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Research Article

Assembly of graphene oxide-formate dehydrogenase composites by nickel-coordination with enhanced stability and reusability

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Featuring unique planar structure, large surface area and biocompatibility, graphene oxide (GO) has been widely taken as an ideal scaffold for the immobilization of various enzymes. In this regard, nickel-coordinated graphene oxide composites (GO-Ni) were prepared as novel supporters for the immobilization of formate dehydrogenase. The catalytic activity, stability and morphology were studied. Compared with GO, the enzyme loading capacity of GO-Ni was enhanced by 5.2-fold, besides the immobilized enzyme GO-Ni-FDH exhibited better thermostability, storage stability and reuse stability than GO-FDH. GO-Ni-FDH retained 40.9% of its initial activity after 3 h at 60°C, and retained 31.4% of its initial relative activity after 20 days' storage at 4°C. After eight times usages, GO-Ni-FDH maintained 63.8% of its initial activity. Mechanism insights of the multiple interactions of enzyme with the GO-Ni were studied, considering coordination bonds, hydrogen bonds, electrostatic forces, coordination bonds, and etc. A practical and simple immobilization strategy by metal ions coordination for multimeric dehydrogenase was developed.

Keywords: Formate dehydrogenase / Graphene oxide / Immobilization / Metal ions coordination / Thermostability

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1 Introduction

Integrating the advantages of facile separation from the product, efficient recovery and enhanced stability, enzymes in immobilized form play an important role in biochemical industry, e.g., medicine, food or energy [1, 2]. A variety of matrixes have been used as the immobilization carriers of enzymes, including chitin [3], silica [4], nanomaterials [5] and biocatalytic membrane [6, 7].

In the past few years, graphene-based nanomaterials have attracted much attention for their excellent physical and biochem-

ical properties [8–10]. Among which, graphene oxide (GO) is an important derivative of graphite created by strong oxidation, with hydroxyl, epoxy, and carboxyl groups distributing on the basal and edge of the plane [11]. The unique structure, large surface area and biocompatibility of GO make it an ideal immobilization scaffold for enzymes. Zhang et al. investigated the physical and catalytic properties of immobilized enzyme GO-HRP, which exhibited better storage stability and thermostability in removing phenolic compound [12]. Zdeněk Sofer et al. showed that lipase immobilized onto graphene oxide is highly resistant to thermal and solvent exposure [13].

Furthermore, the chemical functionalization of GO has been explored to improve the regulation of immobilized enzymes. Horseradish peroxidase (HRP) and oxalate oxidase (OxOx) were

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Abbreviations: FDH, formate dehydrogenase; GO, graphene oxide; GO-FDH, immobilize formate dehydrogenase onto graphene oxide; GO-Ni, nickel coordinated graphene oxide; GO-Ni-FDH, immobilize formate dehydrogenase onto nickel coordinated graphene oxide; SEM, scanning electronic microscopy

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immobilized onto chemically reduced graphene oxide (CRGO) to obtain CRGO-HRP conjugates that showed excellent properties due to the hydrophobic interaction [14]. Chen et al. applied magnetic Fe_3O_4 graphene oxide for alcohol dehydrogenase immobilization with enhanced the enzymatic stability and reusability [15].

Formate dehydrogenases (FDHs) are NAD^+ -dependent enzymes, which can converse formic acid into CO_2 , and reduced NAD^+ to NADH. FDHs are widely used in the regeneration of NADH for the inertness of the substrate and product (CO_2), which is easy to remove from the main product [16]. In order to enhance the stability of FDH, researchers have used different methods to immobilize the enzyme [17].

In this study, two strategies including immobilizing formate dehydrogenase from *Candida boidiniin* onto graphene oxide (GO-FDH) by absorption method and immobilizing FDH onto nickel-coordinated graphene oxide (GO-Ni-FDH) by coordination were compared. Nickel-coordinated graphene oxide was constructed as a novel scaffold for enzyme immobilization. The morphology characterization, catalytic activity and stability were studied. Mechanism insight of the multiple interactions of enzyme with the GO-Ni were studied considering hydrogen bonds, electrostatic forces, coordination bonds, and etc.

2 Materials and methods

2.1 Chemicals

Graphite, NAD^+ and NiSO_4 were purchased from Sigma Chemical Company (Tianjin, China). Kanamycin and isopropyl-beta-D-thiogalactopyranoside (IPTG) were purchased from Trans-Gen Biotech (Shanghai, China). Lysogeny broth (LB media) was purchased from Sangon Biotech (Shanghai) Co. Ltd. All other chemicals were analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). *E. coli* BL21 (DE3) pET-28a (+)-FDH was constructed in our laboratory.

2.2 Strains and culture conditions

FDH gene was obtained from *Candida boidiniin* (190-1284 nucleotide, GeneBank ID: AF004096.1), which was sequenced and synthesized by Sangon Biotech Co. Ltd (Shanghai, China). FDH was expressed in a pET28a, BL21 (DE3) system with an N-terminal His-tag and cultured in LB media supplemented with 50 $\mu\text{g}/\text{mL}$ kanamycin at 37°C until OD_{600} reached 0.5–0.6. Isopropyl-beta-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM and incubated 24–36 h at 16°C, 200 rpm.

2.3 Enzyme extraction and purification

The cells were centrifuged at 8000 rpm for 10 min, washed twice with phosphate-buffered saline solution (PBS) and resuspended. The cells were then pretreated by ultrasonication for 35 times (every time working for 3 s and cooling for 6 s) in an ice bath.

To remove cell debris, samples were centrifuged at 10 000 rpm for 15 min under 4°C.

The crude extract was filtered through a membrane filter (0.22 μm) and loaded onto a 5 mL His-Trap HP affinity column. Ten column volumes of binding buffer (20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4) were applied to wash unbound impurities. The column was equilibrated with binding buffer and eluted with elution buffer (20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4) by one-step elution. The fractions with the desire activity were desalted and concentrated using a Macrosep Advance Centrifugal Device (cut-off 10 kDa, Pall, East Hills, NY, USA). Protein concentrations were determined with a modified Bradford protein assay kit (Sangon Biotech Co. Ltd) using bovine serum albumin as a standard, as described by Bradford [18].

2.4 Enzyme assay

FDH activity was measured by detecting the production of NADH in the oxidative sodium formate at 340 nm using an Infinite 200 PRO spectramicroplate reader (TECAN). One unit (U) of enzyme activity was defined as the quantity of enzyme catalyzing the formation of 1 μmol NADH per min under standard conditions. Specific activity was recorded as units/mg protein. The parallel experiments were repeated four times. In the reaction system, the concentration of the substrate sodium formate and cofactor NAD^+ is 162 and 1.62 mM, respectively and the reaction temperature was 30°C.

To investigate the effect of pH on the free and immobilized FDH, enzyme activities were measured in different buffer (0.2 M potassium phosphate buffer, pH 6–7.5; 0.2 M Tris-HCl buffer, pH 8–9.5; 0.2 M glycine-NaOH buffer, pH 10–11). Temperature effects on the activities of the free and immobilized FDH were conducted over the temperature range of 30–70°C. Thermostability of enzyme was measured by the method of calculating the residual enzymatic activity of free and immobilized FDH after incubating pH 10.0 and 60°C and taking samples every 15 min. The activity was expressed as relative forms (%) with the maximal value of enzyme activity at a certain pH or temperature as 100%.

2.5 Preparation of GO

Graphene oxide (GO) was prepared by the Modified Hummers method. Graphite powder (1.5 g) and sodium nitrate (1.5 g) were first added into a round-bottom flask, and then concentrated sulfuric acid (69 mL, 36 N) was added, which was placed in an ice-water bath and stirred slowly. After that, potassium permanganate (9 g) was added and mixed. The mixture was transferred into water bath at 35°C, and stirred for 60 min. Afterward, the flask was settled at room temperature for 24 h, and then deionized water (120 mL) was added gradually and stirred for 30 min at 90°C. The reaction mixture was then cooled down naturally to room temperature. Subsequently, deionized water (300 mL) and hydrogen peroxide (9 mL, 30%) was added and stirred slowly. By now, a bright yellow solution was obtained; a

precipitate was collected by filtration and washed with diluted hydrochloric acid. After that, the precipitate was put into dialysis tube, changing the distilled water every 2 h. After dialysis, the mixture was centrifuged (4000 rpm, 40 min, 4°C) to remove graphite and obtain graphene oxide.

2.6 Preparation of immobilized enzymes GO-Ni-FDH

GO was exfoliated into GO sheets by ultrasonication in water for 2 h to give colloidal solution with concentration of 1 mg/mL. Then NiSO₄ solution was directly added into the aforementioned GO dispersion under vigorous stirring for 1 h. The coordination product was separated by centrifugation at 10 000 rpm for 15 min and washed three times with DI water. FDH was immobilized on the GO and GO-Ni respectively to investigate the activities of the enzymes before and after immobilization. For the immobilization of FDH on GO, FDH solution was dispersed in the 1 mg/mL GO solution, and then the mixture was incubated at 4°C for 4 h with occasional shaking, followed by being centrifuged at 8000 rpm for 15 min. The supernatant was collected to determine the enzyme loading. While the precipitate was centrifuged and rinsed three times with the Tris-HCl buffer (pH 10) to remove nonspecific adsorbed enzymes. The amount of immobilized enzyme was evaluated by the enzyme concentration change of the solution before and after loading.

2.7 Characterization

Morphology of nanoparticles was observed by scanning electron microscopy (SEM, Zeiss Sigma). To prepare for the samples of SEM, 10 μ L of immobilized enzyme was dropped on a silicon chip and allowed to evaporate and dry overnight at room temperature, and the sample was then coated with platinum (2-nm thickness using a JEOL JFC 1600 (JEOL, Tokyo, Japan) with an electric current of 10 mA for 30 s before imaging with a Zeiss Sigma SEM (Carl-Zeiss AG, Germany). Circular dichroism spectrum (CD) of FDH, GO-FDH and GO-Ni-FDH were determined by a Jasco J-810 CD spectropolarimeter (Biologic, Japan) within the range of 190 and 400 nm with an interval of 1 nm. Fourier transform infrared spectroscopy (FT-IR) (Nicolet 380, Thermo Electron Corporation, U.S.A) was used for detecting the functional groups of materials at the range of 4000–400 cm⁻¹.

2.8 Storage stability and reusability

The free enzyme FDH and immobilized enzyme GO-FDH, GO-Ni-FDH were stored at 4°C, the storage stabilities were studied and compared by measuring the residual activities every 2 days during 20 days.

The reusability of immobilized enzyme GO-FDH and GO-Ni-FDH was explored by repeating the enzymatic reaction for eight times. After finishing each cycle of reaction, the immobilized enzyme was washed by Tris-HCl buffer (pH 10) and re-collected by centrifugation at 10 000 rpm for 15 min (4°C). The first cycle of enzyme activity was set as 100%, and the remaining activity

was counted for percent form (%) compared with that of the first reaction.

3 Results and discussion

3.1 The effect of metal ions on the activity free formate dehydrogenase

To obtain immobilized enzymes with high activities, it was necessary to evaluate the effects of different metal ions on the FDH activity. Metal ions can be activators or inhibitors for enzymes [19]. In the immobilization process, metal ions were selected for GO modification. Twelve divalent metal ions (Ni²⁺, Cu²⁺, Mn²⁺, Co²⁺, Cd²⁺, Zn²⁺, Mg²⁺, Sr²⁺, Ba²⁺, Ca²⁺, Fe²⁺ and Fe³⁺) were selected to investigate the effect of metal ions on FDH activity. The FDH activities were assayed after incubating FDH with metal ions for 1 h at 4°C. Supporting Information Fig. 1 shows that Cu²⁺, Mn²⁺, Co²⁺ and Fe³⁺ inhibited the activity of free FDH, while Fe²⁺ has a slight effect on the activity, while Ni²⁺, Cd²⁺, Zn²⁺, Mg²⁺, Sr²⁺, Ba²⁺ and Ca²⁺ activated the enzyme. Supporting Information Fig. 1A demonstrates that Ni²⁺ stands out for improving the FDH activity by 1.2-fold. Therefore, these properties made Ni²⁺ an outstanding metal ion for coordinated immobilization. As shown in Supporting Information Fig. 1B, the free FDH achieve a maximum activity when the concentration of Ni²⁺ was 0.5 mM.

3.2 GO Coordination with metal ions

GO dispersion solution (pH 7.5) was titrated by Ni²⁺ solution (Supporting Information Fig. 2). The conductivity of the mixture shows a sharp increase when the concentration of Ni²⁺ in the system reached 100 mM at which GO was saturated by Ni²⁺ coordination. It can be calculated that the coordinated capacity of 1 mg GO is 7.11 mM Ni²⁺. The functional groups of GO (hydroxyl, epoxy, and carboxyl) enhanced the adsorption ability of metal ions [20].

3.3 Enzyme immobilization

The effect of enzyme concentration on loading capacity of GO and GO-Ni was studied. FDH was immobilized to GO and GO-Ni at pH 10, respectively. The enzyme loading capacity of GO and GO-Ni is 0.231 and 1.19 mg/mg, respectively (Fig. 1A). The result indicated that metal ions induced coordination provide more binding sites for enzyme immobilization compared with the adsorption by 5.2-fold.

To study the influence of immobilization, the enzymatic activities (U/mg enzyme) of FDH, GO-FDH and GO-Ni-FDH were determined to be 4.65, 3.41 and 4.03 U/mg, respectively. As shown in Fig. 1B, GO-FDH maintained 73.3% of its initial activity, while immobilized enzyme GO-Ni-FDH maintained 86.7% of its initial activity. The loss of activity mainly resulted from steric hindrance and the mass-transfer limitations of the solid supports [21–23]. GO-Ni-FDH maintained high activity may due to the site-specific immobilization of 6 $\times$ His tag of FDH

Figure 1. (A) The effect of FDH concentration on loading capacity of GO and GO-Ni. (B) The relative activity GO-FDH, GO-Ni-FDH. Reaction conditions: sodium formate, 162 mM; NAD^+ , 1.62 mM; pH 10.0; temperature: 30°C. The relative activity is expressed as a percentage of the original activity of free FDH.

Figure 2. Effect of pH and temperature on the activity of free and immobilized FDH. (A) Effect of pH on FDH, GO-FDH and GO-Ni-FDH, enzyme activity was determined in buffer (0.2 M potassium phosphate buffer, pH 6–7.5; 0.2 M Tris-HCl buffer, pH 8–9.5; 0.2 M glycine-NaOH buffer, pH 10–11), reaction conditions: sodium formate, 162 mM; NAD^+ , 1.62 mM; temperature: 30°C. (B) Effect of temperature on FDH, GO-FDH and GO-Ni-FDH at the range of 30–70°C. Reaction conditions: sodium formate, 162 mM; NAD^+ , 1.62 mM; pH 10.0; temperature: 30°C. The activity was expressed as relative forms (%) with the maximal value of enzyme activity at a certain pH or temperature as 100%.

with Ni, which greatly decreased steric hindrance by avoiding the enzyme stacking [24].

Optimal reaction conditions for immobilized FDH and free FDH were studied. The optimum pH of FDH, GO-FDH and GO-Ni-FDH was 10 under the same conditions (Fig. 2A). Figure 2B shows that the optimum temperature of FDH, GO-FDH and GO-Ni-FDH was 60°C. The immobilization did not affect the optimum temperature and pH, which indicated that GO and GO-Ni is biocompatible frameworks for FDH. The results of the enzyme loading and immobilized enzyme activity indicated that GO-Ni is promising for efficient enzyme immobilization. The reaction of pH could change the charge of amino acid residue of the protein, thus effected the conformation of the enzyme.

3.4 Thermostability and storage stability

The thermostability of free FDH and immobilized FDH studied under 60°C by measuring the residual activities every 30 min. After 150 min, GO-FDH and GO-Ni-FDH enzyme activity retained 20.6 and 42.4% of its initial activity, respectively, while

free FDH only retained 4.6% of its initial activity (Fig. 3A). Compared with FDH and GO-FDH, GO-Ni-FDH exhibited a better thermostability.

To investigate the storage stability, the free enzyme and immobilized enzyme were both stored at 4°C and the enzyme activity was determined every two days (Fig. 3B). After 20 days, GO-FDH and GO-Ni-FDH retained 22.8 and 31.4% of its initial activity, respectively, while free FDH only 3.48% of its initial activity. The results indicated that immobilization by GO and GO-Ni greatly improved the storage stability of FDH.

3.5 Reuse stability

The reusability of GO-FDH and GO-Ni-FDH nanoparticles were investigated. Figure 4 shows that the activity of GO-FDH and GO-Ni-FDH gradually decreased over 8 cycles. GO-FDH and GO-Ni-FDH retained 27.28 and 63.81% of its initial activity after cycling through eight successive reactions, respectively. Compared with GO-FDH, activity of GO-Ni-FDH dropped slowly, which indicated the strengthened interaction between

Figure 3. Stability of free and immobilized enzyme. (A) Thermostability of free FDH, GO-FDH and GO-Ni-FDH. (B) Storage stability of free FDH, GO-FDH and GO-Ni-FDH. Reaction conditions: sodium formate, 162 mM; NAD^+ , 1.62 mM; pH 10.0; temperature: 30°C.

Figure 4. The reusability of GO-FDH and GO-Ni-FDH, Reaction conditions: sodium formate, 162 mM; NAD^+ , 1.62 mM; pH 10.0; temperature: 30°C. The relative activity is expressed as a percentage of the original activity of GO-FDH and GO-Ni-FDH.

GO-Ni and enzymes by the coordination bonds. The loss of GO-FDH activity might be caused by the deadsorption of enzyme, while for GO-Ni-FDH, the dissociation of metal ion after multiple operations under continuous stirring may be the main reason. Enzyme conformation change may also be one reason for enzyme activity loss.

3.6 Kinetic parameters

The catalytic activities of the FDH immobilized on GO and GO-Ni were further characterized by turnover number (K_{cat}) and enzyme efficiency (K_{cat}/K_m). K_m and K_{cat} values were obtained according to the Lineweaver-Burk equation (Supporting Information Fig. 3). The values of kinetic parameters K_m and K_{cat} were summarized in Table 1. The similar K_m values for the GO-FDH, GO-Ni-FDH and free FDH indicated that they all have a similar affinity to the reducing substrate. However, the K_{cat} and K_{cat}/K_m of GO-FDH and GO-Ni-FDH were lower than those of free FDH, indicating lower catalytic efficiency of immobilized enzymes. GO-Ni-FDH showed higher K_{cat} and K_{cat}/K_m

Table 1. Kinetic parameters of FDH, GO-FDH and GO-Ni-FDH

Samples	K_m (mM)	K_{cat} (s^{-1})	K_{cat}/K_m ($\text{s}^{-1}\text{mM}^{-1}$)
FDH	5.2770	3.4693	0.6574
GO-FDH	5.6880	3.3820	0.5946
GO-Ni-FDH	5.6314	3.4171	0.6068

* K_m , the Michaelis–Menten constant of sodium formate.

Reaction conditions: sodium formate concentration (5–80 mmol/L); enzyme, 1 mg/L; pH 10.0; temperature, 30°C.

than that of GO-FDH, exhibiting higher catalytic efficiency of GO-Ni-FDH.

3.7 Structural change analysis

Evaluating the extent of the structural changes can be used for the explanation of the interactions of enzyme with carrier and the alteration of the catalytic behavior. The change in enzymes' secondary structure after immobilization was examined using circular dichroism (CD) studies. The process of immobilization changed the structure of enzymes. GO-Ni-FDH with more decreasing of α -helices than GO-FDH (Supporting Information Fig. 4). For GO, the negative charged surface lead to the adsorption of enzyme by electrostatic interaction. Compared with GO-FDH, the strength of coordinated bond of FDH with GO-Ni is much stronger than electrostatic interaction and hydrogen bonds.

Soluble proteins often had less hydrophobic residues exposed on their surface, the structure of enzymes altered to facilitate the immobilization, at least in cases where hydrophobic interactions were the most prominent ones [25–27]. The immobilization procedure led to depletion of α -helices and increases in β -sheets for glucose oxidase [28], catalase [29], and cyt c [30]. Similar results were also observed for the interaction of a *Bacillus subtilis* esterase with several functionalized graphene-based nanomaterials [31].

CD study showed that the secondary structure of FDH had altered after immobilization using GO and GO-Ni. However, the alteration of secondary structure seemed to make little influence in the ability of enzyme to bind with substrate according to kinetic study (Table 1). According to the obtained information, we speculated that the immobilization using GO and GO-Ni could improve FDH stability through the interaction with

Figure 5. SEM micrographs. (A) Immobilized enzyme GO-FDH. (B) Immobilized enzyme GO-Ni-FDH. (C) Immobilized enzyme GO-Ni-FDH after 14 days' storage.

Figure 6. Illustration of assembly of immobilized enzyme GO-Ni-FDH.

the non-active domain of FDH, rather than the active pocket domain.

Furthermore, the FT-IR spectrums of GO, GO-FDH and GO-Ni-FDH were also studied at the range of 4000–400 cm^{-1} . The spectrums exhibit the peak at 3395 cm^{-1} attributing to O–H stretching vibration, the peak at 1715 cm^{-1} attributes to C=O stretching vibration, the peak at 1620 cm^{-1} attributes to C=C stretching vibration. The peak at 1087 cm^{-1} attributes to C–O stretching vibration (Supporting Information Fig. 5).

3.8 Mechanism study of immobilization based on GO and GO-Ni

Morphological study of the immobilized FDH was carried out by scanning electron microscopy. SEM images of GO-FDH and GO-Ni-FDH are presented in Fig. 5, GO-FDH displayed amorphous structure (Fig. 5A), while GO-Ni-FDH formed uniform crystal particles (Fig. 5B).

Mechanism insight of the enzyme immobilization by the metal-ion coordinated GO were investigated based on the analysis of multi-level interactions of enzyme with GO. GO is an oxygen-rich derivative of graphite created by strong oxidation, decorated with hydroxyl, epoxy, and carboxyl groups. These oxygen-containing groups were distributed randomly on the basal planes and edges of the GO sheets. These functional groups provided a negative charged surface. Furthermore, owing to their polarity, weak interactions with protein like hydrogen bonds were formed (Fig. 6). The unmodified areas of the surface maintained their free π -electrons, making any π – π interactions feasible. Therefore, multi-level interaction of GO with enzyme may affect the conformational state and thus the catalytic activity of FDH.

GO-Ni acted as a framework for FDH immobilization by metal ions coordination. $6 \times \text{His}$ tag was usually applied for recombinant protein purification by metal affinity chromatography. FDH was expressed with $6 \times \text{His}$ tag tail which could provide stable binding force with Ni^{2+} ion. The surface chemistry of nanomaterials may influence the interactions with biomolecules and thus affected the immobilization as well as the conformation and biological function of conjugated enzyme. It may explain that GO-Ni-FDH formed uniform crystal structure and with enhanced the stability. Additionally, the oligomeric FDH assemblies were stabilized by the excessive Ni-chelated GOs, which further prevented the disassociation of the FDH subunits. Metal-mediated oligomeric assemblies of enzymes had been reported to improve the crystallizability of proteins and promote protein crystallization by enhancing the interactions of the subunits of the enzyme, which may explain the formation of the inerratic enzyme-metal slice.

Interestingly, the structure of immobilized enzyme GO-Ni-FDH with the extension of storage time of 14 days was determined by the SEM image (Fig. 5C), which showed rectangular structure with about 1 μm wide and 3 μm long. Layer-by-layer assembly of FDH and GO-Ni formed the sandwich structure. The multi-interactions between FDH and GO-Ni enhanced the enzymatic activity and stability compared to GO-FDH.

4 Concluding remarks

A novel carrier based on metal ions coordinated graphene oxide was developed. Metal ions coordination enhanced immobilized capacity of graphene oxide by 5.2-fold compared with adsorption. The thermostability of GO-FDH and GO-Ni-FDH was

enhanced compared with free FDH at 60°C. Morphology characterization indicated the assembly of enzyme with GO-Ni to form the uniform structure. Mechanism insight of the multiple interactions of enzyme with the GO-Ni were studied considering hydrogen bonds, electrostatic forces, coordination bonds, and etc. A novel, practical and simple immobilization strategy based on metal ions coordinated GO as biocompatible supporter for multimeric dehydrogenase was developed.

Practical application

In this work, a novel scaffold based on nickel coordinated graphene oxide (GO-Ni) for the immobilization of unstable enzyme, formate dehydrogenase (FDH), was constructed and characterized. The enzyme loading capacity of GO-Ni was 5.2 folds higher than that of GO. Compared with free FDH and graphene oxide immobilized enzyme (GO-FDH), the immobilized enzyme GO-Ni-FDH exhibited better thermostability, storage stability and reuse stability. Furthermore, GO-Ni-FDH formed uniform crystal structure due to the multi-layer interaction between GO-Ni and FDH. A practical and simple immobilization strategy by metal ions coordination for multimeric dehydrogenase was developed. The application would be useful in areas of cofactor regeneration and biosensors.

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