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Effects of prolonged UV-B enhanced fluorescent radiation on some marine microalgae

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Abstract

The eukaryotic unicellular microalgae *Chlorella salina*, *Dicrateria inornata*, and *Isochrysis galbana* were grown under control (fluorescent 20 W m^{-2}) and UV-B enhanced (UV-B^E, 0.5 W m^{-2}) fluorescent radiation. The growth rate showed marginal increase under UV-B^E. Decrease in protein content was observed in *Dicrateria* cells but in *Chlorella* an initial increase up to 4 d and in *Isochrysis* an increase at days 4 and 5 was noted. The chlorophyll *a* content showed marked increase in *Chlorella* and *Isochrysis* but in *Dicrateria* a decline was found. UV-B^E reduced the photosynthetic activity in all three species, but the reduction was larger in *Chlorella* and *Dicrateria*. Fluorescence excitation spectra for F_{682} in *Chlorella* cells grown for 5 d under UV-B^E showed reduction in all peaks. In contrast to this, in *Dicrateria* and *Isochrysis* cells, the 530 and 590 nm excitation peaks increased with an appreciable decrease in the 466 nm peak. SDS-PAGE analysis revealed significant decrease in the contents of 47, 33, and 23 kDa polypeptides in *Chlorella* cells. In *Dicrateria* cells, significant loss in the content of 55, 38, and 18 kDa polypeptides was observed. The content of low molecular mass polypeptides (15 kDa) remained unaffected. *Isochrysis* cells were more stable in preserving the content of thylakoid polypeptides.

Additional key words: chlorophyll, fluorescence spectra, photosynthetic electron transport, phytoplankton, thylakoid proteins.

Introduction

Marine phytoplankton is the main producer of biomass (104 Gt per year) by photosynthesis in the aquatic ecosystem (Houghton and Woodwell 1989) and the basis of food web in oceans. About 60 % of the total global oxygen production and about 50 % of the global CO_2 fixation are caused by phytoplankton activity. Thus, changes in the quantity and activity of marine phytoplankton are of prime importance for the global climate and the global ecosystem.

High fluence rates of short wavelength UV-radiation may damage phytoplankton by changes of DNA structure (Yammamoto *et al.* 1983), inhibition of motility and orientation systems (photo- and gravitaxis) (Häder 1987, 1993, Häder and Reinecke 1991, Häder and Worrest 1991), disturbances of photosynthesis and biomass production (Smith *et al.* 1980, Zundorf and Häder 1991, Haberlein and Häder 1992), and changes in enzyme activity and in nitrogen incorporation (Döhler 1985, Döhler *et al.* 1986).

The primary objective of this work was to compare the long-term UV-B enhanced changes in microalgae having different thylakoid organization.

Materials and methods

The eukaryotic unicellular microalgae *Dicrateria inornata* Parke, *Isochrysis galbana* Parke (*Chrysophyceae*), and *Chlorella salina* Parke (*Chlorophyceae*), were cultured photoautotrophically at 25 °C in liquid

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Abbreviations: ATP - adenosine triphosphate; Chl - chlorophyll; F_{682} - fluorescence emission at 682 nm; LHCP - light harvesting chlorophyll protein; $NADPH_2$ - reduced form of nicotinamide adenine dinucleotide phosphate; PAGE - polyacrylamide gel electrophoresis; PEPC - phosphoenol pyruvate carboxylase; PS - photosystem; RuBPC - ribulose-1,5-bisphosphate carboxylase; SDS - sodium dodecyl sulphate; UV-B - ultraviolet-B radiation.

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culture medium as devised by Johnson *et al.* (1968). Cultures were maintained under white fluorescent irradiation of 20 W m^{-2}. The age of the culture was counted from the day of inoculation. Cells on the required day were harvested by centrifugation at 5 000 *g* for 10 min, washed twice with the medium and then suspended in a minimum volume of the medium at a final concentration of 100 g (Chl) m^{-3} for immediate use.

Log phase cultures were kept inside an environmental growth chamber maintained at 25 ± 1 °C. Additional UV-B irradiation of 0.5 W m^{-2} was provided by a *Philips TL20/12* lamp (*Philips Gloelampenfabrieken*, Eindhoven, The Netherlands).

Changes in various growth parameters and photochemical activities were followed at regular intervals. The Chl *a* content was determined following the method of Jeffrey and Humphrey (1975). Chl *a* and *b* contents of *Chlorella* were determined according to Arnon (1949). Protein content was estimated following the method of Lowry *et al.* (1951). Photosynthetic O_2 evolution was continuously monitored at 25 °C using a *Hansatech,* King's Lynn, Norfolk (U.K.) oxygen electrode. $^{14}CO_2$ fixation by the whole cells was determined by the modified method of Peschek (1978). Cell free chloroplast particles were prepared following the modified method of Kulandaivelu and Senger (1976). Fluorescence spectra were recorded using a *Hitachi* (Tokyo, Japan) *MPF-4* spectrofluorimeter. Electrophoretic separation of proteins in polyacrylamide gel was performed according to the method of Laemmli (1970).

Results and discussion

Changes in growth rate: To study the long-term effect of UV-B radiation in marine microalgae, cultures of *Chlorella, Dicrateria,* and *Isochrysis* were maintained under fluorescent irradiation with and without UV-B^E. Absorbance at 570 nm (A_{570}) of the culture was taken as the index of growth rate. All the three species exhibited enhanced rate of growth under UV-B^E (Fig. 1). This is in contrast to the observations of Thomson *et al.* (1980), where a marked depression in the growth of the diatom *Malosira nummoloidas* under different doses of UV-B radiation occurred. It was also observed by Calkins and Thordardottier (1980) and Döhler (1984a,b) that the growth of the diatom is sensitive to UV-B radiation. But results of this study are consistent with the general notation that photoadaptation in some microalgae can be accomplished in less than 24 h (Prezelin and Matlick 1980, Steemann-Nielsen *et al.* 1962).

Changes in protein content: The total protein content decreased in *Dicrateria* under UV-B^E and changes observed in other two species are not significant (Fig. 1). There might be several possibilities for a reduction in protein content: UV-B radiation might have affected directly the membranes *via* destruction of proteins and lipids and by that way, the transport system of nitrogen compounds or reduced the supply of ATP due to inhibition of PS2 reaction centre (Kulandaivelu and Noorudeen 1983). UV-B radiation might also have created a diminution in the supply of carbon skeletons for amino acid biosynthesis by reduced photosynthetic CO_2 fixation or respiration and also altered the regulatory mechanism of nitrogen uptake from the pools of amino-acids (glutamine) and/or ammonia, directly affecting the key enzymes of the carbon and nitrogen metabolism.

Changes in pigments: Increase in Chl *a* content occurred in *Chlorella* and *Isochrysis* grown under UV-B^E but not in *Dicrateria* (Fig. 1). This is in contrast to the findings of Döhler *et al.* (1986) and Häder *et al.* (1988) who reported pigment bleaching following exposure to sunlight or UV-B. Our results probably indicate that these cells are adapted to UV-B^E coupled with low fluorescent irradiance (20 W m^{-2}). Pigment bleaching reported in other papers could be due to excessive visible radiation.

Changes in overall photosynthetic electron transport: In all three cell types overall photosynthetic electron transport activity measured as O_2 evolution in the absence of artificial electron acceptor was reduced by UV-B^E (Fig. 1). A 10 to 25 % loss in photosynthetic activity was observed in *Isochrysis* and *Dicrateria* grown under UV-B^E. Numerous reports exist on the target site of UV-B radiation in photosynthetic organisms. Bornman (1989) has extensively reviewed the sites of UV-B damage. Most of the reports have been focused on the PS 2 of the electron transport chain. The effect of UV-B radiation is probably not evenly distributed between the two photosystems. PS 1 mediated cyclic photophosphorylation was inhibited by UV-B radiation (Brandle *et al.* 1977). Iwanzik *et al.* (1983) suggested that PS 2 reaction centres are transformed into dissipative sinks for excitation energy by the action of UV-B radiation. The damage may occur at the level of the primary photochemistry, *i.e.*, at the charge separation reactions leading to Z-P680-Pheo-Q_A^- formation (Noorudeen and Kulandaivelu 1982, Iwanzik *et al.* 1983). The catalytic site of water oxidation was also included (Nedunchezhian and Kulandaivelu 1991) as one of the UV-B targets. Gerber and Häder (1995) showed that the decrease of fluorescence and the inhibition of photosynthetic O_2 production occurred in phytoplankton even after short solar irradiation.

The fluorescence induction experiments showed that F_o values were unaffected except at high UV-B

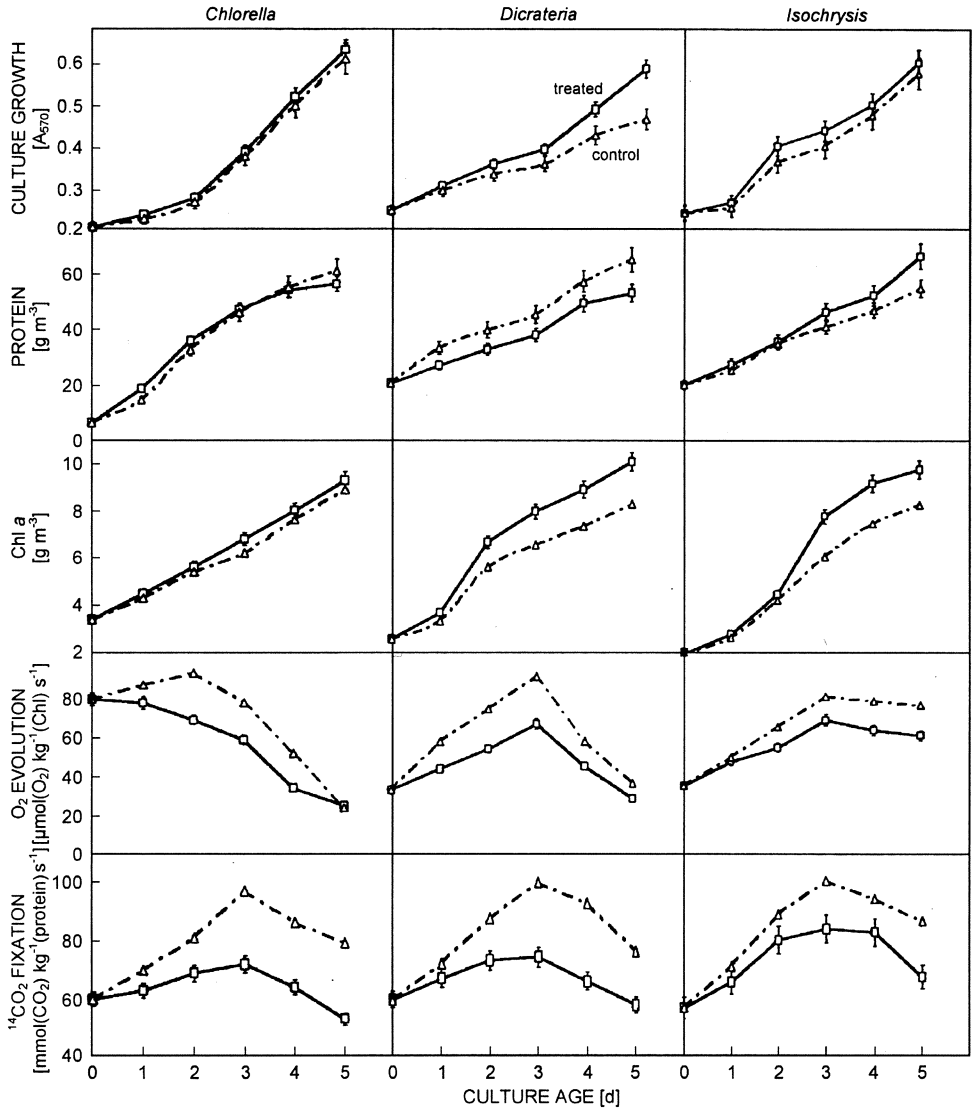

Fig. 1. Changes in the culture growth, protein and Chl *a* contents, and rate of O_2 evolution and $^{14}CO_2$ fixation in *Chlorella, Dicrateria* and *Isochrysis* cultures grown under control and UV-B^E radiation. For details see Materials and methods.

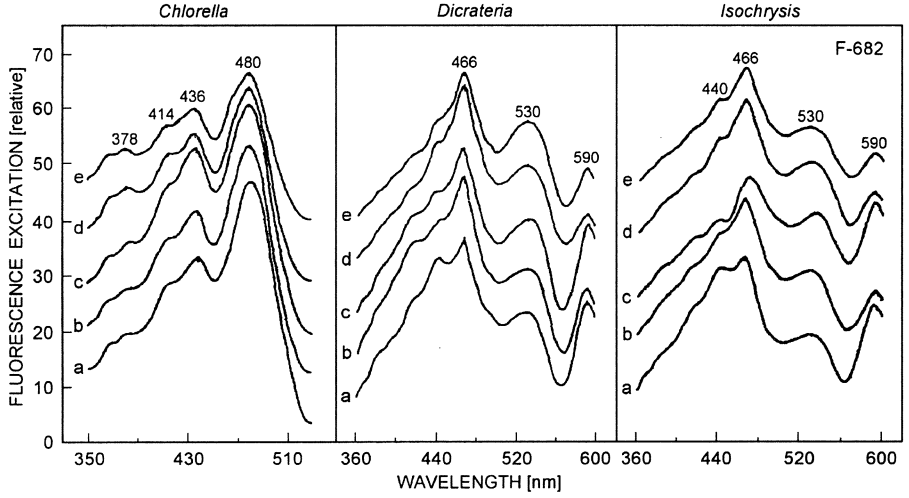

Fig. 2. Changes in fluorescence excitation spectra of *Chlorella, Dicrateria* and *Isochrysis* cells grown under control and UV-B^E radiation (a - 0-d, b - 3-d control, c - 3-d treated, d - 5-d control, e - 5-d treated).

irradiation (Tevini and Iwanzik 1983). High F_0 observed in UV-B treated *Helianthus annuus* cotyledons was notably due to reduced excitation transfer in the antennae or to an increase in the antenna cross section. UV-B irradiation of spinach PS 2 membrane fragments resulted in modification or structural changes in D1/D2 polypeptide complex (Renger *et al.* 1986). The 32 kDa protein (Q_B) degradation under UV-B radiation has also been shown. However, a change in the stoichiometric ratio of PS 1/PS 2 was also proposed (Lingakumar and Kulandaivelu 1993). These findings indicate that the reduced photosynthetic O_2 evolution could be attributed to a reduction in the ATP and $NADPH_2$ supply. In addition to this, UV-B radiation may also directly affect the proteins involved in photosynthetic electron transport systems.

Changes in CO_2 fixation: UV-B enhanced radiation treatment brought about an overall decrease in the rate of CO_2 fixation (Fig. 1). UV-B exposure affects both the photosynthetic electron transport and CO_2 fixation. Enhanced UV-B irradiation depressed the $^{14}CO_2$ fixation rate in *Bellerochea yucatanensis* and *Lithodesmium* (Döhler 1985, Döhler *et al.* 1987). This could be due to a reduction in the supply of ATP. Kulandaivelu and Noorudeen (1983) found specific inhibition of the PS 2 centre by UV-B radiation, which in turn could reduce the formation of ATP *via* non-cyclic electron transport. An inhibition in the transport of inorganic carbon compounds (*e.g.* carbonic anhydrase) through the membrane is also possible under UV-B treatment. Enzymes and proteins might be the direct target for UV-B and as a result biosynthesis of proteins (Döhler 1984b) and activities of key enzymes (*e.g.* PEPC and RuBPC) are affected.

Changes in energy transfer reactions: The room temperature Chl *a* excitation for F_{682} in 3-d UV-B grown *Chlorella* cells did not change. However, continuous UV-B^E for 5 d increased the content of carotenoids. In the UV-B grown *Chlorella* cells absence of a change either in the peak level or in the magnitude of 480 nm peak could be attributed to the efficient functioning of Chl *b* containing LHCP (Fig. 2).

The excitation spectra of 3 and 5 d UV-B grown chrysophytes showed pronounced changes. The Chl *c* peak as well as the fucoxanthin peak increased in the UV-B irradiated cells indicating the presence of an efficient energy transfer from the latter to the former. The long wavelength pigments of PS 1 receive energy from the closely associated LHCP. Thus the enhanced level of 530/590 nm is correlated to the efficient photon capturing of the fucoxanthin complex.

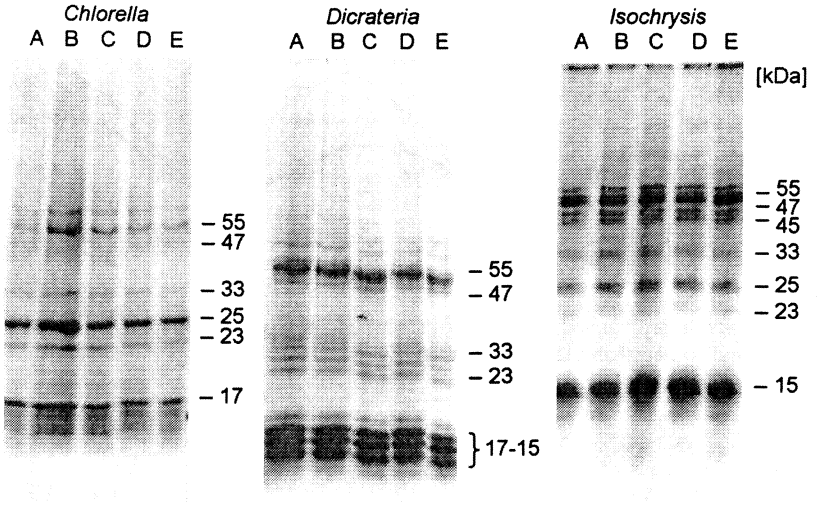

Fig. 3. Coomassie blue stained protein profiles of *Chlorella, Dicrateria* and *Isochrysis* cells grown under control and UV-B^E radiation (A - 0-d, B - 3-d control, C - 3-d treated, D - 5-d control, E - 5-d treated).

Changes in thylakoid proteins: The functioning of energy transducing membrane depends upon the precise structural arrangement of various compounds. Hence the changes in polypeptide pattern were analysed by gel electrophoresis to document any alteration in the protein composition of cells grown under normal and UV-B^E radiation. In each algal class, Chl, β-carotene, and the major xanthophylls are complexed to a few specific polypeptides. These pigment-protein complexes, PS 1 complex (P700-Chl *a* proteins), PS2 complex (P680-Chl *a* proteins), and light harvesting complexes are the main intrinsic proteins of thylakoids (Anderson *et al.* 1981). The main Chl-binding polypeptide of PS 1 complex has a molecular mass of 68 kDa and may give rise to two 60 - 64 kDa polypeptides under strong reducing conditions. Mullet *et al.* (1980) suggest that the 40 - 45 peripheral Chl *a* containing molecules of native PS 1 complexes are bound to 24.5 and 21.5 kDa polypeptides. Delepelaire and Chua (1979) reported that the apoproteins of the PS 2 complex are of 47 - 49 kDa size.

In addition to light-harvesting, the main polypeptides of LHCP are implicated in grana stacking and photon uptake regulation. The 25 and 23 kDa polypeptides are surface exposed and these surface exposed regions are involved in thylakoid stacking *in vivo*. A membrane bound kinase phosphorylates the surface exposed segments of LHCP, altering its interaction with PS 1 and PS 2.

In the present study, in *Chlorella* cells grown under UV-B enhanced fluorescent radiation for 5 d, significant reduction in contents of 47, 33, and 23 kDa polypeptides was observed (Fig. 3). The UV-B treatment might have led to the break of electrostatic forces binding the 24 and 17 kDa polypeptides to 33 kDa polypeptide thereby affecting the overall electron transport. Moreover, the loss of 47 kDa polypeptide might be due to the disruption of the PS 2 complex (Iwanzik *et al.* 1983, Kulandaivelu and Noorudeen 1983).

The main accessory pigments of chromophytes are Chl c_1, Chl c_2 and fucoxanthin. Fucoxanthin is found in intrinsic LHCP. The principal LHC has one of the three polypeptides of the 17 - 24 kDa size. The Chl *a-c*-caroteno-LHC accounts for 70 % of Chl *a* and much of the Chl *c* has a single peptide of 19 kDa (Hiller *et al.* 1993). The >64 kDa fucoxanthin containing complexes of *Nitzschia closterium* and *Isochrysis galbana* had Chl *a*, Chl *c* and fucoxanthin and polypeptide composition dominated by three presumed apoproteins of 18 - 22, 37 - 48 and 55 - 56 kDa size (Boczar and Prezelin 1989). In our study, in *Dicrateria* cells grown for 5 d under UV-B^E, significant loss in the contents of 55, 35 and 18 kDa polypeptides was noticed while the low molecular mass polypeptides (15 - 17 kDa) remained unaffected. This indicates that UV-B has affected only the core PS 2 protein and not the large pool of accessory chromophore. In contrast to *Dicrateria, Isochrysis* cells grown under UV-B^E were more stable in preserving the contents of thylakoid polypeptides. This strongly supports the observed resistance to UV-B damage found in *Isochrysis* cells.

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