Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology B: Biology 78 (2005) 253–258
Journal of Photochemistry and Photobiology B:Biology
www.elsevier.com/locate/jphotobiol

Evidence for the hydration effect at the semiconductor phospholipid-bilayer interface by TiO_2 photocatalysis

M. Suwalsky [a,*], C. Schneider [a,1], H.D. Mansilla [a], J. Kiwi [b]

[a] *Faculty of Chemical Sciences, University of Concepcion, Casilla 160-C, Concepcion, Chile*
[b] *Laboratory of Photonics and Interfaces, Institute of Chemical Sciences and Engineering, EPFL, Lausanne, Switzerland*

Received 19 July 2004; received in revised form 16 November 2004; accepted 23 November 2004
Available online 12 January 2005

Abstract

The interactions of TiO_2 with phospolipid bilayers found in cell membrane walls were observed to perturb the bilayer structure under UVA light irradiation. The structure changes in the phospholipid bilayers upon contact with TiO_2 under light and in the dark were followed by X-ray diffraction. Hydration effects at the semiconductor–phospholipid interface played an important role in the degradation of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) bilayers taken as cell wall lipid bilayer models. Evidence is provided that the fluidity of the phospholipid bilayers plays a significant role when interacting in the dark with the TiO_2 or in processes mediated by TiO_2 under light irradiation.

Keywords: TiO_2; Phospholipid bilayer; Cell membrane; Photocatalysis

1. Introduction

The cell membrane is an assembly of proteins and lipids that separate inside from outside, protecting the cell interior. Furthermore, it is involved in a variety of indispensable cellular functions. It is responsible for the selected transport of molecules and ions into and out of the cell in the extensive network responsible for the traffic between organelles. Without exception, these activities depend on, and are influenced by the physical milieu provided by the molecules making up the membrane bilayers. Changes in the physical and chemical environment of the cell membranes have a direct effect on the membrane structure with serious effects on the cell functions [1]. Most biological membranes possess an asymmetric *trans*-bilayer distribution of phospholipids. Thus, for instance, most eukaryotic plasma membranes present a high percentage of the phospholipids sphingomyelins and phosphatidylcholines in the outer monolayer whereas the inner monolayer is generally richer in phosphatidylethanolamine, phosphatidylserines and phosphatidylinositols. However, the existence of asymmetric plasma membranes is less certain in bacteria than in eukaryotes. Studies of the phospholipid distribution of a Gram-positive bacteria revealed that the outer monolayer is rich in phosphatidylglycerols, the inner one in phosphatidylinositols while cardiolipins are symmetrically distributed between both monolayers. Studies on Gram-negative bacteria such as *Escherichia coli* (*E. coli*) have been able to detect phosphatidylethanolamines in the outer membrane whereas the cytoplasmic membrane has been reported to be rich in phosphatidylglycerols and cardiolipins [2].

The objective of this study is to report the TiO_2 photocatalytic effect on the cell wall membranes structure

* Corresponding author. Tel.: +56 41 204171; fax: +56 41 245974.
E-mail address: msuwalsk@udec.cl (M. Suwalsky).
[1] Present address: Department of Chemistry, Catholic University, Antofagasta, Chile.

1011-1344/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotobiol.2004.11.016

using phospholipid bilayers like dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) as molecular models of cell membranes. Keeping this in mind we address the interaction of DMPC and DMPE with TiO_2 under 365 nm (UVA) light irradiation. These two phospholipid bilayers present sufficient X-ray crystallographic reflections to produce the experimental data which allows interpretation of the structural changes induced in the bilayers by TiO_2 photocatalysis. Since hydration of cell wall membranes plays an essential role in the interaction of the phospholipid bilayers with TiO_2, this effect is also investigated during the TiO_2 mediated photocatalysis. These models subjected to X-ray diffraction methods have been also used in our laboratories to determine membrane perturbation due to metals such as Cu [3], Hg [4], Al [5], Pb [6] and Cd [7].

To our knowledge, no detailed study is available on the interaction of DMPC and DMPE with TiO_2 under band-gap light irradiation. The nature and extent of the interaction with DMPE is of particular importance when trying to understand the time scale of the damage produced by TiO_2 photocatalysis on the cell wall membrane of *E. coli* leading to: (a) injury, (b) the blocking of the normal metabolic functions, (c) the inactivation and finally (d) bacterial death mediated either by TiO_2 suspensions or TiO_2 photocatalytic films.

The photocatalytic properties of TiO_2 have been extensively investigated in fields ranging from solar conversion, metal reductions and for decontamination of air and water [8–10]. When TiO_2 is illuminated with the appropriate light source ($\lambda < 390$ nm) the excitation results in the formation of electron–hole pairs at the surface of the catalyst particle. Since the earlier report of Matsunaga et al. [11], the photocatalysis with TiO_2 has been applied as a way to abate bacteria. Recently Rincón and Pulgarin [12] demonstrated that *E. coli* was almost completely inactivated by irradiation; the degree of inactivation was dependent on the type of irradiation source and operational parameters. In general, it has been assumed that the inactivation of micro-organisms is due to the $OH^{\cdot}$-radicals generated during the photocatalytic process. The photo-inactivation of *E. coli* and *Lactobacillus helveticus* was enhanced when air was passed through the reactor during illumination, indicating the significant role of oxygen in the photokilling of bacteria [13]. Wolfrum et al. [14] demonstrated that TiO_2 surfaces can act as catalyst to degrade microorganisms such as *E. coli*, *Micrococcus luteus*, *Bacillus subtilis*, fungi and bacteria spores and different kind of organic compounds. Phosphatidylethanolamine was used as a model of phospholipid and almost quantitative oxidation was reached in around 12 h irradiation under 50% of relative humidity. TiO_2-photocatalytic destruction of organic compounds and organized matter (e.g., bilayers and vesicles) has been attributed to oxygen radicals, such as hydroxyl and superoxide radicals, which can produce serious alterations of cell membrane structure or DNA degradations. However, a complete and detailed mechanism has not yet been elucidated [15].

2. Materials and methods

2.1. Reagents, sample preparation and irradiation procedures

Synthetic DMPC (lot 49H5156, A grade, MW 677.9), DMPE (lot 49H5189, A grade, MW 635.9) from Sigma (MO, USA) and TiO_2 (Degussa P-25, 56 m^2/g, Germany) were used without further purification. For each experiment, about 2 mg of phospholipid and about 0.2 mg of TiO_2, equivalent to a surface of 100 cm^2, were weighted in a Cahn C-33 microbalance (Cahn Instruments, Cerritos, CA, USA). These mixtures were introduced in 2.0 mm diameter glass capillaries (Glas–Technik and Konstruktion, Berlin, Germany). The samples were then irradiated for different periods of time with a Philips BLB monochromatic light source 160 W ($\lambda = 365$ nm, UVA). Sample-to-lamp distance was 12 cm. The value of the light intensity reaching the TiO_2–phospholipid samples was 1.5–2 mW/cm^2 of sample. Each blank consisted of pure samples of dry DMPC, DMPE, TiO_2, DMPC–TiO_2 and DMPE–TiO_2 mixtures.

2.2. X-ray diffraction analysis of phospholipids

The samples were X-ray diffracted in flat-plate cameras with 0.25 mm diameter glass collimators provided with rotating devices. Specimen-to-film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered Cu Kα radiation from a Philips PW 1140 X-ray generator was used. The relative reflection intensities on film were measured by peak integration using a Bio-Rad GS-700 densitometer (Hercules, CA) and Molecular Analyst/PC image software; no correction factors were applied.

2.3. Effect of the water of hydration in the interaction between TiO_2 and phospholipid bilayers

In order to determine the effect of hydration in the photocatalytic reactions on the phospholipid membranes, about 250 μl of bi-distilled water were added to samples prepared as previously indicated in Section 2.1, agitated with fine glass fibers and kept overnight before irradiation. A different type of DMPE–TiO_2–H_2O sample was also prepared in order to increase DMPE fluidity. It was achieved by heating the mixture DMPE–H_2O up to 58 °C, cooled down to room temperature followed by the addition of TiO_2 and agitation. After light irradiation, the hydrated samples were centrifuged at 2000 rpm