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Immobilization of catalase by using Zr(IV)-modified collagen fiber as the supporting matrix

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ABSTRACT

Collagen fiber (CF), an abundant natural material of biopolymer, was used as supporting matrix for immobilization of catalase. CF was firstly reacted with Zr(IV), then the catalase was immobilized on Zr(IV)-modified CF (Zr–CF) by adsorption. The structures and properties of Zr–CF and the Zr–CF immobilized catalase (Zr–CF-catalase) were characterized by means of DSC, SEM, FT-IR, etc. It was found that the denaturation temperature of CF was remarkably increased from 37 to 75.6 °C after reacting with Zr(IV). The amount of catalase immobilized on Zr–CF was 45.4 mg/g. The K_m and V_{max} values were 23.3 mM and 3.5×10^4 U/mg for free catalase and 30.2 mM and 1.3×10^4 U/mg for immobilized catalase, respectively. Compared with free catalase, the Zr–CF-catalase exhibited higher relative activity in a broad range of pH and temperature (pH 3.0–8.0, 15–75 °C). For example, the relative activity of immobilized catalase is still 70.3% at pH 3.0 and 25 °C and 62.2% at pH 7.0 and 75 °C, which is rather higher than 45.6% and 41.2% of the free ones at the same conditions. Furthermore, Zr–CF-catalase could be reused 70 times before complete deactivation and preserve 86.5% of its original activities after stored at room temperature for 12 days.

1. Introduction

In recent years, biocatalysis has received considerable attention due to its distinct advantages of extremely high activity, selectivity and substrate specificity [1]. Based on the employments of different enzymes, some organic reactions can be easily accomplished under mild conditions (room temperature, aqueous solution and moderate pH) [2]. However, it would be quite difficult to recover the enzymes from reaction systems because they are often water-soluble [3]. Another problem that restrains the application of free enzymes is that they are fragile to operation conditions and need to be utilized within very specific temperatures and chemical environments [4]. To overcome these disadvantages, various strategies have been proposed. Among them, immobilization is one of the most successful methods, where enzymes are commonly immobilized onto water-insoluble supports by chemical or physical interactions [5]. Upon the immobilization of enzyme on supporting matrix, the recovery of enzyme could be easily achieved simply by filtration, and then recycled for another biocatalysis operation [6]. Furthermore, the thermal and chemical stability of enzyme may be considerably improved by the properly designed immobilization process due to the changes of enzyme microenvironment, multipoint immobilization, conformation rigidity, etc. after the interactions between the enzyme and supporting matrix [7,8].

Since the properties of enzyme can be significantly affected by the matrix, it is crucial to select a proper supporting matrix in the enzyme immobilization. In general, the matrix should be nontoxic to the enzyme, and have suitable bioaffinity and hydrophilic–hydrophobic property, which are beneficial for the immobilization of enzyme [9]. From this point, many inorganic supports (silicate, glass, alumina, etc.) and organic biopolymer (chitin, chitosan, gel, etc.) have been used for the immobilization of enzymes [10–16]. Upon them, organic biopolymer with bioaffinity property commonly exhibits relatively high loading amount and stable reusability [17]. Furthermore, some biopolymers from renewable resources have the advantage of low-cost. It can be inferred that the biopolymers with good biocompatibility and amphiphilic property could be very suitably used as the supports for immobilization of enzymes.

Collagen fiber (CF), one of the natural biopolymers, is mainly obtained from the skin of domestic animals. As well documented by the literatures, CF is formed by the aggregation of collagen molecules, of which the collagen molecule is composed of three polypeptide chains with triple helical structure [18]. Collagen has abundant functional groups such as –OH, –COOH, $–CONH_2$ and $–NH_2$, and thus is ready to react with other chemicals. CF has unique biocompatibility to enzymes, which is unparalleled by other inorganic matrix. Importantly, collagen is a kind of "soft" amphiphilic

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material and thus it is able to adjust its conformation when contacted with substrates that have different hydrophilic/hydrophobic properties [19]. Therefore, it can be expected that the activity of enzyme would be well preserved after immobilization on CF, which could adjust its conformation to accommodate the geometrical dimension of enzyme. At present investigation, CF was used as supporting matrix to immobilize catalase.

Catalase is a commonly used enzyme in textile and food industries, as well as in environment protection due to its decomposition effect on hydrogen peroxide [20–22], which also has prospecting applications in the analytical systems as a component of hydrogen peroxide and glucose-biosensor systems [23]. It consists of four subunits, each of which includes ferri-porphyrin as a prosthetic group [24]. As one of the multimeric enzymes, catalase will be first inactivated under certain conditions via subunit dissociation [25]. Therefore, the properly designed catalase immobilization is performed as one of the interesting ways to prevent subunits from dissociation and realize other improvements of the enzyme properties [26].

Before the immobilization, CF firstly reacted with Zr(IV) to increase its stability and affinity to catalase due to the chelating interactions between Zr(IV) and catalase. The reaction of CF with Zr(IV) can be easily achieved in aqueous solution [27]. Subsequently, the immobilization of catalase was performed by adsorption on Zr(IV)-modified CF (Zr–CF). The absorption of enzymes on immobilized metal-affinity chromatography matrixes requires a multipoint enzyme–support interaction. Thereby the stability of the immobilized multi-subunit enzyme may be increased [28]. On the other hand, the multipoint interaction between catalase and Zr–CF can be properly controlled by employing a suitable loading amount of Zr(IV) on CF. To evaluate the catalytic activities of the Zr–CF immobilized catalase (Zr–CF-catalase), the decomposition of H_2O_2 was carried out under different conditions (pH and temperature) by using Zr–CF-catalase as catalyst. The K_m and V_{max} values of free and immobilized catalase were determined. In addition, the reusability, thermal and storage stability of Zr–CF-catalase were also investigated.

2. Materials and methods

2.1. Materials

CF was prepared according to the procedures in our previous work [29]. In brief, bovine skin was cleaned, limed, split, and delimed according to the procedures of leather processing in order to remove non-collagen components. Then the skin was treated by an aqueous solution of acetic acid to remove mineral substances. After the pH was adjusted to 4.8–5.0 with acetic acid–sodium acetate buffer solution, the skin was dehydrated by absolute ethyl alcohol, dried in a vacuum to moisture content ≤10.0%, ground, and sieved. As a result, the collagen fiber was obtained with the moisture content ≤12.0%.

Catalase (hydrogen peroxide oxidoreductase; EC.1.11.1.6; 3120 U/mg) from bovine liver was obtained from Kayon (Shanghai Kayon Biological Technology Co. Ltd., China). $Zr(SO_4)_2$ and other chemicals were analytical grade and used as received.

2.2. Preparation and characterization of Zr–CF

2.0 g CF was soaked in 250 mL deionized water at room temperature for 24 h. The solution pH was adjusted to 1.8–2.0 by using HCOOH and H_2SO_4 solutions. 2.1 g $Zr(SO_4)_2$ was added at 30 °C with constant stirring for 4 h. Then a proper amount of $NaHCO_3$ solution (15%, w/w) was gradually added within 2 h in order to increase the solution pH to 4.0–4.5. Subsequently, the reaction continued at 45 °C for another 5 h. When the reaction was completed, the product was collected by filtration, thoroughly washed with deionized water, dehydrated by acetone and ethanol, and finally dried at room temperature for 24 h.

The morphology of Zr–CF was observed by scanning electron microscopy (SEM, JEOL LTD JSM-5900LV). The heat denaturation temperature of Zr–CF was determined by differential scanning calorimetry (DSC, PC 200DSC, NETZSH Company, Germany) with a heating rate of 10 °C/min. The pH corresponding to zero point of charge (pH_{pzc}) of the Zr–CF was determined by the solid addition method [30]. Other physicochemical properties were determined by common methods.

2.3. Immobilization of catalase on Zr–CF

The immobilization of catalase on Zr–CF was performed by the adsorption method. Briefly, 1 g Zr–CF was mixed with 25 mL of 3 mg/mL catalase solution (9360 U/mL) in distilled water (pH 6.7) or 100 mM phosphate buffer (pH range 5.0–7.5). The mixture was stirred at 25 °C for 30 min, and then filtrated and thoroughly washed with deionized water. Finally, Zr–CF-catalase was obtained after drying at room temperature. CF was also used in the immobilization of catalase for comparison.

Fourier transform-infrared spectroscopy (FT-IR, PerkinElmer, USA) analyses were carried out by using compressed film of KBr pellet and sample powder. All FT-IR spectra were recorded by absorption mode in an interval of 2 cm^{-1} and in the wavenumber range of 4000–500 cm^{-1}.

The catalase concentration in the solution was determined by means of inductively coupled plasma atomic emission spectroscopy (ICP-AES) (ICP, Perkin-Elmer Optima 2100DV, German). Then the loading amount of catalase on Zr–CF was determined by mass balance calculation.

2.4. Enzyme activity assay

To evaluate the activity of catalase, the decomposition of H_2O_2 was carried out using catalase as biocatalyst. The activity was spectrophotometrically determined by direct measurement of decrease in the absorbance of hydrogen peroxide at 240 nm using a specific absorption coefficient of 0.0392 cm^2 μmol $H_2O_2{}^{-1}$ [31]. The activity determination of immobilized catalase was conducted by mixing 0.1 g Zr–CF-catalase with 10 mL phosphate buffer (100 mM, pH 7.0), in which the concentration of H_2O_2 was 100 mM. The reaction was conducted at 25 °C for 5 min and then terminated by the filtration of immobilized catalase from the reaction solution by 400-mesh filter cloth. Zr–CF without adding H_2O_2 was as a blank. The absorbance of the reaction mixture was determined and the activity was calculated. One unit of activity is defined as the decomposition of 1 μmol H_2O_2 per min at 25 °C and pH 7.0.

These activity assays were also carried out over the pH range of 3.0–8.0 in 100 mM phosphate buffer and the temperature range of 15–75 °C to determine the effects of pH and reaction temperature for the free and immobilized catalase, where the highest value of each set was assigned the value of 100%.

2.5. Determination of kinetic parameters

The kinetic studies of free and immobilized catalase were determined by measuring their activities in phosphate buffer (100 mM) at pH 7.0. The concentration range of H_2O_2 was 2, 4, 8, 12.5, 25 and 50 mM. The kinetic parameters were calculated as follows:

$$\frac{1}{\upsilon} = \frac{K_m}{V_{max}} \cdot [S] + \frac{1}{V_{max}} \quad (1)$$

Eq. (1) is the Lineweaver–Burk plot for the Michaelis–Menten equation. Where υ is the reaction rate of the reduction H_2O_2, [S] is the concentration of ethanol. K_m is the Michaelis–Menten constant and V_{max} is the maximum of reaction velocity.

2.6. Thermal inactivation of free and immobilized catalase

0.1 g Zr–CF-catalase or free catalase was added into 10 mL phosphate buffer (100 mM) at pH 7.0. The mixture was kept at different temperatures (15–75 °C) for 5 h. After that, the Zr–CF-catalase was filtrated and then used for catalytic decomposition of H_2O_2 in the same conditions as in Section 2.4. As for free catalase, the activity was directly determined in the phosphate buffer after addition of H_2O_2 (100 mM).

2.7. Reusability of Zr–CF-catalase

The catalytic decomposition of H_2O_2 was initiated by putting 0.1 g Zr–CF-catalase into 10 mL phosphate buffer (100 mM, pH 7.0) at 25 °C. The other reaction conditions were the same as in Section 2.4, and the catalytic activity was calculated. After each reaction, the Zr–CF-catalase was thoroughly washed with deionized water, and then reintroduced into the phosphate buffer for the next reaction.

2.8. The storage stability of Zr–CF-catalase

Free and immobilized catalases were preserved in 100 mM phosphate buffer (pH 7.0) at room temperature for 12 days. Then their catalytic activity for H_2O_2 decomposition was determined as described in Section 2.4. Additionally, the stability of Zr–CF-catalase in air storage at room temperature was also studied.

3. Results and discussion

3.1. Preparation and characterization of Zr–CF

The collagen molecule comprised in skin collagen fiber is mainly type I collagen that contains three polypeptide α-chains, each con-