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Intercalative Route to Heterostructured Nanohybirds

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ABSTRACT

We have successfully synthesized organic-inorganic and bio-inorganic nanohybrids by applying an intercalation technique systematically to Bi-based cuprate superconductors, $Bi_2Sr_2Ca_{m-1}Cu_mO_y$ (m = 1, 2, and 3; BSCCO), and to layered double hydroxides(LDHs), those which are of high importance in terms of basic understanding of intercalation reactions and of their practical applications. The organic-inorganic hybrids were achieved via intercalative complexation of iodine intercalated BSCOO with organic salt of $Py-C_nH_{2n+1}I$ (Py = pyridine). The high-T_c superconducting intercalate with its remarkable lattice expansion can be applied as a precursor for superconducting colloids when dispersed in an appropriate solvent. We were also able to demonstrate that the biomolecules stabilized in the interlayer space of LDH retain their chemical and biological integrity. If necessary, LDH, as a reservoir, can be intentionally removed by dissolving it in an acidic media or interlayer biomolecules in LDH can be released via ion-exchange reaction in electrolyte. It is, therefore, concluded that the inorganic LDH can play a role as a good host lattice for gene reservoir or carrier.

INTRODUCTION

Recently, inorganic/inorganic[1], organic/inorganic[2], and bio-inorganic[3] heterostructured nanohybrids have attracted considerable research interests, due to their unusual physicochemical properties, which cannot be achieved by conventional solid state reactions. In order to develop new hybrid materials, various synthetic approaches, such as vacuum deposition, Langmuir-Blodgett technique, self-assembly, and intercalation method have been explored. Among them, the intercalation reaction technique—that is, the reversible insertion of guest species into two-dimensional host lattice—is expected to be one of the most effective tools for preparing new layered heterostructures because this process can provide a soft chemical way of hybridizing inorganic/inorganic, organic/inorganic, and biological/inorganic compounds. This field appears to be very creative giving the opportunity to invent an almost unlimited set of new compounds (hybrid compounds) with a large spectrum of known or unknown properties. As a consequence of the dual functionality of hybrid materials, this area is also a good field for scouting smart materials. For example, we were able to realize a new inorganic/organic hybrid system with high-T_c superconducting properties. It is well known that the intercalation reaction occurs in highly anisotropic lamellar structures in which the interlayer binding forces are fairly weak,

compared with the strong ionocovalent intralayer ones. The control of the strength of interlayer interactions makes it possible to probe the relation between interlayer coupling and superconductivity.

The layered double hydroxides (LDHs), so-called "anionic clays", have also received considerable attention due to their technological importance in catalysis, separation technology, optics, medical sciences, and nanocomposite materials engineering. LDHs consist of positively charged metal hydroxide, in which the interlayer anions (along with water) are stabilized in order to compensate the positive layer charges. The composition can be generally represented as $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2][A^{n-}]_{x/n} \cdot mH_2O$, where M^{2+} is a divalent cation (Mg^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}), M^{3+} is a trivalent one (Al^{3+}, Cr^{3+}, Fe^{3+}, V^{3+}, and Ga^{3+}) and $A_{x/n}^{n-}$ is an exchangeable anion with charge n. The unique anion exchange capability of LDHs meets the first requirement as inorganic matrices for encapsulating functional biomolecules with negative charge in aqueous media. In this study, we present that the high-T_c superconducting intercalate with its remarkable lattice expansion can be applied as a precursor for superconducting colloids when dispersed in an appropriate solvent[2], also that biomolecules, such as DNA, ATP and antisenses etc. can be incorporated between hydroxide layers by a simple ion-exchange reaction to form bio-LDH nanohybrids. Moreover, the hydroxide layers can play the role not only as reservoir to protect intercalated DNA, but also as nonviral vector to transfer gene or drug to the cell.

EXPERIMENTAL DETAILS.

Organic-inorganic Nanohybrids

The pristine $Bi_2Sr_2Ca_{m-1}Cu_mO_y$ (m = 1 and 2) compounds were synthesized by conventional solid state reaction with nominal compositions of $Bi_2Sr_{1.6}La_{0.4}CuO_x$ (Bi2201) for m = 1 and $Bi_2Sr_{1.5}Ca_{1.5}Cu_2O_y$ (Bi2212) for m = 2, where the Sr ion is partially substituted by the La ion or Ca one to obtain single-phase samples. The intercalation of an organic chain into the pristine material was achieved with the following stepwise synthesis. First, the HgI_2-intercalated $Bi_2Sr_2Ca_{m-1}Cu_mO_y$ (m = 1 and 2; HgI_2-Bi2201 and HgI_2-Bi2212) compounds were prepared by heating the guest HgI_2 and the pristine materials in a vacuum-sealed Pyrex tube, as reported previously. Then, the intercalation of organic chain molecular was carried out by the solvent-mediated reaction between HgI_2 intercalates and alkylpyridinium iodide. The reactants of Py-$C_nH_{2n+1}I$ (n = 1, 2, 4, 6, 8, 10, and 12) were obtained by reacting alkyliodide with 1 M equivalent of pyridine in diethyl-ether solvent. The HgI_2 intercalates were mixed with two excess reactants of Py-$C_nH_{2n+1}I$, to which a small amount of dried acetone was added. Each solvent-containing mixture was reacted in a closed ampoule at 40 °C for 6 hours and washed with a solvent blend of acetone and diethylether (1:1 volumetric ratio) to remove the excess reactant of Py-$C_nH_{2n+1}I$. And finally the resulting products were dried in vacuum[2]. The superconducting colloidal suspension could be obtained by sonicating the organic-salt intercalates in acetone solvent[4], which was then deposited on a film by electrophoretic deposition(EPD) technique and subsequent heating.

Bio-inorganic Nanohybrids.

The pristine Mg_2Al-NO_3-LDH was simply prepared by coprecipitation from aqueous solutions containing metal ions ($Mg(NO_3)_2 \cdot 6H_2O$ and $Al(NO_3)_3 \cdot 9H_2O$; 2:1 molar ratio) with dropwise titration of a base (NaOH) under nitrogen atmosphere. During the titration, the solution pH and temperature were adjusted to 10 ± 0.2 and 60 °C, respectively. The resulting white precipitate was further aged for 24 hrs, then collected by centrifugation and washed thoroughly with decarbonated water. The biomolecule–LDH hybrids were then prepared by ion–exchanging the interlayer nitrate ions in the pristine LDH with various biomolecules such as adenosine–5'–triphosphate (ATP), herring testis DNA, fluorescein 5-isothiocyanate (FITC) or c-*myc* antisense oligonucleotide (As-*myc*; 5' d (AACGTTGAGGG GCAT) 3') at pH =7. Prior to intercalation, the protein free DNA was extracted from the crude materials and then sheared off to the size of 500–1000 base pairs as described in the literature [5]. The pristine LDH was dispersed in a deaerated aqueous solution containing an excess of dissolved, ATP, FITC, DNA, and As-*myc* and reacted for 48 h with a constant stirring. The reaction products were then isolated and washed as described above. The cellular uptake experiments were carried out for FITC-LDH and As-*myc*-LDH hybrid. The 1 µM and 3 µM of FITC-LDH hybrids were added to the 6×10^4 NIH3T3 cells and incubated for 1, 2, 4, 6, and 8 hrs, respectively. All the samples washed with PBS buffer, and fixed with 3.7 % formaldehyde were observed with a laser scanning confocal microscope (Carl Zeiss LSM 410). HL-60 cells were also used to prove that the LDH could act as a drug delivery vector in gene therapy. HL-60 cells were exposed to As-myc or As-*myc*-LDH hybrid at a final concentration of 5, 10, 20 μM, respectively. Cell viability was estimated by spectrophotometry measurement of the samples treated with MTT assay. MTT assay is a colorimetric assay that measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT reagent) by mitochondrial succinate dehydrogenase. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

DISCUSSION

Organic-inorganic Nanohybrids.

We have successfully synthesized organic-inorganic nanohybrids by applying an intercalation technique systematically to Bi-based cuprate superconductors, $Bi_2Sr_2Ca_{m-1}Cu_mO_y$ (m =1, 2, and 3; BSCCO). The synthetic strategy for the organic-inorganic hybrids is based on HSAB (hard-soft-acid-base) interaction between organic guest molecules and inorganic host lattice, which is illustrated in figure 1.

From the particle-size analyses and transmission electron microscopy(TEM) measurement, it is found that the plate-like particles are in the range of 50 – 200 nm(Figure 2). The electron diffraction (ED) pattern for colloidal particle exhibits characteristic pattern of the pristine Bi2212 lattice as shown in Figure 2. All the diffraction patterns can be indexed as *hl* reflections for two-dimensional pseudotetragonal lattice of 5.4 × 5.4 Å, which is consistent with the host structure of

Bi2212. According to the AFM height profiles [6], the vertical distance for the delaminated Bi2212 sheets was determined to be ~20 Å, which is comparable to the thickness of unit building block of the pristine Bi2212. Such a finding can be regarded as an evidence of effective exfoliation of host lattice.
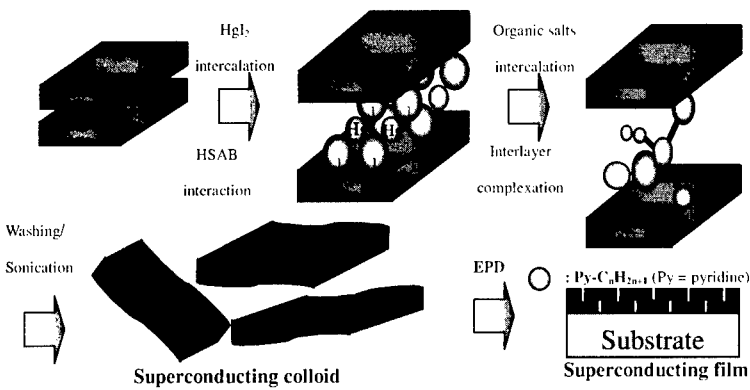

Figure 1. Schematic illustration of synthesis of the organic salt intercalate and superconducting film.

The film morphology plays an important role in determining the superconducting property such as critical current density (J_c). Especially, in order to attain a high J_c, it is important to suppress the volume fraction and grain growth of second phases such as Bi-free phases and Cu-free phases, because such impurity phases can diminish a portion of superconductor and disturb the aligned grain growth of Bi-based superconductor. Therefore, the morphology of representative films was examined by using scanning electron microscopy (SEM). As shown in Figure 3 a, no impurity phases could be seen on the film heat-treated at 850 °C for 5 h. The most important problem in achieving high J_c film is the c-axis orientation, since the c-axis tilt and twist boundaries could act as weak links [7-8]. According to the cross-sectional view of Bi2212 film, we found that the Bi2212 film is highly textured grain along c-axis. Such a result indicates that delaminated Bi2212 nanosheets are expected to be excellent precursor for fabricating the superconducting thin or thick film and wire.
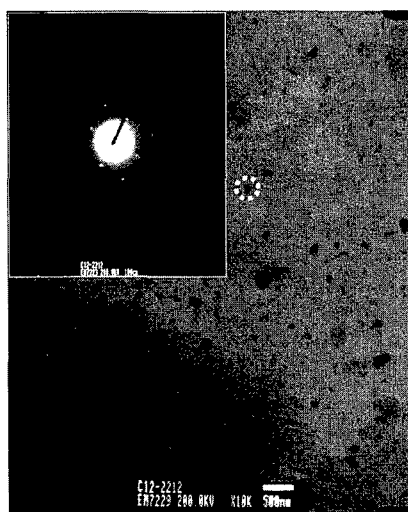
**Figure 2.** TEM and ED pattern of superconducting colloidal particles.
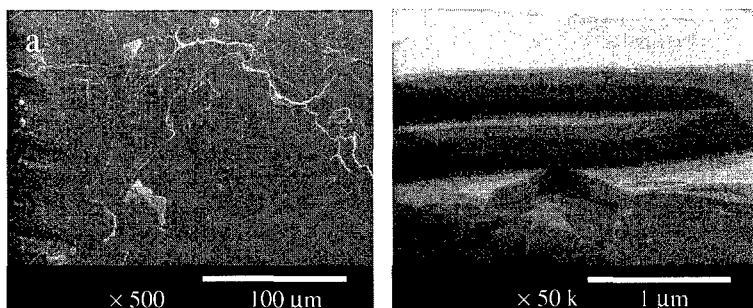
Figure 3. The SEM surface morphology and cross-sectional view of the fabricated superconducting film by electrophoretic deposition.

Bio-inorganic Nanohybrids

The purpose of this study is not only to prepare new bio-inorganic nanohybrids but also to present biotechnological applications of inorganic materials, such as inorganic gene reservoirs or nonviral drug delivery carriers.

Nano-sized inorganic clay, layered double hydroxide (LDH), has been demonstrated as an excellent reservoir and delivery carrier for genes and drugs by hybridizing with adenosine tri-phosphate(ATP), DNA and antisense oligonucleotide (As-*myc*), and also with fluoresceine 5-isothiocyanate (FITC). According to X-ray diffraction pattern, the interlayer distance of LDH increases from 0.87 nm (for NO_3^-) to 2.39 nm (DNA), 1.94 nm (ATP), 1.88 nm (FITC), and 1.71 nm (As-myc), respectively, upon intercalating of biomolecules into hydroxide layers. (Figure. 1)
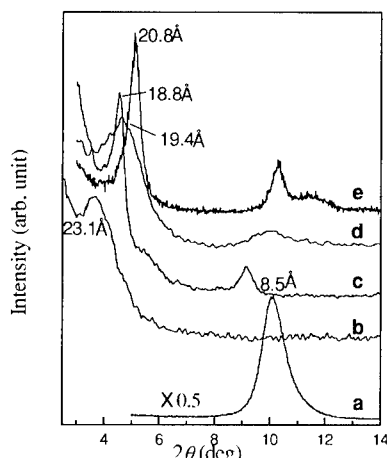

Figure 1. Powder X-ray diffractions for (a) the pristine LDH, (b) DNA-LDH, (c) ATP-LDH, (d) FITC-LDH, and (e) As-myc-LDH.

In the case of DNA-LDH hybrid, it was found that the hybrid has the gallery height of 19.1Å, which is consistent with the thickness of a DNA molecule (~20 Å) in a double helical conformation, with the interlayer DNA molecules arranged parallel to the basal plane of hydroxide layers. From the CD (cyclodichroism) analysis, the CD band of DNA-LDH hybrid was observed at the same wavelength compared with the band of ordinary B-form DNA, which means that the intercalated DNA is stable between the hydroxide layers. Figure 2 represents the electrophoretic analysis of DNA-LDH hybrid, which shows that the DNA-LDH hybrid has pH dependent property. There are no DNA bands beyond pH $\cong$ 3, indicating that the DNA molecules in hybrid system are quite stable even in weak acidic atmosphere. However, the DNA bands appeared when the hybrids are treated in a strong acidic media below pH $\cong$ 2, since the hydroxide layers are dissolved in such acidic condition (lane 1-10). From the DNA elution as shown in lane 11 and 12, it can be deduced that the DNA-LDH hybrid can protect DNA from DNase I enzyme. Consequently, the electrophoretic analysis reveals that the DNA-LDH hybrid plays a role as a gene reservoir.
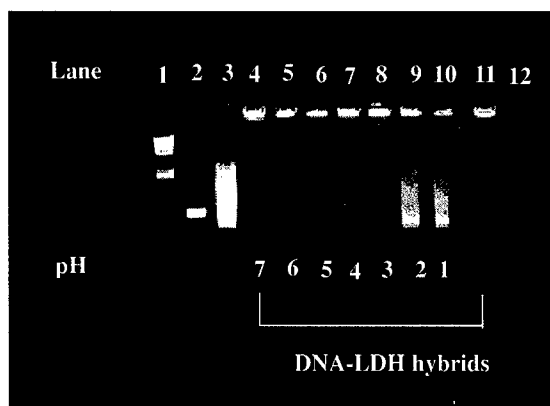
Figure 2. Electrophoresis analyses for the DNA–LDH hybrids with respect to pH. The pH of the solution dispersed with hybrid was adjusted to 7.5, 6.0, 5.0,4.0, 3.0, 2.0 and 1.0, respectively, by adding 1M HCl. Lane 1; λ / Hind III cut DNA marker (descent to 23.1, 9.4, 6.5, 4.3, 2.3, 2.0 kbp), lane 2; 500 bp DNA marker, lane 3; DNA and lane 4-10; DNA–LDH hybrids at pH 7.5, 6, 5, 4, 3, 2, and 1, respectively. lane 11 ; DNA-LDH hybrid treated with DNase I and DNA recovered by acid treatment. Lane 12; DNA only treated with DNase I.
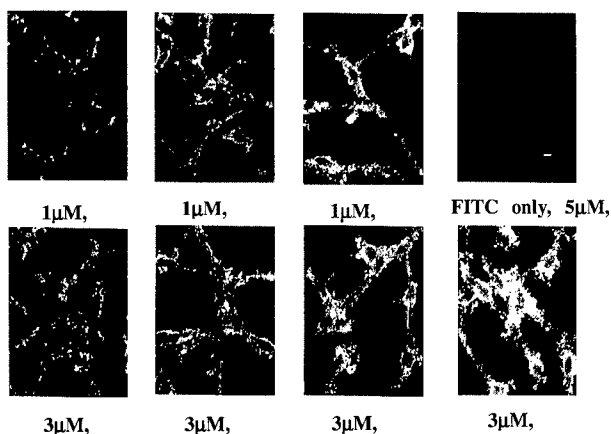

Figure 3. Laser confocal fluorescence microscopy of fluorophore in NIH3T3 cells. 6×10^4cells/well were incubated with (a)1 μM of FITC-LDH for 1, 4, and 8 hrs respectively. (b) with 3 μM FITC-LDH for 1, 2, 6, and 8 hrs respectively. The FITC only fluorescence microphotograph was obtained with 5 μM FITC only. The bar is 10 μm.

Figure 3 represents the cellular uptake experiments of FITC-LDH hybrid, which is compared

with FITC only. The amount of FITC transferred into NIN3T3 cells is measured directly from laser scanning confocal microscopy [9-11]. It is found that the cellular uptake rate increases with respect to the incubation time and the concentration of FITC-LDH hybrid, although FITC itself can not be transferred into the cells. This is evidence that the FITC-LDH hybrid has an advantage in the cellular uptake, which is thought to be influenced by the charge of transferring species. The neutralized FITC-LDH hybrid nanoparticles can be transferred into the cell more effectively than the charged FITC molecules through phagocytosis or endocytosis. So it is obvious that FITC-LDH hybrid can play a role as a nonviral delivery vector.

We also carried out experiments on the suppression of cancer cell division assuming that the biomolecule-LDH hybrid can be utilized in gene therapy. Figure 4 shows the effect of As-myc-LDH hybrid on the growth of cancer cell such as HL-60. The sequence of As-*myc* is 5' d (AACGTTGAGGGGCAT) 3', complementary to the initiation codon and the next four codons of c-*myc* mRNA, which can act as inhibitor for cancer cell. HL-60 cells treated with As-*myc*-LDH hybrids exhibit time-dependent inhibition on cell proliferation, indicating nearly 65 % of inhibition on the growth compared to the untreated cells, after 4 days. In other words, the growth of HL-60 cells treated with As-*myc* hybrid is only about 35 % compared with those treated with As-myc only.
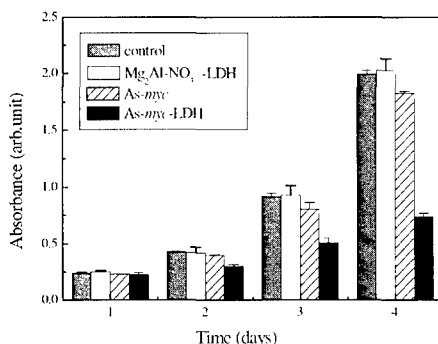
Figure 4. Effect of A-myc-LDH hybrids and As-myc only on the growth of HL-60 cells. Controlled cells are incubated without any treatment. The final concentration of each material was 20μM.

Since Figure 4 reveals that LDH itself does not inhibit the growth of HL-60 cells, the suppression effect of cancer cell is purely affected by As-myc-LDH. These imply that As-*myc*s are incorporated into cells and eventually inhibit the growth of cancer cells thanks to the

hybridization. It was also reported that the growth inhibition effect is time and dose dependent[12]. It is concluded that LDH can protect and deliver the intercalated oligonucleotide, and that interlayer As-myc can be effectively released from the hydroxide layer into cell fluids under physiological salt condition. Based on these findings, it is proved that LDHs can act as a new inorganic carrier in gene therapy that is completely different from existing nonviral vectors.

CONCULSION

New classes of inorganic/inorganic and organic/inorganic heterostructures with high-Tc superconductivity can be synthesized by hybridizing metal halides or organic salts with Bi-based cuprates. These compounds are believed to be promising precursor materials for superconducting nanoparticles, thin or thick films, and wires. And also, we are able to demonstrate that the biomolecules can be intercalated into LDH via ion-exchange reaction to construct bio-inorganic nanohybrid and that inorganic supramolecules, such as the LDHs with nanometer size, can play excellent roles as reservoir for biomolecules and as delivery carrier for gene and drugs.

ACKNOWLDGEMENT

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