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Influence of hydroxypropyl-β-cyclodextrin on photo-induced free radical production by the sunscreen agent, butyl-methoxydibenzoylmethane

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Abstract

The aim of the study was to investigate the effect of hydroxypropyl-β-cyclodextrin (HP-β-CD) on the photo-induced production of free radicals by the sunscreen agent, butyl-methoxydibenzoylmethane (BMDBM). Spin-trapping/electron paramagnetic resonance spectroscopy was used to evaluate the formation of radicals and the extent of BMDBM photodegradation was measured by high-performance liquid chromatography. The stable 2,2,6,6-tetramethylpiperidine-1-oxyl, nitroxide radical (TEMPO) was used as spin-trap. Any free radicals generated during irradiation of the sunscreen agent will couple with the TEMPO radicals giving diamagnetic species and thus a decrease of the signal intensity in the electron paramagnetic resonance spectrum. Following 2-h illumination with simulated sunlight, the solution containing free BMDBM exhibited a 93.9% decrease of the intensity of the TEMPO signal. Under the same irradiation conditions, only a 12.2% reduction of the TEMPO concentration was measured in the sample containing BMDBM complexed with HP-β-CD. Moreover, the decrease of the spin-trap level observed for the HP-β-CD/BMDBM complex was not significantly different from that produced when solutions containing TEMPO only or TEMPO in the presence of HP-β-CD alone were subjected to irradiation. In addition, the photodegradation of the sunscreen agent was reduced by complexation with HP-β-CD (the extent of degradation was 27.6% for the complex compared with 63.1% for free BMDBM). The results obtained indicate that the free radicals generated by BMDBM when exposed to simulated sunlight are effectively scavenged by inclusion complexation of the sunscreen agent with HP-β-CD.

Introduction

It is well recognised that exposure of human skin to sunlight UV radiations (290–400 nm) leads to various types of skin pathologies (National Institute of Health 1989) including acute inflammatory responses (i.e., erythema, oedema) and chronic effects such as actinic aging, immune suppression and cutaneous cancers (National Institute of Health 1989; Ziegler et al 1994; Serre et al 1997; Urbach 1997; Tarras-Wahlberg et al 1999). Awareness of the harmful effects of solar UV rays has promoted the widespread use of topical photoprotective preparations (National Institute of Health 1989; Green et al 1999). These products incorporate UV-absorbing organic chemicals which lessen the dose of sunlight UV radiations reaching human skin. The photoactivated sunscreen molecule dissipates the excitation energy by several mechanisms, including thermal energy, fluorescence, phosphorescence, interaction with neighbouring molecules or photochemical reactions (Damiani et al 1999; Stokes & Diffey 1999). Although the shorter-wavelength portion (UV-B, 290–320 nm) of the solar UV spectrum reaching the earth surface is regarded as the most deleterious (National Institute of Health 1989), protection against the longer-wavelength UV radiations (UV-A, 320–400 nm) by sunscreens has become very important due to increasing evidence about their harmful effects on human skin, such as photoaging and photocarcinogenesis (National Institute of Health 1989; Schwack & Rudolph 1995; Tarras-Wahlberg et al 1999; Damiani et al 2000).

Butyl-methoxydibenzoylmethane (BMDBM; Figure 1A) is the most efficient and frequently used UV-A filter (Roscher et al 1994; Schwack & Rudolph 1995;

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Hayden et al 1998; Tarras-Wahlberg et al 1999; Damiani et al 2000). It is approved by the regulatory authorities of Europe (EEC Directive 1976), USA (US Food and Drug Administration 1999), Japan and Australia (Hayden et al 1998). However, one drawback of this sunscreen agent is its lack of photostability. Published studies have demonstrated that BMDBM undergoes decomposition under solar-simulated radiation, leading to a decrease of its UV-protective capacity (Stokes & Diffey 1999; Tarras-Wahlberg et al 1999). In addition, the photo-induced modification of the sunscreen agent yields an array of breakdown products (Roscher et al 1994; Schwack & Rudolph 1995; Tarras-Wahlberg et al 1999) whose toxicological properties are unknown and which have been associated to photocontact sensitization to BMDBM (Parry et al 1995; Schmidt et al 1998). Reports from Schwack & Rudolph (1995) and Damiani et al (1999, 2000) have demonstrated that BMDBM generates carbon-centred free radicals when illuminated with simulated sunlight and, as a consequence, causes in-vitro strand breaks in DNA (Damiani et al 1999) and oxidative modifications in bovine serum albumin (Damiani et al 2000). Hence, these results indicate that the most dangerous effect of the photolability of this sunscreen agent is the radiation-induced free radical formation rather than the reduction in screening efficiency.

In an earlier investigation (Scalia et al 1998), we demonstrated that the photodegradation of BMDBM was reduced by inclusion complexation of the sunscreen agent with hydroxypropyl-β-cyclodextrin (HP-β-CD). This study has now been extended to evaluate the effect of HP-β-CD as complexing agent on the photo-induced production of free radicals by BMDBM. Spin-trapping/electron paramagnetic resonance spectroscopy was used for the detection of radical formation.

Materials and Methods

Materials

Butyl-methoxydibenzoylmethane (BMDBM) was supplied by Hoffmann-La Roche Ltd (Geneva, Switzerland). Hydroxypropyl-β-cyclodextrin (HP-β-CD; average molar substitution 0.6) was purchased from Aldrich Chimica (Milan, Italy). Methanol, acetonitrile and water were HPLC grade from Merck (Darmstadt, Germany). 2,2,6,6-Tetramethylpiperidine-1-oxyl, nitroxide radical (TEMPO; Figure 1B) was used without further purification as received from Fluka Chemie (Buchs, Switzerland). All other chemicals were of analytical-reagent grade (Sigma, Milan, Italy).

High-performance liquid chromatography

The HPLC apparatus consisted of a modular chromatographic system (Model 980-PU pump and Model 975-UV variable wavelength UV-Vis detector; Jasco, Tokyo, Japan) linked to an injection valve with a 20-μL sample loop (Model 7125; Rheodyne, Cotati, CA). The detector was set at 350 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were effected with a Model 80365 syringe (10 μL; Hamilton, Bonaduz, Switzerland). Separations were performed on a 5-μm Nucleosil C_{18} column (150 × 4.6 mm i.d.; Macherey-Nagel, Düren, Germany) fitted with a guard column and eluted isocratically, at a flow-rate of 1 mL min^{-1} with methanol–acetonitrile–tetrahydrofuran–water (60:15:10:15, v/v). The mobile phase was deaerated on-line by a Model ERC-3311 automatic solvent degasser (Erma, Tokyo, Japan). The identity of the BMDBM peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method.

Preparation of the inclusion complex

The inclusion complex was prepared at a 1:2 molar ratio of BMDBM to HP-β-CD. The complex was obtained by adding 3.5 mL of purified water containing 344.6 mg (0.250 mmol) of HP-β-CD to a solution of BMDBM (38.8 mg, 0.125 mmol) in methanol (4.0 mL). The obtained mixture 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 content of BMDBM in the complex was determined by HPLC after proper dilution.

Thermal analysis

Differential thermal analysis (DTA) and thermal gravimetric analysis (TGA) were carried out on a Netzsch STA 409 simultaneous thermal analyser (Netzsch Italiana, Verona, Italy). The samples (6–7 mg) were accurately weighed in platinum pans (Netzsch) and heated from 30 to 130°C, at a scanning rate of 10°C min^{-1}.

X-ray diffractometry

X-ray diffraction patterns were recorded on a D 5000 powder diffractometer (Siemens, Munich, Germany) using a voltage of 45 kV and a current of 25 mA for the generator, with Cu anode material. The wavelength of the graphite-monocromated radiation was 1.5406 Å. The diffractograms were recorded from 3° (2θ) to 50° (2θ) at an angular speed of 1° (2θ) per minute using 1-1-1-0.15° slits.

UV spectrophotometry

UV spectra of BMDBM alone or its complex with HP-β-CD were recorded in ethanol on a UV/VIS/NIR Spectrometer (Lambda 19; Perkin Elmer, Norwalk, USA).

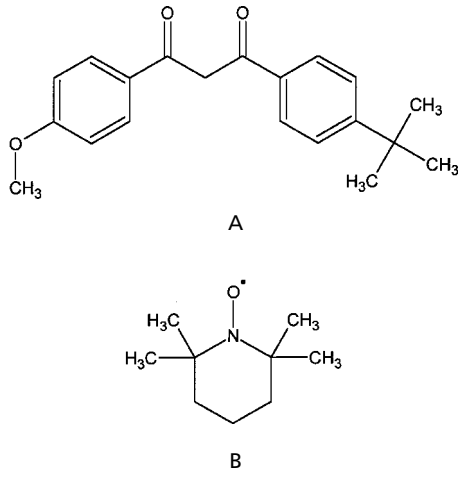

Figure 1 Chemical structures of butyl-methoxydibenzoylmethane (A) and 2,2,6,6-tetramethylpiperidine-1-oxyl, free radical (B).

EPR measurements

Electron paramagnetic resonance (EPR) measurements were performed at room temperature on a Bruker EMX spectrometer (Bruker, Karlsruhe, Germany) operating in the X band (9.71 GHz) equipped with a TE 201 resonator (Bruker OR 4104, 100% optical transmittance), using 2 mW microwave power, 1 G modulation amplitude and 100 KHz field modulation.

Photodegradation studies

Photodecomposition experiments were carried out in 5% (v/v) ethanol–water solutions containing 10 μM TEMPO and 100 μM free or cyclodextrin-complexed BMDBM. After thorough degassing with freeze-pump-thaw technique, 1 mL of the test sample was transferred via cannula under Ar atmosphere into a quartz flat cell and inserted into the microwave cavity of the EPR spectrometer. The samples were fluxed with nitrogen stream and irradiated directly in the cavity with a 350-W medium pressure Hg lamp fitted with focusing lens. Irradiation wavelengths were selected using an Oriel 59812 band pass filter ($290 < \lambda < 410$ nm) coupled with an Oriel IR-block filter to avoid thermal effects. The solar simulator emission was measured by a Goldlux radiometer (Oriel Corporation, USA) and was maintained at about 0.4 mW cm^{-2} and 2.5 mW cm^{-2} for UV-B and UV-A, respectively. Samples were subjected to 2-h illumination and whole EPR nitroxide radical spectra were recorded every 5 min. Concentration values of remaining TEMPO were obtained from double integration of the spectra. Each batch of experiments was repeated at least three times. After the exposure interval, the flat cell was removed and its content quantitatively transferred into a 10-mL calibrated flask, diluted to volume with methanol and assayed by HPLC for BMDBM. All samples were protected from light both before and after irradiation. The degree of photodegradation was measured by comparing the peak areas of BMDBM from the irradiated samples with those obtained by analysis of an equivalent amount of the non-exposed preparation.

Statistical analysis

Statistical analyses were performed by analysis of variance to assess the significance of differences between the sets of data from the spin-trap EPR measurements. A P-value < 0.05 was considered significant. All computations were carried out using the statistical software GraphPad Instat (GraphPad Software, San Diego, CA).

Results and Discussion

In an earlier study (Scalia et al 1998) we reported that the photodegradation of BMDBM in solution was significantly reduced (from 70.4% to 49.2%) by complexation with HP-β-CD. To investigate the influence of this system on the production of free radicals by the UV-irradiated sunscreen agent, the HP-β-CD/BMDBM complex was prepared as previously described (Scalia et al 1998) and characterized using X-ray diffraction and DTA. The absence of both the BMDBM crystalline peaks in the X-ray diffraction pattern and the UV-filter melting peak in the DTA thermogram (data not shown) provided evidence of the inclusion of BMDBM into the HP-β-CD cavity. In addition, UV spectrophotometric analysis of BMDBM and its complex with HP-β-CD showed that the shape of the spectrum and the degree of UV absorption of the sunscreen agent (BMDBM specific absorbance at 356 nm, 1132; BMDBM/HP-β-CD complex specific absorbance at 356 nm, 1138) were not affected by complexation. Solutions containing BMDBM alone or complexed with HP-β-CD were exposed for 2 h to simulated sunlight and the course of photolysis was followed, for the same sample, by spin-trapping/EPR measurements and HPLC determination of the extent of sunscreen degradation. During the light-stability measurements, the applied UV energy corresponded to 20 Minimal Erythemal Doses (MED) which is considered comparable with a daily solar emission (Tarras-Wahlberg et al 1999). The formation of radicals was detected using TEMPO stable nitroxide free radical as spin-trap. Any carbon-centred radicals generated during sunscreen irradiation will couple with the unpaired electron of the nitroxide radical (Figure 1B) giving nonparamagnetic species and thus producing a decrease of its EPR signal. Preliminary photodegradation studies performed in pure methanolic solutions indicated that this solvent interfered with EPR measurements and consequently 5% ethanol in water was used for all subsequent experiments. In the solution containing free BMDBM, the intensity of the EPR signals showed a marked decrease (Figure 2A) with 93.9% of the nitroxide radical lost after 2 h (Figure 3). This indicated the formation of carbon-centred free radicals during illumination of the sunscreen agent with simulated sunlight, in accordance with the data reported by Schwack

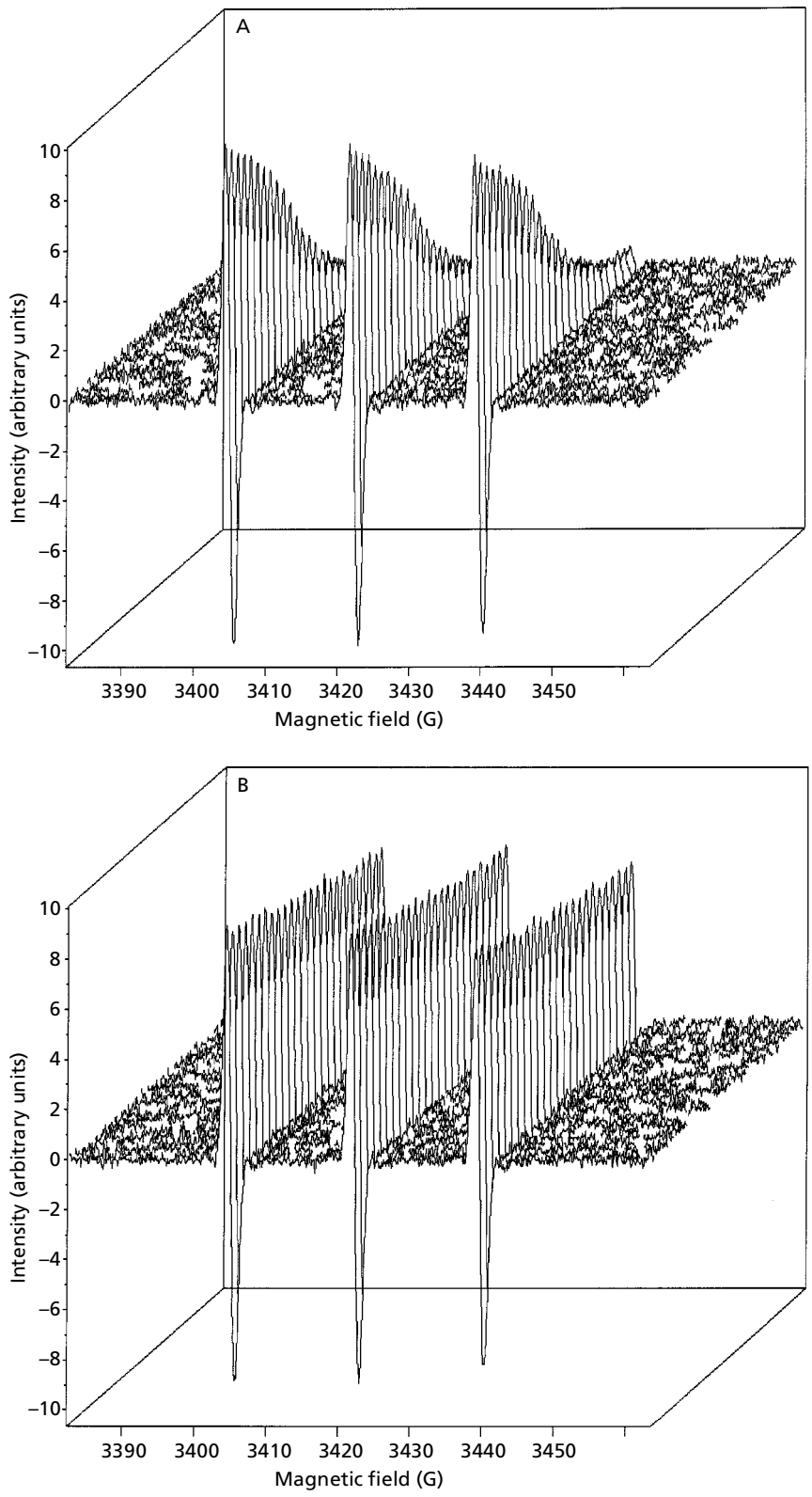

Figure 2 Evolution of TEMPO electron paramagnetic resonance (EPR) spectra in time in the presence of BMDBM (A) or BMDBM/HP-β-CD complex (B). Spectra were recorded every 5 min during 2-h irradiation with simulated sunlight.

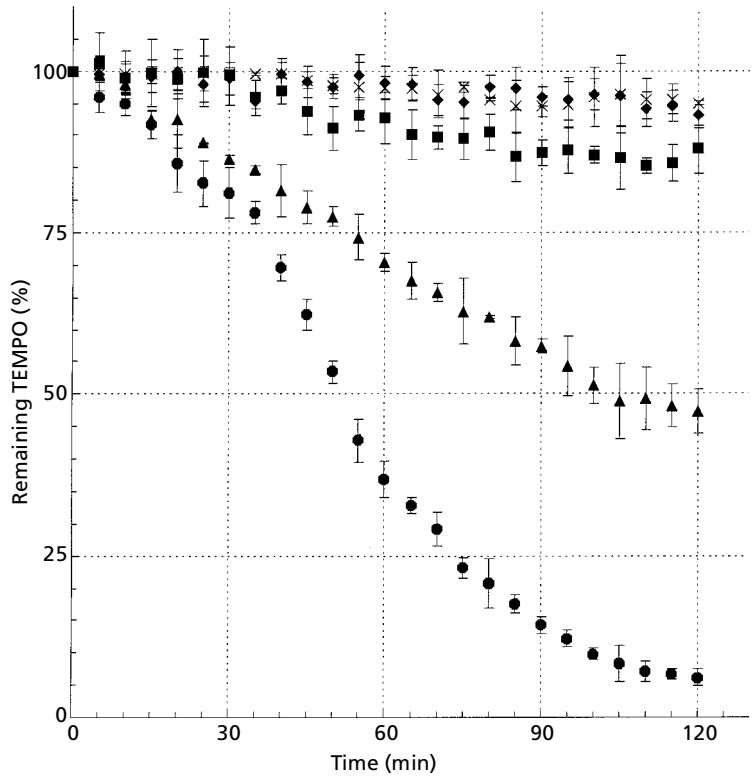

Figure 3 Percent consumption of TEMPO (initial concn, 10 μM) during 2-h irradiation in the presence of BMDBM (●; 100 μM); BMDBM/HP-β-CD inclusion complex (■; 3.4 mg in 10 mL, giving a final concentration of 100 μM BMDBM); BMDBM/HP-β-CD physical mixture (▲; 100 μM BMDBM, 200 μM HP-β-CD); HP-β-CD (◆; 200 μM); ×, solvent only. Each point represents the mean±s.d. of at least three experiments.

& Rudolf (1995) and Damiani et al (1999). The profile of the EPR signal versus time obtained for uncomplexed BMDBM follows a two-state time course (Figure 3), the decrease of the TEMPO concentration being satisfactorily described, in the 40–120 min interval, by a single exponential decay ($r^2 = 0.996$).

Under the same irradiation conditions, the sample containing BMDBM complexed with HP-β-CD exhibited only a small decrease of the EPR peak heights (Figure 2B) corresponding to a 12.2% loss of TEMPO radical after 2 h (Figure 3). The reduction of the spin-trap concentration observed for the HP-β-CD/BMDBM complex was not significantly different (analysis of variance: $F = 2.3$, $df = 7$, $P > 0.05$) from that produced when solutions containing only the nitroxide radical or the nitroxide in the presence of HP-β-CD alone were subjected to irradiation (Figure 3). Futhermore, the EPR spectrum features (g-factor, hyperfine coupling constants and line broadening) were not affected by the presence of the cyclodextrin. Therefore the possible inclusion of the nitroxide radicals into the cyclodextrin cavity can be ruled out. Additional measurements were performed on samples containing uncomplexed BMDBM and HP-β-CD (physical mixture) at the same concentrations used in the previous tests. A decline of the EPR signals (52.7% decrease after 2 h) was observed on UV illumination of the physical mixture (Figure 3), though

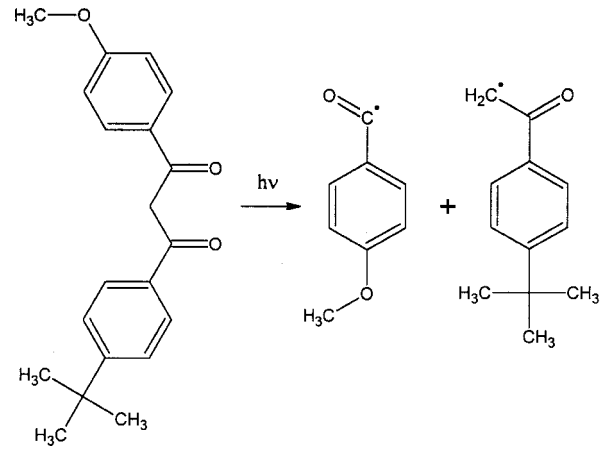

Figure 4 Primary photochemical process in the photodegradation of BMDBM.

the variation was not as marked as in the case of free BMDBM. The difference between free BMDBM and its physical mixture with HP-β-CD can be probably traced to the gradual formation of the complex through an equilibrium process which is established in solution between

the sunscreen and the cyclodextrin during the EPR measurements.

The results shown in Figure 3 demonstrate that the photo-induced free radical production by BMDBM is effectively reduced by inclusion complexation of the sunscreen agent with HP-β-CD.

To gain insight into the action of the cyclodextrin as complexing agent, the samples after spin-trapping/EPR evaluation were analysed by HPLC to measure the percentage loss of the sunscreen agent following irradiation. In the solution containing BMDBM alone, the degree of photodegradation was $63.1 \pm 4.7\%$ (n = 5), which decreased to $27.6 \pm 2.2\%$ (n = 5) in the sample containing the BMDBM/HP-β-CD complex. These results are in agreement with those obtained in a previous study (Scalia et al 1998) and indicate that the effect of HP-β-CD complexation on BMDBM photodecomposition is smaller than that measured on the light-induced free radical generation (Figure 3). Since the photodegradation mechanism of BMDBM (Schwack & Rudolf 1995; Damiani et al 1999) proceeds through the initial formation of carbon-centred free radicals (Figure 4), it is reasonable to assume that these radicals either remain trapped or recombine in the cyclodextrin cavity, or both (Lucarini & Pedulli 2000), and thus their interaction with the nitroxide spin label is hindered, as illustrated by the minor decrease of TEMPO EPR signals (Figures 2B and 3). Moreover, efficient inclusion of the photo-induced carbon-centred radicals by HP-β-CD could account for the enhanced photostability of the complex. Additional research will be necessary to investigate whether the free-radical scavenging achieved by complexation of BMDBM with HP-β-CD would apply to human skin exposed to sunlight.

Conclusions

The results described in this study demonstrate that in addition to enhancing the photostability of BMDBM, its complexation with HP-β-CD represents an effective strategy to scavenge the free radicals produced by the sunscreen agent upon illumination with simulated sunlight, thereby minimizing the photo-induced damage inflicted by BMDBM to important biological macromolecules.

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