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Novel biohybrids of layered double hydroxide and lactate dehydrogenase enzyme: Synthesis, characterization and catalytic activity studies

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ABSTRACT

The present work introduces new biohybrid materials involving layered double hydroxides (LDH) and biomolecule such as enzyme to produce bioinorganic system. Lactate dehydrogenase (Lac Deh) has been chosen as a model enzyme, being immobilized onto MgAl and ZnAl LDH materials via direct ion-exchange (adsorption) and co-precipitation methods. The immobilization efficiency was largely dependent upon the immobilization methods. A comparative study shows that the co-precipitation method favors the immobilization of great and tunable amount of enzyme. The structural behavior, chemical bonding composition and morphology of the resulting biohybrids were determined by X-ray diffraction (XRD) study, Fourier transform infrared (FTIR) spectroscopy and transmission electron microscopy (TEM), respectively. The free and immobilized enzyme activity and kinetic parameters were also reported using UV–Visible spectroscopy. However, the modified LDH materials showed a decrease in crystallinity as compared to the unmodified LDH. The change in activity of the immobilized lactate dehydrogenase was considered to be due, to the reduced accessibility of substrate molecules to the active sites of the enzyme and the partial conformational change of the Lac Deh molecules as a result of the immobilization way. Finally, it was proven that there is a correlation between structure/microstructure and enzyme activity dependent on the immobilization process.

1. Introduction

Bioinorganic systems are nanostructured biohybrid materials in which a biomolecule is assembled to nanosized inorganic solid [1]. They constitute a new generation of materials, at the interface of biology and materials science [2]. These materials have been subjected to intense research not only as ecological materials, but also for other applications including biotechnology such as in biosensor systems [3,4]. Therefore, immobilization of biomolecules, such as DNA, ATP, nucleosides and enzyme with isoelectric point varying in a large pH domain has been extensively studied [5,6]. Bioinorganic research on biohybrids can protect the biomolecules from decomposition or denaturation, which made it useful in safe and targeted.

2D-Layered mineral materials, such as layered double hydroxide (LDH), so called anionic clays or hydrotalcites (HTs), has attracted considerable attention as host structures due to its technological importance in catalysis, separation technology, optics, nanocomposites materials engineering, and medical science [7]. More recently these inorganic materials have been also applied in immobilization of enzyme for biosensor development [8,9]. LDH are synthetic lamellar solids with positively charged brucite-like layers of mixed metal hydroxides separated by interlamellar domains occupied by anions and water molecules, defined by the general formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]^{x+}[(A_{x/n})^{n-}\cdot yH_2O]$ (abbreviated as $M^{2+}M^{3+}$–A, where M^{2+} is a bivalent cation (such as Mg^{2+}, Ni^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+} or Zn^{2+}), M^{3+} is a trivalent cation (such as Al^{3+},

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Cr^{3+}, Ga^{3+} or Fe^{3+}), A is an interlayer anion (such as CO_3^{2-}, SO_4^{2-}, Cl^- or NO_3^-) or an organic species, x represents the molar ratio $[M^{2+}/(M^{2+}+M^{3+})]$ and y is the number of water molecules located in the interlayer region together with anions) (Scheme 1). These compounds, due to its layered structure display a remarkable range of physic-chemical properties make them an attractive choice to immobilize enzymes: (i) adjustable chemical composition layer/interlayer; (ii) variable anionic exchange capacities 1.5–4.5 meq/g; (iii) opened structure which can accommodate large anionic molecules; (iv) and poor adjustable textural properties controlled by the synthesis process and conditions.

Anions such as enzymes are typically immobilized into LDH by three approaches [10,11]. The first approach is the co-precipitation method, which requires the addition of a solution of M^{2+} and M^{3+} ions into a base solution of the desired anions. The second technique is the direct exchange method, where LDH is stirred in a solution of the chosen anions at a suitable concentration. The last method is the rehydration method, where the calcined LDH is added to a solution of desired anions [12].

Several studies showed that the LDHs are a good support for enzyme immobilization, due to their easy synthesis procedure combined with good retention capacity of enzymes [13]. Moreover, they can play a key role not only as matrices for enzyme support, but also to preserve their activity and their interesting properties in charge transport.

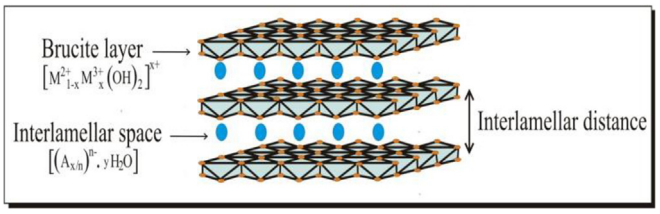
However, lactate dehydrogenase (Lac Deh) enzyme is chosen as a model enzyme, being immobilized in LDH materials. Lac Deh is an enzyme which largely presented in the organs and tissues of vegetal or animal organizations (kidney, heart, muscles, pancreas, skin …) [14], catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to NAD^+ and back. Measurement of lactate using biosensors is of great importance for the clinical analysis as well as for food analysis [15]. For these reasons, several types of enzyme support have been reported in literature [16–20] to meet primarily the need for the development of continuous monitoring techniques. Despite the large number of matrices used as support, no paper concerning the immobilization study into an anionic clay matrix, belonging to the LDH class, has been reported so far.

In the present investigation, we attempted to immobilize Lac Deh on two types of hydrotalcites, MgAl and ZnAl LDH materials via ion-exchange and co-precipitation process. In addition, we systematically studied the merits and demerits of these immobilization methods.

2. Experimental

2.1. Starting materials

Lactate Dehydrogenase (EC 1.1.1.27, molecular weight 140 kD) was purchased from Biomagreb. β-nicotinamide adenine dinucleotide (NAD), $MgCl_2.6H_2O$, $ZnCl_2$, $AlCl_36H_2O$ and NaOH were purchased from Sigma Aldrich. Phosphate-buffered saline (PBS), and distilled water was used during all experiments.



Scheme 1. Schematic representation of the crystal structure of the layered double hydroxide.

2.2. Synthesis of MgAl–Cl and ZnAl–Cl LDHs

The MgAl–Cl and ZnAl–Cl LDH hybrid inorganic materials were prepared by the co-precipitation route. Typically, 50 mL of the magnesium salts $MgCl_2$ or zinc salts $ZnCl_2$ and aluminum salts $AlCl_3$ were prepared in total cationic concentration of 1.0 M. In order to keep a constant M(II)/Al ratio of 2, the amount of $M(II)Cl_2$ and $AlCl_3$ was adjusted for each synthesis. The salt solution was then added drop wise into a reactor with a constant flow of 0.12 mL/min. Throughout this addition, the pH of the solution was then elevated and maintained using 2.0 M NaOH solution, which caused metal co-precipitation until the solution reached a pH of 9 and 7.5 respectively for MgAl and ZnAl phases. The reaction was carried out under N_2 atmosphere to avoid carbonate contamination. The addition of the salt solution was complete within 7 h and the suspensions were immediately centrifuged at 5000 rpm without any ageing in order to quench the crystal growth and therefore obtain small platelets. The solids recovered by centrifugation were washed several times with distilled water until no sodium chloride was present and dried at room temperature for 24 h.

2.3. Adsorption studies

Adsorption of Lac Deh into the MgAl–Cl and ZnAl–Cl LDHs was carried out by direct ion-exchange reaction. Briefly, 5 mL of Lac Deh solutions at different concentrations (Ci) were prepared in a phosphate buffer saline solution (0.1 M and pH = 7.4). Next, these solutions were slowly added to a suspension containing 5 mg of a freshly prepared LDH in 5 mL of distilled water. This system was maintained under magnetic stirring at 25 °C for 2 h. Afterwards, the solid product was isolated by centrifugation, washed thoroughly with distilled water and dried overnight at room temperature. The resulting biohybrid material was denoted LDH/Lac Deh_{ads}. The amount of Lac Deh non-immobilized and present in water of washing was determined by absorbance UV–Vis at 340 nm.

2.4. Synthesis of MgAl–Cl/Lac Deh biohybrid via co-precipitation

The co-precipitation method described above was adapted for the preparation of small amounts of biohybrid materials. Typically, 10 mL of a 0.1 M metallic salts solution (mixed aqueous solution of $MgCl_2$ and $AlCl_3$ with a molar ratio R = Mg^{2+}:Al^{3+} of 2:1) was introduced with a constant flow into reactor containing an aqueous Lac Deh solution under stream of N_2. The pH was maintained constant at 9 by simultaneous addition of a 0.2 M NaOH solution. The co-precipitation was performed for LDH/Lac Deh mass ratio Q equal to 1.0, 98% of the enzyme is immobilized. The resulting material was collected and stored following the same procedure as described above and denoted MgAl–Cl/Lac Deh_{cop}.

2.5. Determination of enzyme activity and kinetics parameters

Activity measurements and kinetic study of free and immobilized lactate dehydrogenase were measured in the direction of oxidation of lactate to pyruvate (Eq. (1)) by monitoring the changes in the absorbance of NADH at 340 nm according to the method described by Sohn et al. [20].

$$\text{Lactate} + NAD^+ \overset{\text{Lac Deh}}{\longleftrightarrow} \text{Pyruvate} + NADH + H^+ \quad (1)$$

Typically, 2 mL of 0.1 mM NAD solution and 3 mL of 1.0 mM lactate solution were added to a test tube containing free enzyme or immobilized enzyme (MgAl–Cl/Lac Deh and ZnAl–Cl/Lac Deh). All assays were conducted at 25 °C in PBS buffer medium (pH = 7.4). After the reaction mixture was stirred shortly by vortexing the