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Algae Extract-Mediated Stimulation and Protection of Proteasome Activity Within Human Keratinocytes Exposed to UVA and UVB Irradiation

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ABSTRACT

Sun exposure is the major environmental influence for epidermal cells; the harmful effect of UV radiation on skin is related to the generation of reactive oxygen species that alter cellular components including proteins. It is now well established that the proteasome is responsible for the degradation of most of oxidized proteins and that impairment of proteasome function is a hallmark of cellular aging. In a previous study, we investigated the effects of UV irradiation on proteasomes in human keratinocyte cultures and showed that all three peptidase activities were decreased 24 h after irradiation of the cells. Increased levels of oxidatively modified proteins were observed in irradiated cells and were found to act as endogenous inhibitors of the proteasome. We report here on the stimulating and protective effects of an algae extract, prepared from *Phaeodactylum tricornutum,* on proteasome peptidase activities of human keratinocytes exposed to UVA and UVB irradiation. In addition, preserving proteasome function resulted in lowering the extent of the irradiation-induced protein oxidative damage, opening up new strategies for protection of epidermal cells against the detrimental effects of UV irradiation. *Antioxid. Redox Signal.* 8, 136–143.

INTRODUCTION

DAMAGE TO MACROMOLECULES resulting from chronic exposure of human skin to UV irradiation constitutes the main characteristic of photoaging (29). UVA radiation (320–400 nm) penetrates more efficiently in the basal layer of the epidermis than UVB (280–320 nm) and represents more than 90% of the terrestrial UV solar energy (4). Upon UVA irradiation of the skin, reactive oxygen species (ROS) are formed through the absorption of photons by endogenous photosensitizers and there is accumulating evidence for the damaging effects of high concentration of ROS generated after UV irradiation of the skin (13, 32, 33). Such ROS as singlet oxygen, superoxide anion, and hydroxyl radical are produced and react with various intracellular targets including lipids, nucleic acids, and proteins (34). Indeed, we have recently shown that UV irradiation of human keratinocytes induces an increased level of oxidized proteins. In addition, concurrent with oxidized protein build-up, inhibition of proteasome peptidase activities have been observed most likely due to the presence of highly oxidatively damaged proteins, such as proteins cross-linked by the lipid peroxidation product 4-hydroxy-2-nonenal (HNE), acting as endogenous inhibitors (7). The proteasome is the main intracellular proteolytic system implicated in the degradation of oxidized proteins. The 20S proteasome is made of 28 subunits arranged as four stacked rings. The two outer rings are formed by α subunits and the two inner rings are formed by β subunits that carry the proteolytic activities. The core 20S

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proteasome can combine with the 19S regulator complex to form a 26S proteolytic complex which is responsible for the ATP- and ubiquitin-dependent proteolysis (18). Previous studies of our laboratory on the effect of aging in human keratinocytes and epidermis have evidenced a decrease of proteasome activity and content and age-related alterations of proteasome subunits (5, 28). These results may explain, at least in part, the accumulation of oxidized proteins in the cytosol of cells during skin aging (27, 29). There is evidence that aging and photoaging have, at least in part, overlapping biochemical mechanisms and we have recently reported inhibition of proteasome activity during UV irradiation of cultured human keratinocytes (7, 27, 29). The current study was undertaken to determine whether natural plant or algae extracts could have a beneficial effect by preventing this UV irradiation-induced inhibition of proteasome. Several extracts were analyzed for their ability to stimulate proteasome activity *in vitro* and to preserve proteasome function after UV irradiation of cultured human keratinocytes. We report here that an extract prepared from the algae *Phaeodactylum tricornutum* can stimulate and protect proteasome peptidase activities from the detrimental effects of UV exposure (25). In addition, as evidenced by assaying the carbonyl content of total protein, oxidized protein level was reduced in the extract treated cells both before and, to an even bigger extent, after UV irradiation.

MATERIALS AND METHODS

20S Proteasome purification and peptidases activities

For *in vitro* experiments aimed at characterizing the effect of the algae extract on purified proteasome, 20S proteasome was purified from human placenta according to the procedure previously described (16) with minor modifications (22). Peptidase activities of the proteasome were assayed using fluorogenic peptides, succinyl-Leu-Leu-Val-Tyr-amidomethylcoumarin (LLVY-AMC) for the chymotrypsin-like activity, *N-t*-butyloxycarbonyl-Leu-Ser-Thr-Arg-amidomethylcoumarin (LSTR-AMC) for the trypsin-like activity, and *N*-benzyloxycarbonyl-Leu-Leu-Glu-β-naphthylamide (LLE-NA) for the peptidylglutamyl-peptide hydrolase activity as previously described (28) with minor modifications. For cellular lysates, the mixture, containing 20 μg of crude homogenate total protein in 25 m*M* Tris-HCl, pH 7.5, was incubated at 37°C with the appropriate peptide substrate (LLVY-AMC at 25 μ*M*, LLE-NA at 150 μ*M*, or LSTR-AMC at 40 μ*M*) in a final volume of 200 μl. Enzymatic kinetics were conducted in a temperature-controlled microplate fluorimetric reader (Fluostar Galaxy, bMG, Stuttgart, Germany). Excitation/emission wavelengths were 350 nm/440 nm and 340 nm/410 nm for aminomethylcoumarin and β-naphthylamine, respectively. Proteasome activities were determined as the difference between total activity and the remaining activity of the crude extract in the presence of 20 μ*M* of proteasome inhibitor *N*-Cbz-Leu-Leu-Leucinal (MG132).

Phaeodactylum tricornutum *extract preparation*

Starting with 250 g (wet weight) of *Phaeodactylum tricornutum* kept frozen at −0°C before proceeding, all operations were carried out as previously described (25) under N_2 to avoid degradation of active molecules. The homogenizing medium (isopropanol) was added in a ratio of at least 1.750 L of isopropanol for 75 g (dry weight) at 83°C. Once homogenization is completed, the final suspension was filtrated to remove all insoluble particles. The filtrate was collected and washed with 1.5 L H_2O and 1.6 L of heptan. The nonaqueous phase was collected and the heptanic wash repeated two times. 7.20 L of nonaqueous phase were collected and the pH adjusted to 2.2 with sulfuric acid. The filtrate was then submitted to a liquid/liquid extraction using 1.58 L of heptan. This step was done five times and the final extract was concentrated; 650 mg of oil from *Phaeodactylum tricornutum* was obtained after a further step of molecular distillation to eliminate all traces of the heptan solvent. The extract final composition was analyzed and showed up to 99% of fatty acids (29% saturated and 71% unsaturated) and less than 1% of xantophylles. Before being used, the algae extract was dissolved in 100% ethanol at a concentration of 50 μg/ml and added to the cells at a final concentration of 0.25 μg/ml in K-SFM culture medium supplemented with 0.1% BSA, 50 μ*M* tocopherol, and 1 m*M* ascorbyl phosphate. The remaining concentration of 0.5% ethanol did not show any effect on proteasome peptidase activities when assayed alone in a control experiment.

Human keratinocyte primary culture

All cell culture media and chemicals were purchased from Gibco Life Technologies (Cergy Pontoise, France) and Sigma (Saint Louis, MO). Epidermal cells were prepared from plastic surgery (mammary gland reductions) from a 60-year-old healthy female donor. Briefly, for the isolation procedure of epidermal cells, skin fragments were rinsed in phosphate buffered saline (PBS), pH 7.4, and cut into thin strips of 2 cm. These fragments were incubated overnight in 0.25% (w/v) trypsin, 100 units/ml of penicillin, and 100 μg/ml streptomycin to remove the epidermis from the dermis. Keratinocytes were suspended, counted, and plated in Dulbecco Minimal Essential Medium containing 10% (v/v) fetal calf serum, 10 ng/ml of mouse epidermal growth factor, 1n*M* of choleric toxin, and 0.4 μg/ml of hydrocortisone at 37°C and 5% CO_2. Twenty-four hours later, medium was changed for K-SFM complemented culture medium. Cells were frozen at –80°C.

Treatment with algae extracts, ultraviolet-irradiation procedure, and preparation of cytosolic fractions

Human keratinocytes were thawed, plated (250.000 cells/dish) and grown at 37°C and 5% CO_2 on a 35 mm plastic culture dishes (Corning Costar, Cambridge, MA). Twenty-four hours later, the algae extract, prepared as described above was first added at a final concentration of 2.5 μg/ml for 7, 24, and 48 hours and the chymotrypsin-like activity of the proteasome was monitored. Since 48 hours treatment was shown to provide a better stimulation of the chymotrypsin-like activity (Table 1), this incubation time was used for the irradiation experiments. To avoid toxicity induced by UV exposure of the culture medium compounds, irradiation was achieved in PBS, pH 7.4. Cells were exposed to sublethal doses: 10 J/cm^2 of

Table 1. Chymotrypsin-like Proteasome Activity in Keratinocytes Treated with Algae Extract for 7, 24 and 48 Hours

	7h	*24h*	*48h*
−Phaeo	100 ± 8	100 ± 16	100 ± 26
+ Phaeo	109 ± 14	124 ± 23	133 ± 9

Keratinocytes were treated for 7, 24, and 48 hours with 2.5 μg/ml of *Phaeodactylum tricornutum* extract or not. The proteasome chymotrypsin-like activity was assayed in cytosolic extracts as described in Materials and Methods and expressed as the percentage of the activity obtained in the presence of extract versus the activity obtained in the absence of algae extract treatment. The values are the average of six different independent experiments ± SE.

UVA with an emission centered at 365 nm and 0.05 J/cm² of UVB radiation with an emission centered at 312 nm, using a Bio-Sun RMX 3W (Vilber-Lourmat, France) as UV source. After irradiation, the PBS was removed and the keratinocytes were incubated in K-SFM medium at 37°C and 5% CO_2 and treated or not with the algae extract for 7 hours. Control cells, either irradiated or not, were treated in the same way. Cells were collected, suspended in 10 m*M* Hepes, pH 8, supplemented with 50 m*M* NaCl, 500 m*M* sucrose, 1 m*M* EDTA, 0.2 % (v/v) Triton X-100, 5 m*M* 2-mercaptoethanol and sonicated 5 × 5 s at 4°C. Cellular debris and organelles were removed from the crude extract by centrifugation at 10,000 *g* for 1 h at 4°C. The supernatant was recovered and protein concentrations were determined by using the Bio-Rad protein assay.

Measurements of cell viability

Cell survival was assayed by measuring mitochondrial activity with XTT reagents kit (Boehringer Mannheim, Germany). This assay is based on the cleavage of the tetrazolium salt XTT to form an orange soluble formazan dye due mainly to the mitochondrial dehydrogenase activities of living cells. Formazan formation was quantified with a spectrophotometer at 450 nm (Fluostar Galaxy, bMG, Stuttgart, Germany). Keratinocytes were plated in a 96-well plate with 7500 cells/well density. After irradiation, cells were incubated for 2 hours at 37°C, 5% CO_2 with XTT.

Gel electrophoresis and immunodetection of carbonyl groups on proteins and of proteasome

SDS/PAGE was done using the Laemmli method on a 12% acrylamide (w/v) separating gel. Immunoblot detection of carbonyl groups was performed with the OxyBlot oxidized protein detection kit (Appligene-Oncor, Illkirch, France), according to the manufacturer. Briefly, 10 μg of proteins were incubated for 15 minutes at room temperature with 2,4-dinitrophenylhydrazine (DNPH) to form the carbonyl derivative dinitrophenylhydrazone before SDS/PAGE separation. After transfer onto Hybond nitrocellulose membrane (Amersham Biosciences, Saclay, France), modified proteins were revealed by anti-DNP antibodies. Detection of bands in immunoblots was carried out with the ECL (Enhanced ChemiLuminescence) Western blotting analysis system of Amersham Biosciences, using peroxidase conjugated anti-rabbit, secondary antibodies. The film was digitized with a JX-330 scanner (Sharp, Hamburg, Germany) and the quantification was done with the Imagemaster 1D Elite software (Amersham Biosciences). Proteasome amount was estimated by Western blot analysis of cytosolic extracts. 20 μg of total proteins were separated on a 12% SDS-PAGE and immunoblot experiments were then performed using the appropriate primary antibodies.

RESULTS

In vitro *activation of proteasome peptidase activities by the algae extract*

Human 20S proteasome was purified from placenta as described in Materials and Methods. The purified proteasome preparation was found to be activated when in the presence of either 0.03% (w/v) SDS or 100 μ*M* linoleic acid, indicating that the proteasome was in its latent form. *Phaeodactylum tricornutum* extract was then assayed for its ability to stimulate proteasome peptidase activities *in vitro.* In the presence of 2.5 μg/ml algae extract, chymotrypsin-like, peptidylglutamyl-peptide hydrolase and trypsin-like activities were activated up to 150%, 180%, and 290% respectively (Fig. 1). No activation effect was observed with two other algae extracts obtained from *Odontella* and *Nutriplancton* (data not shown).

Proteasome peptidase activities in algae extract-treated keratinocytes

Normal human keratinocytes from primary cultures were first either subjected or not to treatment with the algae extract for 7, 24, and 48 hours. The proteasome chymotrypsin-like activity was monitored as described in Materials and Methods and a better activation was observed after treatment with algae for 48 hours (Table 1). Therefore, chymotrypsin-like, peptidylglutamyl-peptide hydrolase, and trypsin-like activities of the proteasome were assayed in lysates of cells that had not been exposed to UV, after 48 hours treatment with the algae extract using appropriate fluorogenic peptide substrates as described in Materials and Methods. As shown in Figure 2A, each of these peptidase activities were significantly increased as a result of the algae extract treatment. Because the measurement of peptidase activities gives no indication as to whether this observed increase is the result of an increased specific activity of the proteasome or an increased content of the enzyme, proteasome amounts were measured by immunoblots using both anti-20S and anti-26S subunits antibodies. No significant increase in either 20S and 26S proteasome subunits content was detected (Fig. 2B), indicating that proteasome peptidase specific activities were stimulated upon treatment with the algae extract.

Proteasome peptidase activities in UV-irradiated keratinocytes

Cells were treated for 48 hours with the algae extract as described in Materials and Methods, and exposed to sublethal doses of UVA (10 J/cm²) and UVB (0.05 J/cm²) and harvested 7 hours after irradiation. Using these doses of UV irradiation, cellular viability was always higher than 90% (data

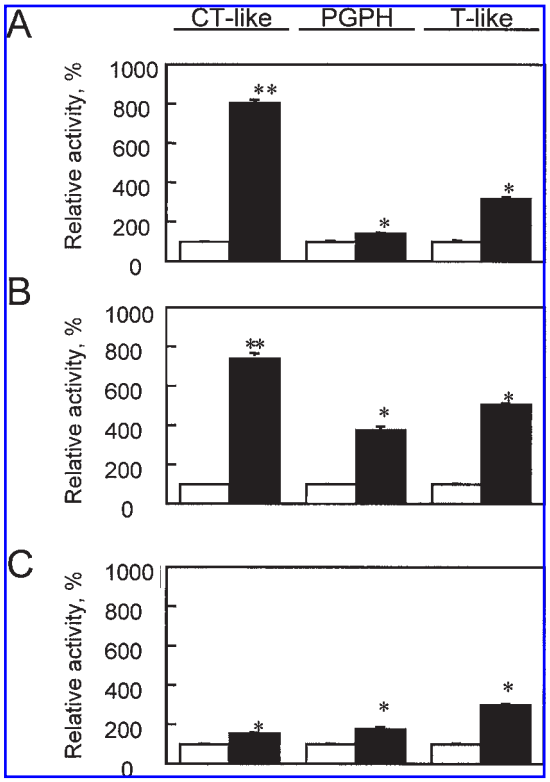

FIG. 1. Effect of extract on peptidase activities of human purified proteasome. Specific proteolytic activities of 20S proteasome purified from human placenta were determined using 2 µg of proteasome (*white bars*). Chymotrypsin-like (CT-like), peptidylglutamyl-peptide hydrolase (PGPH), and trypsin-like (T-like) activities were assayed in the presence of 0.03% SDS (*black bars, panel A*), 100 µ*M* linoleic acid (*black bars, panel B*) or 2.5 µg/ml of *Phaeodactylum tricornutum* extracts (*black bars, panel C*) using fluorogenic peptide substrates, as described in Materials and Methods. The measurement of peptidase activities of untreated proteasome (*white bars*) was taken as 100%. The values are the average of three different independent experiments ± SE. *p* values were obtained with the Student *t* test and $p < 0.05$ was considered as significant and is highlighted by one asterisk (*) on top of the bar graph (** is used when $p < 0.01$).

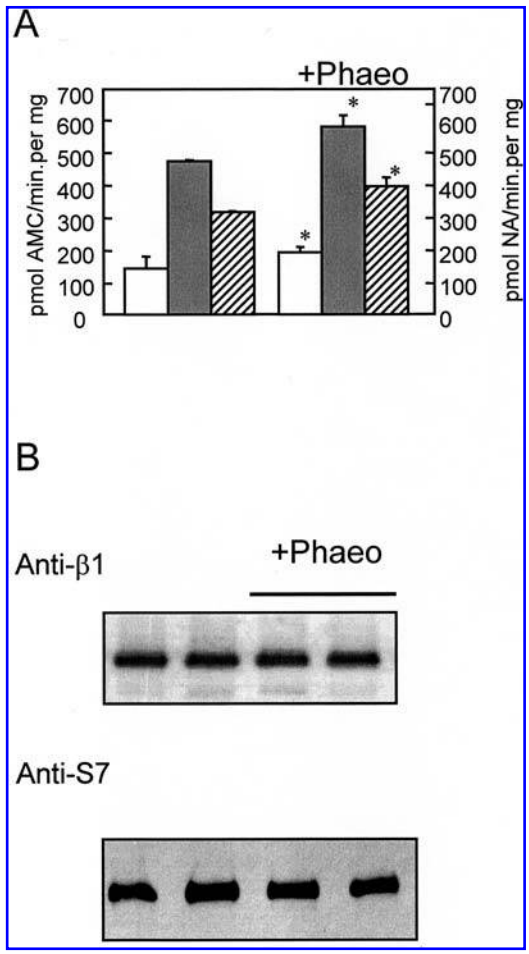

FIG. 2. Proteasome peptidase activities in algae extract-treated keratinocytes. (**A**) Keratinocytes were treated for 48 hours with 2.5 µg/mL of *Phaeodactylum tricornutum* extract. Chymotrypsin-like (*white bars*), peptidylglutamyl-peptide hydrolase (*gray bars*), and trypsin-like (*striped bars*) activities were assayed in cytosolic extracts using fluorogenic peptide substrates, as described in Materials and Methods. Proteasome activities were determined as the difference between total activity and the remaining activity of the crude extract in the presence of 20 µ*M* of proteasome inhibitor MG-132. The values are the average of three different independent experiments ± SE. *p* values were obtained with the Student *t* test and $p < 0.05$ was considered as significant and is highlighted by one asterisk (*) on top of the bar graph (**is used when $p < 0.01$). (**B**) Western blot analysis in duplicate of proteasome subunits content. 20 µg of soluble proteins from control keratinocytes, or treated for 48 hours with 2.5 µg/ml of *Phaeodactylum tricornutum* extract and subjected to SDS/PAGE on a 12% polyacrylamide gel, according to the Laemmli method, electrotransferred onto a nitrocellulose membrane, and immunoblotted with antiproteasome antibodies raised against β1 (catalytic subunit of 20S proteasome) and S7 (subunit of the 19S regulatory particle).

not shown) and irradiated cells presented the same morphology than the control ones (similar cell size, normal size nuclei). Chymotrypsin-like, peptidylglutamyl-peptide hydrolase, and trypsin-like activities of the proteasome were assayed in cell lysates, using appropriate fluorogenic peptide substrates. As shown in Figure 3, each of these peptidase activities were significantly decreased as a result of UV irradiation. Since proteasome peptidase activities were higher in the algae extract-treated cells before irradiation, the proteasome peptidase activities measured for these cells after UV irradiation were not significantly different to those of control cells that had not been treated with the algae extract nor irradiated.

Oxidative modification of cytosolic proteins before and after irradiation of human keratinocytes

Appearance of oxidized forms of cytosolic proteins in keratinocytes has been associated with ROS formation upon UV irradiation. Protein oxidation was assessed through the immunoblot detection and quantification of protein carbonyls derivatized by treatment of protein samples with 2,4-dinitrophenylhydrazine (DNPH), as described in Materials and Methods. As shown in Figure 4, UV irradiation of keratinocytes induced an increase in the level of oxidized cytosolic proteins

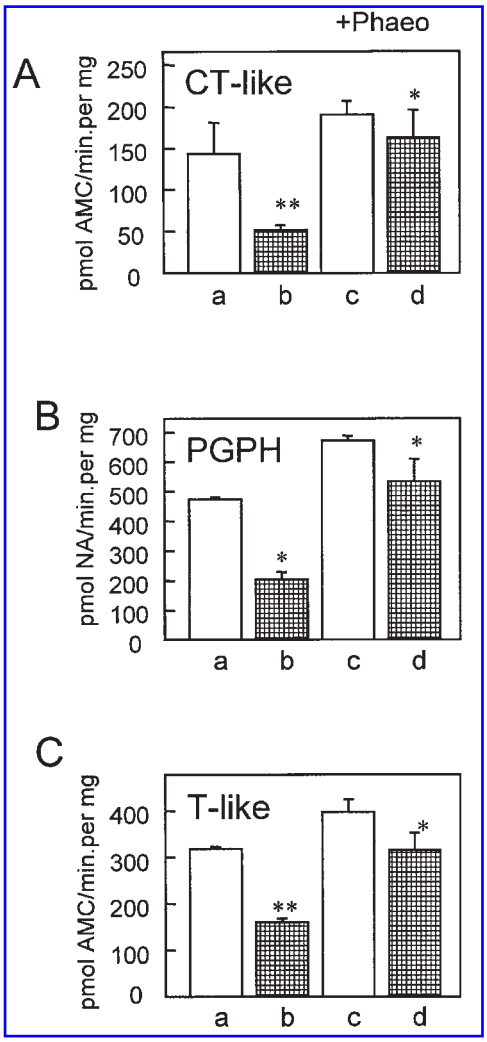

FIG. 3. Effect of UV-irradiation on peptidase activities of human keratinocytes proteasome. Human keratinocytes from primary cultures were irradiated (**b**) or not (**a**) with 10 J/cm^2 of UVA and 0.05 J/cm^2 of UVB radiation in the absence of algae extract. Cells were harvested at 7 hours after irradiation. The chymotrypsin-like (*panel A*), peptidylglutamyl-peptide hydrolase (*panel B*), and trypsin-like (*panel C*) activities were assayed in cytosolic extracts either in irradiated cells (**b**) or in nonirradiated cells (**a**) using fluorogenic peptide substrates, as described in Materials and Methods. Proteasome proteolytic activities were determined as the difference between the total activity of cytosolic extracts and the remaining activity in the presence of 20 μ*M* proteasome inhibitor *N*-Cbz-Leu-Leu-Leucinal (MG132). After treatment with 2.5 μg/ml of *Phaeodactylum tricornutum* extract for 48 hours, human keratinocytes from primary culture were irradiated (**d**) or not (**c**) by 10 J/cm2 of UVA and 0.05 J/cm2 of UVB and then treated again for 7 hours by 2.5 μg/ml of *Phaeodactylum tricornutum* extract. Cells were then harvested and proteasome peptidases activities determined as described above. The values are the average of three different independent experiments ± S.E. p values were obtained with the Student t test and $p < 0.05$ was considered as significant and is highlighted by one asterisk (*) on top of the bar graph (** is used when $p < 0.01$).

in both control and algae extract-treated keratinocytes. However, algae extract-treated cells showed a content of protein carbonyls slightly lower than the control cells and also showed a much more lower increase of protein carbonyls after UV irradiation. Therefore, it appears that the algae treatment increase in proteasome activity is accompanied by a decrease of protein carbonyls content both before but most importantly after UV irradiation.

DISCUSSION

As we previously reported, proteasome activity is compromised by UV irradiation in human keratinocytes (7) since irradiation of the cells by UVA (10 J/cm^2) and UVB (0.05 J/cm^2) results in declines in proteasome chymotrypsin-like, peptidylglutamyl-peptide hydrolase, and trypsin-like activities in cytosolic extracts (Fig. 3). In addition, our results demonstrate that UV irradiation of keratinocytes not only induces a significant decline in proteasome function but also induces an increase in oxidatively modified proteins (Fig. 4). In this study, we describe the ability of an extract from an algae *(Phaeodactylum tricornutum)* to stimulate 20S proteasome peptidases activities both *in vitro* and within human keratinocytes (Figs. 1 and 3) and to reduce the level of oxidized proteins (Fig. 4). Our results show that 48 hours after algae extract addition to keratinocytes in culture, the three peptidase activities increased significantly while the level of oxidatively modified proteins decreased (Figs. 2 and 4). Moreover, incubation of the cells with *Phaeodactylum tricornutum* extract restored significantly the three proteasome peptidase activities and decreased the amount of oxidized protein when the treatment is done before and after UV irradiation (Figs. 3 and 4). Proteasome activation within keratinocytes treated with algae extract cannot be explained by an increased proteasome content as demonstrated by Western blot (Fig. 3). As the proteasome is responsible for the degradation of oxidized protein (15), the keratinocytes treated with *Phaeodactylum tricornutum* exhibited an increased capacity to degrade oxidized proteins (Fig. 4). Indeed, the algae extract is almost exclusively composed of saturated and unsaturated fatty acids that are not antioxidants and hence very unlikely to prevent protein oxidation. Moreover, the algae extract is added to the cells in the presence of a high concentration of antioxidants (50 μ*M* tocopherol and 1 m*M* ascorbyl phosphate) that were not found to protect against UV-induced protein oxidation when assayed alone on control cells. Taken together, our findings provide insight into potential intervention for maintaining proteasome activity after UV irradiation or oxidative stress. A major outcome of this study is that the activation of the proteasome enables the keratinocyte primary culture to cope with an oxidative insult. Through genetic activation of the proteasome upon surexpression of either β1 or β5 catalytic subunits in transformed WI 38-SV40, such an enhancement of cellular resistance to oxidative stress has also been observed (11).

Several lines of investigation have indicated that photoaging when superimposed on intrinsic aging process plays a major role in age-associated degenerative changes of the skin (29, 30). There is also evidence that these processes are at least in part overlapping since they both have a free radical

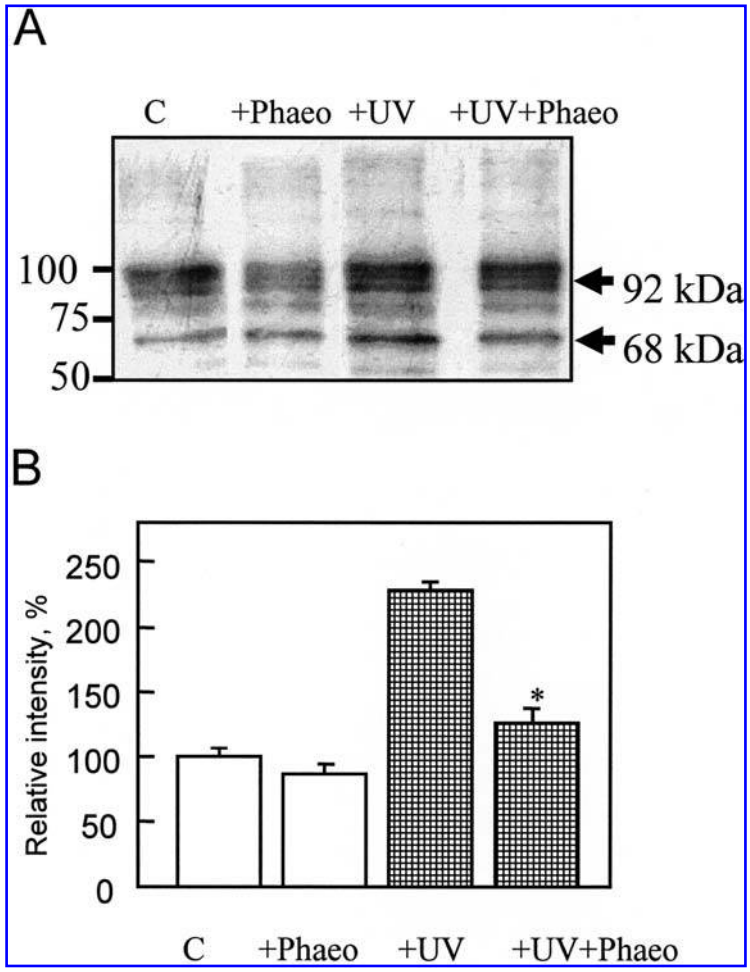

FIG. 4. Detection of oxidatively modified cytosolic proteins. (**A**) Cytosolic extracts were prepared from keratinocytes irradiated with 10 J/cm^2 of UVA and 0.05 J/cm^2 of UVB (+UV) or control keratinocytes (C) and harvested 7 hours after irradiation. Keratinocytes were treated by 2.5 µg/ml of *Phaeodactylum tricornutum* extract for 48 hours and irradiated (+UV+Phaeo) or not (+Phaeo) by 10 J/cm2 of UVA and 0.05 J/cm2 of UVB and then treated again for 7 hours by 2.5 µg/ml of *Phaeodactylum tricornutum* extract. Detection of oxidatively modified proteins was performed using the Oxyblot-kit (Appligene-Oncor, Illkirch, France), as described in Materials and Methods. (**B**) Densitometric analysis was performed (NIH Image software) on Western blots of cytosolic protein isolated from four keratinocytes extracts for each experimental protocol and results are represented as mean ± SE with the mean for control intensity assigned as a value of 100. p values were obtained with the Student t test and $p < 0.05$ was considered as significant and is highlighted by one asterisk (*) on top of the bar graph.

component (24). As expected from the "Free radical theory of aging" and its modification (2, 19, 31), there is an accumulation of oxidation end-products such as oxidized proteins and lipid metabolites during the aging process. We have previously reported a marked decline in the activity of the proteasome in aging, replicative senescence, and oxidative stress (e.g., ischemia-reperfusion) as well as an accumulation of oxidatively modified proteins which are normally removed by the proteasome (5–9, 12, 20, 11, 28, 35). Therefore, our finding showing the use of natural products such as *Phaeodactylum tricornutum* algae extract, to activate the proteasome or to protect the proteasome from oxidants induced inactivation may have important implications not only in protection against oxidative stress but also for developing anti-aging strategies.

The proteasome can be activated by fatty acid and lipids which can be an *in vitro* curiosity with no significance for the *in vivo* regulation of the proteasome (1, 14). Interestingly, the reported lipid activators are localized mainly in the mitochondria (cardiolipin) or in the outer leaflet of the plasma membrane (gangliosides and sulfatides) and the proteasome is located in the nucleus or in the cytoplasm of the cell. Inhibition of the proteasome in cultured cells has been shown to result in the buildup of pro-apoptotic Bcl-2 family member proteins, Bax and tBid, release of cytochrome c from mitochondria, and induction of apoptosis (3, 23). These studies have provided evidence that Bax and tBid are substrates of the 26S proteasome. Based on these findings, a loss of proteasome activity during oxidative stress or UV irradiation is expected to enhance the apoptotic process (17) since the proteasome may function as a checkpoint in the progression of apoptosis regulation. The proteasome function has been also implicated in a diverse array of cellular pathway. For example, it is involved in NFκB activation and p53 degradation which, in turn influence the ratio of pro-apoptotic proteins, the level of antioxidants and the inflammatory response (10, 21). All these interacting factors suggest a central role for radical production and apoptosis (26). Therefore, treatment with proteasome activating compounds such as *Phaeodactylum tricornutum* algae extract may represent an efficient strategy for favorably influencing the outcome of the keratinocyte survival after UV irradiation.

ABBREVIATIONS

CT-like, chymotrypsin-like activity; DNP, 2,4-dinitrophenol; DNPH, 2,4-dinitrophenylhydrazine; HNE, 4-hydroxy-2-nonenal; LLE-NA, *N*-benzyloxycarbonyl-Leu-Leu-Glu-β-naphthylamide; LLVY-AMC, succinyl-Leu-Leu-Val-Tyr-amidomethylcoumarin; LSTR-AMC, *N-t*-butyloxycarbonyl-Leu-Ser-Thr-Arg-amidomethylcoumarin; PGPH, peptidylglutamylpeptide hydrolase

activity; ROS, reactive oxygen species; T-like, trypsin-like activity.

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