Journal of Photochemistry and Photobiology B: Biology 96 (2009) 130–135

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

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

Specific effects of single antioxidants in the lipid peroxidation caused by nano-titania used in sunscreen lotions

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ARTICLE INFO

Article history:
Received 29 January 2009
Received in revised form 30 April 2009
Accepted 11 May 2009
Available online 19 May 2009

Keywords:
TiO_2 nanoparticles
Linoleic acid
Porcine skin
UVB irradiation
Phenylalanine
Ascorbic acid derivatives

ABSTRACT

The effect of some additives, phenylalanine, ascorbyl palmitate and sodium ascorbyl phosphate on the oxidation of linoleic acid and porcine ear skin induced by UV irradiation was investigated, in the absence and in the presence of variously uncoated and coated titania powders. Such additives have, on the one hand, a scavenging activity toward the oxidizing species photogenerated by TiO_2, and on the other one an inhibitory effect toward UVB-induced peroxidation. Sodium ascorbyl phosphate and ascorbyl palmitate displayed a stronger antioxidant effect than phenylalanine toward linoleic acid peroxidation. On porcine skin all the three molecules exhibited both antiradical and antioxidant activity. Their protective effect against peroxidation was higher with porcine skin lipids than with linoleic acid, referable to the chemical differences in the two lipid substrates.

1. Introduction

The skin, being at the interface between the body and the environment, is chronically exposed to both endogenous and environmental pro-oxidant agents, which lead to the harmful generation of reactive oxygen species (ROS). There is evidence that an oxidative stress is involved in the damage of various cellular constituents, such as DNA, cell membrane lipids or proteins. Among the cellular targets of ROS, polyunsaturated fatty acids have recently been studied, and the current hypothesis is that a peroxidation process in cultured human skin cells could be induced either by UVB or by UVA [1]. Lipid peroxidation measured as the release of thiobarbituric-acid reactive substances (TBARS) into the supernatant of cultured human skin fibroblasts was recently reported to be triggered by both UVA and UVB radiation [2].

A variety of products has been formulated to protect the human skin. The lotions employed to absorb or block UV radiation need to be opaque, biologically and chemically inert, stable when applied on the skin, and resistant to water. TiO_2 is an effective opaque material, which reflects and scatters UV radiation, and is the most widely used inorganic chemical component in sunscreens [3]. When in nano-size, TiO_2 is more appropriate because becomes transparent to visible light, while keeping its screening potency toward UV radiation. However, in the presence of UV light, titanium dioxide is activated to produce reactive oxygen species such as hydroxyl radicals, superoxide anions, and singlet oxygen [4–6]. Titanium dioxide and zinc oxide (of cosmetic and drug grade) were reported to induce photo-oxidation of unsaturated lipids under UVB illumination [7]. Different coatings of the titania particles prevented at a larger or lesser extent the photochemical sensitization [7].

A previous study by some of us was devoted to the effect of some uncoated or coated specimens of TiO_2 on the peroxidation under UV irradiation of both linoleic acid – chosen as a model unsaturated substrate – and porcine skin, an appropriate model for human skin. A large variety in peroxidation activity was found among the different coated titania. In some cases the coating was found to be not efficient in inhibiting the oxidative properties of TiO_2 nanoparticles [8].

The skin is protected against oxidant species by a well-organized system of both chemical and enzymatic antioxidants, which work synergistically. Topical application or oral administration of antioxidants has recently been suggested as a preventive therapy for skin photoaging and UV-induced cancer [9].

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doi:10.1016/j.jphotobiol.2009.05.001

Vitamin C is used in cosmetic and dermatological preparations because it has many favorable effects on the skin. As an antioxidant can scavenge aggressive oxidizing agents and free radicals. Ascorbic acid, unfortunately, has a poor chemical stability in heterogeneous systems and may undergo dismutation reactions. Some hydrophilic derivatives of ascorbic acid have been proposed to provide a more persistent protective effect. In particular, sodium ascorbyl phosphate exerts an important antioxidant activity triggered by its conversion into free ascorbic acid upon enzymatic degradation. However, because of its hydrophilic character, sodium ascorbyl phosphate has a low ability to penetrate the skin [10]. Lipophilic derivatives like esters with fatty acids are commonly present in many products. Ascorbyl palmitate applied on the skin decreased the level of formation of free radicals, and its effectiveness depended significantly on the carrier system [11]. The effect of amino acids on the riboflavin-sensitized photo-oxidation of ascorbic acid has also been evaluated. Cysteine showed a strong concentration-dependent antioxidant activity, and an antioxidant effect was also observed with alanine and phenylalanine at 0.1% w/w concentration [12].

The purpose of the present paper is to find systems that could protect the skin when exposed to UV radiation, and to formulate safer sunscreen lotions by associating one amino acid (phenylalanine) and two ascorbic acid derivatives (sodium ascorbyl phosphate and ascorbyl palmitate) to different coated TiO_2 nanoparticles.

2. Materials and methods

2.1. Materials

Titania powders: one uncoated and five variously coated commercial titania nanoparticles have been employed. PW Covasil S-1 was supplied from LCM Trading SpA (Sesto S. Giovanni, Italy), Tego Sun TS plus and Aeroxide P 25 were gifts from Degussa (Vicenza, Italy). T-Lite SF and T-Lite SF-S were from BASF (Cesano Maderno, Italy), Maxlight F-TS20 from Showa Denko K.K. (Tokyo, Japan).

2.2. Chemicals

Hydrochloric acid, 1-butanol, sodium chloride, sodium ascorbyl phosphate (2-phospho-1-ascorbic acid trisodium salt, SAPh), sodium azide, sodium dodecyl sulfate (SDS) and sodium hydroxide are Fluka products (Milan, Italy). Phosphoric acid 85% and dichloromethane were from Carlo Erba (Rodano, Italy). Linoleic acid, ascorbyl palmitate (L-ascorbic acid-6-palmitate 95%; AP) were purchased from Aldrich (Milan, Italy); 2-thiobarbituric acid (4,6-dihydroxy-2-mercaptopyrimidine, TBA), phenylalanine (1,2-amino-3-phenyl propanoic acid; Phe) and 1,1,3,3-tetraethoxypropane (malondialdehyde-bis(diethylacetal)) were purchased from Sigma (Milan, Italy).

2.3. Surface area

The surface area of the powders was measured by means of the BET method based on N_2 adsorption at −196 °C (ASAP 2010 Micrometrics, London, Canada).

2.4. XRD spectroscopy

XRD spectra were collected on a diffractometer (PW1830, Philips, Milan, Italy) using Cu Kα radiation, in the (20–90) 2θ range, with step width $2\theta = 0.05$ and time per step = 1 s. Diffraction peaks have been indexed according to the JPCDS database.

2.5. Photoinduced peroxidation

Photoinduced peroxidation studies were performed by irradiating the samples under UVB lamp (G40T10E, Sankyo Denki, Kanagawa, Japan) with 2.4 W m^{-2} irradiance. During irradiation, the powder suspensions were stirred with a multiple magnetic stirrer (RO 5, IKA, Staufen, Germany). A Micro pH 2001 (Crison, Alella, Spain) was employed to control the pH. A 660/H Transsonic Sonifier (Elma, Singen, Germany) was used to disperse the TiO_2 nanoparticles before irradiation. After irradiation, a 5417 centrifuge (Eppendorf, Milan, Italy) was employed to separate TiO_2 from the samples before analysis. The formation of malondialdehyde (MDA) was followed by a Lambda 2 UV/Vis spectrophotometer (Perkin Elmer, Waltham, MA).

The organic solvent (vide infra) was evaporated under vacuum by RE-111 Rotavapor® (Büchi, Flawil, Switzerland).

2.6. TBA assay

The assay, currently used as an index of lipoperoxidation, is based on the reactivity of MDA, a colorless end-product of degradation, with TBA to produce a pink adduct (TBA–MDA–TBA) that absorbs at 535 nm. MDA was detected spectrophotometrically according to the method described by Bay et al. [13], with some modifications. The sample (0.2 mL) was introduced in a glass tube closed with a screw cap and added with 0.1 mL of water, 0.2 mL of 8.1% w/w SDS, 1.5 mL of 1.0% w/w phosphoric acid, and 1.0 mL of 0.6% w/w TBA. The mixture was stirred and heated in water bath at 95–100 °C for 45 min to favor the formation of the complex. After cooling in ice bath, 4.0 mL of 1-butanol were added to each tube, and the TBA–MDA–TBA complex was extracted upon stirring and centrifugation. The organic supernatant was spectrophotometrically analysed.

To obtain an appropriate calibration curve of the TBA–MDA–TBA complex, a solution of 1,1,3,3-tetraethoxypropane in the concentration range 9.4–208.0 μM was prepared in 8.1% w/w SDS. MDA was produced by the acid hydrolysis of 1,1,3,3-tetraethoxypropane during the reaction with TBA, under the conditions of the TBA assay. Linear least-squares regression was performed to determine the parameters of the calibration equation (slope and intercept) and the correlation coefficient. The final concentration of MDA derived from the reaction was expressed as nmoles of MDA per mg of lipid substrate.

2.7. Calibration curve of the complex TBA–MDA–TBA

The calibration curve was assessed on solutions at different concentrations of a MDA precursor (1,1,3,3-tetraethoxypropane) in 8.1% w/w SDS. The precursor, in a similar way as MDA, reacts with TBA to form a pink colored chromophore.

2.8. Irradiation runs

Irradiation was carried out in Pyrex® glass cells (10.0 mL) under a UVB lamp. All the samples were irradiated for 120 min under magnetic stirring at a distance of 10 cm from the lamp.

2.9. UVB-induced linoleic acid peroxidation

The sample was prepared by dispersing the unsaturated acid (1.0% w/w) in 4.0% w/w SDS aqueous solution, and it was magnetically stirred for 24 h in the dark. Before irradiation, the pH was adjusted to 4.0. An aliquot (10.0 mL) of the sample was UVB irradiated as reported above, then centrifuged (12000 rpm) for 15 min. An aliquot (0.2 mL) of supernatant was taken to determine MDA through the TBA assay.