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Phosphorus dendrimers and photodynamic therapy. Spectroscopic studies on two dendrimer-photosensitizer complexes: Cationic phosphorus dendrimer with rose bengal and anionic phosphorus dendrimer with methylene blue

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ABSTRACT

Dendrimers due to their unique architecture may play an important role in drug delivery systems including chemotherapy, gene therapy and recently, photodynamic therapy as well. We investigated two dendrimer-photosensitizer systems in context of potential use of these systems in photodynamic therapy. The mixtures of an anionic phosphorus dendrimer of the second generation and methylene blue were studied by UV–vis spectroscopy while that of a cationic phosphorus dendrimer (third generation) and rose bengal were investigated by spectrofluorimetric methods. Spectroscopic analysis of these two systems revealed the formation of dendrimer-photosensitizer complexes *via* electrostatic interactions as well as π stacking. The stoichiometry of the rose bengal-cationic dendrimer complex was estimated to be 7:1 and 9:1 for the methylene blue-anionic dendrimer complex. The results suggest that these polyanionic or polycationic phosphorus dendrimers can be promising candidates as carriers in photodynamic therapy.

1. Introduction

Dendrimers are well-defined hyperbranched polymers characterized by low polydispersity. The structure of a dendrimer consists of a core molecule, branches, internal cavities and many terminal groups. Due to such an architecture, these polymers are attractive carriers of drugs, imaging or transfection agents and can be used for diverse ways of administration (Klajnert and Bryszewska, 2001; El Kazzouli et al., 2012; Mignani et al., 2013a,b).

Photodynamic therapy (PDT) is an alternative cancer treatment method whereby cancer cells are destroyed by reactive oxygen species (ROS) and singlet oxygen ($^1O_2$) generated via a photodynamic effect. The photodynamic effect is a result of use of photosensitizer (PS) which under the influence of a specific wavelength of visible light induces a cascade of reactions leading to oxidative stress and cell death (Dolmans et al., 2003). However, PDT has limits similar to conventional chemotherapy. It involves, among others, poor PS selectivity, long-lasting skin sensitivity to light and rapid PS destruction. It is related to inappropriate biodistribution of photosensitizers (O'Connor et al., 2009). Therefore, to overcome these problems the challenge of modern PDT is creation of more selective and effective PS. This can be achieved by use of drug delivery systems. Recently dendrimers have gained some attention as promising transport systems for PS that potentially improve photodynamic therapy efficiency (Klajnert et al., 2012).

Abbreviations: PDT, photodynamic therapy; ROS, reactive oxygen species; PS, photosensitizer; PAMAM, polyamidoamine dendrimer; PPI, polypropyleneimine dendrimer; PEG, polyethylene glycol; PpIX, protoporphyrin IX; ALA, aminolevulinic acid; MB, methylene blue; RB, rose bengal; 1cat, cationic phosphorus dendrimer of generation 3; 1an, anionic phosphorus dendrimer of generation 2.

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PS molecules can be covalently bound to the core of the dendrimer, to the interior of the molecule or attached to the terminal groups. The presence of internal cavities allows for encapsulation of PS molecules in the dendrimer (Ihre et al., 2002; Patri et al., 2005; Bhadra et al., 2003). In several studies dendrimers were used as photosensitizer carriers. Polyamidoamine (PAMAM) and polypropyleneimine (PPI) dendrimers modified with polyethylene glycol (PEG) have been reported to be efficient in encapsulation of rose bengal and protoporphyrin IX (PpIX). PpIX encapsulated in PEG-PPI dendrimers revealed higher phototoxicity in comparison with a free photosensitizer (Kojima et al., 2007). A different approach to use dendrimers in PDT is based on the synthesis of photosensitizer dendrimers possessing porphyrin or phtalocyanine molecules in the core. Dendritic photosensitizers were also incorporated into polyion complex micelles through PEG-polyelectrolyte block copolymers. Polymeric micelles encapsulating dendrimer phthalocyanine showed high phytotoxicity in vitro and in vivo (Herlambang et al., 2011; Nishiyama et al., 2009; Zhang et al., 2003). Dendrimer conjugates containing 18 molecules of aminolevulinic acid (ALA) have been reported to efficiently transport ALA to tumor cells and induce porphyrin production in vitro (Battah et al., 2007).

As mentioned above, several types of dendrimers, including PPI and PAMAM, have been studied in the context of use in PDT. In this paper, we propose a novel approach to use phosphorus dendrimers as potential photosensitizer carriers. Phosphorus dendrimers exhibit interesting biological properties that make them promising drug carriers, transfection, anti-prion, imaging (Caminade et al., 2010), anti-inflammatory (Poupot et al., 2006) or anti-tumoral agents (El Brahmi et al., 2015). However, phosphorus dendrimers have not been studied as potential photosensitizer carriers. In designing new drug delivery systems it is crucial to determine the potential interactions between drug molecules and nanocarriers. In this paper we present studies on the interaction of two dendrimer-photosensitizer systems in order to evaluate whether phosphorus dendrimers can be used as carriers in PDT. We chose two phosphorus dendrimers that differ in generation and electrostatic charge and two photosensitizers: methylene blue (MB) and rose bengal (RB). Methylene blue is a monocationic phenothiazine dye with potential to be a promising photosensitizer (Tardivo et al., 2005). Rose bengal, on the other hand, is an dianionic xanthene dye that is also reported to possess good PDT efficacy (Wachter et al., 2003). Both PSs upon irradiation generate singlet oxygen (Tardivo et al., 2005; Wachter et al., 2003). The aim of the study was therefore to check whether dendrimers are capable to form complexes with PSs possessing opposite charges using absorption and fluorescence spectroscopy in the case of methylene blue and rose bengal, respectively. The results allow estimating the potential role of phosphorus dendrimers in PDT as carriers of photosensitizers.

2. Materials and methods

2.1. Materials

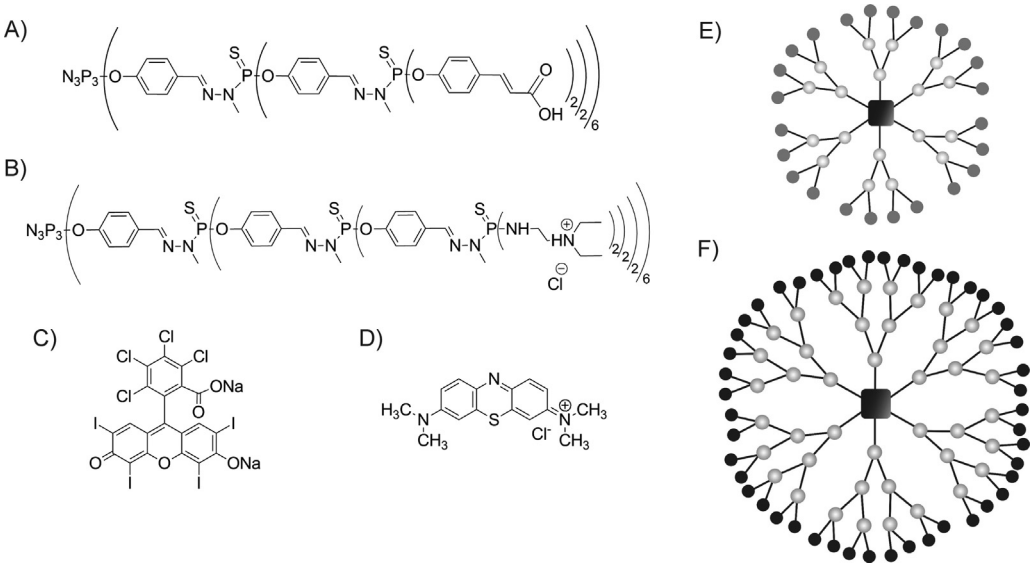
Two phosphorus dendrimers were used: cationic phosphorus dendrimer of generation 3 (MW = 15150.25 g/mol) possessing 48 ammonium terminal groups (**1cat**), and anionic phosphorus dendrimer (**1an**) of generation 2 (MW = 7851 g/mol) with 24 carboxyl terminal groups. Both dendrimers possess cyclotriphosphazene core. Dendrimers were synthesized in Laboratoire de Chimie de Coordination du CNRS, Toulouse, France. Rose bengal (RB) and methylene blue (MB) were purchased from Sigma–Aldrich. All solutions were made in phosphate-buffered saline (PBS), pH 7.4 using distilled water from Mili-Q system (Millipore). Scheme 1 depicts chemical characterization of the compounds used in this study.

2.2. Methods

2.2.1. UV–vis spectroscopy: study on the interaction between anionic phosphorus dendrimer (*1an*) and methylene blue (MB)

Absorption spectra were recorded on a Jasco V-650 spectrophotometer. All measurements were performed in 100 mM PBS pH 7.4, at room temperature. Spectra were recorded in a wavelength range from 550 nm to 700 nm. Optical path length was 1 cm. PBS was used as a reference for all measurements.

In titration experiments methylene blue (MB) was used at constant concentration of 10 μM. The MB solution was titrated with anionic dendrimer using different stocks of dendrimer in order to maintain specific molar ratios (1–20) of MB molecules per



Scheme 1. Chemical structures of dendrimers and photosensitizers used in this study. (A) anionic phosphorus dendrimer of the second generation—**1an**; (B) cationic phosphorus dendrimer of the third generation—1cat; (C) rose bengal; (D) methylene blue; (E) schematic structure of the second generation of phosphorus dendrimer; (F) schematic structure of the third generation of phosphorus dendrimer.