Available online at www.sciencedirect.com
ScienceDirect
Microporous and Mesoporous Materials 109 (2008) 12–20
MICROPOROUS AND MESOPOROUS MATERIALS
www.elsevier.com/locate/micromeso

Co-intercalation of myoglobin and Eu^{3+} ions into the gallery of layered titanate: Preparation, structures as well as enzymatic and photoluminescent properties

Yihua Wu, Qiuming Gao *, Lu Gao

State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, Shanghai 200050, PR China

Received 25 September 2006; received in revised form 4 April 2007; accepted 5 April 2007
Available online 17 May 2007

Abstract

Myoglobin (Mb) and Eu^{3+} ions were co-intercalated into the galleries of layered titanate $H_{0.7}Ti_{1.825}\square_{0.175}O_4 \cdot H_2O$ ($\square$ = vacancy) by the exfoliation assembly method. The structure, composition and morphology of the Mb,Eu^{3+}/titanate composites were characterized by many techniques, such as XRD, HRTEM, ICP-AES, UV–vis spectra and SEM. No structural change of the immobilized Mb could be observed based on the FT-IR and UV–vis spectra analyses. The activity studies show that the intercalated Mb retained enzymatic peroxidase activity. The Mb,Eu^{3+}/titanate composites with mass ratios (Mb: titanate) of 1.8 retained 53% initial rates of free Mb. Meanwhile, intensely red emission was exhibited in the PL spectra for the intercalated Eu^{3+} ions at room temperature, which may be due to the band gap excitation in the titanate nanosheets.

Keywords: Myoglobin and Eu^{3+} ions; Co-intercalation; Layered titanates; Enzymatic activity; Photoluminescence

1. Introduction

Enzymes are excellent biological catalysts with exquisite selectivity; but their applications are severely limited, since they are expensive, lack of long-term stability and unstable in organic media or under other extreme conditions. The disadvantages may be overcome by immobilizing the enzymes onto a matrix [1,2]. Additionally, when used in the medical fields, such as diagnosis and treatment of various diseases [3], the enzymes which are generally foreign proteins are antigenic and can elicit an immune response. The immobilized enzymes could block the formation of antibody through isolating the enzymes from the body fluid. Besides, the immobilization of enzymes has enormous applications in the fields of biocatalysis, biosensor, bioreactor, medical implant and biological fuel cell, etc. [4–9]. So far, many materials have been used to immobilize enzymes, such as polymers [10,11], sol gels [12], mesoporous materials [13,14] and self-assembly films [8], etc.

Recently, a series of inorganic layered materials were found to be the competitive matrixes for immobilization of enzymes. There are several advantages for selecting them as the support matrixes. First, they can be readily expanded to accommodate enzymes with varied dimensions because of their flexible interlayer distances. Second, these materials may provide a matrix stable to extreme conditions of chemical, thermal and mechanical stresses. The protective environment of the matrix can make the immobilized enzymes more stable than that of the free ones. Last but not least, they can be further tailored to obtain optimally biochemical functions or desired physical properties. For instance, Kumar and co-workers [15–17] reported the immobilization of enzymes by reassembly of the exfoliated α-zirconium phosphates with guest molecules under mild

* Corresponding author. Tel.: +86 21 52412513; fax: +86 21 52413122.
E-mail address: qmgao@mail.sic.ac.cn (Q. Gao).

1387-1811/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.micromeso.2007.04.057

conditions (pH 7.2) and within a short reaction time. Besides, many other inorganic layered materials, such as manganese oxides [18], γ-zirconium phosphates [19], delaminated zeolites [20], were successfully used as supporting matrixes to immobilize enzymes by the similar methods. Layered titanates [21,22], niobates [23] and magadiites [24] were studied for the immobilization of enzymes in our laboratory.

However, most of those inorganic materials are just used as inert matrixes to provide protective environments for the enzymes. Their own functions have not been utilized. If the immobilized enzymes had photoluminescent properties, we could track the path of the enzymes with several complementary techniques when they were used in industry, such as diagnoses and therapies. Recently, the synthesis and photoluminescence of the layered titanates intercalated with the Eu^{3+} ions by electrostatic self-assembly methods were reported [25,26]. The bright light emission at room temperature is mainly contributed by the band gap excitation in the host titania nanosheets. The ability of the titania nanosheets accommodating enzymes and the Eu^{3+} ions, intrigues us to co-intercalate both of them into the titanate interlayers. Consequently, we successfully prepared an immobilized enzyme with photoluminescent properties in this paper.

Mb, a standard globular protein for oxygen carriage, has strong spectroscopic signatures that the structure and activity are easy to monitor by UV–vis absorption spectra. The Mb contains one heme prosthetic group with a molecular weight of about 17,000 and an isoelectric point of about 6.8. Thus, the novel bifunctional composites were prepared by choosing Mb and Eu^{3+} ions to co-intercalate into the titanate layers in our experiments and the photoluminescent properties could be adjusted by changing the lanthanide ions. To the best of our knowledge, it is the first time that the photoluminescent metal ions and the enzymes have been co-intercalated into the inorganic layered material. Not only the layered titanate sheets are utilized as inert matrixes, but also their semiconductor properties are necessary for the utilization of immobilized enzyme composites. The emission from Eu^{3+} by exciting the host with UV light could be observed at room temperature. Other enzymes or biomolecules can be applied in this system with a similar preparation method.

2. Experimental

2.1. Synthetic methods

2.1.1. Preparation, protonation and exfoliation of $CsTi_{1.825}\square_{0.175}O_4$

The starting material of Cs^+-type titanate $CsTi_{1.825}\square_{0.175}O_4$ was prepared by heating a stoichiometric mixture of Cs_2CO_3 and TiO_2 at 1027 K for 40 h [27]. The protonated derivative of $H_{0.7}Ti_{1.825}\square_{0.175}O_4\cdot H_2O$ was prepared by ion-exchanging the corresponding alkali compound in 1.0 M of HCl solution for 3 days with replacement of acid every day. Then, the protonic titanate (1.0 g) was shaken vigorously with an aqueous solution (100 mL) of 2.5 mM tetrabutylammonium hydroxide (TBAOH, Acros Organics), which produced a translucent colloidal suspension [28,29].

2.1.2. Intercalation of Mb and Eu^{3+} ions

The Mb intercalated titanate (named as Mb/titanate) composite was prepared according to our published procedures [21,22], by mixing stock solution of Mb (1.0 mg/mL) and colloidal suspension of the exfoliated titanate (1.0 mg/mL) with volume ratios varied from 4:1 to 1:1. Previous to the reaction, the pH of the colloidal suspension was carefully adjusted to 6.0 with 0.1 M of acetic acid solution. The solid was separated by centrifugation and lyophilization under reduced pressure. At the same time, the residual Mb content in the aqueous solution could be determined with UV–vis spectra by recording the corresponding absorbance at 408 nm.

The Eu^{3+} ions intercalated titanate (Eu^{3+}/titanate) composite was prepared according to the literatures [25,26] with minor modification. First, the pH of the colloidal suspension was carefully adjusted to 7.7 ± 0.2 with 0.1 M of acetic acid solution. The initial pH of the aqueous Eu^{3+} solution was about 5.5 without pH adjustment. Then, the colloidal suspension (10 mL, 1.0 mg/mL) was slowly added to the Eu^{3+} aqueous solution (10 mL, 1.1 mM). The precipitated white product was filtered, washed with distilled water and dried in air.

The Mb and Eu^{3+} ions co-intercalated titanate (Mb,Eu^{3+}/titanate) composite was prepared as follows. Typically, $EuCl_3\cdot\chi H_2O$ (10 mL, 1.1 mM) and Mb (10 mL, 1.8 mg/mL) were mixed in an aqueous solution. The pH of the solution is 5.8 ± 0.1. Then, the colloidal suspension (20 mL, 0.5 mg/mL, pH 7.7 ± 0.2) was slowly added to the solution under stirring at room temperature. Red solid was precipitated from the solution after 2–3 h. The solid was separated by centrifugation and lyophilization under reduced pressure. The residual Mb content in the solution could be determined with the same method described above.

2.2. Characterization

Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku D/MAX-2200 diffractometer with Ni-filtered Cu Kα radiation ($\lambda = 1.5418$ Å). For the XRD pattern measurement, the wet solid was directly spread on the glass slide and air-dried. The Ti and Eu contents were determined on a Perkin–Elmer model P40 ICP-AES after heat nitric acid decomposition. The FT-IR spectra between 4000 and 400 cm^{-1} was recorded with 4 cm^{-1} resolution on a Thermo Nicolet nexus by the KBr disk method. UV–vis absorption spectra were measured on a Shimadzu UV-3101PC spectrophotometer. The high-resolution transmission electron microscopy (HRTEM) observations were performed on a JEOL EM-2100 transmission electron