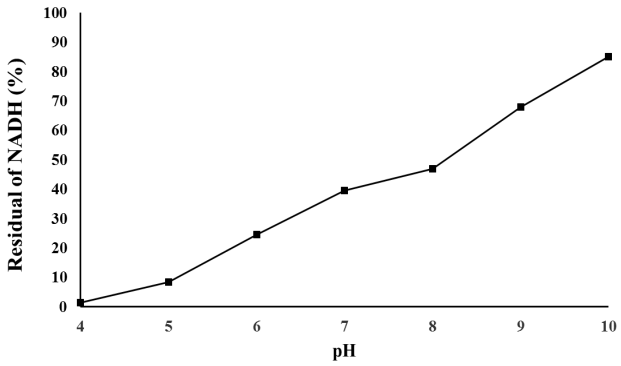

Figure 4. The effects of pH to the conversions of NADH.

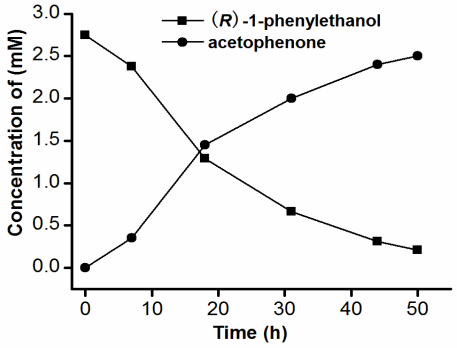

Figure 6. The concentrations of (R)-1-phenylethanol and acetophenone during the reaction process.

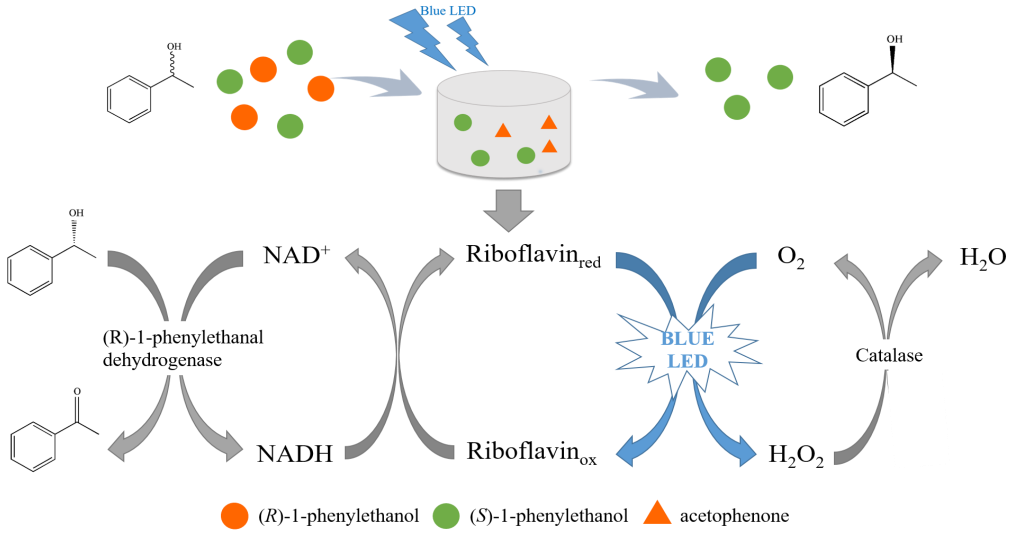

Figure 5. The integrated diagrammatic sketch of coupling enzymatic catalysis and photocatalysis

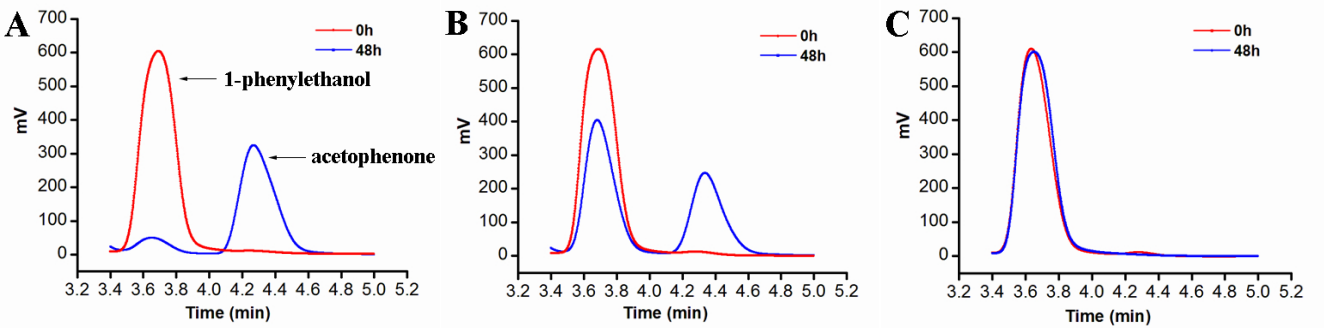

Figure 7. The content of (R)-1-phenylethanol and acetophenone when (R)-1-phenylethanol added (A); The content of (R, S)-1-phenylethanol and acetophenone when racemic 1-phenylethanol added (B); The content of (S)-1-phenylethanol and acetophenone when (S)-1-phenylethanol added (C)

An equal concentration of (*R*)-1-phenylethanol, (*S*)-1-phenylethanol, and (*R,S*)-1-phenylethanol was added to the solution respectively. The result was showed in Figure 7. when racemic 1-phenylethanol added, half of (*R,S*)-1-phenylethanol was oxidized to acetophenone. When (*S*)-1-phenylethanol added, the content of (*S*)-1-phenylethanol was not decreased and no acetophenone generated. According to the results, substrate specificity of the coupling reaction was determined. (*R*)-1-phenylethanol was the optimum substrate to the reaction and (*S*)-1-phenylethanol was not acted. It is because the (*R*)-1-phenylethanol dehydrogenase used was a kind of chiral oxidoreductase, so the (*S*)-1-phenylethanol could not be convert by the coupling catalysis. The photocatalysis did not influence the substrate specificity of (*R*)-1-phenylethanol dehydrogenase. We expect that the coupling will improve and apply in further applications in synthetic organic chemistry.

4. Conclusion

In this study, VB_2 was employed as an efficient photosensitizer for the NAD^+ regeneration of enzymic catalysis under blue light. VB_2 exhibited a high capacity to drive the NADH to NAD^+ in pH 8.0 at 37 °C. The photoregenerated NAD^+ was used for production chiral 1-phenylethanol by NAD^+-dependent (*R*)-1-phenylethanol dehydrogenase.

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