Reactive & Functional Polymers 87 (2015) 1–6

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Quantification of singlet oxygen generation from photodynamic hydrogels

Rebecca A. Craig, Colin P. McCoy *, Áine T. De Baróid, Gavin P. Andrews, Sean P. Gorman, David S. Jones

School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK

ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form 12 November 2014
Accepted 24 November 2014
Available online 6 December 2014

Keywords:
Porphyrin
Hydrogel
Singlet oxygen
Quantification

ABSTRACT

Recently, we described a series of novel porphyrin-impregnated hydrogels capable of producing microbicidal singlet oxygen ($^1O_2$) on photoactivation. Indirect assessment of the efficacy of $^1O_2$ production from such hydrogels has been previously described using microbiological techniques, but here we report a novel, direct method of quantification. Anthracene-9,10-dipropionic acid (ADPA) is known to irreversibly form an endoperoxide on reaction with $^1O_2$, causing photobleaching of its absorbance band at approximately 378 nm. Here, the reaction of this probe is exploited in a novel way to provide a simple, inexpensive, and convenient measurement of $^1O_2$ generation from the surface of porphyrin-incorporated photosensitising hydrogels, with the ability to account for effects due to hydrogel porosity. Ingress of the probe into the materials was observed, with rates of up to 3.83×10^3 s^{-1}. This varied by up to 200-fold with material composition and surface modification. Rates of $^1O_2$ generation in these porphyrin-incorporated hydrogels, after compensating for ADPA ingress, ranged from 1.86×10^3 to 5.86×10^3 s^{-1}. This work demonstrates a simple and straightforward method for direct $^1O_2$ quantification from porous materials, with general utility.

1. Introduction

The high reactivity of photosensitiser-generated singlet oxygen ($^1O_2$) has led to its exploitation for photodynamic therapy and photodynamic antimicrobial chemotherapy, and it therefore plays an important role in a number of clinical and antimicrobial treatments and areas of research [1–3]. Recently, we have prepared photosensitiser-incorporated hydrogels for ocular applications [4]. On application of visible light to these hydrogels, $^1O_2$ is generated through the reaction of the excited state of the photosensitiser with molecular oxygen via what is often referred to as a type II pathway. This reaction is catalytic, with the photosensitiser unconsumed in the process. Additional reactive oxygen species such as superoxide anions and hydroxyl radicals may be generated via a type I pathway, but it is known that antioxidant enzymes in bacteria, which can protect against a number of reactive oxygen species, are ineffective against $^1O_2$ [5,6]. As it is widely accepted and has been experimentally determined that $^1O_2$ predominates in the mechanism of photosensitised cell death [7–9], the quantification of $^1O_2$, particularly from porphyrin photosensitisers, is of great significance, and is becoming more necessary as the study and use of photosensitiser-incorporated systems grows.

The detection and quantification of $^1O_2$ from photocatalytic surfaces, however, remains in many ways problematic. To date, only indirect microbiological methods have been used, whereby the adherence of micro-organisms is characterised following irradiation. While this provides a useful assessment of the antimicrobial properties of the materials, it does not distinguish if these properties are due solely to $^1O_2$ generation, and as such cannot provide accurate or quantitative information regarding the rate of $^1O_2$ generation. A previously-reported procedure for the direct physical detection of $^1O_2$ from hydrogels involves measurement of the near-infrared luminescence of $^1O_2$ at 1270 nm [10]. Due to the short lifetime of $^1O_2$ in aqueous solution, its poor solubility, and the low quantum yield of the transition back to ground state, detection by this method is challenging. We previously described a method for directly measuring $^1O_2$ generation from hydrogel surfaces, involving a liquid-nitrogen-cooled Indium Gallium Arsenide detector [11], which is complex, expensive, and custom-designed, due to the insensitivity of most conventional photomultipliers at the wavelength of $^1O_2$ fluorescence emission. Furthermore, data deconvolution due to competing processes is complex. It is therefore desirable to find an inexpensive, rapid, and effective alternative means of testing in aqueous media to provide a general

* Corresponding author. Tel.: +44 28 9097 2081; fax: +44 28 9024 7794.
E-mail address: c.mccoy@qub.ac.uk (C.P. McCoy).

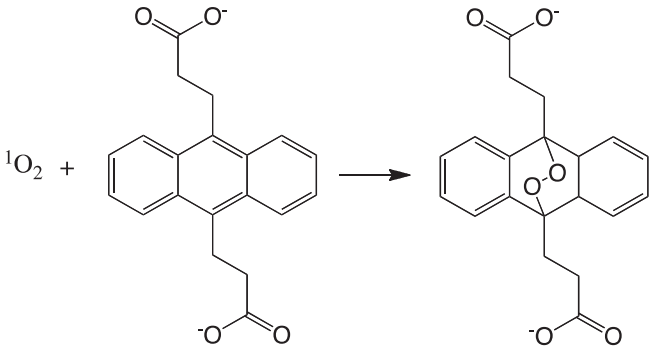
http://dx.doi.org/10.1016/j.reactfunctpolym.2014.11.009
1381-5148/

method of quantification of $^1O_2$ generation from materials surfaces, including porous materials such as hydrogels. Given the potentially numerous biological applications of these anti-infective materials, a method which is readily applicable to aqueous systems is particularly relevant.

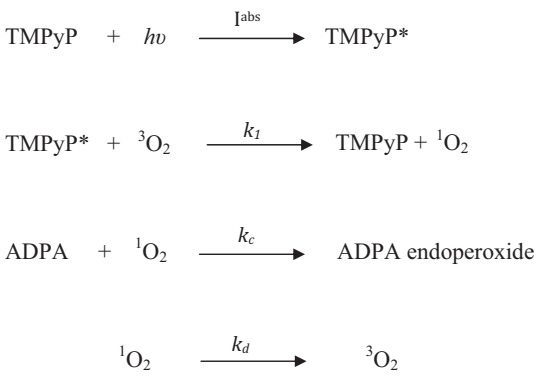
A number of chemical methods to detect $^1O_2$ have been described, involving the use of a chemical probe that reacts with $^1O_2$ to form an endoperoxide. Generally, the probe itself can be spectroscopically characterised in terms of sensitive UV–visible absorbance or fluorescence emission, but the endoperoxide does not possess absorbance or emission in the same wavelength range as the parent molecule, as its formation breaks an extended π-system found in the parent molecule. The ability to monitor this reaction spectrophotometrically allows sensitive and convenient measurement. Some compounds used include 9,10-dimethyl anthracene [12], 2,5-dimethylfuran [13], anthracene-9,10-diyldiethyl disulfate (EAS), bis-9,10-anthracene-(4-trimethyl-phenylammonium)dichloride (BPAA) [14], anthracene-9,10-divinylsulfonate (AVS) [15], anthracene-9,10-bisethanesulfonic acid (AES) [15,16], and anthracene-9,10-dipropionic acid (ADPA) [17]. EAS, AVS, and AES are anionic and therefore may bind with cationic photosensitisers. As TMPyP is tetracationic, they are unsuitable for this study. 2,5-dimethylfuran is only soluble in lipid matrices, also rendering it unsuitable for use in the aqueous systems in which biologically-active polymers are used, and BPAA is cationic which may lead to interaction between it and any unbound anionic methacrylic acid groups at the polymer surface. Singlet Oxygen Sensor Green (SOSG), a commercially available sensor, has also been widely used but it is associated with a number of drawbacks including photodecomposition, intersystem crossing, and $^1O_2$ generation, thus interfering with quantification of photosensitiser-generated $^1O_2$ [17].

The use of ADPA in the detection of $^1O_2$ was first described by Lindig et al. [18]. It has since been employed to assess $^1O_2$ generation by various photosensitiser systems including methylene blue-containing polyacrylamide and silica nanoparticles [19], butadiyne-bridged bisporphyrin for photodynamic therapy (PDT) [20], and meta-tetra(hydroxyphenyl)-chlorin containing silica nanoparticles for PDT [21], and a polymer-based porphyrin system [22]. It has also been used to quantify $^1O_2$ generation from porous hydrogel particles, but without taking into account ingress into the hydrogel [23]. ADPA ingress into the bulk material is the major hurdle to be overcome in quantification of $^1O_2$ generation from porous materials, and is addressed in the present study as such as method is currently absent from the literature.

ADPA reacts very rapidly and irreversibly with $^1O_2$ to produce an endoperoxide, resulting in bleaching of the absorbance maximum of ADPA at approximately 378 nm [18,22] (Scheme 1). The endoperoxide formed is thermally stable at room temperature [23]. It is a useful method of quantification as, even when $^1O_2$ generation rates are low, the concentration of endoperoxide produced remains proportional to the cumulative amount of $^1O_2$ generated [12]. In addition, it has a high rate constant for reaction with $^1O_2$ [18,24] and high water solubility, which allows testing in aqueous media, and in systems with biological applications.



Scheme 1. The conversion of ADPA to an endoperoxide on reaction with $^1O_2$.



Scheme 2. The series of reactions that occur in solution, resulting in photobleaching of ADPA. I^{abs} indicates intensity of absorbance, k_1, k_c and k_d are rate constants.

This reaction is demonstrated here to provide a quantitative measure of the role of $^1O_2$ generation at the surface of a series of porphyrin-incorporated hydrogels, and therefore provide an indication of their bactericidal activity. This method is distinctive from previously reported uses of ADPA in that it can account for ingress of ADPA into the materials under evaluation. This is the first use, to our knowledge, of this probe for detection of $^1O_2$ generation from porous bulk hydrogels.

2. Materials and methods

2.1. Materials

Anthracene-9,10-dipropionic acid disodium salt (ADPA) was obtained from Chemodex (St. Gallen, Switzerland). Tetrakis(4-N-methylpyridyl)porphyrin (TMPyP), 2-hydroxyethyl methacrylate 97% (HEMA), methyl methacrylate 99% (MMA), methacrylic acid 99% (MAA), ethylene glycol dimethacrylate 98% (EGDMA), and benzoyl peroxide 70% (BPO) were obtained from Aldrich (U.K.) and used as supplied.

2.2. Preparation of solutions

ADPA solutions were prepared in 20:80 (v/v) methanol: deionised water. All UV–visible absorption measurements were obtained using a PerkinElmer Lambda 650 UV–visible spectrophotometer with a tungsten-halogen lamp, and viewed using UVWinLab software (PerkinElmer, USA).

For solution studies, TMPyP, at various concentrations, and ADPA were dissolved in 20:80 (v/v) methanol: deionised water, with a final ADPA concentration of 5.22×10^{-5} mol/dm^3. A control solution of ADPA (5.22×10^{-5} mol/dm^3) in 20:80 (v/v) methanol: deionised water, containing no TMPyP was also tested. Solutions were exposed to white light (8.91 mW/cm^2) generated from 25 W halogen lights (Halolux Ceram, Osram, Germany), at a fixed distance of 11 cm for time periods ranging from 5 to 20 min, with the use of a fan to maintain temperature at 27 °C, or maintained in dark conditions. Absorption spectra were recorded between 320 and 550 nm, allowing visualisation of the main absorption peaks of ADPA, and the Soret band of TMPyP.

To quantify the $^1O_2$ generated by TMPyP on irradiation, the reactions occurring in solution require consideration. Scheme 2 describes the processes that occur when ADPA and TMPyP are irradiated together in solution (modified from [19,21]):