Impact of UV-B Radiation on the Lipid and Fatty Acid Composition of Synchronized *Ditylum brightwellii* (West) Grunow

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Z. Naturforsch. **49 c,** 607–614 (1994); received April 25/May 30, 1994

Dedicated to Professor Hartmut Lichtenthaler on the occasion of his 60th birthday

Lipid and Fatty Acid Composition, UV-B Effects, Synchron Cultures, Marine Diatoms

The marine diatom *Ditylum brightwellii* (West) Grunow isolated from the Baltic Sea could be synchronized by a light/dark rhythm of 6.5:17.5 h (white light intensity 8 W m^{-2}) at 18 °C and 0.035 vol.% CO_2. Content of protein, DNA and RNA increased linearly up to the end of the cell cycle. Pigments (chlorophyll *a*, chlorophyll c_1+c_2, carotenoids) and galactolipids were synthesized in the light period only. A lag phase of 2 h was observed in the biosynthesis of sulphoquinovosyl diacylglycerol and phosphatidylglycerol. Formation of phosphatidylglycerol and phosphatidylcholin continued in the dark period (30% and 28%, respectively). The pattern of major fatty acids ($C_{14:0}$, $C_{16:1}$, $C_{16:0}$, $C_{18:1}$ and $C_{20:5}$) varied during the cell cycle of *Ditylum*.

Biosynthesis of acyl lipids was reduced in dependence on the UV-B dose. The most sensitive lipid was digalactosyl diacylglycerol (total inhibition at 585 J m^{-2}), whereas phosphatidylcholin was less affected (20% reduction). UV-B radiation during the dark period had no effect on the lipid and pigment content. Strongest inhibitory effect of UV-B on cell division, synthesis of protein, pigments, sulphoquinovosyl diacylglycerol and phosphatidylglycerol was found after UV-B radiation at the beginning of the cell cycle (0.–2. h). An exposure time at the end of the light period (4.–6. h) led to a marked damage on the synthesis of monogalactosyl diacylglycerol and phosphatidylglycerol. These findings indicate a stage-dependent response of *Ditylum* to UV-B irradiance. The impact of UV-B resulted in an increase of unsaturated long chained fatty acids (C_{18}, C_{20}) and in a diminution of short chained fatty acids (C_{14}, C_{16}). Content of ATP was not affected by UV-B radiation under the used conditions. The inhibitory effect of UV-B on synthesis of DNA, RNA, protein and acyl lipids was mainly reversible. Results were discussed with reference to UV-B damage on the enzymes involved in the biosynthesis of acyl lipids and by a reduction of available metabolites.

Introduction

Fatty acid composition of microalgae was shown recently to be altered by environmental changes, especially by different nutrient supply. Lipid synthesis and fatty acid composition of *Nannochloropsis* were studied under a 12:12 h light/dark regime (Sukenik and Carmeli, 1990). Cellular components, such as pigments, proteins, carbohydrates and lipids increased in the light period and decreased during the dark period. Changes in the lipid content were associated with variations in the fatty acid composition. The fatty acid distribution of *Nannochloropsis* can be regulated by light intensity (Sukenik *et al.*, 1989). Fatty acid composition of phytoplankton species changed considerably at different photon flux densities (Thompson *et al.*, 1990) and is a dynamic component of cellular physiology. Patterns of fatty acid biosynthesis of marine microalgae were related to nutrient limitations: phosphorus deficiency (Siron *et al.*, 1989) and nitrogen limitation (Parrish and Wangersky, 1990; Suen *et al.*, 1987). Nutrient regime affected the neutral lipid composition while changes in physiological state were reflected in the polar lipid composition (Mayzaud *et al.*, 1990). Fatty acid variations could be attributed to species composition of a complex assemblage of phytoplankton.

A few publications are known about variations in acyl lipid and fatty acid composition during the division cycle (Anderson and Sweeney, 1977; Beck and Levine, 1977; Döhler and Th. Biermann, 1988; Döhler and Datz, 1989). This paper presents data from the acyl lipid and fatty acid content during

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0939–5075/94/0900–0607 $ 06.00

the cell cycle of the marine diatom *Ditylum brightwellii* under the influence of UV-B radiation.

Materials and Methods

Growth and irradiation conditions

The marine diatom *Ditylum brightwellii* (West) Grunow isolated from the Baltic Sea was grown in an artificial sea-water medium according to v. Stosch and Drebes (1964). Synchronization of the cell division could be obtained by a light/dark regime of 6.5:17.5 h (white fluorescent lamps Osram L 40 W/25-1; white light intensity 8 W m^{-2}; 5 klux). Experiments were performed with *Ditylum* cells harvested from the 3. cycle. Algae were exposed to UV-B irradiance in special quartz tubes under the same culture conditions. An aliquot of the suspension was placed into glass tubes (not UV-B-treated cells = control). Philips lamps (TL 40 W/12) and cut-off filters (WG 305) have been used for UV-B irradiation experiments. UV was measured with a spectroradiometer (Optronic Model 742) from Prof. Dr. Tevini, Karlsruhe in connection with a computer (Hewlett-Packard, Model 85 B). The different doses of UV-B were obtained by changing the exposure time. However, the usual dose was 780 J m^{-2} weighted after Caldwell (1971) or 17.38 mW m^{-2} calculated to DNA damage. For transmission spectra see Döhler *et al.* (1991).

Analytical procedures

Algae harvested after different periods of the cell cycle were reduced to a small volume by filtration and ruptured with a Branson Sonifier (Model S-75). Extraction and measurement of DNA were carried out after the colorimetric method of Sponholz (1970) and RNA according to Ceriotti (1955). Chlorophyll content was estimated after Jeffrey and Humphrey (1975) and the carotenoids according to Myers and Kratz (1955). The method of Bradford (1976) was used for measuring the protein value. Lipids were extracted similar to the method of Tevini (1971) and separated by thin-layer chromatography. The quantities of the lipids were determined by estimation of the glycolipid sugar content (Roghan and Batt, 1968) and the PG-bound phosphorus as described by Debuch *et al.* (1968). Fatty acid composition of the acyl lipids were separated and analyzed by gas liquid chromatography (GC) using a Varian 3700 Model with a 2 m glass column (10% DEGS on Chromosorb G, 80–100 mesh). For further details see Datz and Döhler (1981). Extraction and measurement of ATP have been carried out according a modified method of Falkowski (1977).

Results

Growth and cell components

The marine diatom *Ditylum brightwellii* was isolated from phytoplankton samples of different habitats of the North and Baltic Sea. The isolated species from the Baltic Sea (klon "S") was presynchronized after Darley and Volcani (1971) and used for our experiments in the 3. cell cycle. Algae were presynchronized by exposure up to 24 h in the dark; after this time no further cell division could be observed. The generation time was 12 h and cell division lasted from the end of the light to the beginning of the dark period (see Fig. 1). The percentage of biprotoplastic cells has been used as a degree of synchronization (73%) which is in agreement with Eppley *et al.* (1967). Biprotoplastic cells are characterized by a special develop-

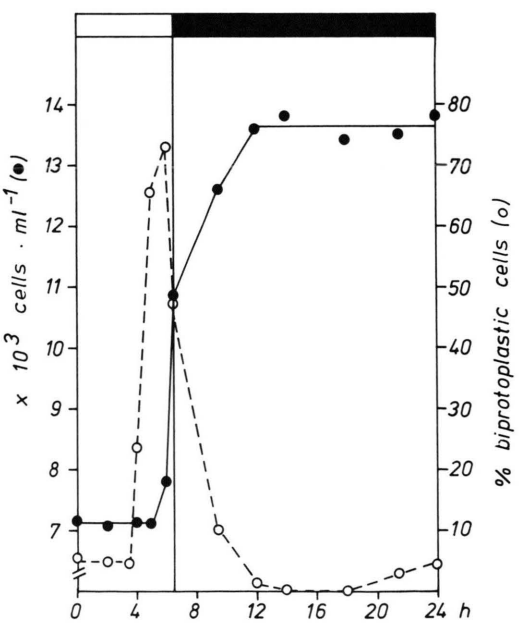

Fig. 1. Time course of cell division of *Ditylum brightwellii* grown synchronously during the 3. cycle. Further details in Materials and Methods.

mental state: at the end of cytokinesis and at the beginning of the release of daughter cells. All studies of the cell components have been performed at the 3. cell cycle after presynchronization. The standard error was about 5%. Content of DNA and RNA increased to the double value whereas synthesis of soluble proteins was threefold higher at the end of the cell cycle. Biosynthesis of chlorophylls, carotenoids and all the tested lipids (MGDG, DGDG, SQDG, PG and PC) run during the light period and reached the double values at 6.5 h. Main components of fatty acids are: $C_{20:5} > C_{16:1} > C_{16:0} > C_{18:1} > C_{14:0}$; linolenic acid ($C_{18:3}$) could not be detected.

The dose effect curve of UV-B irradiance showed that 780 J m^{-2} led to a total but reversible inhibition of the formation of biprotoplastic cells (data not shown). Therefore, algae were exposed to this dose in our experiments. Generally, UV-B exposure resulted in a reduction of the cell number. A total depression of cell division was found after UV-B radiation at the beginning of the cell cycle up to 4 h. Plasmolysis of the cells could be observed in all tested developmental stages.

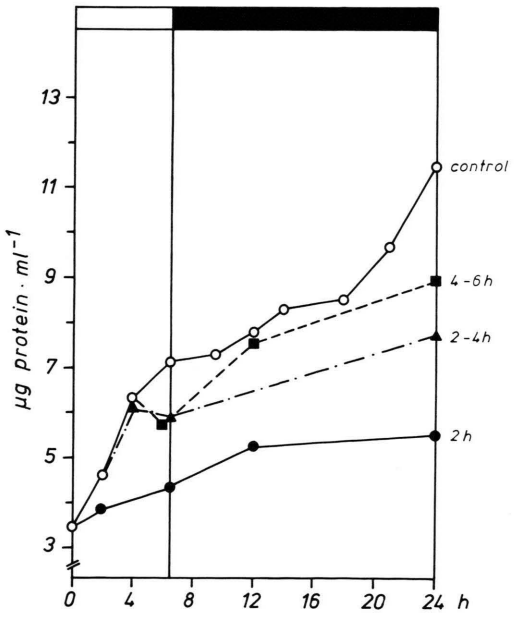

Fig. 2. Increase of the protein content of *Ditylum brightwellii* after UV-B irradiance during 0.–2. h (●), 2.–4. h (▲), 4.–6. h (■) and of not UV-exposed cells (○).

Fig. 2 contains results of the impact of UV-B on protein synthesis. Independent on the irradiation time during the cell cycle the protein content increased, however, to a lower level compared to not UV-B-exposed cells: UV-B radiation during 0.–2. h up to 44.8%, 2.–4. h to 27% and 4.–6. h to 57%. A reduction of 10–15% after UV-B radiation during the dark period (14.–16. h) was observed, only. Generally, the exposure time to UV-B was 2 h and changed over the cell cycle. Cell components were estimated after 2 h UV-B irradiance and at different times during the cell cycle. Cell number was 7.2×10^3 per ml.

In an other series of experiments a possible damaging effect of UV-B on chlorophylls and carotenoid (β-carotene) synthesis has been studied. Data of Fig. 3 indicate a significant reduction of the pigment contents independent on the time

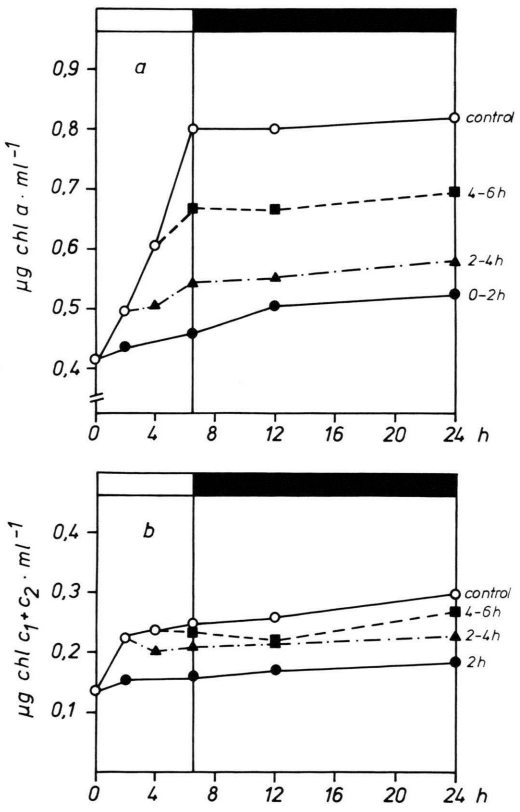

Fig. 3. Effect of UV-B radiation (780 J m^{-2}) on chlorophyll *a* (a) and $c_1 + c_2$ (b) content of *Ditylum brightwellii* during the division cycle. Exposure time was: 0.–2. h (●), 2.–4. h (▲), 4.–6. h (■) and not UV-B-treated cells (○).

of UV-B exposure at the division cycle. Contents of chlorophyll *a* and carotenoids (data not shown) increased during the cell cycle to about 2.5–13%. Synthesis of chlorophyll c_{1+2} was nearly inhibited by UV-B irradiance during 2.–6. h of the cell cycle. No influence of UV-B on all the tested pigments was observed after illumination within the dark period (14.–16. h).

Lipid composition

The biosynthesis of MGDG, DGDG, SQDG and PG were reduced dependent on the dose of UV-B irradiance. UV was given from the beginning of the light period up to 2 h. Fig. 4 indicate a different response of the individual acyl lipids. The most sensitive lipid was DGDG (100% inhibition at 585 J m^{-2}) whereas PC was less affected after UV-B irradiance (20% reduction). A linear depression – in dependence on the UV-B dose – was found at the synthesis of SGDG, MGDG and PG.

The impact of UV-B radiation on acyl lipid content of *Ditylum brightwellii* was investigated at a UV-B dose of 780 J m^{-2} exposed at different times during the light period (Fig. 5 and 6). Fig. 5 presents values of MGDG (a), DGDG (b) and PG (c) of synchronized *Ditylum* cells after UV-B exposure and over the cell cycle. Synthesis of

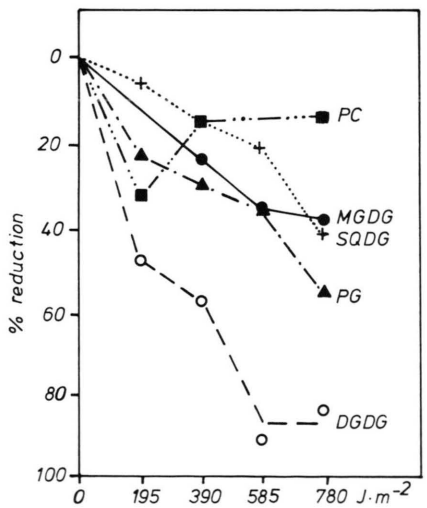

Fig. 4. Impact of different UV-B doses on biosynthesis of acyl lipids of *Ditylum brightwellii*. UV-B radiation was performed at the beginning of the light period. Values are calculated to those of not UV-B-treated cells and expressed as % reduction.

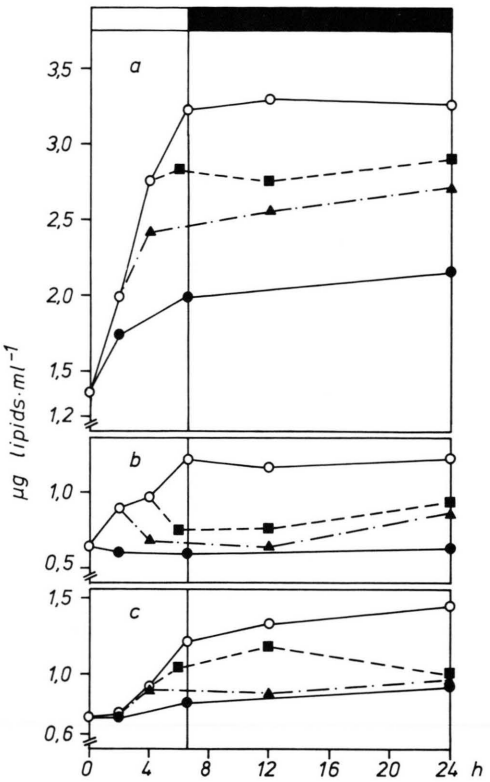

Fig. 5. Effect of UV-B radiation on acyl lipid content of *Ditylum brightwellii* during the cell cycle after UV-B irradiance (780 J m^{-2}). Exposure time was: 0.–2. h (●), 2.–4. h (▲), 4.–6. h (■) and not UV-B-treated cells (○). a, MGDG; b, DGDG; c, PG content.

DGDG and PG was markedly reduced or totally inhibited whereas an increase of the MGDG content could be observed up to the end of the cell cycle. A similar effect was found in DGDG after UV-B radiation from 2.–6. h. An UV inhibitory effect on SQDG synthesis exists independent on the exposure time during the light period (Fig. 6b). No damage on PC content was detected by UV-B radiation from 0.–4. h whereas an enhancement was measured after UV-B exposure at 4.–6. h of the cell cycle (Fig. 6a). UV-B radiation in the dark period (14.–16. h) had no influence on all the tested acyl lipid biosynthesis (data not shown).

Fatty acid composition

The impact of UV-B radiation on possible variations in the pattern of fatty acids of all tested

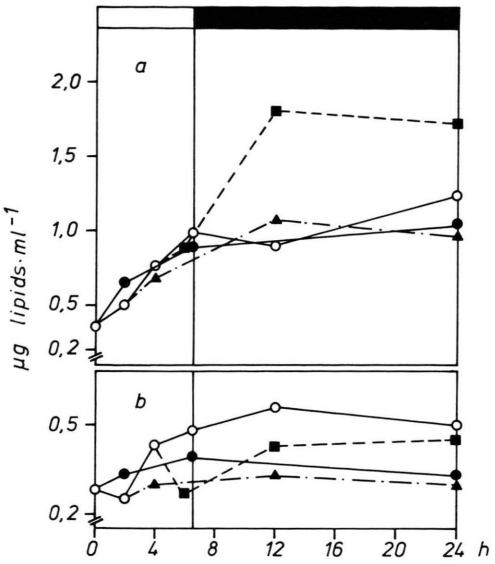

Fig. 6. Effect of UV-B radiation on acyl lipid content of *Ditylum brightwellii* during the cell cycle after UV-B irradiance (780 J m^{-2}). Exposure time was: 0.–2. h (●), 2.–4. h (▲), 4.–6. h (■) and not UV-B-treated cells. a, PC; b, SQDG content.

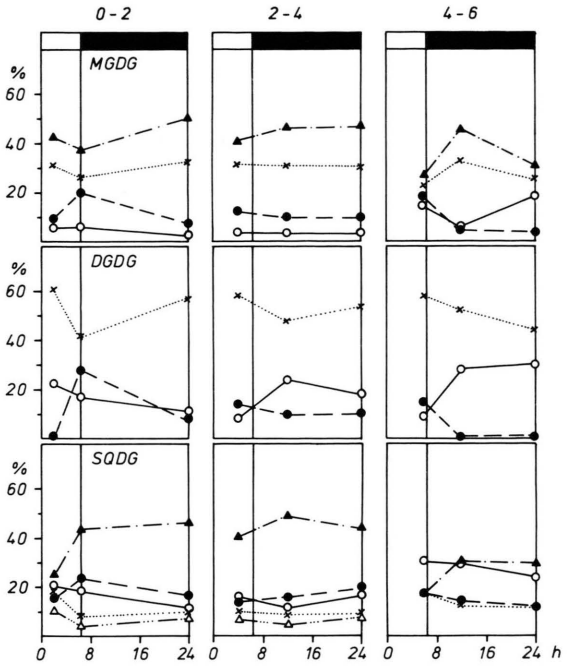

Fig. 7. Changes in percentage proportion of glycolipid-bound major fatty acids after UV-B radiation of synchronized *Ditylum brightwellii* cells at different times of the light period (0.–2., 2.–4. and 4.–6. h). $C_{20:5}$ (▲), $C_{18:1}$ (×), $C_{18:0}$ (△), $C_{16:1}$ (●) and $C_{16:0}$ (○).

lipids was studied in *Ditylum* cells after different exposure times during the light period. The analysis of the fatty acids of all the lipids showed that palmitic ($C_{16:0}$), hexadecenoic ($C_{16:1}$), octadecenoic ($C_{18:1}$) and eicosapentaenoic ($C_{20:5}$) acid as major components accounted for more than 90% of total fatty acids in MDGD and DGDG. SQDG, PC and PG contains also amounts of myristic ($C_{14:0}$), stearic ($C_{18:0}$) and hexadecadonoic ($C_{16:2}$) acid. Percentage proportion of major fatty acid components varied after UV-B radiation (Fig. 7 and 8) and synthesis of other fatty acids was induced, too. $C_{16:0}$ content was reduced in MGDG and enhanced in DGDG whereas the opposite reaction could be observed in $C_{18:1}$ content. As a result of UV-B irradiance, the fatty acid $C_{20:5}$ increased in all acyl lipids. Pattern of fatty acids of the phospholipid PG was similar to that of MGDG. The amount of $C_{14:0}$ from the sulfolipid and of PC decreased markedly from 30 to 5%. The influence of UV-B radiation on the pattern of fatty acids was similar after exposure from 0.–4. h of the cell cycle whereas a change in the distribution of the fatty acids was observed after irradiation with UV-B at 4.–6. h in the following dark period. Proportion of unsaturated fatty acids of MGDG,

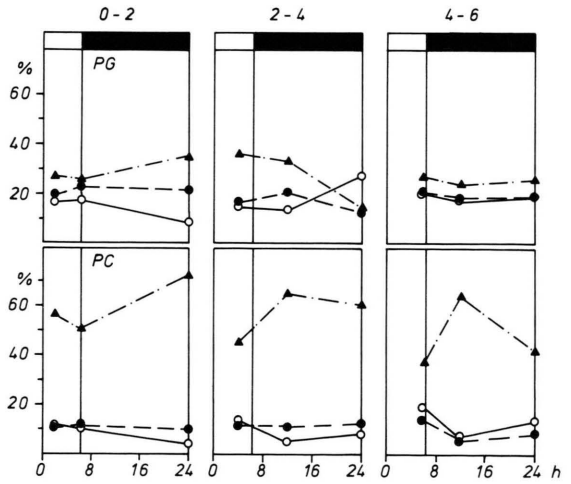

Fig. 8. Changes in percentage proportion of phospholipid-bound major fatty acids after UV-B radiation (780 J m^{-2}) of synchronized *Ditylum brightwellii* cells at different times of the light period (0.–2., 2.–4. and 4.–6. h). $C_{20:5}$ (▲), $C_{16:1}$ (●) and $C_{16:0}$ (○).

DGDG, SQDG and PC increased after UV-B radiation during 4.–6. h. Additionally, experiments showed that total $^{14}CO_2$ fixation was significantly depressed after UV-B exposure at the beginning of the light period (0.–2. h) and no damaging effect of UV-B on ATP content was found (not UV-B-irradiated cells: 9.4 and (UV-B-exposed cells 8.9×10^{-7} M ATP ml^{-1}).

Summarizing, the impact of UV-B on biosynthesis of the cell components was dependent on the exposure time (cell developmental stage): 1. synthesis of proteins was most sensitive after UV-B radiation at the beginning of the light period (0.–2. h), 2. strongest inhibition of chlorophyll *a*, chlorophyll c_1+c_2, SQDG and PC was mainly observed after exposure from 2.–4. h and 3. lowest contents of carotenoids, MGDG and PG were found after UV-B irradiance at the end of the light period of the cell cycle of *Ditylum brightwellii* (4.–6. h).

In another series of experiments a possible reactivation or repair of the UV-B damage were tested after an exposure time from 0.–2. h followed by further 3 untreated cell cycles. In the 3. cell cycle after UV-B radiation (780 J m^{-2}) an increase in the cell number but no synchroneous cell division was found. Inhibition of DNA, lipid and RNA synthesis was reversible: 40% respectively 80% of the values were measured in the 5. cycle. No variations in the percentage proportion of the individual acyl lipids could be detected as a result of UV-B irradiance.

Discussion

Synchronized growth of diatoms could be induced by silica deficiency or precultivation of 24 h in the dark (Darley and Volcani, 1971). Synchronization was dependent on the temperature and the light/dark regime (Eppley *et al.*, 1967; Paasche, 1967). The best synchronized growth (73%) was available under our used culture conditions compared to the results of Paasche (50%) and Eppley *et al.*, (25%). It could be demonstrated that the light/dark rhythm is necessary for induction of a synchronous growth; no further synchronization was possible in the following period of continuous illumination. Our findings showed that doubling of the cell numbers was parallel to DNA synthesis and finished in the dark period. A correlation between cell division and DNA replication of diatoms has been described by Darley *et al.* (1976) and Paul and Volcani (1976). Total inhibition of DNA and RNA synthesis by UV-B was reversible within the following cell cycles.

The observed reduced protein content after UV-B exposure of *Ditylum* can be attributed to a reduction of protein synthesis *via* inhibition of RNA formation and photosynthetic CO_2 fixation. It was found that the diminution of pigment biosynthesis by UV-B radiation was dependent on the species and UV-B dose (Döhler, 1984).

The findings of Thompson *et al.* (1990) suggest that fatty acid composition is a dynamic component of cellular physiology and responds to light conditions: Eicosapentaenoic acid ($C_{20:5}$) increased from 6 to 15% of the total fatty acids of *Chaetoceros simplex* grown under high and low light conditions, respectively. Fatty acid composition of *Ditylum* was nearly identical with that described by Chuecas and Riley (1969). The relative high amount of $C_{18:1}$ might be characteristic for this marine diatom. The percentage proportion of the fatty acid composition from the main lipids without of PG varied during the cell cycle of *Ditylum.* Biosynthesis of pigments and galactolipids as well as photosynthetic $^{14}CO_2$ fixation was parallel during the light period. Similar results have been described by Beck and Levine (1977) and Döhler and Datz (1989). This indicate a correlation between galactolipids and the orientation of the pigments in the thylakoid membrane.

The impact of UV irradiance on polar acyl lipids has been investigated on higher plants, only (Tevini *et al.*, 1981). A preliminary study of UV-B on lipid and fatty acid composition of the marine diatom *Ditylum* was presented by Döhler and Th. Biermann (1988). No further data are available up to now. Our findings indicate a different damaging effect of UV-B in dependence on the dose and the developmental state of *Ditylum brightwellii.* The possible effect of UV-B on lipid biosynthesis might be: 1. a reduced supply with metabolites and ATP; 2. inhibition of enzyme activities or *de novo* synthesis and 3. degradation of lipids.

A correlation between photosynthesis and lipid biosynthesis of *Ditylum* could be demonstrated by experiments with 3-dichlorophenyl-1-dimethyl urea (DCMU). Application of 10^{-6} M DCMU led to a reduction of the contents of MGDG, PC

and SQDG up to 64 or 78%, respectively. Synthesis of DGDG and PG was totally inhibited. The damage of UV-B radiation on lipid biosynthesis of *Ditylum* can probably not be attributed to a reduction of ATP. No reduction of the ATP content could be measured after UV-B exposure of *Ditylum*.

Another reason for the observed UV-B effects can be the impact on enzyme activity or *de novo* synthesis of enzymes involved in lipid biosynthesis. We assume that one of the main damages are on the enzymes. It could be demonstrated that enzymes of the carbon and nitrogen metabolism are sensitive to UV-B irradiance (Döhler, 1990). Therefore, a reduction of the supply with metabolites from the photosynthetic CO_2 fixation must be considered. The marked depression of the DGDG content as a result of UV-B irradiance can be probably attributed to a degradation of the lipid. On the other hand, UV-B exposure during the dark period had no effect on the lipid contents. Results of Mantai *et al.* (1990) obtained from experiments with *Scenedesmus* and spinach chloroplasts don't indicate a degradation of the lipids, too.

More recently, Goes *et al.* (1994) described the impact of UV-B radiation on the fatty acid composition of the marine chlorophyte *Tetraselmis* in relation to the cellular pigments: In presence of UV-B the absolute concentrations decreased and polyunsaturated fatty acids were suppressed. They found a selective suppression of $C_{16:4}$ after UV-B exposure. In agreement with our findings, the absolute concentrations of fatty acids were lower in *Tetraselmis* cells after UV-B irradiance. The observation on the reduction of $C_{16:4}$ under UV-B radiation of *Tetraselmis* was used by the authors as an indicator of UV-B stress in green algae. Therefore, it appears to us that the increase of $C_{20:5}$ in *Ditylum* after UV-B exposure might be an indicator for UV-B damage in diatoms.

Acknowledgement

Thanks are expressed to the Bundesministerium für Forschung und Technologie, Bonn for the financial support.

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