Lipid vesicles as model membranes in photocatalytic disinfection studies

O.K. Dalrymple, W. Isaacs, E. Stefanakos, M.A. Trotz, D.Y. Goswami*

Clean Energy Research Center, University of South Florida, Tampa, FL 33620, USA

ABSTRACT

The potential use of solar-powered photocatalytic disinfection water systems is an attractive concept and has generated much research over the last two decades. Photocatalytic inactivation of a wide range of water pathogens has shown promise to provide an effective alternative to traditional disinfection methods. However, in order for photocatalysis to be effectively used as a water disinfection process, its inactivation kinetics must be well established. Recent literature points to the peroxidation of phospholipid membranes as the main mechanism for photocatalytic inactivation of bacteria. To test the peroxidation hypothesis, researchers utilized free lipids, particularly lipids with the ethanolamine polar group which is dominant in the cell membrane of *Escherichia coli*. Although these experiments yielded useful information about byproducts, they did not provide information on the kinetics of lipid peroxidation in cells exposed to photocatalytic treatment.

In this work, lipid vesicles were prepared with a mixture of natural *E. coli* phospholipids and appropriately sized to be comparable to real cells. The vesicles and *E. coli* cells were photocatalytically treated in a test tube batch reactor using TiO_2 (Degussa P25) and UVA lamps. The rate of phospholipid membrane degradation was determined by measuring the production of malondialdehyde (MDA) and lipid hydroperoxide (LOOH), byproducts of lipid peroxidation. Thiobarbituric Acid Reactive Species (TBARS) and Ferrous Oxidation of Xylenol (FOX) assays were used to assess each byproduct respectively. The fatty acid content of *E. coli* cells was also modified by adding oleic (C18:1 n-9) and α-linolenic (C18:3 n-3) acids to the growth media. Byproduct formation and oxidation kinetics were compared for all experiments. The results show that the oxidation kinetics of lipid vesicles closely matched the oxidation of *E. coli* cells in photocatalytic systems proving that the vesicles are useful model systems to study the interaction of cell membranes with TiO_2. However, differences in monosaturated fatty acids in *E. coli* did not appear to affect the overall disinfection kinetics. While these findings further validate membrane peroxidation as an important process in the mechanism of photocatalytic disinfection, they suggest that overall inactivation results from a far more complex collection of processes.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Over the past decade the mechanism of photocatalytic disinfection has been heavily debated among researchers. Increasing evidence suggests that the oxidation of cell membrane lipids plays an important role in the photocatalytic inactivation of bacterial pathogens in water [1–5]. The general hypothesis is that the unsaturated fatty acids, mainly polyunsaturated fatty acids, present in the phospholipid membranes are very sensitive to oxidation by radical species, particularly the hydroxyl radical. The repeating arrangement of lipids in the membrane allows cell injury to occur at sites relatively distant from the initiation source due to radical-induced chain reactions (Fig. 1).

During photocatalysis hydroxyl radicals are generated on the surface of a solid semiconductor catalyst, such as titanium dioxide (TiO_2), when exposed to light of the appropriate wavelength [6]. The hydroxyl radical is known to oxidize all macromolecules found in cells including proteins [7,8], polysaccharides [9], lipids [10–12], and nucleic acids [13,14]. However, the disinfection process is mainly characterized by an interaction between the cell membrane and the photocatalyst [15]. The phospholipid components of the cell are localized in the cell membrane. TiO_2 is capable of initiating an irreversible oxidation of the fatty acids present in the membrane of the pathogen when hydroxyl radicals extract H-atoms from unsaturated lipids. The initiation process is followed by a propagation cycle in which the newly formed lipid radical reacts with oxygen to produce a lipid peroxyl radical. The propagation cycle continues as the lipid peroxyl radical reacts with a nearby unsaturated lipid producing a new lipid radical and a lipid hydroperoxide (LOOH). The process is terminated when two radicals react forming a non-radical species. The lipid hydroper-

* Corresponding author. Tel.: +1 8139740956; fax: +1 8139746438.
E-mail addresses: odalrymp@mail.usf.edu (O.K. Dalrymple), goswami@usf.edu (D.Y. Goswami).
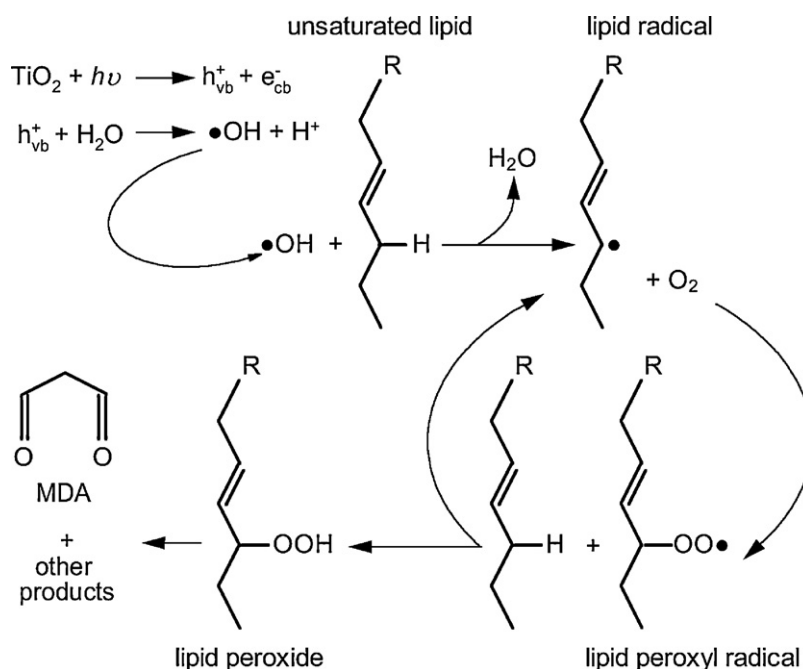
Fig. 1. Schematic of radical-induced lipid peroxidation.

oxide can further be oxidized by lipid radicals to form aldehydes, especially MDA. The latter is often used as a biomarker for lipid peroxidation in cells. The process of lipid peroxidation leads to the destruction of the cell membrane, disrupting its functions and eventually leading to cell inactivation [10,16].

The lipid composition of bacterial membranes depends very much on the species and even on the culture conditions and stage in the growth cycle. Membrane fractions usually contain 10–30% lipid. In Gram-positive bacteria phosphatidylglycerol (PG) is present, but phosphatidylethanolamine (PE) is more common in Gram-negative species. Phosphatidylethanolamine can form 75% of the total phospholipids in *E. coli*. The other lipids are PG and cardiolipin; the proportion of each depends on the growth phase [17,18]. Phospholipids have a fatty acid tail, which have the potential to serve as initiation sources for membrane peroxidation. This is particularly true for polyunsaturated fatty acids, even though they are often present in smaller proportions than monosaturated fatty acids. In *E. coli* unsaturated fatty acids can account for as much as 50% of all fatty acids, and generally include palmitoleic acid (C16:1 n-7) and *cis*-vaccenic (C18:1 n-7) [17,19–22]. It is therefore suspected that the kinetics of peroxidation of the cell membrane may be affected by the fatty acid content of the cell, mainly the unsaturated species.

In previous studies of photocatalytic disinfection, lipid peroxidation was confirmed by comparing MDA production during photocatalytic oxidation of PE and *E. coli* cells [1,4]. However, in these studies PE was used in a dissolved form in a homogenous solution. While this approach yielded useful information about byproduct formation, it does not offer much information on the kinetics of cell membrane oxidation because chain reactions in a compartmentalized membrane system can follow very different kinetics and mechanisms from those observed in homogeneous solutions [23]. In addition, unlike other phospholipids which spontaneously form lamellar phases in aqueous media, pure PE solutions or mixtures enriched in PE are notable for being unstable and adopt a hexagonal phase [24,25]. They often require a stabilizing agent to maintain a bilayer structure similar to biological membranes.

The current study goes further to establish lipid peroxidation in cells during photocatalysis by using lipid vesicles as model *E. coli* membranes. Lipid vesicles of PE were prepared with the addition

of PG, which served as a stabilizing agent, but also represented a more realistic and natural *E. coli* membrane. The vesicles were also sized to be comparable to real cells to mimic the colloidal nature of the solution. In addition, the effect of unsaturated fatty acid enrichment in *E. coli* on peroxidation kinetics was also tested. Enrichment was achieved by supplementing the growth media with oleic and α-linolenic acids and validated by fatty acid methyl ester (FAME) analysis. The cells and vesicles were then exposed to illumination with TiO$_2$ and the evolution of MDA and LOOH was measured during the experiments to assess membrane peroxidation.

2. Materials and methods

2.1. Cell culture

E. coli ATCC 25922 was grown aerobically in 100 mL of Luria broth at 37 °C in an incubator shaker (250 rpm) for 6 h (to log phase). The cells were harvested from the broth by centrifugation at 1380 × g for 10 min in a 15-mL polypropylene centrifuge tube. The cell pellet was washed and re-suspended in sterile deionized water (resistivity >16 Mohm-cm). This process was performed twice to ensure that most of the broth solution was removed. The turbidity of the suspension was measured at 550 nm with a DR/2000 spectrophotometer (Hach Company). The cell suspension was diluted to the required final concentration for all experiments based on a standard curve that correlated turbidity with cell concentration (CFU mL^{-1}). Lipid modification of the cells was achieved by supplementing the Luria broth base with 32 μM of oleic (C18:1 n-9) and α-linolenic (C18:3 n-3) acids obtained from MP Biomedicals (Solon, OH).

2.2. Fatty acid analysis

At least 20 mg of cells was harvested and twice pelletized by centrifugation at 1380 × g for 15 min in a 15-mL tube after successive washing of the cells with sterile deionized water. The cell pellets were sent to Microbial ID (Newark, DE) for fatty acid methyl ester (FAME) analysis. The general steps in a FAME analysis included extraction of the fatty acids by a procedure which consisted of