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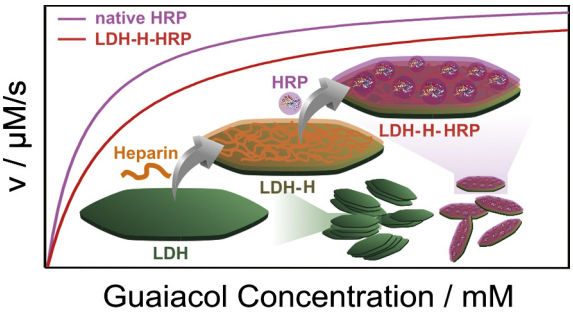
Horseradish peroxidase-nanoclay hybrid particles of high functional and colloidal stability

Marko Pavlovic [a], Paul Rouster [b], Zoltan Somosi [c], Istvan Szilagyi [c,d,*]

[a] Department of Inorganic and Analytical Chemistry, University of Geneva, CH-1205 Geneva, Switzerland
[b] Institute of Condensed Matter and Nanosciences - Bio and Soft Matter, Université Catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium
[c] MTA-SZTE Lendület Biocolloids Research Group, University of Szeged, H-6720 Szeged, Hungary
[d] Department of Physical Chemistry and Materials Science, University of Szeged, H-6720 Szeged, Hungary

GRAPHICAL ABSTRACT



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ABSTRACT

Highly stable dispersions of enzyme-clay nanohybrids of excellent horseradish peroxidase activity were developed. Layered double hydroxide nanoclay was synthesized and functionalized with heparin polyelectrolyte to immobilize the horseradish peroxidase enzyme. The formation of a saturated heparin layer on the platelets led to charge inversion of the positively charged bare nanoclay and to highly stable aqueous dispersions. Great affinity of the enzyme to the surface modified platelets resulted in strong horseradish peroxidase adsorption through electrostatic and hydrophobic interactions as well as hydrogen bonding network and prevented enzyme leakage from the obtained material. The enzyme kept its functional integrity upon immobilization and showed excellent activity in decomposition of hydrogen peroxide and oxidation of an aromatic compound in the test reactions. In addition, remarkable long term functional stability of the enzyme-nanoclay hybrid was observed making the developed colloidal system a promising antioxidant candidate in biomedical treatments and industrial processes.

1. Introduction

Enzymes are important natural biocatalysts in various catalytic processes as they exhibit high efficiency and substrate specificity as well as provide green and sustainable manufacturing processes [1,2]. On the other hand, their high sensitivity to the environmental conditions (e.g., temperature [3], pressure [4] and pH [5]) and difficult separation from the reaction mixture [6] prevent their widespread use. The most efficient way to overcome these challenges is the enzyme immobilization, which became one of the most important fields in bio-relevant catalysis in the past decade [7].

* Corresponding author at: Department of Physical Chemistry and Materials Science, University of Szeged, H-6720 Szeged, Hungary.
E-mail address: szistvan@chem.u-szeged.hu (I. Szilagyi).

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Immobilization techniques include binding to surfaces [8,9], catalyst entrapment [10] and cross-linking [11]. The first method relies on the affinity of the enzymes to the surface of the carrier particles and the attachment may take place through electrostatic and hydrophobic interactions or through primary and secondary chemical bonds [12,13]. The most commonly used supports for enzyme immobilization involve inorganic particles [14–16], synthetic or natural polymers [17] and various hybrid materials [18,19].

Among the first ones, clay materials including layered double hydroxides (LDHs) of dimensions in the nanoscale range represent a resourceful type of solid support to immobilize enzymes on their surface by physical adsorption or between the lamellae by intercalation [20–22]. Given the fact that the majority of the enzymes and other biomolecules are negatively charged at physiological pH, the anion exchange capacity of the LDHs has been widely utilized during the immobilization processes [23,24]. Other advantages in using LDHs as solid supports include high enzyme loading due to large surface area, which can even be further improved by delamination into unilamellar nanosheets, and their ease of synthesis as well as high compositional diversity [25,26]. Moreover, it has been shown that LDHs are biocompatible providing the opportunity to use them in biomedical applications [27–29]. Functionalization of LDHs with polymeric substances is a versatile tool to further extend the range of possible biomolecules that can be used to prepare LDH-based hybrid nanomaterials [30,31]. In biomedical applications, one of the aims is to obtain dispersions of stable functionalized particles, since their aggregation may lead to thrombosis [32].

Horseradish peroxidase (HRP) is a member of the vast group of peroxidases that can use hydrogen peroxide as an electron acceptor and further oxidize organic molecules [33]. HRP contains a heme group with an iron cation in its active center that can interact with hydrogen peroxide and form an intermediate state of enzyme that can oxidize organic molecules like guaiacol in the most typical HRP assay [34]. Immobilization of HRP has been in the focus of numerous research groups to develop hybrid materials for various applications including water treatment [35], sensing [36] and catalysis [37]. A wide range of solid supports was applied in the immobilization procedures. Accordingly, physical adsorption of HRP on quantum dots [16], titania [38] and nanocomposites [39] has been reported. Intercalation between oxide layers was also investigated to improve the enzyme sensitivity [40]. Another advantageous method to achieve strong enzyme binding to the carrier is the covalent linkage, which was achieved in the case of mesoporous silica [37] and polymer beads [41]. Coprecipitation of copper(II) salts and HRP resulted in nanoparticles of high surface area with enhanced catalytic activity and increased functional stability [42]. HRP containing hybrid materials were developed by preparing LDH-carbon nanodot composites first and by co-adsorbing them with the enzyme on an electrode to obtain selective and reproducible biosensor for hydrogen peroxide detection [18]. In addition, certain nanoparticles also showed significant HRP-like activity even without additional catalysts immobilized on their surface [43–45].

The previously mentioned studies mainly focused on the structural characterization, electrochemical properties and enzymatic activity of the hybrid materials, however, limited attention has been paid to the colloidal stability of the enzyme-particle systems. Given the fact that the majority of the applications take place in dispersions, aggregation processes and their effect on the efficiency in peroxide decomposition reactions are critical issues for obtaining highly active biohybrids. Therefore, in order to achieve stable composite dispersions having significant enzymatic function, the charging and aggregation processes under the application conditions have to be investigated in detail. The main goal of such investigations must be to develop fine colloidal dispersions of primary nanocomposite particles of high specific surface area.

In the present work, HRP enzyme was immobilized on the surface of LDH particles previously modified by adsorption of heparin (HEP) polyelectrolyte (Scheme 1). The enzyme loading was determined and optimized, in addition, the surface charge properties and aggregation processes were followed in the systems to obtain highly stable colloids. The efficiencies of the bionanohybrids were tested and compared to the bare enzyme. Finally, the long term functional and colloidal stability of the dispersions and the hydrogen peroxide consuming ability were assessed.

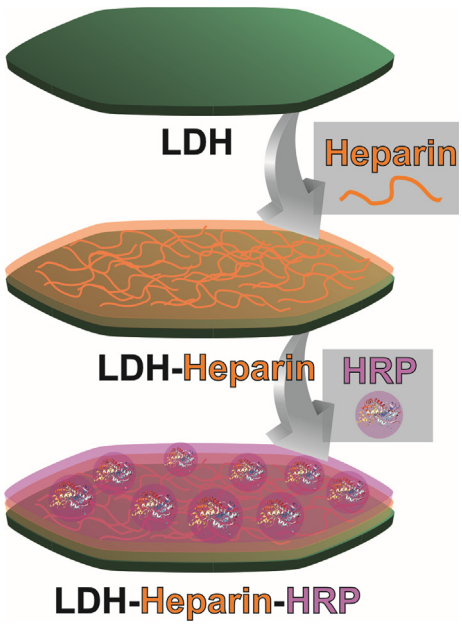
2. Materials and methods

2.1. Chemicals

Sodium heparin of low molecular weight (12–15 kg/mol) was obtained from Acros Organics. HRP (type VI, EC 1.11.1.7) was bought from Sigma-Aldrich (P8375) in the form of salt-free lyophilized powder (250 units/mg solid, Reinheitszahl value is 2.5–4.0) and used without further purification. H_3PO_4 (99.99%), HCl (99%), NaOH (97%), NaCl (99.5%), Na_2CO_3 (99.5%), $Al(NO_3)_3{\cdot}9H_2O$ (98%), $Mg(NO_3)_2{\cdot}6H_2O$ (99.99%), NaH_2PO_4 (99%), Na_2HPO_4 (99%) and Guaiacol (98%) were purchased from Sigma-Aldrich. The experiments were performed at 25 °C in aqueous medium using ultrapure Milli-Q water (Millipore) at pH (7.0 ± 0.5).

2.2. LDH synthesis

LDH nanoparticles composed of Mg^{2+} and Al^{3+} layer forming metal ions and CO_3^{2-} intercalated anions were synthesized by the "flash" coprecipitation method and their particle size distribution was improved by hydrothermal treatment, as described earlier [46]. In brief, $Mg(NO_3)_2{\cdot}6H_2O$ and $Al(NO_3)_3{\cdot}9H_2O$ salts were dissolved together in a stoichiometric ratio of 2:1 in 100 mL ultrapure water. A second solution composed of 20 mL of 1 M NaOH and Na_2CO_3 in a stoichiometric ratio to $Mg(NO_3)_2{\cdot}6H_2O$ of 1:1 was prepared. These two solutions were rapidly mixed together and the



Scheme 1. Representation of the heparin functionalization of LDH particles and the HRP immobilization process.