Oxybenzone Oxidation Following Solar Irradiation of Skin: Photoprotection *versus* Antioxidant Inactivation

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We used noninvasive Fourier transform (FT) Raman spectroscopy to follow the fate of the broadly used ultraviolet UVA sun blocker, oxybenzone, after topical application to the skin. Our results showed that oxybenzone is rapidly photo-oxidized, yielding oxybenzone semiquinone, a potent electrophile, which reacts with thiol groups on important anti-oxidant enzymes and substrates, such as thioredoxin reductase and reduced glutathione, respectively. Although oxybenzone is an excellent broad spectrum UVA filter, its rapid oxidation followed by the inactivation of important antioxidant systems indicates that this substance may be rather harmful to the homeostasis of the epidermis. Furthermore, these results demonstrate that FT-Raman spectroscopy is a useful method for studying the transport and metabolism of active ingredients in topical preparations. ***Key words: oxybenzone/free radical defense. J Invest Dermatol 106:583–586, 1996***

Oxybenzone (2-hydroxy-4-methoxybenzophenone) is a broad-range ultraviolet UVA filter with λmax 288 and 325 nm and extinction coefficients of 14,000 and 9,400 $cm^{-1}M^{-1}$ (Shaath *et al*, 1990; Rünger *et al*, 1995). The strong UVA and partial UVB absorbing properties allowed the selection of this compound as a major component of sun protection creams and lotions (Rünger *et al*, 1995). Sundaram *et al* showed, however, that topical application of oxybenzone in Sun Science cream (SPF-24) (E. Arden, New York) caused UVA-induced photo-inactivation of the important antioxidant enzyme, thioredoxin reductase (TR), in skin biopsies obtained from 15 healthy volunteers (Sundaram *et al*, 1990). Based on this study, it was proposed that oxybenzone may undergo photo-oxidation to a highly reactive semiquinone intermediate, which could have the capacity to interact with the thiolate active site of TR to covalently inactivate the enzyme by Michael addition (Sundaram *et al*, 1990). More recently, TR and its substrate, thioredoxin, have been shown to be induced in the guinea pig and human epidermis by superoxide anion radical (O_2^-) generating systems (i.e., UVB light, x-rays and xanthine oxidase activities); meanwhile, the other antioxidant defense systems superoxide dismutase, catalase, and glutathione reductase are inhibited under the latter conditions (Schallreuter *et al*, 1994; Yodoi and Uchiyama, 1992; Buckman *et al*, 1993). The induction of TR and thioredoxin occurs in parallel with increased melanogenesis in the human epidermis, guinea pigs, and melanoma cells (Schallreuter *et al*, 1994; Matsuda *et al*, 1991; Tagaya *et al*, 1989).

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Abbreviations: TR/T, thioredoxin reductase/thioredoxin; FT, Fourier transform.

Fourier transform (FT) Raman spectroscopy has been used to study human and reptilian skin both *in vitro* and *in vivo* (Williams *et al*, 1993, 1994a, 1994b). With this technique, the epidermis can be studied noninvasively to examine lipid, water, redox-status, and protein domains such as α-helix, β-pleated sheet structures, etc. Recently, the preserved skin of the so called "Ice Man", the 5,200-y-old Ötzi, was examined by this technique; the results showed that considerable protein degradation had occurred, but the lipid component was largely unaltered compared with contemporary controls (Williams *et al*, 1995). This technique is quantitative and has the potential to assign functional groups in both small molecular weight substances and in macromolecules such as proteins and lipids (Williams *et al*, 1993, 1994a, 1994b, 1995). Since the earlier experiments on oxybenzone metabolism were carried out on skin biopsies, we wished to reexamine these results directly on the living skin surface using FT-Raman spectroscopy in a noninvasive study.

MATERIAL AND METHODS

Human Probands Seven healthy age-matched controls with skin types I–VI (Fitzpatrick classification) (Pathak *et al*, 1987) served for the *in vivo* experiments (type I, n = 1; type II, n = 2; type III, n = 2; type V, n = 1; type VI, n = 1). The male/female ratio was 5/2. In addition, one female proband with a congenital nevus and skin type II was examined. Clinically, the nevus presented as 1 cm in diameter, was slightly elevated, and showed an evenly distributed red-brown color. The margins were sharply circumscribed.

Laser Raman Spectroscopy Fourier transform Raman spectra were recorded using a Bruker FRA 106 Raman module on a Bruker IFS 66 optics system. A Nd: YAG Laser operating at 1.064 μm was used as the excitation source, and the laser beam was focused to a 100-μm spot on the skin surface of the inner arm of each proband. A laser power of 20 mW was used with increments of 200–1,000 scans at 4 cm^{-1} being collected. The Raman band positions are quoted in terms of wave numbers (cm^{-1}), and are scientifically correct, as frequency has the units S^{-1} or Hz. A liquid nitrogen–cooled

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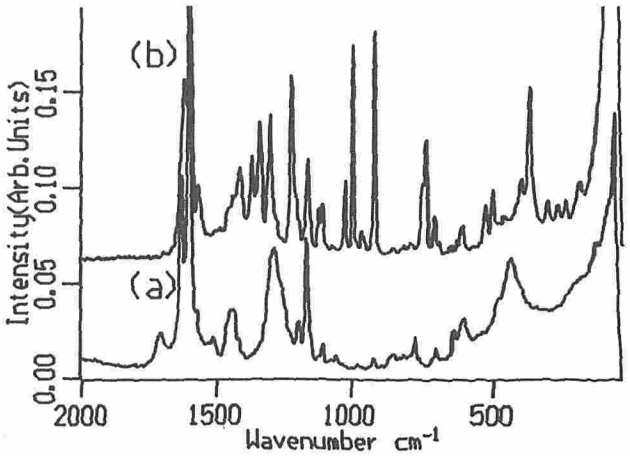

Figure 1. Raman spectra of an oxybenzone standard and oxybenzone in Soltan facial cream. FT-Raman spectrum (1,000 scans) of Soltan Facial Cream (SPF 25) containing oxybenzone as UVA filter (*a*) and the spectrum from oxybenzone in acetonitrile (*b*). The carbonyl group (C=O) is characterized by the doubled peak at 1,606 and 1,635 cm^{-1}.

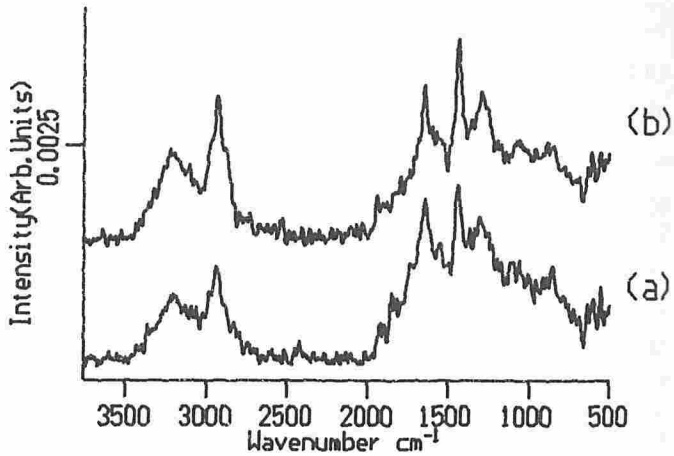

Figure 2. Raman spectra of photo-skin types I and III. FT-Raman spectra (1,000 scans) from skin types I (*a*) and III (*b*). Stratum corneum N-H stretch = 3,208 cm^{-1}; lipid = 2,943 cm^{-1}; amide band of α-helical proteins = 1,652 cm^{-1}; C-S to S-S = 644–526 cm^{-1}. Spectra were obtained noninvasively on the inner forearm at a standard distance from the laser beam.

germanium detector with an extended bandwith was used for measuring the range $\Delta\nu$ = 100–3,750 wavenumbers cm^{-1}.

Experimental Protocol FT-Raman spectra were obtained from pure solutions of oxybenzone (10% in acetonitrile), reduced glutathione (25% in distilled water), and the Michael addition complex formed from oxybenzone semiquinone and reduced glutathione (vol/vol:1/1). Soltan Facial Cream (SPF 25) containing oxybenzone was applied to a metal plate, and the spectrum was recorded at 0 time and after 15 min of natural sun exposure (May, midday, in England, 54° latitude). In addition, the normal epidermis of the inner arms of eight probands including one congenital nevus was examined *in vivo* before and directly after application of SPF25 (Soltan) and after 15–30 min of sun exposure.

Materials Oxybenzone and reduced glutathione were from Sigma Chemical Co. (St. Louis, MO). Soltan Facial Cream (SPF 25) containing oxybenzone was obtained over the counter and came from Boots Company, plc, (Nottingham, U.K.). Other listed ingredients of the cream were provitamin B5, cocoa butter, and Vitamins A and E in liposomes.

RESULTS

Raman Spectroscopy of Oxybenzone and Normal Human Epidermis The Raman spectrum of oxybenzone (10%) in acetonitrile is presented as a reference to oxybenzone in the proprietary cream **(Fig 1)**. The carbonyl group (>C=O) gives the strongest peak at 1,606 cm^{-1} and one of a lower intensity at 1,635 cm^{-1} similar to that reported for p-benzoquinone, which is used as reference (Schrader, 1989). FT-Raman spectra with 1,000 scans were collected for the human epidermis *in vivo* for each proband (n = 8). The spectra presented essentially the same features, but pigmented epidermis (skin type VI, Fitzpatrick classification) yielded poorer resolution of the major peaks due to quenching by melanin. Spectra from skin types I and III, presented in **Fig 2**, are similar to the results previously reported by Williams *et al* (1994b). The broad band at 3,208 cm^{-1} represents the N-H stretching vibration of the stratum corneum, whereas tape-stripped epidermis lacks this peak. The peak at 1,652 cm^{-1} has been assigned as C=O in the amide bonds of α-helical protein domains (Williams *et al*, 1994b). The peak centered at 2,943 cm^{-1} represents the lipid component, and peaks at 644–526 cm^{-1} represents C-S to S-S bonds (Williams *et al*, 1995). The latter assignments were established by examination of the spectra of nail and hair (Williams *et al*, 1994a).

Photo-Oxidation of Oxybenzone by Sunlight The stability of oxybenzone to sunlight in Soltan Facial Cream (SPF 25) was first tested by the application of a thin layer of this preparation to an aluminum plate followed by exposure to natural sunlight for 15 min. The spectra presented in **Fig 3*A*** show the C=O stretches from oxybenzone at 1,606 and 1,635 cm^{-1}. After exposure to sunlight, there was a significant increase in the C=O concentration indicative of the oxidation of the 2-OH-group to a second C=O group, in the same structural environment as the first C=O group, indicating oxidation of oxybenzone to its semiquinone.

Photo-Oxidation of Oxybenzone and the Depletion of Antioxidants **Figure 3*B*** shows a representative result of the fate of oxybenzone on the skin type III epidermis. The lower trace (*a*) presents the spectrum of the untreated epidermis, the middle trace (*b*) is the spectrum after the application of the cream revealing the C=O stretch, and the upper trace (*c*) shows the spectrum after 15 min of exposure to natural sunlight. The results of this experiment showed a significant quantitative increase in C=O at 1,606 and 1,635 cm^{-1}. Similar results were obtained with the skin of the probands except for one with skin type I and red hair, where the increased band intensity at 1,606 and 1,635 cm^{-1} appeared transiently and was followed by a rapid increase of the intensity in the C-S region of the spectrum between 630 and 540 cm^{-1}. This result suggests that the semiquinone of oxybenzone reacts rapidly with the excess thiols such as cysteine and reduced glutathione, which are increased in the epidermis of fair-skinned people who synthesize more pheomelanin. In addition, this observation supports inactivation of the thiolate active site of TR by this semiquinone (Sundaram *et al*, 1990). **Figure 3*C*** presents the spectra of a congenital nevus, clinically appearing with red-brown color following the formation of oxybenzone Michael addition complexes at 642 and 617 cm^{-1}, and the reduced glutathione complex at 557 cm^{-1}, before, immediately after application, and after 30 min of sun exposure. **Figure 4*A*** shows the increased band intensity of the C-S stretches at 642, 617, and 557 cm^{-1} immediately after exposure to the cream. The band at 447 cm^{-1} appears to represent the Michael addition complex between oxybenzone and reduced glutathione. After 15 min of natural sunlight exposure, the C-S stretch is significantly more marked. A "kinetic analysis" of C-S bond formation with time after the application of Soltan Facial Cream (SPF 25) on skin type 1 is presented in **Fig 4*B***.

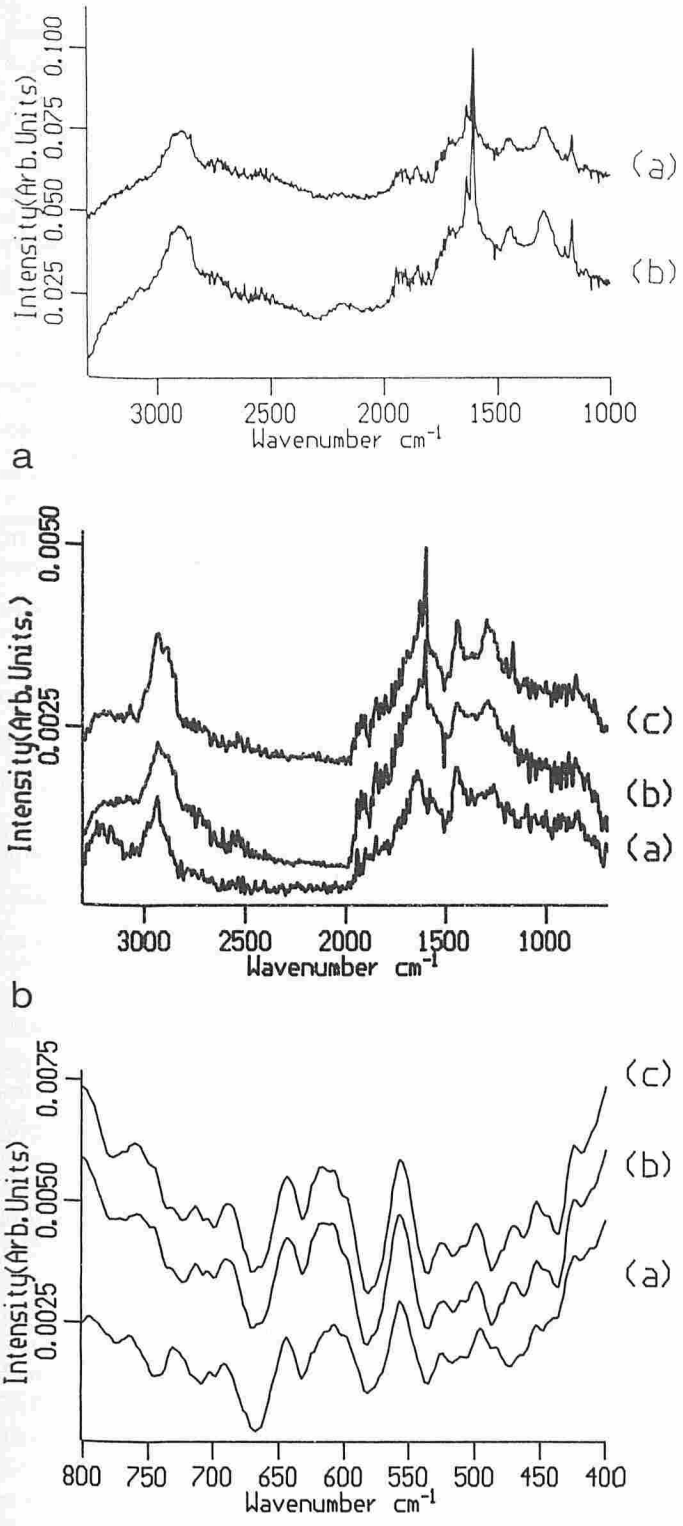

Figure 3. Raman spectra showing the photo-oxidation of oxybenzone both at the surface of an aluminum plate and on the epidermis *in vivo*. *A*) FT-Raman spectra (1,000 scans) from Soltan Facial Cream (SPF 25) after application on an aluminum plate at 0 time (*a*) and after 15 min of natural sun exposure (*b*). *B*) FT-Raman spectra (500 scans) of skin type III before (*a*), after application of Soltan Facial Cream (SPF 25) at 0 time (*b*), and after 20 min of natural sun exposure (*c*). *C*) FT-Raman spectra (1,000 scans) obtained from a congenital nevus before (*a*), after application at 0 time (*b*), and after 30 min of natural sun exposure (*c*). These results indicate that oxybenzone is unstable to normal sunlight being photo-oxidized to a highly reactive semiquinone.

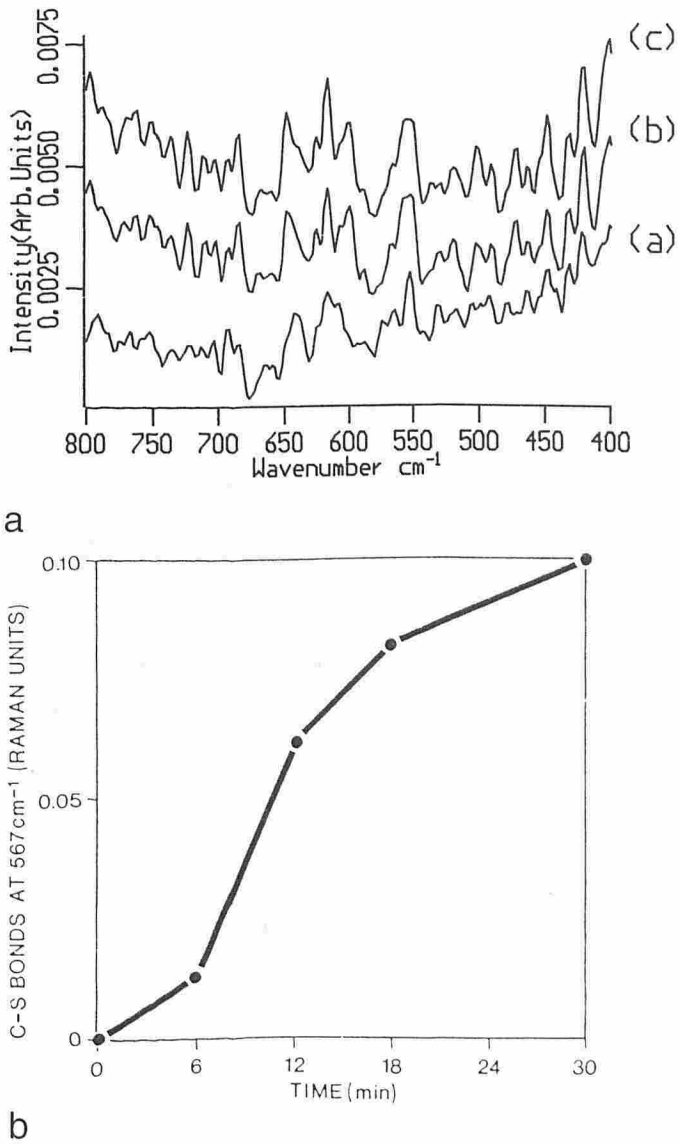

Figure 4. Evidence for the reaction of the photo-oxidation product oxybenzone semiquinone with thiol groups in the epidermis. *A*) FT-Raman spectra (1,000 scans) of skin type III before (*a*), after application of cream at 0 time (*b*), and after 20 min of natural sun exposure (*c*). *B*) Time dependent formation of the Michael addition complex between reduced glutathione and oxybenzone semiquinone in the sun exposed epidermis of skin type I. The C-S band was recorded at 557 cm^{-1} at 200, 400, 600, and 1,000 scans. These results indicate that the photo-oxidation of oxybenzone at the surface of the epidermis by normal sunlight leads to inactivation of important antioxidant processes by Michael addition to important thiolate groups.

DISCUSSION

It is well established that enzymes and coenzymes with thiol active sites play a critical role in the cellular defense against toxic oxygen species generated in the course of normal metabolism and UV light exposure in the human epidermis (Schallreuter and Wood, 1989). The thioredoxin reductase/thioredoxin (TR/T) system is especially

significant because it is induced by oxidative stress. This system functions as an anti-oxidant both in association with plasma membranes and in the cytosol of keratinocytes and melanocytes (Schallreuter *et al*, 1986). Recent experiments with human keratinocytes *in vitro* using a monoclonal antibody for thioredoxin showed that UVB exposure promotes a rapid increase in cytosolic thioredoxin and is followed by its migration into the nucleus upon severe oxidative stress.[1] These results suggest that thioredoxin must play a major protective role against DNA damage by reactive oxygen radicals and hydrogen peroxide. Earlier, Spector *et al* also demonstrated that lens epithelial cells are protected from hydrogen peroxide cytotoxicity by infusion of genetically engineered thioredoxin (Spector *et al*, 1988). In addition to the TR/T system, the glutathione reductase/glutathione/glutathione peroxidase system is required specifically for the reduction of hydrogen peroxide to water (Buckman *et al*, 1993). It has been demonstrated in the human skin that the heme active site of catalase is inactivated by UVB light generated by hydroxyl radicals from hydrogen peroxide (Aronoff, 1965; Schallreuter *et al*, 1991). Therefore, the TR/T and glutathione reductase/glutathione/glutathione peroxidase systems are critically important (Buckman *et al*, 1993; Schallreuter and Wood, 1989). Earlier experiments with 15 normal healthy probands suggested that the sun protection factor oxybenzone inhibited membrane-associated TR activities after UVB exposure (Sundaram *et al*, 1990). For these experiments, 3-mm punch biopsies of skin were used. It has been shown that oxybenzone could be rapidly metabolized upon photoactivation compromising membrane-associated TR (Sundaram *et al*, 1990). In this report, we present further *in vivo* evidence for the rapid photo-oxidation of oxybenzone to its semiquinone followed by Michael addition to active thiolate groups in the epidermis. **Figure 5** presents the reaction pathway with photo-oxidation preceding Michael addition to form C-S-R complexes. The latter reaction is most rapid in photo skin types I and II (Fitzpatrick classification). Based on the increased sun sensitivity of this group, SPF 25 will be very likely used in order to protect these skin types against solar rays, but oxybenzone as the UVA filter would most likely trigger an increased threat from reactive oxygen species in their epidermis. Our results on the direct fate of oxybenzone in the human epidermis of different skin types strongly suggest that the use of this widespread UVA filter in many different preparations warrants careful reassessment.

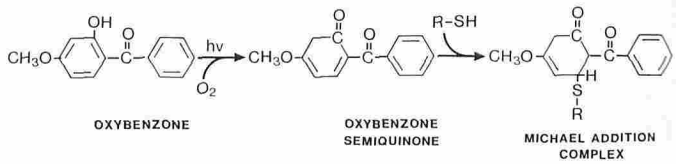

Figure 5. Proposed reaction pathway for the photo-oxidation of oxybenzone by normal sunlight to oxybenzone semiquinone followed by Michael addition to thiolate groups in the epidermis to compromise the anti-oxidant defense processes.

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