

Review

Potential applications of porphyrins in photodynamic inactivation beyond the medical scope



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ABSTRACT

Although the discovery of light-activated antimicrobial agents had been reported in the 1900s, only more recently research work has been developed toward the use of photodynamic process as an alternative to more conventional methods of inactivation of micro(organisms). The photoprocess causes cell death through irreversible oxidative damage by reactive oxygen species produced by the interaction between a photosensitizing compound and a light source.

With great emphasis on the environmental area, photodynamic inactivation (PDI) has been tested in insect eradication and in water disinfection. Lately, other studies have been carried out concerning its possible use in aquaculture waters or to the control of food-borne pathogens. Other potential applications of PDI in household, industrial and hospital settings have been considered.

In the last decade, scientific research in this area has gained importance not only due to great developments in the field of materials chemistry but also because of the serious problem of the increasing number of bacterial species resistant to common antibiotics. In fact, the design of antimicrobial surfaces or self-cleaning materials is a very appealing idea from the economic, social and public health standpoints. Thus, PDI of micro(organisms) represents a promising alternative.

In this review, the efforts made in the last decade in the investigation of PDI of (micro)organisms with potential applications beyond the medical field will be discussed, focusing on porphyrins, free or immobilized on solid supports, as photosensitizing agents.

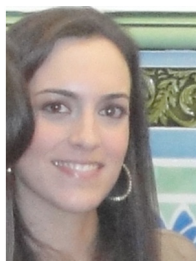
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1. Introduction

Photodynamic therapy refers to the use of a light source (visible light of an appropriate wavelength), an oxidizing agent (molecular oxygen, O_2) and an intermediary agent (named photosensitizer, PS) able to absorb and transfer the energy of the light source to molecular oxygen leading to the formation of highly cytotoxic species (singlet oxygen [1O_2], hydrogen peroxide [H_2O_2], and/or free radicals, such as superoxide [$O_2^{\cdot-}$] and hydroxyl radical [HO^{\cdot}]), causing a multi-targeted damage and destruction of living tissues [1,2]. The generation of these reactive oxygen species (ROS) can occur *via* two mechanisms or pathways, known as type I and type II, which require the presence of O_2 (Fig. 1). In the presence of light ($h\nu$), the photosensitizer in the singlet ground state absorbs a photon, affording the excited singlet state. Then, it can lose energy by returning to the singlet ground state with fluorescence emission (F) or, through an intersystem crossing (ISC) process, it can be converted in the long-lived triplet state. This excited triplet-state PS can decay to ground state by phosphorescence emission (P) or can react with a substrate, namely an electron donor molecule. In this case the formation of radical ions can occur giving rise to radical ions which react with ground state oxygen (3O_2), originating ROS (type I mechanism). Alternatively, the excited triplet-state PS can transfer energy directly to molecular oxygen affording the excited singlet state (1O_2) (type II mechanism). Both photoprocesses may occur simultaneously but type II is, in general, the predominant one. The cytotoxic species can cause irreversible damage to proteins, nucleic acids and lipids [3,4].

The advantage of being a process without a specific cell target renders photodynamic inactivation (PDI) effective in the oxidation of different biomolecules with the consequent destruction of several cell types. In fact, this methodology has a broad spectrum of activity and, using the same PS, is able to destroy human cells [1], viruses [5], bacteria [6], molds [7], yeasts [8], protozoa [9], helminths [10] and insects [11].

Moreover, the ability to structurally tailor the PS as well as to successfully link it to other molecules, with a high degree of specificity (e.g., antibodies, enzymes, nucleic acids), or to solid supports gives this therapy a multiplicity of clinical and non-clinical applications.

The discovery that positively charged PS could effectively inactivate Gram-negative bacteria without the addition of permeabilizing agents [12,13] brought a new impetus to the investigation on the PDI of microorganisms as a new therapeutic modality.

The difference in susceptibility between the two types of bacteria, Gram-negative and Gram-positive, is explained on the basis of the structural features of their cell wall (Fig. 2). Gram-positive bacteria have a cell wall composed of lipoteichoic and teichoic acids organized in multiple layers of peptidoglycan, which confers a degree of porosity to bacteria so as to facilitate the anchoring and entry of PS into the cell [14,15]. In Gram-negative bacteria, the presence of a complex outer membrane in the cell wall, consisting of phospholipids, lipopolysaccharides, lipoteichoic acids and lipoproteins creates an impermeable barrier to antimicrobial agents [14,15]. The interaction between the cationic PS and the constituents of the Gram-negative cell wall generates electrostatic interactions that promote destabilization of the native organization of the wall, allowing the binding and eventual entry of the PS molecules into the cell [14,15]. In the case of fungi, the cell wall contains chitin, glucans and lipoproteins that represent a barrier with intermediate permeability in comparison to Gram-positive and Gram-negative bacteria [16]. With regard to viruses, enveloped viruses are more easily inactivated than non-enveloped ones, but some studies show that non-enveloped viruses can also be efficiently inactivated by the phototoxic action of cationic PS [17],

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