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Influence of complexation with cyclodextrins on photo-induced free radical production by the common sunscreen agents octyl-dimethylaminobenzoate and octyl-methoxycinnamate

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Received February 11, 2005, accepted March 23, 2005

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Pharmazie 61: 41–45 (2006)

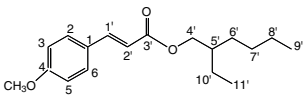
The influence of complexation with hydroxypropyl-β-cyclodextrin (HP-β-CD) or β-cyclodextrin (β-CD) on the photo-induced production of free radicals by the sunscreen agents octyl-dimethylaminobenzoate (ODAB), oxybenzone (OB) and octyl-methoxycinnamate (OMC) was investigated. The formation of radical species during irradiation was detected by spin-trapping electron paramagnetic resonance (EPR) spectroscopy. 2,2,6,6-tetramethylpiperidine-1-oxyl, nitroxide radical (TEMPO) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) were used a spin-traps. Following the 4-h illumination with simulated sunlight, OB did not generate radicals. On the other hand, photoexcitation of solutions containing ODAB or OMC produced a marked decrease (>40%) of the TEMPO signal intensity, demonstrating the formation of carbon-centred radicals. In addition, the results obtained on irradiation of ODAB solutions containing DMPO as spin-trap indicated the generation of oxygen-centred radicals. Complexation of ODAB with HP-β-CD and OMC with β-CD markedly inhibited (>64%) the formation of free radicals generated by the sunscreens on exposure to simulated sunlight. Therefore, inclusion of ODAB and OMC into the cyclodextrin cavities minimizes their photosensitising potential.

1. Introduction

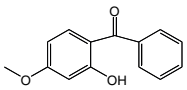
Because of the expanding knowledge of the acute and chronic damage (e.g, erythema, oedema, cutaneous photoaging, skin cancer) of the skin by the exposure to sunlight UV radiation (National Institute of Health 1989; Ziegler et al. 1994; Tarras-Wahlberg et al. 1999), the use of topical sunscreening preparations has increased markedly (Gasparro et al. 1998; Green et al. 1999). The most common active sunscreen ingredients are organic chemicals which attenuate the transmission of the sun UV rays to the skin by absorbing the radiation (Gasparro et al. 1998; Hayden et al. 1998). The photoactivated sunscreen compound can dissipate the excitation energy through several mechanisms including heat, fluorescence, phosphorescence, interaction with neighbouring molecules or photochemical reactions (Broadbent et al. 1996; Damiani et al. 1999).

An essential requirement for the efficacy of UV filters is their photostability (Berset et al. 1996; Maier et al. 2001). However, published studies have demonstrated that during sunlight exposure, several sunscreen agents undergo modifications, including fragmentation and isomeration (Broadbent et al. 1996; Tarras et al. 1999; Scalia et al. 1999; Scalia et al. 2002) which reduce the concentration of the active UV filter thereby decreasing the photoprotective power of the suncare preparation (Maier et al. 2001; Serpone et al. 2002). In addition, some sunscreens dissipate the absorbed solar energy through photochemical processes that yield reactive intermediates, such as free radicals and active oxygen species which can induce harmful effects on important biomolecules (Damiani et al. 1999; Serpone et al. 2002; Scalia et al. 2004). Photoproducts generated in suncare lotions exposed to simulated sunlight have been shown *in vitro* to cause damage to DNA and lead to cell mutations and cell death (Knowland et al. 1993; Damiani et al. 1999; Stevenson et al. 1999; Inbaray et al. 2002). Moreover, reported cases of phototoxic and photoallergic reactions resulting from the use of commercial sunscreens have been traced to photodegradation and photo-induced singlet oxygen formation (Allen et al. 1996; Ricci et al. 2003). Therefore there is a need to develop sun protection systems that, while providing the required broad-band UV absorption, will be photochemically stable and non-toxic to human skin.

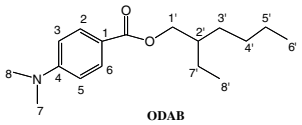
Sunscreen agents like octyl-dimethylaminobenzoate (ODAB), oxybenzone (OB) and octyl-methoxycinnamate (OMC) produce free radicals under sunlight irradiation and sensitize the formation of active oxygen species (Knowland et al. 1993; Allen et al. 1996; Serpone et al. 2002; Ricci et al. 2003). In previous investigations (Scalia et al. 2002; Scalia et al. 2004) we demonstrated that complexation of the sunscreen agents, butyl-methoxydibenzoylmethane and phenylbenzimidazole sulphonic acid with cyclodextrins efficiently inhibited the formation of free radicals generated by these two UV filters on exposure to simulated sunlight. The present study was undertaken to examine the effect of β-cyclodextrin (β-CD) or hydroxypropyl-β-cyclodextrin (HP-β-CD) as complexing agents on the photo-induced production of free radicals by ODAB



OMC



OB



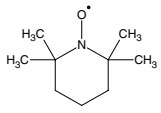
ODAB

and OMC. In addition, the feasibility of using the electron paramagnetic resonance (EPR) spin-trapping technique for detection of the radical species generated during photochemical excitation of the sunscreen agents was also investigated.

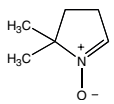
2. Investigations, results and discussion

The photo-induced production of free-radicals by the UV filters OB, ODAB and OMC was examined by the EPR spin-trapping technique, using two different spin-traps namely, 2,2,6,6-tetramethylpiperidine-1-oxyl, nitroxide radical (TEMPO) and 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The former is a stable nitroxide free radical (A) whose EPR spectrum consists of a 1 : 1:1 triplet. All carbon-centred radicals generated during sunscreen irradiation are expected to couple with the unpaired electron of TEMPO giving a non-paramagnetic species and, thus, causing a decrease of its EPR signal (Damiani et al. 1999; Scalia et al. 2002). Photogenerated radical species might be also detected as a consequence of their reaction with DMPO (B). In this case, radical species ($R^{\bullet}$) formed under UV radiation and characterised by very short lifetime react with the DMPO diamagnetic molecule to give more stable paramagnetic adducts according to the Scheme (Janzen and Haire 1990).

Fig. 1 shows that photoexcitation of ODAB solutions produced a marked decrease of the EPR signal intensity of TEMPO at a fixed field position. In particular, about 47% of this signal was lost after 4-h exposure to the solar simulator, independently of the presence of O_2. This is a clear indication that carbon-centred free radicals were

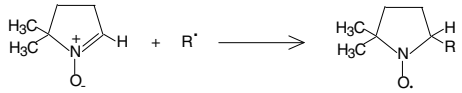


A



B

Scheme



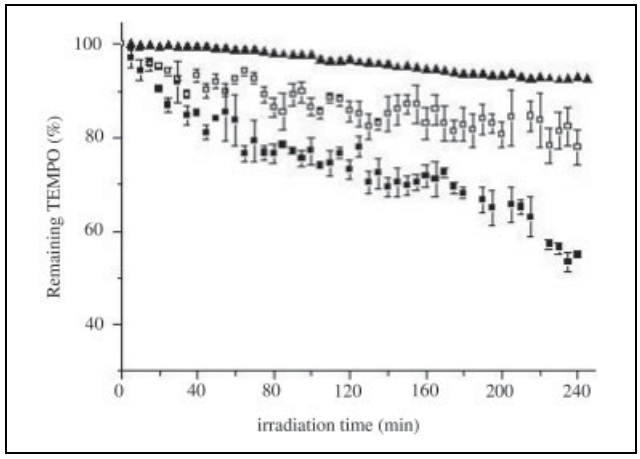


Fig. 1: Percent consumption of TEMPO during 4-h irradiation in the presence of ODAB (squares); ODAB/HP-β-CD inclusion complex (open squares); solvent only (triangles). Each point represents the mean ± s.d. of at least three experiments

formed during illumination of the sunscreen agent with simulated sunlight. The EPR spectrum obtained upon irradiation of ODAB aerated solutions containing DMPO instead of TEMPO as spin-trap, is illustrated in Fig. 2A. The main signals of this spectrum consist of a triplet, which can be traced to the photoinduced fragmentation of

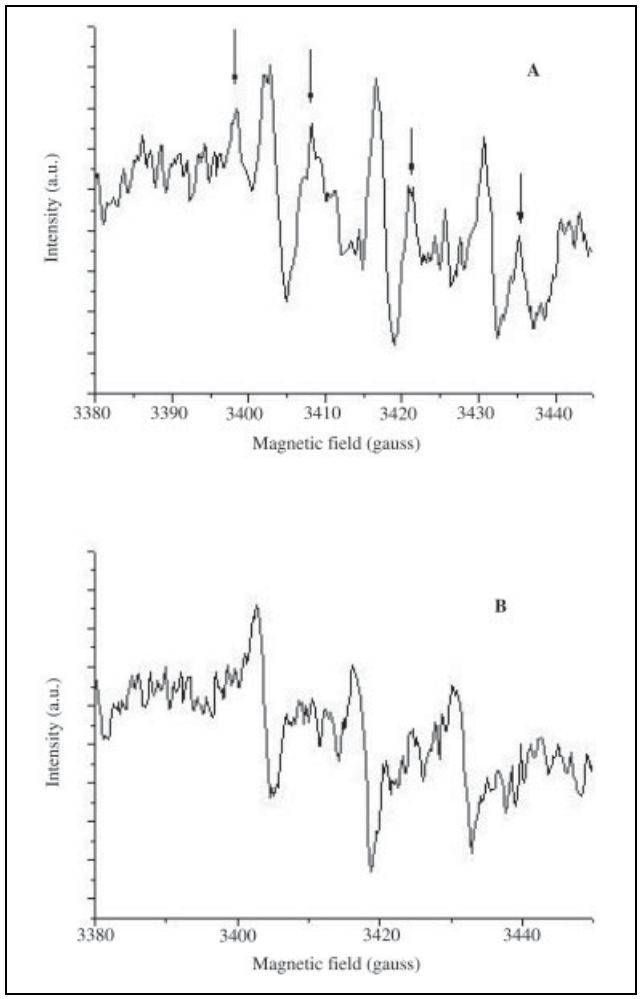


Fig. 2: EPR spin-trapping spectrum of the adduct obtained after irradiation of solutions containing DMPO in the presence of free ODAB (A) or the ODAB/HP-β-CD inclusion complex (B)

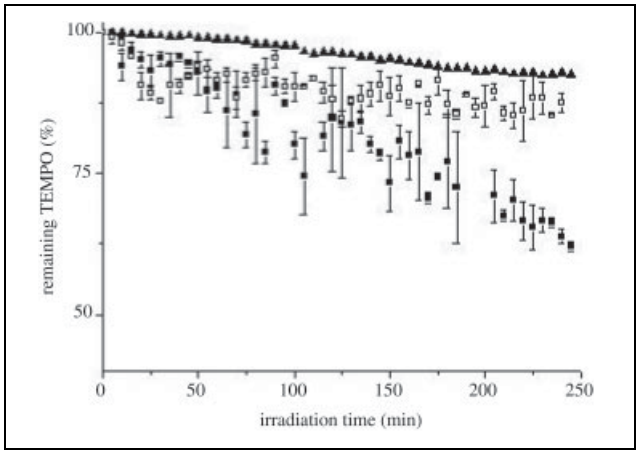


Fig. 3: Percent consumption of TEMPO during 4-h irradiation in the presence of OMC (squares); OMC/β-CD inclusion complex (open squares); solvent only (triangles). Each point represents the mean ± s.d. of at least three experiments

the spin-trap (Janzen and Haire 1990). Moreover, the photoexcitation of ODAB was also accompanied by the growing of four lines with a 1 : 2 : 2 : 1 intensity ratio and with hyperfine splitting constants $a_N = a_H = 14.9$ G (Fig. 2A). This spectrum can be ascribed to the presence of the DMPO-OH• adduct (Buettner 1987). Since this species reflects the formation of both singlet oxygen (Jones and Wilson 1980) and superoxide radical anion (Finkelstein et al. 1979), we can conclude that energy or electron transfer processes from the photoexcited ODAB to O_2 may occur. This is in agreement with previous investigations (Knowland et al. 1993; Allen et al. 1996).

Irradiation of OMC solutions with simulated sunlight produced a significant decrease of the EPR intensity signal of TEMPO (Fig. 3), indicating that also the photochemical excitation of OMC leads to the formation of carbon-centred free radicals. About 40% of the EPR signal was lost after 4-h irradiation. On the other hand, no evidence for sensitised processes was obtained, since no EPR signal due to the trapping of OH• radicals was detected using DMPO as spin-trap in OMC aerated solutions.

At variance with ODAB and OMC, experiments carried out in the presence of TEMPO or DMPO demonstrated that the irradiation of OB did not generate any detectable amounts of radicals, in accordance with an earlier report (Allen et al. 1996). However, controversial data have also appeared in the literature suggesting that this UV filter produces hydroxyl radicals on illumination (Serpone et al. 2002).

In order to examine the effect of cyclodextrin complexation on the light-induced production of free radicals by ODAB and OMC, the complexes of these sunscreen agents with HP-β-CD or β-CD were prepared as previously described (Scalia et al. 1999, 2002). HP-β-CD and β-CD were selected because they have been shown to interact more strongly than other available cyclodextrins with ODAB or OMC (Scalia et al. 1999, 2002). Solutions containing the sunscreen agents complexed with HP-β-CD or β-CD were exposed to simulated sunlight, under the same conditions reported for the uncomplexed UV filters, and the course of photolysis was followed by spin-trapping/EPR measurements.

The EPR spectra features (g-factor, hyperfine coupling constants and line broadening) were not affected by the presence of the cyclodextrins, thus demonstrating that the possible inclusion of spin-traps into the cyclodextrin cavity can be ruled out. In order to acquire evidence of complex formation in the medium used for the EPR measurements, ^{1}H NMR analyses were performed. Table 1 lists the major changes in the chemical shift values of selected protons of ODAB and OMC induced by the presence of HP-β-CD or β-CD, respectively. The observed chemical shift variations indicated the occurrence of interactions between the UV filters and the cyclodextrins in the ethanol-water solvent system. In addition, UV spectrophotometric analysis showed that the shape of the spectrum and the degree of absorption of the sunscreen agents were not significantly affected by complexation (spectra not shown).

Table: ^{1}H NMR chemical shift changes (Δδ, ppm) for OMC and ODAB in the presence of cyclodextrins

OMC protons	$\frac{\Delta\delta^a}{\beta\text{-CD}}$	ODAB protons	$\frac{\Delta\delta^a}{\text{HP-}\beta\text{-CD}}$
H2, H6	0.004	H2, H6	−0.029
H3, H5	0.007	H3, H5	−0.008
OCH_3	0.008	H7, H8	0.017
H2′	−0.010	H1′	0.030
H4′	−0.003	H2′	0.044
H5′	0.004	H6′, H8′	0.010

[a] $\Delta\delta = \delta_{\text{with cyclodextrin}} - \delta_{\text{free}}$

As illustrated in Fig. 1, inclusion of ODAB into the HP-β-CD cavity inhibited the generation of photo-induced carbon-centred radicals, by up to 64.5%. In addition, when DMPO was used as spin-trap, no EPR signal due to the trapping of OH• radicals was detected (Fig. 2B). This indicates that also the production of oxygen-centred radicals by the irradiated ODAB is suppressed when the sunscreen is complexed with HP-β-CD.

In the sample containing OMC complexed with β-CD, only a small reduction of the EPR signal intensity of TEMPO was observed after 4-h irradiation (Fig. 3). The decrease in the spin-trap concentration measured for the OMC/β-CD complex was not significantly different (analysis of variance, $P > 0.05$) from that produced by illumination of the nitroxide radical alone. The results shown in Fig. 3 demonstrated that the photo-induced free radicals production by OMC is effectively reduced by inclusion complexation of the sunscreen agent with β-CD.

The observed marked effect of complexation on the photosensitising activity of ODAB and OMC can be ascribed to steric constraint factors which favour radical pair recombination within the confined environment of the cyclodextrin cavity. Moreover, hindered interaction of photoactivated complexed sunscreens with oxygen or reduction of the life-time of the photogenerated triplet state may account for the lower photoreactivity of the sunscreen agents complexed with cyclodextrins. It is conceivable that the enhanced stability of the inclusion complexes under solar simulated radiation (Scalia et al. 1999, 2002) could also be due to the trapping of radical intermediates in the cyclodextrin cavity.

In conclusion, EPR spin-trapping analyses demonstrated that exposure of the sunscreen agents ODAB and OMC to simulated sunlight induced the formation of carbon-centred radicals. Moreover, irradiation of ODAB led also to the generation of oxygen reactive species. Complexation with cyclodextrins represents a simple and effective strategy to decrease the photo-induced production of free radicals by these sunscreen agents, thereby minimizing the related toxic effects on biosubstrates. Additional re-

search is being carried out to investigate whether the free-radical scavenging activity achieved by cyclodextrin complexation of OMC and ODAB would apply to conditions that parallel those encountered in the usage of suncare preparations.

3. Experimental

3.1. Materials

Oxybenzone, octyl-dimethylaminobenzoate, and octyl-methoxycinnamate were supplied by Merck (Darmstadt, Germany). Hydroxypropyl-β-cyclodextrin (HP-β-CD; average molar substitution 0.6) and β-cyclodextrin (β-CD) were purchased from Aldrich Chimica (Milan, Italy). TEMPO was used without further purification as received from Fluka Chemie (Buchs, CH). DMPO was obtained from Aldrich Chimica. Methanol, acetonitrile and water were HPLC grade from Merck (Darmstadt, Germany). All other chemicals were of analytical-reagent grade (Sigma, Milan, Italy).

3.2. High-performance liquid chromatography

The HPLC apparatus comprised a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 20 µl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV-Vis detector (Jasco, Tokyo, Japan) set at 305 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developments, Le Fontanil, France). Sample injections were effected with a Model 701 syringe (10 µl; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-µm Zorbax SB-CN column (150 × 4.6 mm i.d.; Agilent Technologies, Waldbronn, Germany) eluted isocratically, at a flow-rate of 1.0 ml/min, with methanol-acetonitrile-tetrahydrofuran-water (40 : 10 : 10 : 40, v/v/v/v). Chromatography was performed at ambient temperature. The identity of ODAB and OMC peaks was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method.

3.3. Preparation of the complexes

The inclusion complexes were prepared at a 1 : 1 molar ratio of sunscreen agent to HP-β-CD or β-CD. The complex with HP-β-CD was obtained as follows: ODAB (69.3 mg, 0.25 mmol) was dissolved in methanol (4 ml) and added to 3.0 ml of purified water containing an equimolar quantity of HP-β-CD (345 mg, 0.25 mmol). The obtained suspension was maintained under stirring for 24 h at room temperature and shielded from light. The solvent was then evaporated under vacuum at 40 °C by rotary evaporation and the residue was kept in a desiccator until used. The OMC/β-CD complex was prepared by adding an equimolar concentration of OMC (72.6 mg, 0.25 mmol) to a solution of β-CD (283.7 mg, 0.25 mmol) in purified water (20 ml). The mixture was stirred for 24 h at room temperature and shielded from light. A precipitate was obtained which was collected by filtration and stored under vacuum in a dessicator until used. The content of OMC or ODAB in the complexes was determined by HPLC after proper dilution.

3.4. NMR spectroscopy

^{1}H NMR spectra were recorded on a Varian Mercury Plus (400 MHz). Samples were solubilized in C_2D_5OD-D_2O (60 : 40, v/v) at a concentration of ca. 10 mM. Typical parameters for the ^{1}H NMR spectra were: 0.35 Hz/pt resolution, 18 s relaxation delay, 90° pulse.

3.5. UV spectrophotometry

UV spectra were recorded in EtOH-H_2O (60 : 40, v/v) on a UV/VIS/NIR Spectrometer (Lambda 19; Perkin Elmer, Norwalk, USA).

3.6. EPR measurements

Electron paramagnetic resonance (EPR) measurements were performed with a X-band Bruker spectrometer (Bruker, Karlsruhe, Germany) equipped with a TE 201 resonator (Bruker OR 4104, 100% optical transmittance). Spectra were recorded with the following instrumental settings: 2 mW microwave power, 1 G modulation amplitude and 100 kHz field modulation. A quartz flat cell was used as a reaction vessel and samples were irradiated directly inside the microwave cavity employing a 350-W medium pressure Hg lamp. Irradiation wavelengths were selected using an Oriel 59814 (Oriel Corporation, USA) band pass filter ($290 < \lambda < 410$ nm) coupled with an Oriel IR-block filter to avoid thermal effects. EPR spin-trapping experiments were performed on ethanol/water (60 : 40, v/v) solutions containing 10 µM TEMPO and 100 µM free or cyclodextrin-complexed sunscreen agent. After thorough degassing with freeze-pump thaw technique, 1 ml of the test sample was transferred via cannula under Ar atmosphere into the quartz flat cell and inserted in the EPR spectrometer cavity. The solutions were fluxed with a nitrogen stream and subjected to 4-h irradiation, with the whole EPR nitroxide radical spectra being recorded every 5 min. Concentration values of remaining TEMPO were obtained from double integration of the spectra. Additional experiments were performed in oxygen saturated ethanol-water (60 : 40, v/v) solutions using DMPO (100 mM) as spin-trap with the uncomplexed or complexed sunscreen agent (10 mM). The samples were irradiated in the EPR cavity for 25 min and spectra of the paramagnetic adducts were accumulated and recorded at appropriate time intervals. EPR signal intensities of the obtained paramagnetic adducts were measured at a fixed field position.

Each series of experiments was repeated at least three times. Analysis of variance was performed to assess the significance of differences recorded for the spin-trap EPR measurements. Significance was taken as $P < 0.05$. All calculations were carried out using the statistical software GraphPad Instat (GraphPad Software, San Diego, CA).

Acknowledgements: The authors are grateful to MIUR for financial support.

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