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Monitoring the bactericidal effect of UV-A photocatalysis: A first approach through 1D and 2D protein electrophoresis

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ABSTRACT

The bactericidal effect of UV-A photocatalysis is studied through the 1D and 2D protein electrophoresis biochemical approach over model and bacterial proteins, which shows that a majority of proteins are heavily and non-specifically damaged. This suggests that the emergence of resistance to this treatment should be almost impossible.

1. Introduction

The report of biocidal effects of TiO_2 in 1985, with the photoelectrochemical killing of microbial cells by semiconductors, opened the door to the use of photocatalysis for the control of pathogenic agents [1]. Photocatalysis has attracted great attention since decades and acts mainly through oxidative photogenerated holes or $OH^{\cdot}$ radicals [2–4]. The UV-A irradiation of semiconductors leads to the oxidation of the organic constituents of microorganisms like for liquid and gas phase organics, and thus inactivates bacteria, viruses, spores, yeasts, etc. [5]. Recent worldwide damage caused by pathogenic microorganisms, gave rise to a stimulating research mainly dealing with self-decontaminating surfaces, or water disinfection and potabilization using TiO_2 suspensions [6–8], mainly targeting bacteria, viruses, fungi, algae and protozoa. Recently, due to major public health problems, the treatment by UV-A photocatalysis of contaminated air received a growing interest inside both industrial and academic communities involved in innovative sustainable environmental research [9–12].

Although the bactericidal effect of photocatalysis is well-documented, notably using TiO_2 suspensions and *Escherichia coli* bacteria as model target [6–8], the molecular mechanism of the photocatalytic attack over microorganisms is still a blackbox. Studies of the oxidative attack on both the cellular and the molecular levels are necessary not only for fundamental purposes, but also because noticeable differences in sensitivity towards photocatalysis among biomolecules may forecast the development of resistances due to the generalisation of photocatalytic bio-applications, as it has been observed with the overuse of silver in hospitals [13]. Moreover, it can give some clues about the future of the by-products generated during the photocatalytic processes.

Viability numerations for evaluating the photocatalytic coating efficiencies towards microorganisms are up to now unsuitable for getting more insight on the necessary molecular understanding of the attack mechanisms. Therefore, although the targets were biological objects and the study of the impact of UV-A photocatalysis towards such biological targets usually implied biological numeration methods and techniques, replacing a biological approach by a biochemical one was necessary for progressing on the mechanistic knowledge at the molecular level taking place in photocatalysis over microorganisms.

To achieve this goal, we focused on the bacterial proteins, since these molecules play important roles in both the structure and the functioning of the cell, and they account for at least 50% of the total cell molecules (except water). Indeed, *e.g.* for Gram negative bacterial cells such as *E. coli* and *Legionella pneumophila*, both inner cytoplasmic and outer membranes (this latter being a part of the cell wall with a peptidoglycan layer) are composed of proteins, phospholipids and various sugars. At the cell surface, these structures act as protective barrier, contributing to maintain vital functions, and constitute the first targets for photocatalysis. This complex layered assembly of high molecular weight compounds

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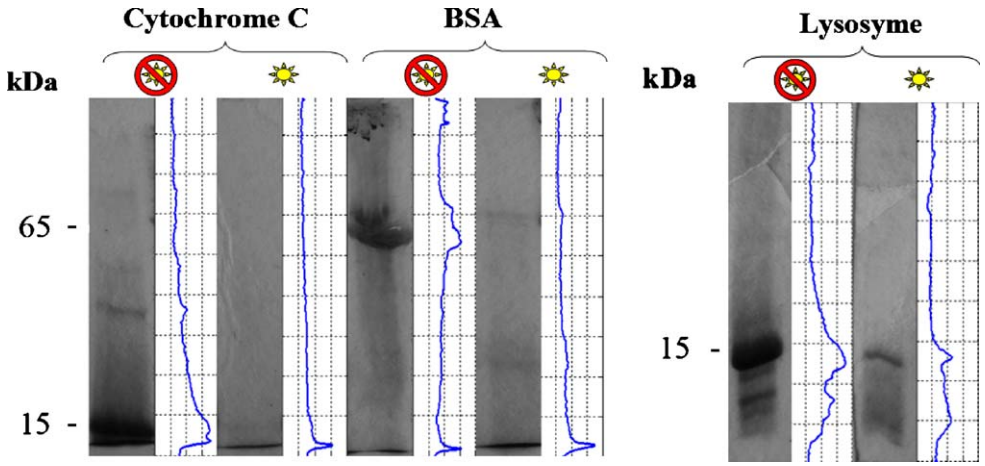

Fig. 1. SDS–PAGE electrophoresis of three purified proteins, BSA (65 kDa), lysozyme (15 kDa) and cytochrome *C* (13 kDa) before and after a 1-h photocatalytic treatment (scanned electrophoresis and corresponding intensity curves, in arbitrary units a.u.). BSA without TiO_2 was also submitted to UV-A and was used as control. without UV-A; with UV-A.

(MW > 1000 kDa; kDa = 10^3 g mol^{-1}) highlights the necessity of also working from a biochemistry point of view over the biological targets to degrade. Moreover, one can note that pioneer valuable works already showed the drastic influence of singlet oxygen over peptidic bindings for fragmenting purified proteins.

This short article is a first step in the design of a biochemical approach of photocatalysis over microorganisms as a complement to a pure biological one, and aims to emphasize on the first use of 1D and 2D SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) technique as a promising and powerful tool for visualising the effect of photocatalysis on proteins.

2. Experimental

The lyzozyme, cytochrome *C* and bovine serum albumin (BSA) purified model proteins were purchased from Sigma (>95% purity).

E. coli bacteria was chosen because of the wide knowledge concerning its protein content (structure, function and localisation). *E. coli* was grown at 37 °C in LB medium. Bacterial cells were harvested in late exponential growth phase and washed twice in isotonic buffer. Cells were disrupted by sonication and membranes were recovered by ultracentrifugation (45,000 × *g*, 1 h, 4 °C).

Proteins (1.25 mg or otherwise indicated) were mixed with 10 mg TiO_2 P25 powder (Degussa) and coated onto a glass substrate (0.5 mg TiO_2/cm^2 coverage) and dried in the dark. Illumination was obtained by a usual Philips actinic BL 40W UV-A lamp 2 cm apart (with a spectral peak centred on 365 nm and corresponding to a measured 2.8 mW/cm^2 illumination). After the photocatalytic treatment, the recovered sample was re-suspended in sample buffer containing 2% SDS/5% β-mercapto-ethanol and boiled for 1D 12% SDS–PAGE or in urea 8 M, CHAPS 2% for 2D SDS–PAGE. After centrifugation at 13,200 rpm, an aliquot supernatant was submitted to 1D or 2D SDS–PAGE.

The SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gel electrophoresis) technique was used in 1D and 2D modes as a powerful tool enable to separate model purified proteins and both membrane and cytoplasm proteins on a polyacrylamide gel according to their electrophoretic mobility (mainly a function of length of polypeptide chain or molecular weight, *i.e.* globally of their size). The Laemmli method was used to perform SDS–PAGE analysis [14]. Each protein migrates according to its molecular weight and forms a band which can be visualised after staining. The intensity of each band is directly proportional to the protein quantity. Gels were stained using Coomassie blue G-250 stain, and further scanned with a Magic Scan apparatus (600 dpi resolution) and analyzed using Matlab Software.

SDS–PAGE can be coupled to proteins isoelectric focusing (IEF) for separating in 2 dimensions (both IEF and molecular weight). 2D-SDS–PAGE analysis was performed according to the O'Farrell method using an immobilized pH gradient (IPG-PHOR) system (Amersham Pharmacia Biotech Inc., Piscataway, NJ) [15].

3. Results and discussion

First, we challenged the lyzozyme (15 kDa), cytochrome *C* (13 kDa) and bovine serum albumin (BSA, 65 kDa) purified model proteins to TiO_2 UV-A treatment. In order to avoid artefacts linked to the adsorption of molecules on TiO_2 particles which have been pointed out by Gogniat et al. [16,17], we used protein concentrations in excess (125 μg protein per mg of catalyst). To evaluate the effects of photocatalysis on model proteins, SDS–PAGE analysis was performed. Fig. 1 presents the results obtained with the model proteins treated or not with photocatalysis. Electrophoretic gels and intensity profiles show that after 1 h treatment with TiO_2/UV-A, the intensity of the protein band was much lower compared to the fresh sample, suggesting a drastic protein degradation. No large aggregates were evidenced since comparable results were observed when samples were submitted to sonication or not, and whatever the acrylamide gel concentration was (7.5% and 12%; data not shown). In control experiments (not shown), the proteins remained unaffected, after the samples were submitted up to 100 °C for 1 h, showing that the degradation was not linked to the heat caused by UV-A lamp.

The kinetic of the photocatalytic effect was studied with BSA. Fig. 2 shows a drastic BSA degradation since the first minutes of TiO_2/UV-A exposure. This degradation reaches a limit after 30–45 min of illumination due to the BSA not being in contact with TiO_2 (as a result of the large amount of BSA used) and to the non-illuminated underlying part of the dried proteins–TiO_2 mix. It should be noted that the use of large amounts of BSA was necessary for recovering enough molecules after photocatalysis for performing and interpreting the SDS–PAGE analysis.

The kinetic of the BSA denaturation was followed by looking on the integration of the colour intensity of the clump at 65 kDa, taking into account the 100% and 0% reference intensities corresponding to the starting protein quantity estimated after 60 min in the dark, and to protein-free lane. Thus, the intensity profile analysis led to evaluate at 40 ± 15% the part of proteins mixed with TiO_2 grains able to react after 30–45 min of exposure, *i.e.*

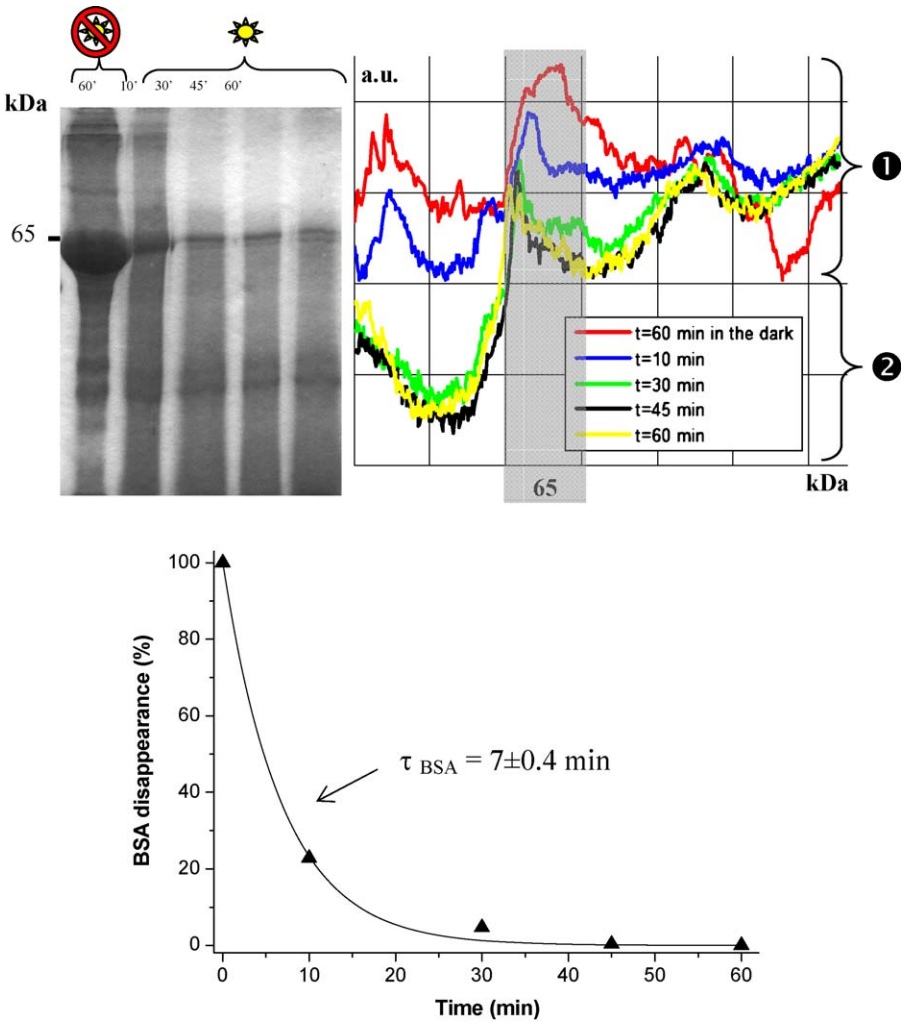

Fig. 2. (Top) SDS–PAGE time analysis of the BSA photocatalytic denaturation with unchallenged proteins for reference. (1) Proteins–TiO_2 available for photocatalytic degradation (40 ± 15%). (2) Proteins–TiO_2 masked (60 ± 15%). (Down) First order kinetic of the BSA disappearance for the 65 kDa protein, obtained on the basis on proteins–TiO_2 mixture available for photocatalytic degradation. without UV-A; with UV-A.

the fraction of reacting mixture. Therefore, the apparent characteristic time constant of the first order disappearance kinetic was thus calculated by taking this 60 ± 15% limit level as asymptotic 0% reference intensity. It should be mentioned that the ±15% value corresponded to the 95% confidence interval for the 60% limit value resulting from the apparent first order disappearance fit. Considering only the fraction allowed to react and assuming a first order disappearance kinetic with a decreasing exponential modelling, the integration of the BSA peaks at 65 kDa allowed an apparent characteristic time constant of 16 ± 2.2 min to be obtained for the BSA degradation in the presence of air.

However, since we used huge protein amounts compared to protein bacterial content (1.25 mg *vs.* 12 μg protein for 10^7 bacterial cells for Gogniat et al. [16,17]) we can assume a much higher efficiency in photocatalytic processes on bacterial cells. Therefore, in a second step, the SDS–PAGE protein analysis was further done on proteins from fresh whole cells as well as on proteins still embedded in the membrane fraction extracted from *E. coli* bacteria. It should be noted that membrane proteins represent about a quarter of whole cell total proteins. On fresh bacterial cells, as clearly shown in Fig. 3, left, treatment of intact *E. coli* bacteria by TiO_2/UV-A photocatalysis resulted in the partial or complete disappearance of protein bands suggesting that all of the proteins were attacked during photocatalysis. A similar effect of

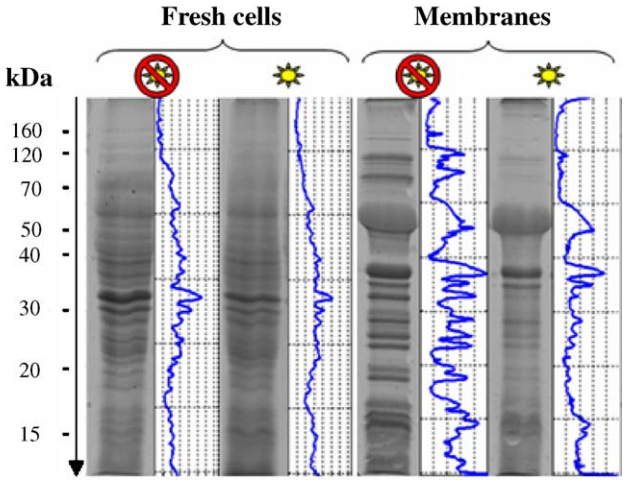

Fig. 3. SDS–PAGE analysis of fresh *E. coli* cells (left) and of the membrane fraction (right) before and after a 1-h photocatalytic treatment (scanned gels and corresponding intensity profiles, in a.u.). 800 μg membranes proteins and 2×10^9 whole fresh cells were used. without UV-A; with UV-A.

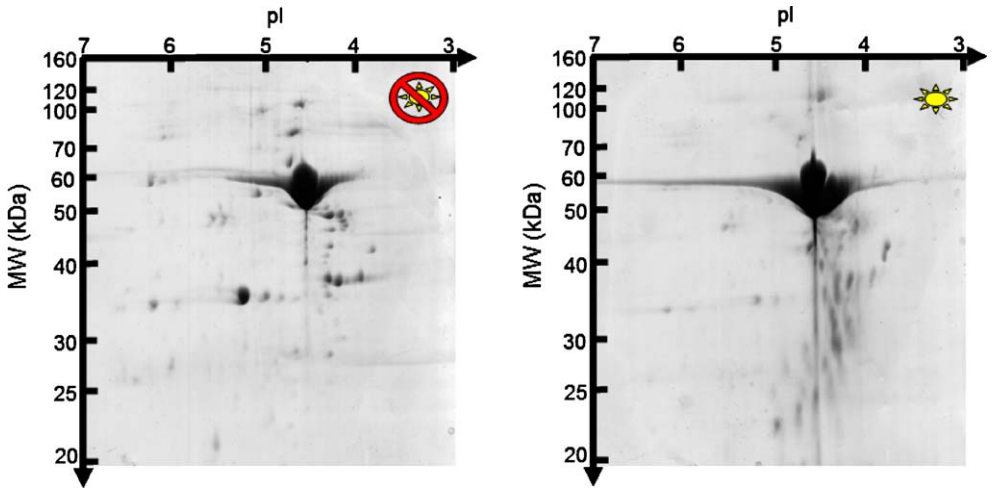

Fig. 4. 2D SDS–PAGE analysis of the membrane fractions before and after 1-h TiO_2/UV-A photocatalysis. Proteins are separated following their molecular weight (MW) and their isoelectric point (p*I*). without UV-A; with UV-A.

photocatalysis-mediated proteins degradation was observed on bacterial membranes (Fig. 3, right).

These results were confirmed by using the 2D SDS–PAGE technique using urea chaotropic agent and chaps as mild detergent. This accurate technique allows separating proteins according both to their molecular weight and their isoelectric point. Thus each delimitated spot corresponds to only one protein. After treatment with TiO_2/UV-A the appearance of smears and the disappearance of the great majority of the protein spots was observed, suggesting that complete degradation of most of the proteins was achieved. As shown in Fig. 4, all spots—except one corresponding to the *E. coli* major membrane protein (due to saturation phenomena)—disappeared, pointing out the non-selectivity of the attack. Since the degradation is proportional to the major protein initial quantity in the sample, the apparent resistance of this protein to the photocatalytic attack is probably related to its high proportion among the total membrane proteins.

Bacterial proteins from whole cells or embedded in the membranes were affected in the same manner during the photocatalytic treatment. Most of them were quickly degraded during photocatalysis, leading to a loss of cell activity and finally to death. The fact that no definite protein target was found out, suggests that the occurrence of resistance mechanisms is almost impossible. This is all the most important since UV-A photocatalysis treatments will thus not be polluted by the emergence of resistant microorganisms, like it can be the case with the widespread UV-C or chemical treatments that can act on specific sites, resulting for bacteria in developing treatment resistance. This confirms that UV-A photocatalysis is a way of disinfecting surfaces and preventing water and air contamination.

4. Conclusion

1D and 2D electrophoresis was used as a promising tool for evidencing the effect of UV-A photocatalysis on purified and bacterial proteins. We hope this biochemical approach for investigating UV-A photocatalysis over microorganisms, could be a first step – in complement to a pure biological one – for apprehending the understanding of the cellular and molecular mechanisms involved during the UV-A photocatalytic attack on TiO_2, also in order to control eventual microorganism resistance phenomena.

This preliminary work only deals with the reference *E. coli* bacteria. However, since all bacteria have proteins at the surface and we proved that the photocatalytic attack on proteins is non-specific, our results can be very probably transposed to other bacteria such as pathogenic *E. coli* or *L. pneumophila*, also *Bacillus anthracis* spores, and even on virus since their capsid is composed of proteins. In parallel to proteins, works over bacterial phospholipids are ongoing for providing global information on the UV-A photocatalytic attack on both protein and lipid targets from the bacterial membranes.

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References

[1] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, FEMS Microbiol. Lett. 29 (1985) 211.
[2] D. Bahnemann, in: P. Boule (Ed.), Environmental Photochemistry, vol. 2, Springer, Berlin, 1999, pp. 285–351.
[3] P. Pichat, in: M.A. Tarr (Ed.), Chemical Degradation Methods for Wastes and Pollutants, vol. 2, Marcel Dekker Inc., New York, 1999, pp. 77–119.
[4] A. Fujishima, K. Hashimoto, T. Watanabe, TiO_2 Photocatalysis—Fundamentals and Applications, BKC, Inc., Tokyo, Japan, 1999.
[5] S. Josset, N. Keller, M.-C. Lett, M.J. Ledoux, V. Keller, Chem. Soc. Rev. 37 (2008) 744.
[6] K. Sunada, Y. Kikuchi, K. Hashimoto, A. Fujishima, Environ. Sci. Technol. 32 (5) (1998) 726.
[7] A.G. Rincon, C. Pulgarin, Appl. Catal. B: Environ. 49 (2004) 99.
[8] D.M. Blake, P.C. Maness, Z. Huang, E.J. Wolfrum, J. Huang, W.A. Jacoby, Sep. Purif. Methods 28 (1999) 1.
[9] D.Y. Goswami, D.M. Trivedi, S.S. Block, J. Sol. Energy. Eng. 119 (1997) 92.
[10] V. Keller, N. Keller, M.J. Ledoux, M.C. Lett, Chem. Commun. (2005) 2918.
[11] S. Josset, T. Taranto, N. Keller, V. Keller, M.C. Lett, M. Ledoux, V. Bonnet, S. Rougeau, Catal. Today 129 (2007) 215.
[12] A. Pal, S.O. Pehkonen, L.E. Yu, M.B. Ray, Ind. Eng. Chem. Res. 47 (2008) 7580.
[13] S.L. Percival, P. Bowler, D. Russell, J. Hosp. Infect. 60 (2005) 1.
[14] U.K. Laemmli, Nature 227 (1970) 680.
[15] P.H. O'Farrell, J. Biol. Chem. 250 (1975) 4007.
[16] G. Gogniat, PhD Dissertation, EPFL, Lausanne, Switzerland, 2006.
[17] G. Gogniat, M. Thyssen, M. Denis, C. Pulgarin, S. Dukan, FEMS Microbiol. Lett. 258 (2006) 18.