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Biocompatible protoporphyrin IX-containing nanohybrids with potential applications in photodynamic therapy

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Abstract

Protoporphyrin IX was immobilized in the interlayer region of Mg–Al layered double hydroxides in order to produce biocompatible nanohybrids that could find applications in photodynamic therapy. Protoporphyrin IX and perfluoroheptanoic acid were also cointercalated to produce nanohybrids that combine the oxygen dissolving properties of perfluorocarbons with the photodynamic effect of the porphyrin. The various nanohybrids were characterized by using X-ray diffraction, Fourier transformed infrared spectroscopy, ultraviolet-visible spectroscopy and thermogravimetric analysis. In addition to the intercalation of protoporphyrin IX in the interlayer region of the layered double hydroxides, the X-ray diffraction pattern also showed that intercalation of perfluoroheptanoic acid resulted in the formation of a bilayer between the inorganic layers. Photooxidation experiments using substrates such as imidazole, 2,3-dimethyl-2-butene or linoleic acid, demonstrated the generation of singlet oxygen by these nanohybrids.

Keywords: Layered double hydroxide; Protoporphyrin IX; Photodynamic therapy; Perfluoroheptanoic acid

1. Introduction

Conventional cancer therapies include radiation, chemotherapies, surgery or combinations of them, that usually have important side effects. An alternative is photodynamic therapy (PDT), a treatment modality that is becoming very common against various forms of cancer [1]. In PDT cancer cells are destroyed by reactive oxygen species, mostly singlet oxygen, which is produced with the aid of a photosensitizer and light. Thus, the success of PDT is based on the correct regulation of these three factors: photosensitizer, light and oxygen.

Photosensitization provides a simple and controllable method for the production of singlet oxygen [2]. The photosensitizer molecule is excited by light at high quantum yields and then produces singlet oxygen via energy transfer to molecular oxygen. Porphyrin derivatives, chlorins and phthalocyanines are the most frequently used photosensitizers in PDT [3]. Protoporphyrin IX (ppIX) is a second generation photosensitizer and it is used as an endogenous photosensitizer that is produced in the body by the conversion of topically applied 5-aminolevoulinic acid [4].

The availability of oxygen is also crucial for the efficacy of PDT [5,6]. Under hypoxic conditions cell photoinactivation is totally abolished. As oxygen concentration is a rate limiting factor for the production of singlet oxygen it is important to maintain high oxygen concentrations in solution. A way to achieve this involves the use of perfluorocarbons (PFCs), which are compounds with all aliphatic protons substituted by atoms of fluorine. PFCs can dissolve 20 times more oxygen than water and two to three times more than corresponding hydrocarbons [7]. Their remarkably high gas dissolving capacities along with their chemical and biological inertness make them unique carriers for oxygen delivery in tissues [7–9]. Among perfluorinated fatty acids with different chain lengths, perfluoroheptanoic acid (PFHA) is less toxic and is rapidly eliminated by the body [10,11].

Layered double hydroxides (LDHs) or hydrotalcite-like compounds are synthetic clay materials that form successive layers of metal hydroxides, separated by layers consisting of anions and water [12]. The metal hydroxide layers are positively charged and have similar structure to brucite with a thickness of 4.8 Å. LDHs are represented by the general formula

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$[M_x{}^{2+}M_y{}^{3+}(OH)_{2x+2y}]A_{y/n}{}^{n-}\cdot zH_2O$, where M^{2+} and M^{3+} are di- and tri-valent metal cations, respectively, and A^{n-} is the interlayer anion. Several organic–inorganic hybrid materials based on LDH have been produced by a simple ion exchange reaction of the interlayer anion [13–15]. These materials are often used as catalysts for chemical conversions [16]. Porphyrins and phthalocyanines have also been intercalated in LDHs [17] and other clays, like smectite, for catalytic purposes [18,19] or for the formation of photocatalytic assemblies [20]. Additionally, hemin was immobilized on smectite to produce a heme-protein model as it could bind oxygen and carbon monoxide [21].

Besides their ion-exchange and catalytic properties, LDHs have been proven to be promising drug delivery vectors [22,23]. LDHs can enter cells and then release the interlayer anions either by ion exchange with cellular anions or by dissolution of the metal hydroxide layers in the acidic environment of the lysosome. Additionally, surface modification could lead to targeted drug delivery to specific cells or organs. Thus, LDHs have been used as carriers in drug delivery and controlled release systems for several anticancer drugs like camptothecin [24], folinic acid and methotrexate [25] and for anti-inflammatory drugs like ibuprofen [26] and naproxen [27].

In this study ppIX was immobilized on layered double hydroxides in order to produce biocompatible nanohybrids that could find application in photodynamic therapy. The immobilized ppIX was able to produce singlet oxygen when it was irradiated with visible light and was catalytically active against various substrates. Nanohybrids containing both ppIX and PFHA were also synthesized. These materials combine the drug delivery properties of LDHs with the photodynamic effect caused by ppIX and the high oxygen solubility of O_2 in perfluorocarbons. As a result, improved PDT can be achieved if these nanohybrids successfully deliver their contents into tumor cells.

2. Experimental

2.1. Materials

Magnesium nitrate hexahydrate 98%, 4-(1,1,3,3-tetramethylbutyl)phenyl–polyethylene glycol (Triton X-100) and 6-O-(*N*-heptylcarbamoyl)-methyl-α-D-glucopyranoside 90% (Hecameg) were purchased from Sigma. Protoporphyrin IX disodium salt, perfluoroheptanoic acid 99% and 2,3-dimethyl-2-butene 98% were purchased from Aldrich. Sodium hydroxide pellets 99%, aluminum nitrate nonahydrate 98.5% and $CDCl_3$ 99.8% for NMR were purchased from Merck. Dichloromethane 99.9% and linoleic acid 90% were purchased from Fluka. Imidazole 99% was purchased from Riedel-de Haen. All chemicals were used without further purification.

2.2. Synthesis of LDH and LDH nanohybrids

Mg–Al–NO_3 layered double hydroxides with Mg/Al ratio 2/1 and 3/1 were prepared by coprecipitation at constant pH and at room temperature according to Miyata [28]. A solution containing $Mg(NO_3)_2\cdot 6H_2O$ (30.8 g, 120 mmol) and $Al(NO_3)_3\cdot 9H_2O$ (22.5 g, 60 mmol) in deionized water (200 cm^3) and a solution containing NaOH (16.0 g, 400 mmol) in deionized water (200 cm^3) were added dropwise to deionized H_2O (200 cm^3) so as the pH was maintained at 10 ± 0.5 at room temperature. After the addition was completed, the resulting slurry was aged for 1 day at room temperature. The suspension was then centrifuged at $12{,}000 \times g$ for 15 min and washed several times with deionized water. LDH was stored as a suspension in order to prevent crystal aggregation. All solutions during synthesis were decarbonated under N_2 flow for 20 min.

LDH nanohybrids were synthesized via anion exchange. For the preparation of the nanohybrid of LDH with ppIX (LDH–ppIX), ppIX (91 mg, 0.15 mmol) and $[Mg_3Al(OH)_8]$-NO_3 (50 mg) were added in decarbonated water (50 cm^3). The pH was adjusted to 9.0 and the mixture was stirred at room temperature for 2 days. The resulting nanohybrid was collected by centrifugation at $12{,}000 \times g$ for 15 min and after two washing steps with deionized water it was filtered under vacuum and dried in air.

PpIX and PFHA were co-immobilized on LDH with Mg/Al ratio 2/1 at the same conditions as above. The reaction mixture contained ppIX (91 mg, 0.15 mmol) and PFHA (164 mg, 0.45 mmol) in decarbonated water (50 cm^3) and the resulting nanohybrid (LDH–ppIX–PFC) was collected by centrifugation after 4 days.

The nanohybrid of LDH with PFHA (LDH–PFC) was prepared by dissolving PFHA (0.36 g, 1 mmol) in an aqueous solution (50 cm^3) containing Hecameg (0.25 g, 7.5 mmol). After the addition of $[Mg_3Al(OH)_8]NO_3$ (0.20 g) the pH was adjusted to 10.0 and the solution was stirred for 2 days.

2.3. Characterization

Powder X-ray diffraction (XRD) patterns were recorded on a Rigaku RINT 2000 powder diffractometer, using Cu Kα radiation ($\lambda = 1.54$ Å) at 40 kV and 178 mA. Fourier transformation infrared (FT-IR) spectra were recorded using KBr disks on a Perkin-Elmer 1760X FT-IR spectrometer. Ultraviolet-visible (UV–vis) spectra were recorded using a SLM-Aminco DW2000 spectrometer. The ^1H NMR spectra were recorded on a 300 MHz MSL Bruker spectrometer. Thermogravimetric analysis was carried out using a Perkin-Elmer Pyris Diamond TG/DTA, at 10 °C/min. Immobilized ppIX was quantified by dissolving a known amount of each nanohybrid in ethanol/1 M HCl 4/1 and then recording the absorbance at 558 nm.

2.4. Photocatalytic activity measurement

The activity of the immobilized ppIX was assayed by monitoring the O_2 uptake during the photooxidation of imidazole, 2,3-dimethyl-2-butene or linoleic acid. Oxygen concentration was monitored by a Yellow Spring Instruments (model 5300) Clark-type electrode. A small amount of ppIX, LDH–ppIX or LDH–ppIX–PFC was dissolved in deionized water (15 cm^3) containing Triton X-100 (0.15 cm^3) and the solution was equilibrated with pure O_2 for 15 min. The exact concentration of ppIX