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Photobactericidal films from porphyrins grafted to alkylated cellulose – synthesis and bactericidal properties

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ABSTRACT

Two series of porphyrinic cellulose laurate esters plastic films, where the photosensitizers are covalently linked to the cellulosic polymer have been synthesised by using a "one-pot, two-step" esterification reaction. The photosensitizers were first covalently bounded to the cellulosic polymer using either 4- or 11-carbon spacer arms. The porphyrinic plastic films were then obtained by a second esterification with lauric acid. The reaction was studied according to reaction time, temperature, lauric acid amount, pyridine playing the role proton trapping base. *Para*-toluenesulfonylchloride has been proved to be a powerful activating agent for this reaction. The drawback of the steric hindrance of the porphyrinic macrocycle towards cellulosic hydroxyl groups has been overcome by increasing the number of carbon of spacer arms from 4- to 11-carbons. The photobactericidal activity of these materials was evaluated against Gram positive and Gram negative strains bacteria. First results show that these new plastic films display photobactericidal activity for porphyrin grafting percentage higher than 0.16, whereas the non-porphyrinic control allowed full growth of bacteria. These materials could be an alternative in order to overcome the growing bacterial multiresistance to classical antibiotics.

1. Introduction

Sever health hazards and diseases can be induced by the adhesion and proliferation of bacteria on the surfaces of numerous materials [1]. Nosocomial infections are one of the most important known effects of this survey. For example, in the United States, two million of nosocomial infections are diagnosed every year [2]. Sources of nosocomial infections are various including textile (bedding, surgeons gowns) and polymeric materials as syringes, bags, catheters and gloves. As a consequence of this global concern, research of bactericidal and bacteriostatic surfaces in the fields of medical and hospital equipments are investigated [3–13]. The most studied way is the incorporation of biocidal molecules into materials [14–17]. According to this approach, phenol derivatives [18], antibiotics [19], phosphonium salts [20], quaternary ammonium salts [21,22], heavy metal ions [18] and *N*-halamines [23] have been incorporated into nylon fibers and polymeric structures to provide bactericidal properties. The resulting materials process via different mechanisms and their antimicrobial activities diverge considerably inhibiting or destroying the metabolic or enzymatic cellular processes. During last 20 years, the photosensitizing properties of porphyrins have been intensively studied for their used as photosensitizing agent in Photodynamic Antimicrobial Chemotherapy (PACT) [24–27]. PACT relies on the accumulation of a photosensitizing agent into cells followed by illumination with visible light. The photodynamic process involves energy absorption by the photosensitizer which, brought to its excited triplet state, either activates ground state molecular oxygen into singlet oxygen or generates free radicals. Although the cellular mechanism of the photodynamic process is not yet fully understood, it is presently admitted that phototoxicity primarily relies on the formation of singlet oxygen ($^1O_2$) after illumination [28,29]. This

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highly reactive species is able to react with almost every cellular component, bringing irreversible damages that ultimately lead to cell death [30]. This strong reactivity, combined with a low specificity gives rise to a promising approach for the photoinactivation of microorganisms, such as bacteria.

A requisite of PACT is the binding of the photosensitizer to the bacterial cell wall prior to its penetration into cells [31,32]. However, this prerequisite frequently stays unfulfilled since a number of bacteria prove impermeable to most photosensitizers; this drawback can be bypassed by the use of additives that increase bacterial cell permeability [33,31]. Photobactericidal surfaces constitute a second way to overcome the permeation challenge. Illumination of a photosensitizer present on the surface of materials such as plastics generates a singlet oxygen flow that induces damages to adhering cells [34–36]. Efficient materials are often obtained by coating or dyeing, although bioactive molecules are progressively leached from the coated material by different mechanisms such as erosion of the binder. Covalent binding of bioactive molecules to the surface appears as an effective way to overcome this problem. Tetrapyrrolic π-systems like porphyrins have already shown their effectiveness for the photoinactivation of microorganisms [37–39]. On the other hand, we have shown that esterification of cellulose, by fatty acid allows the formation of plastic materials [40,41]. In connection with our research program on photodynamic antimicrobial chemotherapy [42], we have combined these two properties to give rise to a new photobactericidal surface.

In this paper, we describe the synthesis of two news photobactericidal plastic films (Scheme 1) obtained by "one-pot, two-step" esterification reaction starting from cellulose and porphyrins, followed by preliminary results on the photoinactivation of *Escherichia coli* and *Staphylococcus aureus* strains by these new polymers.

2. Experimental

2.1. Materials

All solvents and reagents were purchased from SDS and Acros, respectively, and cellulose powder (20 μm) from Aldrich. Pyrrole was distilled over CaH_2 under reduced pressure immediately before use. Analytical thin-layer chromatography (TLC) was performed on silica gel Merck $60F_{254}$. Column chromatography was carried out with silica gel (60 ACC, 20–40 μm, Merck). Infrared spectra were recorded on a Perkin–Elmer spectrum 1000 by direct transmission through the plastic films. UV–visible spectra were recorded on a Perkin–Elmer Lambda 25 double-beam spectrophotometer using 10 or 50 mm quartz cells. ^{1}H NMR spectroscopy was performed with a Brucker DPX-400 spectrometer. Chemical shifts are reported as δ ppm, downfield from internal TMS, and are listed according to the standard numbering of *meso*-arylporphyrins. Mass spectrometry (MALDI) was performed by the University of Paris VI. MALDI mass spectra were recorded with a Voyager Elite time of flight mass spectrometer. The two strains used for antimicrobial experiments were obtained from 'Institut Pasteur, Paris': *E. coli* (Gram negative, CIP 368548, class 2) and *S. aureus* (Gram positive, CIP 35053156, class 2).

2.2. Methods

2.2.1. Synthesis of photosensitisers

2.2.1.1. Synthesis of 5-[4-(3-ethoxycarbonylpropyloxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin (2). Hydroxyphenyltritolylporphyrin **1** synthesised according to Little method (150 mg, 0.22 mmol, 1 equiv) dissolved in anhydrous DMF (8 ml) was reacted with K_2CO_3 (616 mg, 4.4 mmol, 20 equiv). The mixture was stirred for 15 min. at room temperature. Ethyl-4-bromobutanoate (318 μl, 2.2 mmol, 10 equiv) was then added and the solution stirred during 12 h in the dark at room temperature. DMF was then evaporated under vacuum and the crude product was dissolved in methylene chloride (50 ml). The organic layer was washed with water (3 × 25 ml) and dried with $MgSO_4$. After solvent evaporation and purification by column chromatography performed with $CHCl_3$, porphyrin ester **2** was obtained in 92% yield (161 mg).

R_f : 0.56 (SiO_2, $CHCl_3$). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C, ppm): −2.71 (br s, 2H), 1.36 (t, J = 7.2 Hz, 3H), 2.31 (m, 2H), 2.66 (m, 2H), 2.69 (s, 9H), 4.25 (q, J = 7.1 Hz, 2H), 4.30 (t, J = 6.0 Hz, 2H), 7.26 (d, J = 8.6 Hz, 2H), 7.56 (d, J = 7.8 Hz, 6H), 8.11 (d, J = 7.7 Hz, 2H), 8.12 (d, J = 7.7 Hz, 6H), 8.88 (s, 8H). UV–vis ($CHCl_3$) λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^{-3}): 420 (354.0), 516 (14.3), 552 (7.2), 592 (4.7), 648 (4.2). MALDI MS (*m/z*): calcd for $C_{53}H_{46}N_4O_3$, 786.36; found, 787.90 $[M+H]^+$.

2.2.1.2. Synthesis of 5-[4-(10-methoxycarbonyldecanoxy)-phenyl]-10,15,20- tri(4-methylphenyl) porphyrin (3). Compound **3** was synthesised according to the same method described for compound **2** starting from hydroxyphenyltritolylporphyrin **1** (150 mg, 0.22 mmol, 1 equiv) and methyl-11-bromoundecanoate (538 μl, 2.2 mmol, 10 equiv). Porphyrin ester **3** was obtained in 90% yield (175 mg).

R_f : 0.57 (SiO_2, $CHCl_3$). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C, ppm): −2.76 (br s, 2H), 1.63 (m, 10H), 1.97 (m, 2H), 2.32 (m, 4H), 2.70 (s, 9H), 3.34 (s, 3H), 3.49 (m, 2H), 4.24 (t, J = 6.4 Hz, 2H), 7.26 (d, J = 7.7 Hz, 2H), 7.55 (d, J = 7.8 Hz, 6H), 8.10 (d, J = 7.7 Hz, 2H), 8.10 (d, J = 7.7 Hz, 6H), 8.87 (s, 8H). UV–vis ($CHCl_3$) λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^{-3}): 419 (444.0), 518 (13.3), 553 (8.3), 593 (5.6), 648 (4.8). MALDI MS (*m/z*): calcd for $C_{59}H_{58}N_4O_3$, 870.45; found, 871.50 $[M+H]^+$.

2.2.1.3. Synthesis of 5-[4-(3-carboxypropyloxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin) (4). Porphyrin ester **2** (150 mg) was dissolved in DMF (8 ml) and molar KOH/EtOH (2 ml) was added. The mixture was stirred under reflux for 2 h. After cooling, solvent was evaporated under vacuum and the residue was dissolved in methylene chloride (50 ml). The solution was neutralized by addition of molar HCl (10 ml) washed with water (2 × 25 ml) and dried over $MgSO_4$. After purification by column chromatography performed with $CHCl_3$ and increasing amounts of ethanol (0–10%), porphyrin acid **4** was isolated with quantitative yields (142 mg).