Intercalative Nanohybrids of Nucleoside Monophosphates and DNA in Layered Metal Hydroxide

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Received May 26, 1998
Revised Manuscript Received November 5, 1998

Recently there has been considerable interest in the nanohybrids which often exhibit extraordinarily high synergetic and complementary behavior between two component materials.[1,2] In particular, the combination of two-dimensional layered materials and intercalation technique offers new areas for developing new hybrids with desired functionality.[3] One of the layered materials, the layered double hydroxides (LDHs), also called "anionic clays", have received considerable attention due to their technological importance in catalysis, separation technology, optics, medical science, and nanocomposite materials engineering. LDHs consist of positively charged metal hydroxide layers, in which the anions (along with water) are stabilized in order to compensate the positive layer charges. Various kinds of inorganic or organic anions have been introduced between the hydroxide layers by simple ion-exchange reaction or coprecipitation.[4−7] In the present study, we attempted, for the first time, to synthesize novel LDH nanohybrids intercalated with biomolecular anions. Since the nucleoside monophosphates and deoxyribonucleic acid (DNA) are negatively charged,[8] they can also be incorporated between the hydroxide layers as the charge-compensating anion through the ion-exchange route. The purpose of this study is not only to prepare new bioinorganic nanohybrids but also to provide basic data for developing new inorganic gene reservoirs or carriers.

First, the pristine $Mg_2Al(NO_3)$−LDH was prepared by coprecipitation under N_2 atmosphere following the conventional route. A mixed aqueous solution containing Mg^{2+} (0.024 M, from $Mg(NO_3)_2$) and Al^{3+} (0.012 M, from $Al(NO_3)_3$) was titrated dropwise into a NaOH solution with vigorous stirring. During the titration, the solution pH was adjusted to 10 ± 0.2, and the temperature was controlled to 25 °C. The resulting white precipitate was collected by centrifugation and washed with deionized water thoroughly.

The biomolecule−LDH hybrids were then prepared by ion-exchanging the interlayer nitrate ions in the pristine LDH with nucleoside monophosphates such as adenosine-5′-monophosphate (AMP), guanosine-5′-monophosphate (GMP), and cytidine-5′-monophosphate (CMP), and herring testis DNA (Sigma D-6898) 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.[9] The pristine LDH was dispersed in a deaerated aqueous solution containing an excess of dissolved AMP, GMP, CMP, and DNA and reacted for 48 h with a constant stirring. The reaction products were then isolated and washed as described above. The stoichiometry of each biomolecule−LDH hybrid was determined by elemental analysis (CHN), thermogravimetry, and inductively coupled plasma spectrometry (ICP) as shown in Table 1.

The X-ray diffraction patterns[10] for the pristine LDH and its biomolecule−LDH hybrids are shown in Figure 1A. The diffrac-

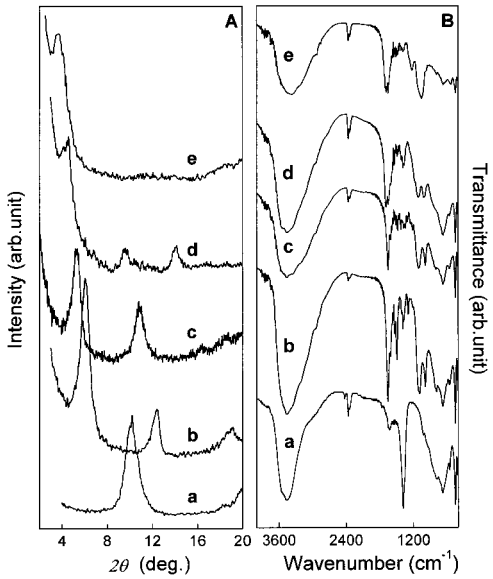

Figure 1. (A) Powder X-ray diffraction patterns and (B) Infrared spectra for (a) the pristine LDH, (b) CMP−LDH, (c) AMP−LDH, (d) GMP−LDH, and (e) DNA−LDH.

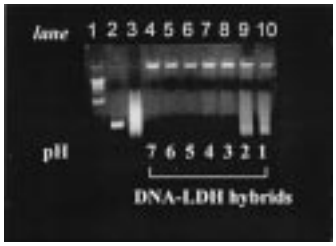

Figure 2. Electrophoresis analyses for the DNA−LDH hybrids with respect to pH. The pH of the solution dispersed with hybrid was adjusted to 7.5, 6.0, 5.0, 4.0, 3.0, 2.0, and 1.0, respectively, by adding 1 M HCl. After 1 h incubation of mixtures at room temperature, each sample was analyzed by 0.7% agarose gel electrophoresis and stained with ethidium bromide. 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.

Table 1. Compositional Data for the Pristine LDH and Biomolecule−LDH Hybrids[a]

sample	chemical composition
pristine LDH	$Mg_{0.68}Al_{0.32}(OH)_2(NO_3)_{0.32}\cdot 1.2H_2O$
AMP−LDH	$Mg_{0.66}Al_{0.34}(OH)_2(AMP)_{0.14}(OH)_{0.20}\cdot 0.9H_2O$
CMP−LDH	$Mg_{0.66}Al_{0.34}(OH)_2(CMP)_{0.19}(OH)_{0.14}\cdot 0.7H_2O$
GMP−LDH	$Mg_{0.66}Al_{0.34}(OH)_2(GMP)_{0.14}(OH)_{0.20}\cdot 0.9H_2O$
DNA−LDH	$Mg_{0.66}Al_{0.34}(OH)_2(bp)_{0.17}\cdot 0.5H_2O$

[a] AMP, CMP, GMP:monoanion, bp (= base pair of DNA):dianion.

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10.1021/ja981823f CCC: $18.00
Published on Web 02/03/1999

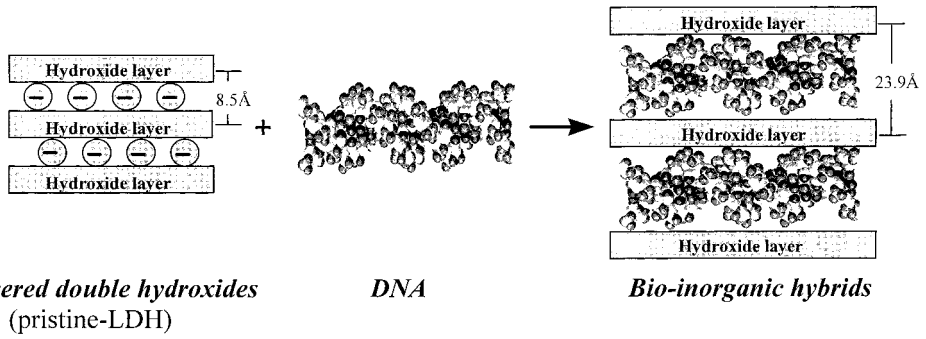

Figure 3. The schematic illustration of DNA intercalated into LDH inorganic layer.

tion peak at 8.5 Å for the pristine LDH corresponds to the basal spacing of NO_3^- incorporated in hydroxide layers. Upon substituting NO_3^- ions with biomolecules, the (*00l*) reflections shift to lower angles, indicating that the hydroxide layers are further expanded upon intercalation of bulky anionic biomolecules. In addition, well ordered (*00l*) series imply that the anion-exchange reaction occurs topotactically without any reconstruction of the lamellar structure of the pristine hydroxide layers. Taking into account the brucite-like LDH sheets (4.8 Å), the gallery heights of biomolecule−LDH hybrids were estimated to be 9.7 Å (for CMP), 12.1 Å (for AMP), and 13.6 Å (for GMP), which means that the monophosphates tend to have a monolayer arrangement. It is thought that the anionic substituents (phosphate groups) are oriented toward the LDH layers to maximize the electrostatic attraction as observed in organic-pillared LDHs and other organoclays.[11] An increase in the interlayer spacing (19.1 Å) of the DNA−LDH complex corresponds to the thickness of the intercalated DNA molecules when it adopts a helix oriented parallel to the basal plane of LDH.[8] Circular dichroism (CD) technique[12] is quite effective for probing the changes of the secondary structure of DNA because the CD spectrum is very sensitive to conformational variations of natural nucleic acid. While the free herring testis DNA only exhibits a typical CD curve of the B-form,[13] the DNA−LDH hybrid shows a weakening of longest wavelength CD band at ~275 nm. Such a collapse of the CD band for this hybrid is very similar to that of DNA in a salt solution with high concentration due to the efficient charge neutralization of phosphate groups in DNA.[14] It is therefore concluded that the negative charge of DNA (particularly that of the phosphate backbone) is compensated for by the positive surface charge of LDH forming the DNA−LDH hybrid through electrostatic interaction.

Figure 1B shows the infrared spectra[15] for biomolecule−LDH hybrids. All of the characteristic bands, corresponding to biomolecules, appear with the superimposition on those of LDH. At first, the absorption band at 1360 cm^{-1} due to the stretching vibration of NO_3^- in the pristine LDH completely disappears after the ion-exchange reaction, suggesting that the interlayer NO_3^- ion is completely replaced by biomolecules. The absorption bands at 1000−1100 cm^{-1} correspond to the ν_1,ν_3 (P−O) modes of PO_3^{2-} group in biomolecules.[16] When the bands are compared with the spectra of native biomolecules, however, they are relatively broad owing to the interaction between biomolecules and hydroxide sheets. The other characteristic bands due to various functional groups such as the aromatic C═C or C═N and the conjugates C═O and −N−C═O in biomolecules are also clearly observed as shown in Figure 1B.

According to the UV−vis[17] absorption spectra, the intercalated samples exhibit characteristic absorption bands in the range of 250−300 nm, which are attributed to $\pi \rightarrow \pi^*$ or n $\rightarrow \pi^*$ transitions in the cytosine, adenine, and guanine groups.[18] Here, the absorption maximums are almost identical with those of native biomolecules. This strongly suggests that the biomolecules are intercalated in the hydroxide layers and preserve their integrity.

It is worthy to note here that the biomolecules can be stored within the LDH layers and, if necessary, recovered very easily in an acidic condition. Such a unique feature of LDH can be applicable as a reservoir of genes or other biomolecules. Thus, we attempted to test the pH-evolving stability of DNA−LDH hybrids by gel retardation experiments. The DNA−LDH hybrids were treated with an acidic solution and fractionated by 0.7% agarose gel electrophoresis (Figure 2). The DNA−LDH hybrids did not move from the wells above pH = 3, while the LDH segments of the hybrids were disintegrated below pH = 2 with a release of DNA without any degradation. This indicates that biomolecule−LDH hybrids are pH-sensitive and applicable to a gene reservoir.

In summary, it has been clearly demonstrated, for the first time, that the biomolecules such as CMP, AMP, GMP, and even DNA could be hybridized with an inorganic layer compound like LDH, giving rise to a heterostructured nanohybrid which consists of one biomolecular layer and inorganic hydroxide layer alternatively. Comparing XRD, CD, IR, and UV−vis spectra among the present biomolecule−LDH hybrids and the chemically well-defined biomolecules and LDH, it is concluded that the biomolecules stabilized in the interlayer space of LDH retain their chemical and biological integrity as schematically illustrated with an example of DNA−LDH hybrid in Figure 3. And also, this hybrid is pH-sensitive and able to be recovered in acidic conditions without any degradation. Therefore, it is expected that the inorganic LDH can be a good host lattice for a gene reservoir.

Acknowledgment. This work was in part supported by the Korea Science and Engineering Foundation through the Center for Molecular Catalysis, by the Korean Ministry of Education (BSRI-97-3413), and by the Research Institute of Molecular Science in Seoul National University (1998).

Supporting Information Available: Figures and experimental data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA981823F

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