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Intercalation of hydrophilic and hydrophobic antibiotics in layered double hydroxides

Markos Trikeriotis, Demetrios F. Ghanotakis *

Department of Chemistry, University of Crete, PO Box 2208, GR-71003 Voutes, Heraklion, Crete, Greece

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Abstract

Four pharmaceutically active molecules, each representing a different class of antibiotic, were intercalated in layered double hydroxides. Two of them, gramicidin and amphotericin B, are hydrophobic, surface active drugs that were incorporated in artificial membranes formed in the interlayer of the inorganic host. The other two, ampicillin and nalidixic acid, are water soluble, commonly used antibiotics that were directly intercalated by using simple ion exchange reactions. The synthetic nanohybrid materials were characterized by various methods, as X-ray diffraction, infrared spectroscopy and ultraviolet–visible spectroscopy that verified the successful intercalation of the antibiotics and provided information regarding the interlayer structure of the nanohybrids. The reversible interaction of the antibiotic molecules with the inorganic host leads to release of the active drugs under the appropriate conditions. The release studies showed that the synthetic nanohybrids can successfully serve as controlled release systems for different kinds of antibiotics.

Keywords: Layered double hydroxide; Gramicidin; Amphotericin B; Ampicillin; Nalidixic acid; Drug delivery

1. Introduction

Systemic drug administration results in distribution of the drug throughout the patient's body through blood circulation. This can lead to elevated drug concentrations in undesired parts of the body that cause severe side effects. Additionally, there are many cases where conventional drug administration methods do not provide satisfactory pharmacokinetic profiles because the drug concentration rapidly falls below desired levels. Drug delivery and controlled release systems are more sophisticated drug administration systems designed to overcome such problems (Kidane and Bhatt, 2005). These systems utilize carriers that slowly release their contents in order to maintain drug concentrations at the desired levels for a longer period of time. Moreover, drug carriers can be surface modified in order to target specific cells or tissues and thus reduce the risk of toxic side effects (Brannon-Peppas and Blanchette, 2004; Petrak, 2005). Drug carriers are usually polymers or various types of lipid vesicles, like liposomes, that form micro- or nano-particles (Brannon-Peppas, 1995; Zimmer and Kreuter, 1995; Labhasetwar et al., 1997; Müller et al., 2000). Recently, biocompatible inorganic materials, like layered double hydroxides, are being used in drug delivery and controlled release systems. These materials are more stable and less toxic than conventional drug carriers.

Layered double hydroxides (LDHs), commonly known as hydrotalcites or anionic clays, are a family of natural and synthetic materials represented by the general formula $[M_{(1-x)}^{II}M_x^{III}(OH)_2][A^{n-}]_{x/n}\cdot zH_2O$ where M^{II} and M^{III} are a divalent and trivalent metal, respectively, and A^{n-} is the interlayer anion (Cavani et al., 1991). These materials form successive positively charged metal hydroxide layers and negatively charged anion layers. The metal hydroxide layers have a structure similar to brucite and are 4.8 Å thick, while the thickness of the intermediate layers depends on the size of the anion. Among the properties of LDHs, anion-exchange provides a simple method to replace the interlayer anion and thus synthesize a variety of different layered materials (Meyn et al., 1990). These materials have been used as anion-exchangers and catalysts (Cavani et al., 1991; Pinnavaia et al., 1995), but recently medical applications of LDH-biomolecule nanohybrids have gained attention.

* Corresponding author. Tel.: +30 2810 545034; fax: +30 2810 545001.
E-mail address: ghanotakis@chemistry.uoc.gr (D.F. Ghanotakis).

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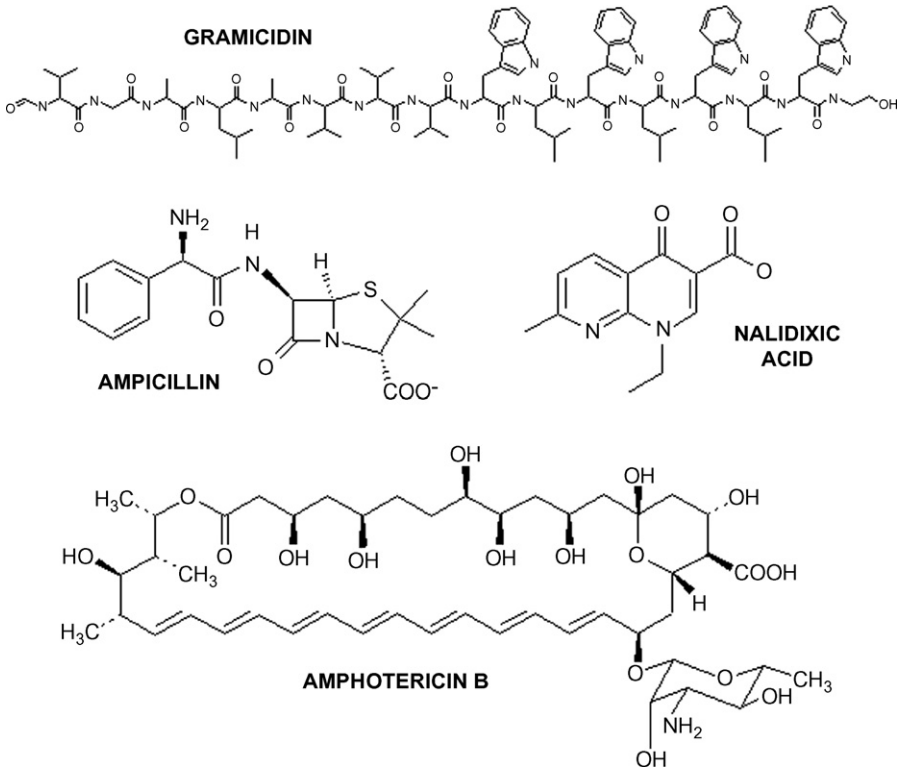

Fig. 1. Chemical structures of the four antibiotics that were used.

LDH became an attractive material for drug delivery and controlled release applications when Choy et al. intercalated DNA in Mg–Al LDH (Choy et al., 1999) and showed that the LDH–DNA nanohybrids can deliver the DNA into cells (Choy et al., 2000). Furthermore, it was shown that these nanohybrids can be injected intravenously in rats without toxic side effects (Kwak et al., 2004). Even the gene of the green fluorescent protein was intercalated in LDHs and was then delivered and expressed in various cell lines (Tyner et al., 2004a). Additionally, the possibility of surface modification of the inorganic layers, leading to targeted drug delivery to specific cells or organs, makes LDH a very attractive drug carrier. Anti-inflammatory drugs like ibuprofen (Ambrogi et al., 2001; Khan et al., 2001) and naproxen (Khan et al., 2001; Wei et al., 2004) and anti-cancer drugs like camptothecin (Tyner et al., 2004b) and folate derivatives (Choy et al., 2004) have been intercalated in LDH. In this study, four different antibiotics, gramicidin, amphotericin B, ampicillin and nalidixic acid (Fig. 1), were intercalated in layered double hydroxides.

Gramicidin is a polypeptide ionophore antibiotic produced by the bacterium *Bacillus brevis* (Wallace, 1998) that is active against Gram-positive bacteria. It is a hydrophobic peptide consisting of 15 amino acids that forms dimeric channels across biological membranes. These channels are permeable to monovalent cations but are blocked by divalent cations. Amphotericin B is a polyene antibiotic that is used for treatment of fungal infections in immunodepressed patients (Schreier et al., 2000). It forms pores in fungal membranes by complexing with ergosterol. However, due to severe side effects, amphotericin B is usually administered in lipid complexes (Espuelas et al., 1997; Hargreaves et al., 2006) and was one of the first drugs that were commercially available in liposomal form (AmBisome®).

Ampicillin belongs to the family of penicillins or β-lactam antibiotics that are widely used against bacterial infections. It inhibits the synthesis of peptidoglycan in bacterial cell walls (Tipper and Strominger, 1965) and thus is more active against Gram-positive bacteria. Some drug delivery and controlled release systems for ampicillin have been developed using polymethacrylate (Fernández Degiorgi et al., 1995) or hydroxyapatite (Queiroz et al., 2001) as carriers. A similar antibiotic, phenoxymethylpenicillin has been intercalated in LDH and the activity of the intercalated material against *Staphylococcus aureaus* was demonstrated (Li et al., 2006). Quinolone antibiotics, such as nalidixic acid, are inhibitors of bacterial DNA gyrases (Shen et al., 1989; Kampranis and Maxwell, 1998). They are more active against Gram-negative than Gram-positive bacteria and find clinical application mostly in the treatment of urinary tract infections. Nalidixic acid (Clerc and Barenholz, 1995) and enoxacin (Fang et al., 2001) have been loaded into liposomes in order to produce drug delivery systems.

In the present study two hydrophobic antibiotics, gramicidin and amphotericin B, and two hydrophilic antibiotics, ampicillin and nalidixic acid, where intercalated in layered double hydroxides. Surface active drugs like gramicidin and amphotericin B have been intercalated for the first time in LDHs by incorporating them in artificial membranes formed in the interlayer space of the inorganic host. Moreover, the LDH–cholate–gramicidin nanohybrid can be used as a model for membrane protein immobilization in LDHs as it is the first hydrophobic polypeptide