Effect of UV-B Radiation on Biomass Production, Pigmentation and Protein Content of Marine Diatoms

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UV-B Stress, Pigment and Protein Content, Pure Cultures of Marine Diatoms

Several species of marine diatoms were grown at +18 °C and +22 °C under normal air conditions (0.035 vol.% CO_2) at a light/dark alteration of 14:$\overline{8}$ h. Intensity of white light was 1 mW (~5000 lux). An artifical nutrient solution of 35‰ salinity was used. Algae – harvested during exponential growth – were exposed to different intensities of UV-B radiation (439, 717 and 1230 $J \cdot m^{-2} \cdot m^{-1}$) for 2 days. UV-B radiation depressed the growth of all tested marine diatoms. Low levels of UV-B resulted in a slight increase of the biomass production (dry weight) compared to not UV-B treated cells. Enhanced UV-B doses caused a diminution of the primary productivity in all species. Algae exposed to UV-B stress showed a marked decrease in the protein and pigment content (chlorophyll *a*, chlorophyll $c_1 + c_2$ and carotenoids). In +22 °C grown cells of *Lauderia annulata* and *Thalassiosira rotula* were more sensitive to UV-B radiation than those cultures grown at +18 °C. *Bellerochea yucatanensis* cells grown at +22 °C were less affected after UV-B exposure than at +18 °C grown algae. The UV-B sensibility and growth of the individual species varied in a mixture of several marine diatoms. Results were discussed with reference to the UV-B effect on metabolic processes.

Introduction

Recent atmospheric studies showed that a partial depletion of the stratospheric ozone layer could result in an increased solar UV-B radiation. The effect of ultraviolet irradiance on several processes of higher plants, isolated chloroplasts and microorganism has been investigated more recently in detail [1–6]. The UV-B component of solar irradiation is capable to damage *e.g.* pigments, photosynthesis, DNA and protein molecules [7, 8]. Primary production of the marine phytoplankton near the surface waters was reduced by UV-B [9, 10]. It could be shown in isolated chloroplasts that UV-B specifically inactivates the photosystem II centre [5, 11]. Many UV-B responses in higher plants have much in common with the impact observed in marine phytoplankton [3, 6]. In the present study of the UV-B radiation on several parameters of marine diatoms demonstrates the species dependant sensibility of these organisms.

Materials and Methods

The following 12 species of marine diatoms from different families which we have obtained from Prof. Dr. H. v. Stosch, Marburg; Dr. G. Drebes, Sylt and Dr. E. Hagmeier, Helgoland, were grown in pure cultures at +18 °C or +22 °C under normal air conditions (0.035 vol.% CO_2). A light/dark alteration of 14:$\overline{10}$ h (light intensity: 1 mW; ~5000 lux) and an artificial nutrient solution (35‰) of v. Stosch and Drebes [12] was used.

Coscinodiscaceae

Thalassiosira angstii
Thalassiosira rotula
Skeletonema costatum

Biddulphiaceae

Biddulphia regia
Biddulphia sinensis
Ditylum brightwellii
Bellerochea spinifera
Bellerochea yucatanensis

Chaetoceraceae

Chaetoceros debilis

Leptacylindraceae

Lauderia annulata

Fragilariaceae

Asterionella glacialis
Synedra planctonica

Marine diatoms harvested during exponential growth were exposed to UV-B radiation for 2 days

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Table I. Effect of different UV-B doses (439, 717 and 1230 J · m^{-2} · d^{-1}) on chlorophyll *a*, chlorophyll $c_1 + c_2$ and carotenoid concentration of several marine atoms. Algae grown at +18 °C and 0.035 vol.% CO_2 were exposed to UV-B irradiation for 2 days at the same temperature using a white light/dark rhythm of 16:8 h and an intensity of 3500 lux (0.8 mW). % presents calculations to values of the not UV-B-irradiated cells (control). For further details see Materials and Methods.

Species	Control	439 J · m^{-2} · d^{-1}		717 J · m^{-2} · d^{-1}		1230 J · m^{-2} · d^{-1}	
	[µg/ml]	[µg/ml]	[%]	[µg/ml]	[%]	[µg/ml]	[%]
				Chlorophyll *a*			
Asterionella glacialis	21.38	13.02	60.9	7.56	35.4	2.56	12.0
Bellerochea spinifera	12.33	3.61	29.3	3.41	27.7	2.04	16.5
Bellerochea yucatanensis	42.99	28.88	67.2	19.57	45.5	17.33	40.3
Biddulphia regia	18.99	18.02	94.9	14.55	76.6	11.94	62.9
Biddulphia sinensis	5.92	4.47	75.5	3.14	53.0	1.90	32.1
Chaetoceros debilis	9.70	3.26	33.6	2.96	30.5	0.73	7.5
Ditylum brightwellii	18.01	6.14	34.1	5.44	30.2	4.87	27.0
Lauderia annulata	23.56	15.90	67.5	10.00	42.4	4.65	19.7
Skeletonema costatum	5.53	2.82	51.0	1.89	34.2	–	–
Synedra planctonica	25.07	5.68	22.7	4.88	19.5	1.07	4.3
Thalassiosira angstii	29.76	26.76	89.9	19.00	63.8	14.26	47.9
Thalassiosira rotula	26.53	25.38	95.7	14.92	56.2	10.33	38.9
				Chlorophyll $c_1 + c_2$			
Asterionella glacialis	4.68	2.43	51.9	1.78	38.0	0.62	13.2
Bellerochea spinifera	2.03	1.27	62.6	0.74	36.5	0.67	33.0
Bellerochea yucatanensis	8.19	7.14	87.2	4.78	58.4	3.54	43.2
Biddulphia regia	2.94	2.81	95.6	2.42	82.3	2.01	68.4
Biddulphia sinensis	1.76	1.77	100.4	1.22	69.1	0.78	44.1
Chaetoceros debilis	2.54	1.32	52.0	1.22	48.0	0.62	24.4
Ditylum brightwellii	4.99	2.03	40.7	1.79	35.9	1.77	35.5
Lauderia annulata	5.23	3.39	64.8	1.97	37.7	1.60	30.6
Skeletomena costatum	1.03	–	–	0.88	85.5	–	–
Synedra planctonica	4.08	1.17	28.7	1.19	29.2	0.54	13.2
Thalassiosira angstii	4.56	4.39	96.3	3.43	75.2	2.51	54.9
Thalassiosira rotula	5.06	5.37	106.1	3.39	67.0	2.18	43.1
				Carotenoids			
Asterionella glacialis	19.58	11.98	61.1	6.95	35.5	2.18	11.1
Bellerochea spinifera	12.00	4.25	35.4	4.00	33.3	2.08	17.3
Bellerochea yucatanensis	47.00	34.50	73.4	23.75	50.5	22.00	46.8
Biddulphia regia	19.00	18.80	98.9	16.18	85.2	13.03	68.6
Biddulphia sinensis	6.02	4.53	75.3	3.20	53.2	2.01	33.4
Chaetoceros debilis	9.28	3.20	34.5	3.20	34.5	0.57	6.1
Ditylum brightwellii	22.00	8.00	36.4	7.00	31.8	6.33	28.8
Lauderia annulata	26.75	17.05	63.7	11.00	41.1	4.53	16.9
Skeletonema costatum	4.24	2.31	54.5	1.62	38.2	–	–
Synedra planctonica	25.33	6.05	23.9	5.29	20.9	0.78	3.1
Thalassiosira angstii	26.13	24.33	93.1	17.55	67.2	13.80	52.8
Thalassiosira rotula	22.42	21.88	97.6	14.13	63.0	9.16	40.9
				Protein content			
Asterionella glacialis	0.203	0.123	60.6	0.094	46.3	0.002	1.0
Bellerochea spinifera	0.136	0.064	47.1	0.072	52.9	0.0087	6.4
Bellerochea yucatanensis	0.432	0.302	69.1	0.183	42.4	0.151	35.0
Biddulphia regia	0.127	0.123	96.9	0.085	66.9	0.063	49.6
Biddulphia sinensis	0.050	0.034	68.0	0.012	24.0	0.0032	6.4
Chaetoceros debilis	0.122	0.071	58.2	0.023	18.9	0.0016	1.3
Ditylum brightwellii	0.170	0.061	35.9	0.042	24.7	0.0191	11.2
Lauderia annulata	0.335	0.185	55.2	0.089	26.6	0.027	8.1
Skeletonema costatum	0.086	0.039	45.3	0.037	43.0	–	–
Synedra planctonica	0.260	0.058	22.3	0.0503	19.3	0.0215	8.3
Thalassiosira angstii	0.160	0.130	81.3	0.084	52.5	0.062	38.8
Thalassiosira rotula	0.437	0.512	117.2	0.218	49.9	0.027	6.2

Table I (continued)

Species	Control	439 J · m^{-2} · d^{-1}		717 J · m^{-2} · d^{-1}		1230 J · m^{-2} · d^{-1}	
	[µg/ml]	[µg/ml]	[%]	[µg/ml]	[%]	[µg/ml]	[%]
				Dry weight			
Asterionella glacialis	19.46	9.82	50.5	9.68	49.7	9.34	48.0
Bellerochea spinifera	1.91	1.84	96.3	1.82	95.3	1.62	84.8
Bellerochea yucatanensis	4.21	4.88	115.9	4.35	103.3	3.58	85.0
Biddulphia regia	4.76	5.09	106.9	5.84	122.7	4.34	91.2
Biddulphia sinensis	2.82	3.16	112.1	2.72	96.5	2.59	91.8
Chaetoceros debilis	–	–	–	–	–	–	–
Ditylum brightwellii	3.75	3.12	83.2	3.69	98.4	2.08	55.5
Lauderia annulata	3.68	3.76	102.2	3.55	96.5	2.87	78.0
Skeletonema costatum	9.47	10.28	108.6	9.36	98.8		–
Synedra planctonica	4.20	4.35	103.6	4.88	116.2	3.06	72.9
Thalassiosira angstii	4.28	4.33	101.2	4.38	102.3	3.63	84.8
Thalassiosira rotula	3.91	4.76	121.7	3.25	83.1	2.07	52.9

in a special quartz tube at +18 °C or +22 °C. Further conditions as described by Döhler [8]. Irradiation was supplied by Philips TL 40/12 (UV-B) and white fluorescence lamps (Osram L 36 W/11). Cut-off filters (Schott, WG 305, 3 mm thickness) were used. Different UV-B irradiances (439, 717 and 1230 J · m^{-2} · d^{-1}; weighted according Caldwell [13] as biological effectiveness) were obtained by changing the distances from the lamps to the cultures (100, 60 and 20 cm). Daily irradiation time was 5 h.

For the analytical procedures the algae were concentrated by filtration on paper filters or by low centrifugation. Dry weight of the cells was estimated after the method of Worrest *et al.* [14] on glass filters (Whatman GF/C, 4.7 cm) or in small glass tubes after heating at +450 °C. Cells were disrupted by sonification (2 × 30 s; 20 kHz) with a Branson Sonifier (Model S-75). The procedure of Bradford [15] was used for determination of the protein content. Quantitative estimation of chlorophyll *a* and *c* was carried out in 80% aceton after the method of Jeffrey and Humphrey [16]. The carotenoid content we have measured according to the procedure of Myers and Kratz [17]. Cells numbers were counted be using a plankton microscope (Zeiss).

Results

UV-B radiation (290–320 nm) has a significant effect on biological systems (*e.g.* higher plants, microorganism) and organic molecules (DNA, proteins) which can absorb UV-B. Calkins and Thordardottir [18] could demonstrate that an exposure of a few hours to ambient solar UV-B levels during summer can be lethal to some marine diatoms from the North Atlantic. It could be shown by several researchers that the growth rates of marine diatoms were depressed by UV-B irradiance [19–21]. Growth of the algae tested in our study were significantly reduced in dependence on the UV-B dose. Enhanced UV-B levels (1230 J · m^{-2} · d^{-1}) were usually lethal to all tested species mainly at a temperature of +22 °C.

Table I presents the effect of UV-B radiation upon biomass (dry weight). Exposure to low levels (439 J · m^{-2} · d^{-1}) caused mainly an increase of the primary production – here dry weight – compared to the values of not UV-B-irradiated cells. Enhanced UV-B doses resulted in a reduction of the biomass productivity in all marine diatoms. *Asterionella glacialis* and *Ditylum brightwellii* were more sensitive to UV-B radiation than the other species. Our findings are in agreement with a study on primary productivity of seven species of marine phytoplankton [22].

Alterations in the level of the protein content in 12 species of marine diatoms exposed to different intensities of UV-B radiation are shown in Table I. Generally, a significant depression was found in all species. An increase in protein content was observed at low UV-B dose (429 J · m^{-2} · d^{-1}) in *Biddulphia regia* (*B. r.*) and *Thalassiosira rotula* (*Th. r.*). Enhanced UV-B radiation affect significantly the protein content in all tested algae. *Biddulphia regia*, *Bellerochea yucatanensis*, and *Thalassiosira angstii* are less sensitive to UV-B than the other diatoms.

The content of photosynthetic pigments (chlorophyll *a*, chlorophyll $c_1 + c_2$ and carotenoids) were

reduced to a similar extent as the protein content. A species dependant diminution of the total pigment content was found, too. *Biddulphia regia*, *Thalassiosira angstii* and *Thalassiosira rotula* were less sensitive than *Bellerochea spinifera* and *Synedra planctonica*. Our results are in agreement with the published results of higher plants and microorganisms [1, 3, 6, 8, 21–23]. The light/dark rhythm of 16:8 during UV-B irradiation seems to be sufficient for the repair mechanisms of the UV-B-induced damage in marine diatoms. Values presented in Table I indicate that normally chlorophyll *a* and the carotenoids were more sensitive to UV-B-radiation than chlorophyll $c_1 + c_2$. Chaetoceros debilis and *Synedra planctonica* were the most sensitive species in this study. A reduction of the chlorophyll *a* content after UV-B radiation was found in an autotrophic plankton community by several workers [6].

Preliminary results obtained from a mixture of 4 diatoms (*Asterionella*, *Biddulphia*, *Ditylum* and *Thalassiosira*) showed that UV-B radiation (439 and 717 $J \cdot m^{-2} \cdot d^{-1}$) depressed biomass production, protein and pigment content (data not shown). Chlorophyll *a* was more reduced than chlorophyll $c_1 + c_2$ and the carotenoids. Cell number arose after 2 days exposure to 717 $J \cdot m^{-2} \cdot d^{-1}$ UV-B dose. Under these conditions growth of *Asterionella glacialis* and *Ditylum brightwellii* increased compared to the not UV-B irradiated cells and to that exposed to low UV-B intensity (439 $J \cdot m^{-2} \cdot d^{-1}$). These findings are surprizing, both species were very sensitive to UV-B radiation when algae were grown in pure cultures and exposed to UV-B. In this "artifical ecosystem" the cell number of *Thalassiosira rotula* decreased with increasing UV-B dose (data not shown). Long term UV-B irradiance should give more information.

In other series of experiments the impact of UV-B radiation on dry weight, protein and pigment content was studied with diatoms grown at +22 °C. The tropical diatom *Bellerochea yucatanensis* was found to be less sensitive to UV-B stress at +22 °C than *Lauderia annulata* and *Thalassiosira rotula*. Biomass production, protein and pigment content of *Bellerochea* cells grown at +22 °C and exposed to UV-B radiation for 2 days at the same temperature were less affected by UV-B than cells grown at +18 °C before exposed to UV-B. On the other hand, UV-B radiation at +22 °C caused a significant decrease in biomass production, protein and pigment content of *Lauderia annulata* and *Thalassiosira rotula* in comparison to algae grown at +18 °C. Enhanced levels of UV-B (1230 $J \cdot m^{-2} \cdot d^{-1}$) were lethal for both species and lead to cell death. Other species collected from the North Sea were not able to grow at +22 °C (*e.g. Asterionella glacialis*, *Biddulphia sinensis*, *Chaetoceros debilis*, and *Ditylum brightwellii*). After plasmolysis and shrinkage of the cells contamination with bacteria were found under these conditions. But in spite of this, growth of in +18 °C cultivated diatoms (*Asterionella*, *Biddulphia*, *Chaetoceros* and *Ditylum*) could be observed also under UV-B stress at +22 °C for 2 days. Effect of UV-B radiation on the individual pigments of diatoms grown at +18 °C and exposed to UV-B at +22 °C showed that chlorophyll $c_1 + c_2$ was less affected by UV-B than the other pigments of *Asterionella*, *Chaetoceros*, and *Thalassiosira*. No significant differences in the diminution of the individual pigments after UV-B irradiance of *Bellerochea*, *Biddulphia*, and *Ditylum* were observed. Chlorophyll $c_1 + c_2$ was the most sensitive pigment of UV-B irradiated *Lauderia* cells. Summarizing, the impact of UV-B (290–320 nm) on marine diatoms was species dependant especially at different temperatures.

Discussion

Microscopic studies have shown a significant reduction in the growth of the tested marine diatoms which was dependant on the species, temperature and the UV-B fluence (data not presented). A total inhibition of the growth was found in species collected from the North Sea after exposure to 1230 $J \cdot m^{-2} \cdot d^{-1}$ at +22 °C. Growth of the tropical diatom *Bellerochea yucatanensis* was markedly decreased by UV-B at low temperature (+18 °C) in comparison to +22 °C. Growth of *Ditylum* and *Asterionella* was less affected by UV-B radiation in a mixture of 4 diatoms than in the uni-algae cultures. The behavior of the individual species to UV-B stress at different temperatures can be explained by the different natural distribution of the diatoms. Thomson *et al.* [20] found also an inhibition in the growth of the diatom *Melosira nummoloides* by UV-B radiation of different doses. In agreement with our results a species dependant behavior to UV-B stress was observed in a natural phyto-

plankton population [22]: *Thalassiosira* was relatively resistant compared to *Melosira* and *Chaetoceros*.

Biomass production (dry weight), protein and pigment content of all diatoms were depressed by UV-B radiation – mainly at high levels. It was found a species dependant behavior: *Bellerochea yucatanensis* was less sensitive to UV-B at high temperature (+22 °C) than at +18 °C; *Thalassiosira rotula* was more affected by UV-B at +22 °C than at +18 °C. Impact of UV-B stress upon protein and pigment content was more pronounced than on the biomass production (dry weight). Reduction of photosynthetic CO_2 fixation by UV-B radiation ($717\ J \cdot m^{-2} \cdot d^{-1}$) can be explained by a diminution of supply with ATP and $NADPH_2$ and by an inhibition of the enzyms involved in the Calvin cycle. On the other hand, it could be demonstrated in a fluorescence study that UV-B inhibits photosystem II activity by inactivating the photosystem II centre [4, 5 and 11]; the water splitting enzyme was not affected. By that way primary productivity of the diatoms can be also depressed.

The photosynthetic apparatus contains structural proteins, enzymes and pigments closely associated with proteins. Proteins absorb in the 290–320 nm waveband and can be easily destroyed by UV-B radiation as shown in this paper, too. Since proteins are parts of all photosynthetic pigments UV-B damage to protein may directly destructive to photosynthetic reactions. On the other hand, it can be suggested that UV-B radiation result in a variation of the arrangement of the photosynthetic apparatus in the membranes. This may inhibit numerous photochemical and biochemical processes. The investigation of the impact of UV-B radiation on incorporation of ^{14}C- and ^{15}N-labelled compounds and enzymatic studies should give more information on UV-B damage in marine diatoms.

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