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Layered double hydroxides: A review

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Combination of two-dimensional layered materials and intercalation technique offers a new area for developing nanohybrids with desired functionality. Layered double hydroxides (LDHs) are mineral and synthetic materials with positively charged brucite type layers of mixed metal hydroxides. Exchangeable anions located in interlayer spaces compensate for positive charge of brucite type layer. Since most biomolecules are negatively charged, can be incorporated between LDHs. A number of cardiovascular, anti-inflammatory agents are either carboxylic acids or carboxylic derivatives and could be ion exchanged with LDHs to have controlled release. LDHs have technological importance in catalysis, separation technology, medical science and nanocomposite material engineering.

Keywords: Anticancer drugs, Intercalation, Layered double hydroxides (LDHs), Nanobiohybrides, Nanotechnology

Introduction

Since living matter is composed of biological nanomachines and nanostructures, biology and medicine could be prime field for application of nanotechnology[1]. In particular, combination of two-dimensional layered material and intercalation technique offers new area for developing nanohybrids with desired functionality. Nanohybrids have composites function and most biomolecules (nucleoside monophosphates and ATP) that are negatively charged can be incorporated between hydroxide layers as charge compensating anions through ion exchange. Layered double hydroxides [LDHs] are also called anionic clays; mineral of this family is Hydrotalcite (Mg-Al-CO_3). LDHs have technological importance in catalysis, separation technology, optics, medical science and nanocomposite material engineering[2].

Layered Double Hydroxides (LDHs)

Chemical composition of LDH (Fig. 1) is generally expressed as

$$M(II)_{1-x} M(III)_x (OH)_2 (A^{n-})_{x/n} \times yH_2O,$$

where, M (II) =divalent cation, M (III) =trivalent cation, A =interlayer anion, n- =charge on inerlayer ion, and x and y are fraction constants.

Inorganic or organic anions can be introduced between hydroxide layer by ion exchange or precipitation[3]. LDHs containing magnesium and aluminum have already been used as an antacid and antipepsin agent; therefore, LDH is quite biocompatible. Novel biohybrids of LDH and biomolecules [ATP or nucleoside monophosphate] are designed and organized artificially on nanometer scale to provide opportunities for reservoir and delivery carriers of functional biomolecules in gene therapy and drug delivery.

LDHs can act as soluble inorganic vectors for different genes and DNA biomolecules. Negatively charged biomolecules intercalated in gallery spaces would gain extra stabilization energy due to electrostatic interaction between cationic brucite like layers and anionic biomolecules. Such biomolecules incorporated between hydroxide layers can be intentionally dissolved in an acidic media, which offers a way of recovering encapsulated or intercalated biomolecules[5]. Hosting of biologically active molecules inside LDHs can act as a 'chemical flask-jacket', protecting host from degradation. Additionally,

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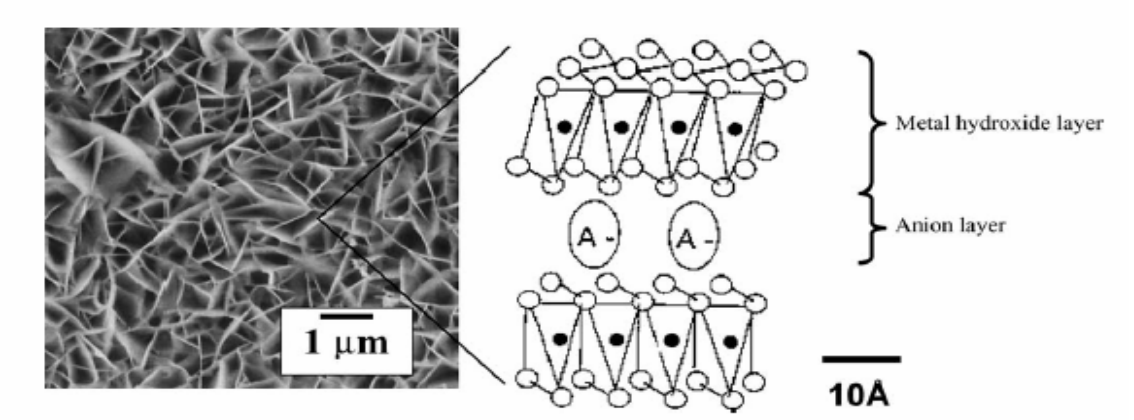

Fig. 1 - A schematic illustration of LDHs structure (Metal hydroxides layer located on top and bottom layers while anion layer located in middle[4])

hosting of a negatively charged species could provide improved ways of drugs and genetic material to be introduced into cells. If ingested, biomolecules-LDH nano-hybrid can move across mucous membrane of intestine into bloodstream. Neutral hybrid can then enter cells by moving across negatively charged cell membrane without repulsive electrostatic interactions that would be experienced by guest anion alone. Once inside the cell, LDH is broken down by lysosomes resulting in intercalate release.

LDH materials, being unstable in acidic conditions, do not survive for long in stomach. However, given a suitable enteric coating, slow-release of drugs into small intestine could be realized leading to effective delivery of fragile genetic materials into cells.

Antisense Therapy

Antisense DNA, a potential gene specific therapeutic agent, can be intercalated in LDH to form Bio –LDH nanohybrid (Fig. 2), which protects intercalated antisense molecule from degradation and also improves cell penetration. Bio-LDH nanohybrid also avoids specific aptameric effects (leading to non-specific binding of antisense oligonucleotides). Once LDH-antisense hybrids entered cell, hydroxide layers are removed by dissolution in lysosomes, where pH is slightly acidic and encapsulated biomolecules are released into cell[6].

Preparation of Layered Double Hydroxides (LDHs)

Reconstruction Method

Metal salts are calcinated at 500°C for 4 h in nitrogen at a heating rate of 5°C/min. This solid is then added to solution containing decarbonated water with guest molecule. pH (7-8)is adjusted by NaOH. Then, precipitate aged at room temperature, filtered, washed with decarbonated water thoroughly and finally dried under vacuum[7].

Co-precipitation Method

Typically, a mixed solution of two different metal salts in decarbonated water is added dropwise over hours to an aqueous solution containing organic guest species under nitrogen atmosphere with vigorous stirring. During titration, solution pH (7-8) is adjusted with 0.1 N NaOH to induce co-precipitation. Then precipitate, aged at room temperature for 24 h, is filtered, washed with decarbonated water thoroughly and finally dried under vacuum[7]. Biomolecules LDH hybrids can be prepared by ion-exchanging interlayer anion of LDH with biomolecules. Co precipitation method is more useful (yield, 3 times) than reconstruction method.

Characterization of Layered Double Hydroxides (LDHs)

Stoichiometry of each biomolecule–LDH hybrid can be determined by elemental analysis (CHN), thermogravimetry (TG), and inductively coupled plasma spectrometry (ICP). Synthesis of each hybrid can be confirmed by XRD measurement using Ni-filtered Cu-Ka radiation with a graphite diffracted beam monochromator. Infrared spectra (IR) can be obtained with FT-IR spectrometer by standard KBr disk method. Crystal structure of biomolecule–LDH hybrids can be studied by X-ray diffraction carried on biomolecules (CMP, AMP, GMP and ATP). Taking into account brucite-like LDH sheets (4.8 A°), gallery heights of biomolecule–LDH hybrids were estimated

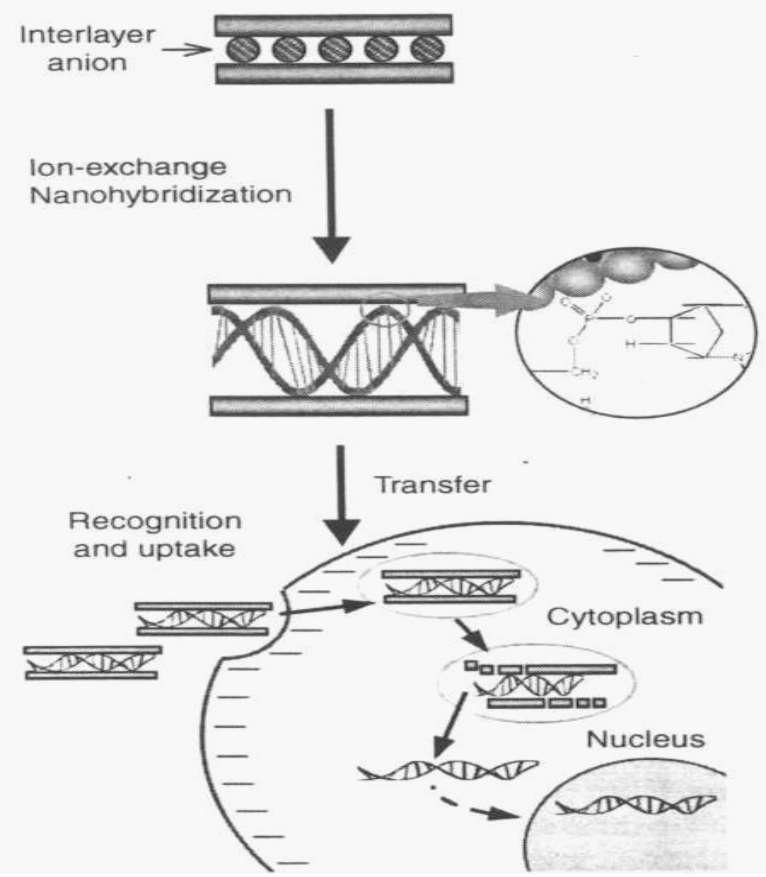

Fig. 2 - Schematic illustration of hybridization and expected transfer mechanism of bio LDH nanohybrid into a cell[6]

to be: CMP, 9.7; AMP, 12.1; GMP, 13.6; and ATP, 14.6 A°. It means that nucleotides tend to have a monolayer arrangement. Anionic substituents (phosphate groups) are reported[8] to orient towards LDH layers to maximize electrostatic attraction. Considering charge density of layers, about 22 intercalates are perpendicularly arranged to hydroxide layer. Schematic molecular arrangement in interlayer of LDH is based on basal spacing and molecular size of corresponding intercalates.

Transfer Efficiency and Cellular Uptake of LDH-Biomolecule Hybrid

Biomolecules are well stabilized in LDH lattice, and can be, if necessary, deintercalated by ion-exchange reaction with other anions or atmospheric CO_2. These features will allow LDHs to be applied as new drug or gene carriers if transfer efficiency of biohybrids to target organs or cells is proved. To elucidate transfer efficiency, isotope-labeled [^{32}P] ATP–LDH hybrid was prepared by ion exchange and uptake of such hybrids by eukaryotic cells was monitored with respect to incubation time. Exogenously introduced ATP–LDH hybrid can enter into HL-60 cells effectively within a relatively short time. Transfer efficiency was found to be higher (up to 25-fold) after 2 h of incubation, than that of ATP only, where after 4 h of incubation, uptake amount of hybrids becomes lower (below 12-fold). Triphosphate group of [^{32}P] ATP has a negative charge, which inhibits [^{32}P] ATP from being internalized in cell through negatively charged cell walls. In contrast, hybridization between ATP and LDH neutralizes surface charge of anionic phosphate groups in ATP due to cationic charge of LDH, which leads to favorable endocytosis of cells, and results in enhanced transfer efficiency[9].

Longer the incubation time in a CO_2 atmosphere, more ATP will be released from interlayer space of hydroxide lattice. In spite of this, transfer efficiency

of hybrid remains higher than that of ATP only (up to 4-fold) after 24 h of incubation. Thus hybridization between cationic layers and anionic biomolecules greatly enhances transfer efficiency of biomolecules to mammalian cells or organs.

Controlled Drug Delivery using LDHs

Addition of one of LDHs to a solution of chosen pharmaceutical in water at room temperature results in intercalation of these molecules between sheets of host. LDHs are able to swell by up to 20 A° to accommodate size of new guest molecules. LDHs possess antacid and antipepsin properties. Proprietary antacids products (TALCID™ and ALTACITE™) contain LDH $[Mg_6 Al_2 (OH)_{16}]CO_3^3$. Drugs [Diclofenac (DIC), Ibuprofen, Naproxen, Gemfibrozil, 2-Propylpentonoic acids, 4-Biphenylacetic acid, and Tolfenamic acid] are reversibly intercalated into LDHs. A number of cardiovascular, anti-inflammatory agents are either carboxylic acids or carboxylic derivatives, could be ion exchange intercalated in a LDH to have controlled release.

Apart from the potential of using these materials to deliver drug *in-vivo*, it will be possible to control the point of release and pharmacokinetic profile by selection of metal ions in host layers. Antacid performance and pH stability is also controllable by choice of metal layers, which restricts molecular interactions and dynamics and should improve long-term stability. In addition, improved taste qualities of formulation are predicted.

Intercalating into hydrotalcite (HTIc) modifies DIC release. Interlayer region of this matrix can be considered a micro vessel, in which drug may be stored and released by a deintercalation process due to the ions present in small intestine[10-12]. Release of DIC depends on diffusion through particle and not on drug concentration. *In vitro* studies show that drug is released by a deintercalation process due to exchange of drug with ions present in dissolution medium. At pH 7.5, drug release from HTIc-DIC is slower than that from physical mixture and is complete after 9 h. Kinetic analysis shows importance of diffusion through particle in controlling drug release rate. Hence, reversible intercalation of number of active cardiovascular and anti-inflammatory agents into LDHs can lead to novel tune able drug delivery system.

Anticancer Drug Therapy using LDHs

Folic acid derivatives [Folinic acid and Methotrexate (MTX)] have been hybridized with LDHs by ion-exchange reaction. MTX is used in therapy for different forms of cancers. But, very short plasma half-life of MTX necessitates administering a high dose that could lead to drug resistance and nonspecific toxicities in normal proliferating cells. Intercalation of MTX into LDH protects MTX from deterioration during transportation, whereas anion exchange along with acid dissolution may result in controlled release. LDH also probably affect permeability of MTX through cell barrier, leading to significant enhancement. X-ray diffraction patterns and spectroscopic analysis indicate that these molecules intercalate into hydroxide interlayer. Cellular uptake test of MTX-LDH hybrid is carried out in fibroblast (human tendon) and SaOS-2 cell (Osteosarcoma, human) by *in vitro* MTT [3-(4, 5-dimethyl thiazol-2-yl) 2-diphenyl tetrazolium bromide] assay. Initial proliferation of SaOS-2 cell is more strongly suppressed by MTX-LDH hybrid than with MTX alone. Thus LDH acts as biocompatible delivery matrix for drugs and facilitates a significant increase in delivery efficiency[13].

Campothecin (CPT), an inhibitor of topoisomerase I (enzyme involved in replication of DNA), has been studied as a treatment for several forms of cancer. CPT is pentacyclic indole alkaloid, with terminal ring converting readily between lactone in acidic environments (pH < 5) to carboxylate (pH < 8) form. For CPT to be active, lactone form must dominate. Active form, however, is only slightly soluble in water, leading to poor dispersions in physiological solutions as well as difficulties in efficient dose delivery. Study[14] demonstrated new delivery for non-ionic, insoluble drugs such as Campothecin. Drug is loaded into a micelle, which is then intercalated into nanometer galleries of LDHs. This complex provides similar cytotoxic characteristics to naked drug, but nanohybrids can be administered in a dose-controlled fashion due to good dispersion of complexes in water. Also, threefold increase in solubility is observed as compared to naked drug. Ability to attach targeting biomolecules to outside surface of hybrids as well as potential controlled release properties of complexes indicate that these hybrids may be used for specific delivery of poorly water-soluble, non-ionic drugs.

Improved stability of Vitamins

Vitamins [retinoic acid (Vit A), ascorbic acid (Vit C) and tocopherol (Vit E)] that are very sensitive to light,

temperature, oxygen etc. can be stabilized by intercalating into LDHs[15].

Avoids Side Effects of Drugs

Organic UV ray absorbents, used as sun care products, may pose a safety problem in high concentration use, when they tend to be absorbed in body through skin. This problem may be solved by intercalation of organic UV ray absorbents in nanospaces of LDHs[16]. Nonsteroidal anti-inflammatory drugs, used in rheumatic treatment, produce side effects such as gastric-duodenal ulcer formation. Intercalation of Indomethacin with LDHs reduces gastric damage[17].

Intercalation of Amino Acids and Peptides in LDHs

DNA is anionic macromolecule and is expected to be intercalated by ion-exchange method[5,6]. Amino acids exist as zwitterions and are neutral (pH 7). Therefore, intercalation of amino acids and protein are expected to be difficult. By using co precipitation method and reconstruction method, it is sometimes possible to intercalate neutral molecules, which could not be intercalated by ion-exchange method. Recently, intercalation of some amino acids into Zn–Al and Mg–Al LDHs by co precipitation method has been reported[18].

Because amino acid exits as zwitterions in interlayer space of LDH, another anion (OH^- or CO_3^{2-}) must be intercalated at the same time for electrical neutrality of LDH-amino acid that is co intercalation. Therefore, amino acid would be interacted with positive LDH layer not by Coulomb force but by hydrogen bonding. MASS-NMR spectrum of LDH-glycine and LDH-leucine suggested no deformation of LDH layer by reconstruction reaction, and distance between layers just changed. In amino acids with larger hydrophobic group, molecule arranges as bilayer structure in LDH as evidenced by XRD. In other amino acids, long axis of amino acid molecule is parallel to LDH. XRD pattern and MASS-NMR spectrum of LDH-aspartame showed interlayer distance of 2.2 nm, and a sharp signal at 9 ppm, suggesting bilayer structure of aspartame in interlayer space. This bilayer structure is reasonable because phenylalanine also shows bilayer structure because of large hydrophobic phenyl group. Amino acid was easily deintercalated in H_2O, because interaction between positive LDH layer and zwitterions is not so strong hydrogen bonding. This co-intercalation mechanism was also true for peptides. Although easy release of amino acid in H_2O suggests the problem for controlled release formulation, it shows that LDH/amino acid could be used as amino acid reservoir and adsorbent[19].

Conclusions

Hybridization of drug or a biomolecule with LDH results in remarkable transfer efficiency and stability. So, LDH hybrids can be useful as reservoirs and carriers for genes, drugs, and other functional molecules. LDH will allow many diseases to be monitored, diagnosed and treated in minimally invasive way and it thus holds great promise of improving health and prolonging life. LDH might very well be next breakthrough in drug delivery system.

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