Available online at www.sciencedirect.com
ScienceDirect
Solar Energy 82 (2008) 1107–1117
SOLAR ENERGY
www.elsevier.com/locate/solener

Involvement of both Type I and Type II mechanisms in Gram-positive and Gram-negative bacteria photosensitization by a *meso*-substituted cationic porphyrin

Karim Ergaieg [a,*], Martine Chevanne [b], Josiane Cillard [b], René Seux [a]

[a] *Laboratoire d'Etude et de Recherche en Environnement et Sante, National School of Public Health, Av. Pr. Leon Bernard, CS 74312, Rennes 35043, France*
[b] *Laboratoire de Biologie Cellulaire et Vegetale, UPRES 3891, UFR des Sciences Pharmaceutiques et Biologiques, University of Rennes 1, 2 Av. Pr. Leon Bernard, CS 34317, Rennes 35043, France*

Received 28 April 2007; accepted 16 May 2008
Available online 17 June 2008

Communicated by: Associate Editor Gion Calzaferri

Abstract

A *meso*-substituted cationic porphyrin (TMPyP) showed a photocytotoxicity against Gram-positive and Gram-negative bacteria. In order to determine the mechanism involved in the phototoxicity of this photosensitizer, electron paramagnetic resonance (EPR) experiments with 2,2,6,6-tetramethyl-4-piperidone (TEMP), a specific probe for singlet oxygen, and the spin-trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were carried out with illuminated TMPyP. An EPR signal characteristic of TEMP-singlet oxygen (TEMPO) adduct formation was observed, which could be ascribed to singlet oxygen ($^1O_2$) generated by TMPyP photosensitization. The signal for the DMPO spin adduct of superoxide anion (DMPO-OOH) was observed in DMSO solution but not in aqueous conditions. However, an EPR spectrum characteristic of the DMPO-hydroxyl radical spin adduct (DMPO-OH) was observed in aqueous conditions. The obtained results testify a primary hydroxyl radical ($^{\cdot}OH$) generation probably from superoxide anion ($O_2^{\cdot-}$) *via* the Fenton reaction and/or *via* Haber-Weiss reaction. Gram-positive and Gram-negative bacteria inactivation by TMPyP photosensitization predominantly involved Type II reactions mediated by the formation of $^1O_2$, as demonstrated by the effect of quenchers for $^1O_2$ and scavengers for $^{\cdot}OH$ (sodium azide, thiourea, and dimethylsulphoxide). Participation of other active oxygen species cannot however be neglected since Type I reactions also had a significant effect, particularly for Gram-negative bacteria. For Gram-negative bacteria the photoinactivation rate was lower in the presence of superoxide dismutase, a specific $O_2^{\cdot-}$ scavenger, and/or catalase, an enzyme which specifically eliminates H_2O_2, but was unchanged for Gram-positive bacteria. The generation of $^1O_2$, $O_2^{\cdot-}$ and $^{\cdot}OH$ by TMPyP photosensitization indicated that TMPyP maintained a photodynamic activity in terms of Type I and Type II mechanisms.

Keywords: TMPyP; Photosensitization; Active oxygen; EPR; Photoinactivation

1. Introduction

Sensitized photo-oxidation, also referred as photosensitization or photodynamic action, is an indirect photochemical reaction very similar to photocatalytic oxidation (Cooper and Goswami, 1998). This process may offer an advantage over the photocatalytic process because the sensitizers can absorb light in the visible spectrum, allowing for use of a greater percentage of available sunlight. Photosensitization can represent a useful approach for the killing of microbial cells since it has been shown that several porphyrins and related compounds display phototoxicity against bacteria (Merchat et al., 1996a,b; Minnock et al., 1996, 2000), yeast (Lambrechts et al., 2005), and Helminth eggs (Alouini and Jemli, 2001). Photosensitization seems to

* Corresponding author. Tel.: +33 2 9902 2918; fax: +33 2 9902 2929.
E-mail address: kergaieg@yahoo.fr (K. Ergaieg).

0038-092X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.solener.2008.05.008

be a promising ecologically-friendly water disinfection technique (Jiménez-Hernández et al., 2006) which employs the visible light (or even sunlight) as energy source and the oxygen dissolved in water as the oxidizing agent. This procedure is based on the exposure of cells to a photosensitising agent which is activated by irradiation with visible light of wavelength compatible with its absorption spectrum. Upon light activation, the photosensitizer generates active oxygen species which can modify many biological molecules and eventually lead to cell death (Moan and Peng, 2003). The excited photosensitizer may undergo a photoinduced electron transfer (reductive or oxidative) and/or exchange an hydrogen atom, producing radicals and active oxygen species such as the superoxide ($O_2^{\cdot-}$) and the hydroxyl ($^{\cdot}OH$) radicals. This pathway is termed as a Type I process. The excited state can also transfer energy to dioxygen, in a so-called Type II mechanism generating singlet oxygen ($^1O_2$) (Foote, 1991). Singlet oxygen is thought to play a key role in photosensitized inactivation of bacteria by porphyrins (Henderson and Dougherty, 1992). However, even in cases where Type I reactions may occur for these compounds, the Type II reactions will usually take place in tandem and it is difficult to differentiate the photobiological effects that are exclusively due to radical species. Thus, the question of whether a singlet oxygen or a free radical mechanism is involved in the photodamage is still unsettled (Tanielian et al., 2000).

Regarding bacterial inactivation, it has been shown that Gram-positive bacteria are sensitive to photosensitization by many different dyes, while Gram-negative bacteria are more resistant, being destroyed only after increasing the permeability of the outer membrane either by pre-treatment with different chemical (Bertoloni et al., 1990) or biological agents (Nitzan et al., 1992) or by employing cationic photosensitizers (Merchat et al., 1996a). Jori and Brown (2004) suggest that the positive charge favours the binding of the photosensitizer molecule at critical cellular sites that once damaged by exposure to light cause the loss of cell viability. In this context, a *meso*-substituted cationic porphyrin (TMPyP), whose photocytotoxicity against bacteria has been proved previously, has retained our attention.

Over the last decade, photosensitized inactivation mechanisms of Gram-positive and Gram-negative bacteria by cationic *meso*-substituted porphyrins have been well studied but none of these studies gave importance to the mechanisms of formation of active oxygen species (Merchat et al., 1996b; Reddi et al., 2002; Salmon-Divon et al., 2004). In this paper, we have attempted to determine the nature of the oxygenated intermediates which occur during the photosensitization of a *meso*-substituted cationic porphyrin (TMPyP) in either air-saturated buffered and aqueous conditions, or DMSO solution. We used Electron Paramagnetic Resonance (EPR) spectroscopy to detect and/or study short-lived intermediates such as $^1O_2$ and the free radicals $O_2^{\cdot-}$ and $^{\cdot}OH$. In order to determine the mechanism involved in the phototoxicity of TMPyP, cell photosensitization studies on Gram-positive and Gram-negative bacteria were performed in the presence of specific scavengers and quenchers of active oxygen species.

2. Materials and methods

2.1. Chemicals

Meso-tetra (*N*-methyl-4-pyridyl) porphyrin tetra-tosylate (TMPyP) was obtained from Frontier Scientific Inc. (Logan, UT, USA). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2,6,6-tetramethyl-4-piperidone (TEMP), deuterium oxide (D_2O), sodium azide, thiourea, β-carotene, superoxide dismutase (SOD), catalase (CAT), bovine serum albumin, xanthine, xanthine-oxidase, dimethylsulphoxide (DMSO), ethanol, mannitol, desferrioxamine (DFO), and hydrogen peroxide (H_2O_2) were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Tetrahydrofuran (THF) was provided by Carlo Erba-SDS (Val de Reuil, France). Histidine was provided by Hoffmann-La Roche Ltd. (Bâle, Switzerland). All these products were used without purification, with the exception of DMPO which was purified before use by filtering through activated charcoal and was stored according to the method described by Floyd et al. (1984).

2.2. EPR studies of the photogeneration of $^1O_2$ and free radicals ($O_2^{\cdot-}$, $^{\cdot}OH$)

2.2.1. Irradiation procedure

The photosensitizer (TMPyP) was exposed to a 300 W high-pressure arc xenon lamp (ILLUM 4000, Eurosep, Cergy Pontoise, France) for different durations and strengths of illumination. The spectral emission of this lamp mimics sunlight. This is obtained by using a colored glass filter (Melles Griot Inc., Irvine, California, USA) to cut the wavelengths below 305 nm. The distance between the illuminated samples (300 μL) placed in an EPR spectrometer cavity and the light source was ~20 cm. For the detection of DMPO-OH adducts, irradiation of the samples was carried out outside the microwave cavity in a 300 μL aqueous flat cell as described previously (Viola et al., 1996; Hadjur et al., 1997). The illuminated photosensitizer was placed at 10 cm from the source of illumination with a light intensity ranging from 0.095 to 0.285 W cm^{-2}, and then immediately transferred into quartz capillaries for EPR analysis. Samples were illuminated for periods range from 1 to 15 min. The intensity of illumination was measured by a luxmeter (TES-1339, TES Electrical Electronic Corp., Taipei, Taiwan).

2.2.2. Measurement by EPR

EPR spectra were recorded with a Bruker Model ESP 106 spectrometer operating at room temperature (22–24 °C). Two types of EPR spectrometer cavities were used. The first was specially designed for direct irradiation of the samples (300 μL) inside the microwave cavity. EPR was performed under the following conditions: magnetic field,