Impact of UV-B Radiation on Photosynthetic Assimilation of ^{14}C-Bicarbonate and Inorganic ^{15}N-Compounds by Cyanobacteria

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The cyanobacteria *Anabaena cylindrica* and *Synechococcus leopoliensis (= Anacystis nidulans)* were grown at different levels of UV-B radiation (439, 717, 1230 and 1405 J m^{-2}d^{-1}, weighted according Caldwell, 1971) for 2 days. Dry weight was hardly affected but phycocyanin content of both species decreased linearly to the level of UV-B radiation. Contents of protein, carotenoids and chlorophyll a were reduced only after exposure to high doses (1230 J m^{-2}d^{-1}) of UV-B radiation. Photosynthetic ^{14}CO$_2$ fixation of *Anabaena* cells was reduced linearly with increasing UV-B dose whereas no effect could be observed in *Synechococcus*. A depression of photosynthetic ^{15}N-nitrate uptake was found after UV-B stress in both species. UV-B irradiance caused an increase of ^{15}N-incorporation into glutamine, but no effect was noted for incorporation into alanine or aspartic acid. An increase of ^{15}N-excess in glutamic acid linear with the UV-B dose was observed in *Synechococcus*, only. Patterns of ^{14}C-labelled photosynthetic products were either less affected by UV-B radiation *(Anabaena)* or an enhancement of ^{14}C-label in total amino acids was detected *(Synechococcus)*. The amount of total free amino acids increased parallel to the level of UV-B radiation. Only, the high dose of UV-B (1405 J m^{-2}d^{-1}, weighted) results in a decrease of the glutamine pool. Our results indicate an inhibition of glutamate synthase by UV-B irradiation in *Anabaena*, only. Results were discussed with reference to the damage of the photosynthetic apparatus.

Introduction

Photosynthetic organisms have been exposed to changing levels of ultraviolet irradiation during the course of evolution and the formation of the earth's atmosphere. Cyanobacteria − a phylogenetically old group with procaryotic cellular organization − were probably faced with more intense ambient solar UV radiation than other organisms *e.g.* algae or higher plants appearing at a later period [1]. A depletion of the stratospheric ozone layer by human activities may result in enhanced levels of solar UV-B radiation on the earth which may damage the biological ecosystems. It is well known that UV-B stress affects several metabolic processes, pigmentation and community composition of biological system [2−6]. More recently, the effect of ultraviolet B (280−320 nm) irradiation on physiological activities of several species of cyanobacteria has been studied. Newton *et al.* [7] found that the nitrogen fixing enzyme in cyanobacteria is more sensitive to UV-B than other metabolic processes.

Our studies show that ultraviolet-B radiation leads to a marked reduction of phycocyanin and a diminution of the photosynthetic ^{14}CO$_2$ fixation and assimilation of inorganic ^{15}N-compounds. In this report we describe the influence of UV-B on carbon and nitrogen metabolism of the cyanobacteria *Synechococcus leopoliensis* and *Anabaena cylindrica* in more detail.

Materials and Methods

The cyanobacteria *Synechococcus leopoliensis* (Raciborski) Komarek (strain 1402-1; former *Anacystis nidulans*) and *Anabaena cylindrica* Lemmermann (strain 1403-2) were obtained from the Algal Collection, Göttingen. Both species were grown under normal air conditions (0.03 vol.% CO$_2$) and a light/dark regime of 14:10 (light intensity 1 mW cm^{-2}). Growth temperature of *Synechococcus* was 32 °C and that of *Anabaena* 23 °C, respectively. A nutrient medium of Döhler and Koch [8] has been used for the cultures of *Synechococcus* and for *Anabaena* cells BG 11 according to Allen and Arnon [9]. Cyanobacteria were harvested during exponential growth and exposed to different UV-B levels (439, 717, 1230 and 1405 J m^{-2}d^{-1}, weighted according to Caldwell [10]) for 2 days (5 h per day) in spe-

cial quartz tubes. Different doses of UV-B radiation were obtained by variation of the distances of the culture vessels to the UV lamps (Philips, TL 40/12) and cut-off filters from Schott & Gen., Mainz; WG 305 of 3 mm thickness were used. A control suspension was put into a glass tube (cells not irradiated with UV-B). An additional irradiation with white fluorescent lamps (Osram L 36 W/II, light intensity 1 mW cm^{-2}) was applied, too.

Dry weight was estimated with small glass vessels. Contents of chlorophyll a and phycocyanin were measured after the method of Jones and Myers [11] and that of the carotenoids according to Myers and Kratz [12]. The separation and analysis of amino acids and amides have been carried out by reversed-phase high performance liquid chromatography (HPLC), after precolumn derivatization with o-phthaldialdehyde, using an equipment of Beckman Instruments (Model 342). The mobile phases of the gradient solvent system were a sodium acetate 0.05 N, pH 6.8:methanol:tetrahydrofurane (80:19:1) and methanol:sodium acetate (80:20). For more details see [13].

Cyanobacteria harvested after treatment with UV-B were resuspended in a nitrogen-free medium for ^{15}N-experiments and in a normal fresh nutrient solution for ^{14}C-experiments. For the experimental procedures a special assimilation chamber of plexiglass was used. ^{14}C- and ^{15}N-compounds were added

under steady state conditions. Samples have been collected by a syringe after different photosynthetic periods. ^{14}C-labelled products were separated by thin-layer chromatography. The used procedures have been described in more detail by Döhler [14]. The assimilation of ^{15}N-nitrate has been studied in parallel experiments using the same suspension. Combustion of the samples was carried out according to the method of Dumas [15] with a special equipment after Dr. G. Hentschel, Stuttgart-Hohenheim. ^{15}N-analysis was performed with a Zeiss Statron NOI-5 atomic emission spectrophotometer. For further details see [16, 17].

Results

Table I presents the data of dry weight, pigment and protein contents of *Anabaena* and *Synechococcus* cells grown for 2 days without (control) and with additional UV-B irradiation (5 h per day). It is clearly shown that phycocyanin was strongly affected by UV-B in both cyanobacteria, even after exposure to a low UV-B dose. The contents of chlorophyll a, carotenoids and protein in *Anabaena* were markedly reduced after exposure to the highest UV-B dose, only. Similar results have been obtained in experiments with cultures of *Synechococcus leopoliensis*. Biomass production (dry weight), contents of protein and carotenoids of both species were slightly en-

Table I. Effect of different doses of UV-B radiation (439, 717, 1230 and 1405 J m^{-2}d^{-1}, weighted according to Caldwell, 1971) on dry weight, protein, chlorophyll a, phycocyanin and carotenoid content of *Anabaena cylindrica* and *Synechococcus leopoliensis* grown under normal air conditions (0.035 vol.% CO$_2$) at 23 °C and 32 °C, respectively. Experiments were performed 3-fold, but results of a representativ experiment are shown. For other details see Materials and Methods.

	Dry weight [mg/ml]	[%]	Protein [mg/ml]	[%]	Chlorophyll a [mg/ml]	[%]	Phycocyanine [mg/ml]	[%]	Carotenoids [mg/ml]	[%]
Synechococcus leopoliensis										
Control	14.49	100	0.261	100	0.025	100	0.111	100	0.022	100
439 J m^{-2}d^{-1}	15.31	105.66	0.235	89.98	0.025	100	0.080	72.17	0.024	110.0
717 J m^{-2}d^{-1}	14.89	102.76	0.274	105.01	0.025	100	0.061	54.85	0.025	111.11
1230 J m^{-2}d^{-1}	15.09	104.14	0.245	93.73	0.020	80	0.039	35.82	0.022	100
Anabaena cylindrica										
Control	17.39	100	0.788	100	0.049	100	0.293	100	0.024	100
439 J m^{-2}d^{-1}	18.15	104.4	0.849	107.7	0.047	95.2	0.253	86.3	0.024	100.4
717 J m^{-2}d^{-1}	18.33	105.4	0.895	113.5	0.052	106.9	0.264	90.1	0.028	116.5
1230 J m^{-2}d^{-1}	18.34	105.5	0.921	116.8	0.042	84.8	0.150	51.3	0.025	102.4
1405 J m^{-2}d^{-1}	17.18	98.8	0.582	73.8	0.029	59.2	0.098	33.6	0.017	68.5

hanced after exposure to relative low levels of UV-B (439 and 717 J m^{-2}d^{-1}, weighted according to Caldwell, [10]. But enhanced levels of UV-B (> 1230 J m^{-2}d^{-1}) caused a reduction of these tested parameters. Summarizing, chlorophyll *a* was only affected by UV-B radiation using doses of > 717 J m^{-2}d^{-1}. Phycocyanin was the most sensitive compound to UV-B irradiance tested; low doses can cause a significant reduction. Enhanced levels of UV-B (717 J m^{-2}d^{-1}) led to a strong depression of the phycocyanin content (see Table I). Growth of *Anabaena* and *Synechococcus* was affected by high doses of UV-B irradiance (> 1230 J m^{-2}d^{-1}), only.

In further experiments the effect of UV-B radiation on pool sizes of amino acids and primary amides has been tested. Table II presents data of the pool sizes of free amino acids of both cyanobacteria without and with UV-B treatment (717 and 1405 J m^{-2}d^{-1}, weighted). After UV-B radiation the total amino acid content was enhanced with increasing UV-B doses for both cyanobacteria. But each amino acid showed a different behaviour with respect to UV-B exposure. It should be emphasized that glutamine being the most abundant free amino acid was reduced markedly after exposure to high levels of UV-B (1405 J m^{-2}d^{-1}). Low dose of UV-B radiation (717 J m^{-2}d^{-1}) resulted in an enhanced pool of glutamine. Pool sizes of the other amino acids have

been either affected by UV-B stress or were significantly enhanced, *i.e.* alanine, aspartic acid, glutamic acid.

Parallel to the study of the pool sizes of amino acids we have also estimated the ^{14}C-bicarbonate and ^{15}N-nitrate assimilation of both cyanobacteria after different photosynthetic periods. A depression of photosynthetic assimilation of ^{15}N-nitrate has been found under enhanced levels of UV-B radiation in both tested cyanobacteria. Fig. 1 shows the effect of UV-B stress on nitrate assimilation in *Synechococcus leopoliensis*. The time course of nitrate uptake into cells not irradiated with UV-B can be characterized by a rapid assimilation within 1 min followed by a lower increase to a steady state. A markedly pronounced lag phase in ^{15}N-nitrate assimilation was observed in *Synechococcus* suspensions after exposure to a relatively low UV-B dose (717 J m^{-2}d^{-1}, weighted), while a significant decrease of nitrate uptake at longer photosynthetic periods tested (5 min) could be found in cells treated with a high UV-B dose (1405 J m^{-2}d^{-1}, weighted). Under the same conditions a similar behaviour was observed using *Anabaena* cells.

UV-B radiation also affects the distribution of ^{15}N-incorporation into the amino acids (see Fig. 2). Both species of the cyanobacteria showed, when exposed to UV-B, an enhanced ^{15}N-excess in glutamine

Table II. Effect of UV-B irradiance (717 and 1405 J m^{-2}d^{-1}, weighted) on pool sizes of amides and free amino acids in *Anabaena cylindrica* and *Synechococcus leopoliensis* extracts. Values are given in μg ml^{-1} and % of total amino acid concentration. Control = cells not irradiated with UV-B. Further details in Materials and Methods.

Amino acids	Anabaena cylindrica						Synechococcus leopoliensis			
	Control [%]		717 J m^{-2}d^{-1} [%]		1405 J m^{-2}d^{-1} [%]		Control [%]		1405 J m^{-2}d^{-1} [%]	
aspartate	3.35	7.3	4.31	8.1	8.68	10.6	3.05	7.4	5.78	8.2
glutamate	4.96	10.8	7.88	14.8	12.39	15.2	7.48	18.1	15.31	21.7
asparagine	0.62	1.4	0.63	1.2	0.35	0.4	0.65	1.6	1.46	2.1
serine	4.71	10.3	3.57	6.7	9.92	12.2	5.02	12.1	9.42	13.4
glutamine	11.91	26.0	18.59	35.0	6.73	8.2	9.99	24.1	6.94	9.8
glycine	4.46	9.7	4.52	8.5	8.32	10.2	4.19	10.1	6.56	9.3
threonine	2.48	5.4	1.79	3.4	4.25	5.2	1.38	3.3	2.67	3.8
arginine	1.86	4.1	2.10	4.0	4.25	5.2	0.76	1.8	1.76	2.5
alanine	3.35	7.3	3.68	6.9	7.08	8.7	2.32	5.6	5.32	7.5
tyrosine	2.23	4.9	1.58	3.0	4.25	5.2	1.54	3.7	2.51	3.6
valine	1.61	3.5	1.37	2.6	3.19	3.9	1.76	4.2	2.73	3.9
phenylalanine	1.36	3.0	0.73	1.4	2.66	3.3	0.22	0.5	1.62	2.3
isoleucine	1.24	2.7	0.95	1.8	2.13	2.6	1.30	3.1	2.48	3.5
leucine	1.24	2.7	1.16	2.2	2.48	3.0	1.27	3.1	2.40	3.4
lysine	0.50	1.1	0.42	0.8	4.96	6.1	0.51	1.2	3.59	5.1
Total amino acids	45.76		53.15		81.62		41.44		70.55	

Synechococcus leopoliensis
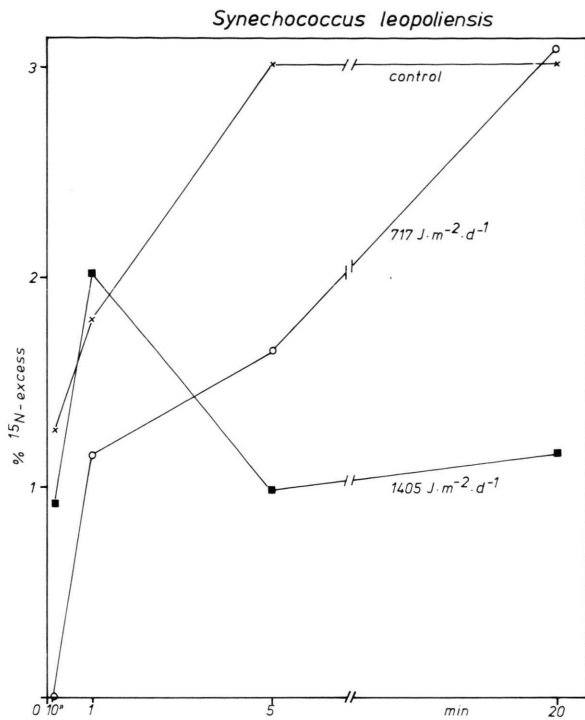
(Gln). UV-B stress caused an increase of ^{15}N enrichment into alanine and glutamic acid of *Synechococcus leopoliensis*. Pronounced differences in ^{15}N-labelling of the other tested amino acids of control and UV-treated algae couldn't be found.

After UV-B treatment a linear depression of photosynthetic $^{14}CO_2$ fixation has been observed in *Anabaena* cells whereas no effect could be observed with *Synechococcus* cells (data not shown here). The impact of UV-B irradiance on the pattern of ^{14}C-labelled photosynthetic products is presented in Figs. 3 and 4. Percentage distribution of radioactivity into photosynthetic products of *Anabaena* was only slightly affected by UV-B radiation using levels of 717 and 1405 J $m^{-2}d^{-1}$ (weighted). No significant ef-

Fig. 1. Effect of UV-B radiation (717 and 1405 J $m^{-2}d^{-1}$, weighted) on uptake of ^{15}N-nitrate ($K^{15}NO_3$, 96 atom %; 1 mM final concentration in the suspension) of *Synechococcus leopoliensis*. After UV-B exposure cells were resuspended in a nitrogen-free nutrient medium; ^{15}N-nitrate was added under steady state conditions after 15 min photosynthesis. The data represent the percentage of ^{15}N of the notal nitrogen content. For further details see Materials and Methods.
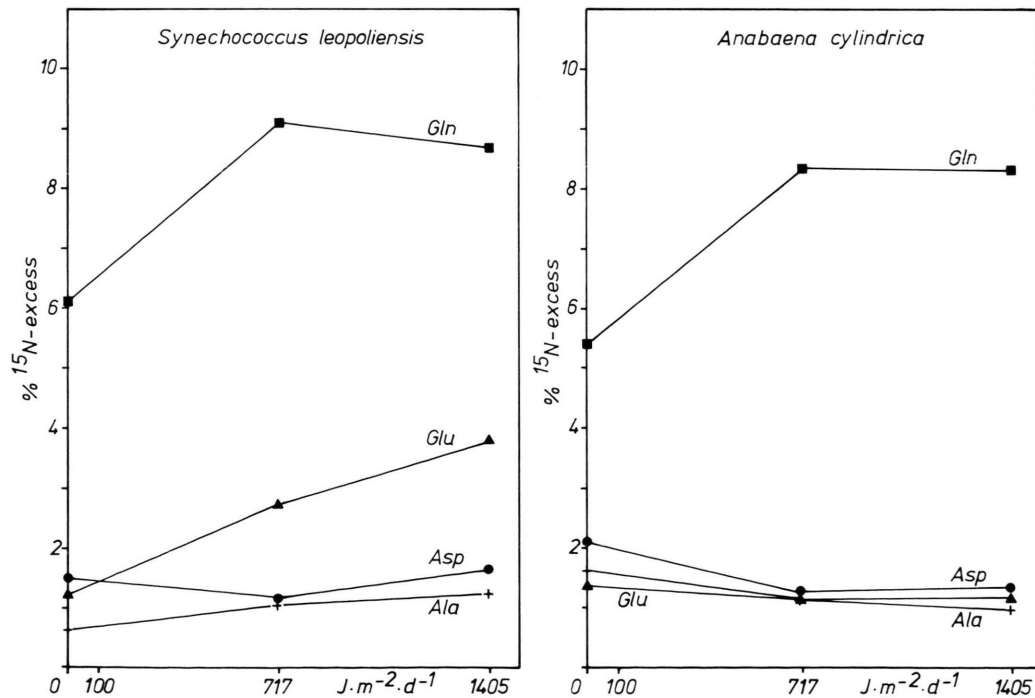

Fig. 2. Effect of UV-B irradiance (717 and 1405 J $m^{-2}d^{-1}$, weighted) on distribution of ^{15}N excess into several amino acids of *Anabaena cylindrica* and *Synechococcus leopoliensis* after 5 min photosynthesis. ^{15}N-nitrate was added after a preillumination period of 15 min. For more details see Materials and Methods.
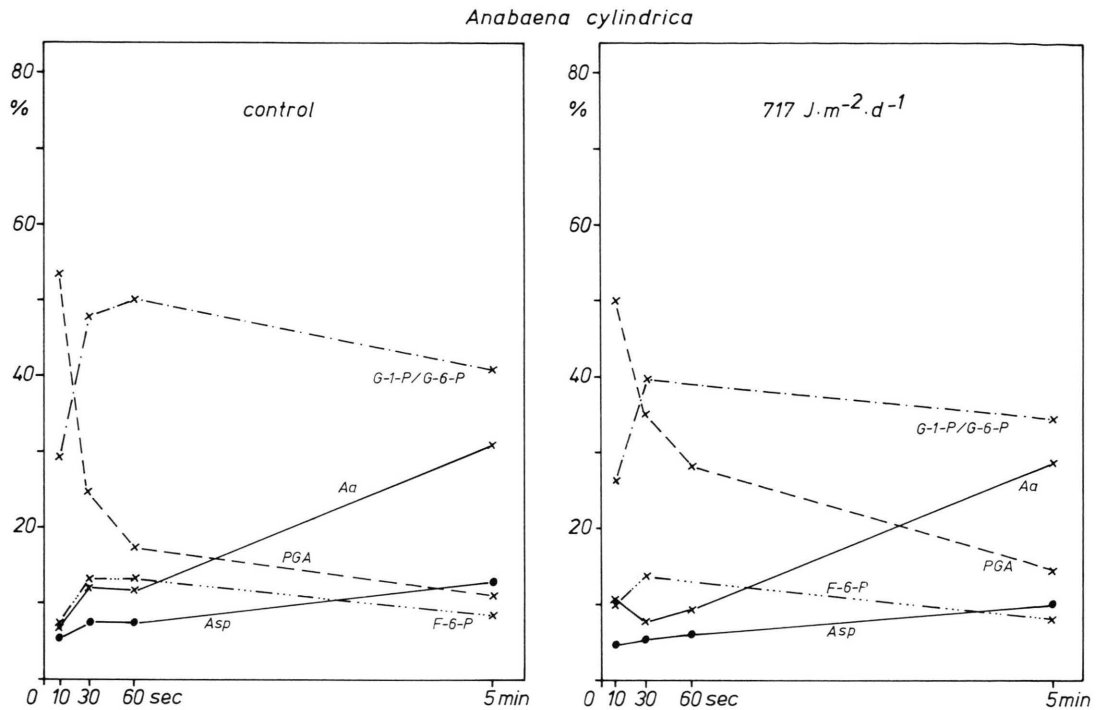
Fig. 3. Effect of UV-B radiation (717 J $m^{-2}d^{-1}$, weighted) on time course of ^{14}C-incorporation into several soluble photosynthetic products (% distribution of total ^{14}C-labelled products) of *Anabaena cylindrica*. ^{14}C-bicarbonate was added after 15 min photosynthesis. *Aa* total amino acids, *Asp* aspartic acid, *F-6-P* fructose-6-phosphate, *G-1-P/G-6-P* glucose-1-phosphate/glucose-6-phosphate and *PGA* 3-phosphoglyceric acid. Further details in Materials and Methods.

fect could be found at ^{14}C-labelling of sugar monophosphates (G-1-P/G-6-P and F-6-P), total amino acids (Aa) and of aspartic acid (Asp), too. The percentage proportion of 3-phosphoglyceric acid (PGA) was enhanced after UV-B stress of *Anabaena* cells (Fig. 3). However, the time course of ^{14}C-incorporation into the photosynthetic products of the coccoid cyanobacterium *Synechococcus* differed from that of *Anabaena* (Fig. 4). An increase of ^{14}C-label in aspartic acid and total amino acids has been found in *Synechococcus* after exposure to UV-B. An enhanced radioactivity of glucose-1-/glucose-6-phosphat (G-1-P/G-6-P) within 1 min photosynthesis was detected in this cyanobacterium after treatment with UV-B. ^{14}C-label of 3-phosphoglyceric acid (% distribution of total soluble products) was significantly reduced in *Synechococcus* cells after exposure to UV-B. Summarizing our results, the impact of UV-B on the pattern of ^{14}C-labelled photosynthetic products was species-dependent and relative independent on the UV-B dose (717 or 1405 J $m^{-2}d^{-1}$).

Discussion

The results presented in this paper show, compared to the results already presented for diatoms [2, 3], that cyanobacteria are less sensitive to UV-B radiation. Biomass production (dry weight) of both tested cyanobacteria species was enhanced after exposure to UV-B up to a dose of 1230 J $m^{-2}d^{-1}$ (weighted [10]); under these conditions a strong reduction (up to 40%) could be observed using marine diatoms [2]. This different behaviour might be explained by the sequence of phylogenetic evolution; diatoms appeared later than cyanobacteria and therefore show more pronounced damage to UV-B. Protein content and pigmentation of cyanobacteria were also less sensitive to UV-B than that of marine diatoms. Only the phycocyanin content was strongly affected (Table I). This dramatically effect can be interpreted by the arrangement of the phycobilisomes near the photosynthetic membranes and the surface of the cyanobacteria cells [18]. The phycobilisomes attached to the thylacoids are more exposed to
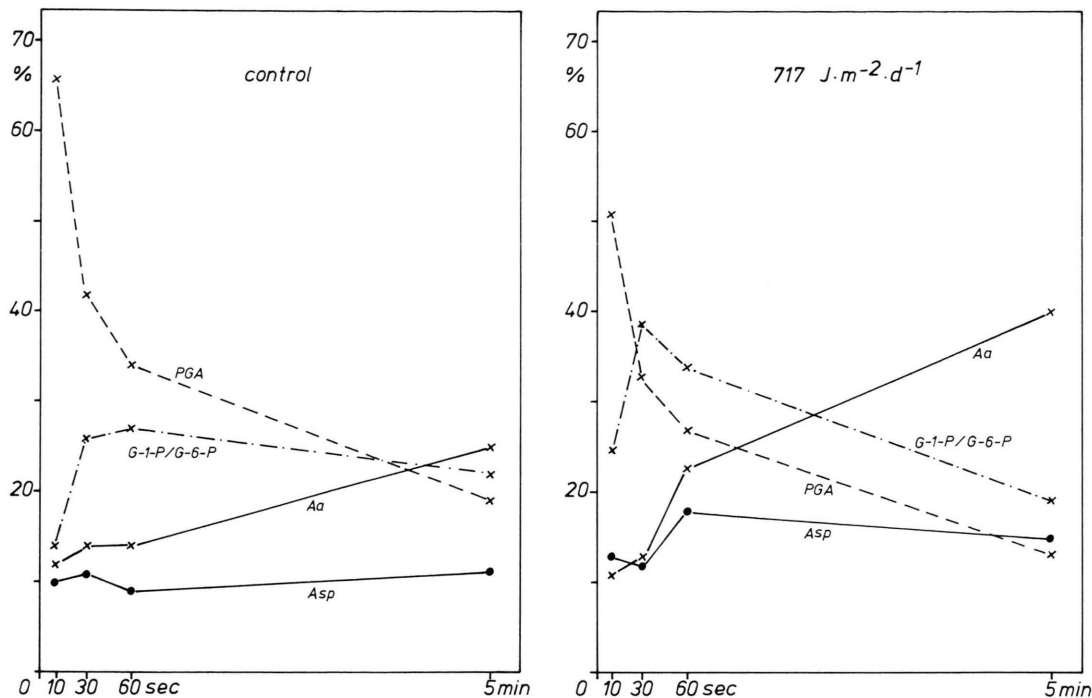
Synechococcus leopoliensis

Fig. 4. Effect of UV-B radiation (717 J m^{-2}d^{-1}, weighted) on time course of ^{14}C-incorporation into various soluble photosynthetic products (% distribution of total ^{14}C-labelled compounds) of *Synechococcus leopoliensis*. For abbreviations and more details see Fig. 3 and Materials and Methods.

UV radiation than chlorophyll *a* and the carotenoids. Therefore, the UV-B damage can be primarily seen in phycocyanin. A significant depletion of the phycocyanin content has also been described in high white light grown *Anabaena* and *Synechococcus* cultures [19, 20].

The observed marked increase of total amino acid content which followed linearly the level of UV-B radiation can be explained by an inhibition of the protein biosynthesis, especially for at 1405 J m^{-2}d^{-1} grown cells. But the enhanced pool of free amino acids of cyanobacteria irradiated with 717 J m^{-2}d^{-1} UV-B can not be interpreted by that way because we found a slightly enhanced protein content under this condition for both tested cyanobacteria. A decomposition of proteins caused by UV-B stress also doesn't seem probably for the lower UV doses.

The pattern of the pool sizes of free amino acids was changed after UV-B radiation; the main variation could be detected in the pools of glutamic acid and glutamine. The enhanced incorporation of ^{15}N

into glutamine at 717 J m^{-2}d^{-1} UV-B coincident with a higher glutamine pool so that an increased synthesis of glutamine could be considered. In contrast to these findings, the amount of glutamine decreased and ^{15}N-incorporation into glutamine increased in cyanobacteria after exposure to high levels of UV-B (1405 J m^{-2}d^{-1}) compared to cells not irradiated with UV-B. A further surprising result of the amino acid metabolism can be seen in the ^{15}N incorporation into the amino acids. The high ^{15}N-labelling of glutamine in both species suggests a possible disturbed transport of the amino group to other amino acids. A damage or inhibition of the glutamate synthase and/or other amino transferases may be involved in cyanobacteria exposed to UV-B radiation, especially in *Anabaena* cells. ^{15}N-labelling of glutamic acid was low in this species whereas ^{15}N-label of glutamine increased with the UV-B dose.

The depression of photosynthetic ^{14}CO$_2$-fixation of cyanobacteria after exposure to enhanced levels of UV-B may be attributed to a damage in the photo-

synthetic apparatus and in a reduction in the ATP and $NADPH_2$ supply. It is known by fluorescence studies using spinach chloroplasts that UV-B radiation affects the photosystem II centre and has no influence on the water-splitting enzyme [5, 21]. A reduction of photosynthetic CO_2 uptake and $^{14}CO_2$ fixation was found in high white light grown *Anabaena* and *Synechococcus (= Anacystis nidulans)* cells, too [19, 20]. This could be attributed to variations in the activities of the key enzymes of the carbon metabolism. Enhancement and depression of the biomass production in marine diatoms – caused by different levels of UV-B radiation – were due to changes in the enzymatic activities. In agreement with these findings UV-B may affect directly the enzymes of the carbon metabolism of the cyanobacteria. Preliminary experiments using marine diatoms have demonstrated such a relationship.

In addition to the results presented in this paper variation in illumination conditions during growth (*e.g.* strong white light) led to a significant increase of ^{14}C-incorporation into amino acids (percentage distribution of soluble products) of *Anabaena* [20]. Similar data were obtained with *Synechococcus* cells especially after exposure to UV-B (see Fig. 4). The enhanced ^{14}C-label of aspartic acid indicates a stimulation of the phosphoenolpyruvate carboxylation. The different behaviour in carbon and nitrogen metabolism (see comparison of time course of ^{14}C- and ^{15}N-incorporation between both cyanobacteria) can be explained by the possibility that *Anabaena* is able to fix molecular nitrogen.

Photosynthetic uptake of ^{15}N-nitrate can also be inhibited by a reduced supply of ATP in UV-B exposed cyanobacteria. On the other hand, the enhanced levels of amino acids in cells irradiated with UV-B (see Table II) may cause a reduction of nitrate uptake, too. It was found in marine diatoms that stress conditions (*e.g.* hypo- and hypertonic nutrient solution during growth, high light intensities and low UV-B dose) result in an increase of the glutamine pool and glutamine synthesis (Fig. 2). Glutamine may act as an effector in the inhibition of nitrate utilization [22]. Therefore, the depression of nitrate uptake of cyanobacteria exposed to UV-B could be attributed to the increased intracellular levels of glutamine. However, it may also be suggested that UV-B directly affects the transport of nitrate via an inhibition of the permease. Further short-term kinetics experiments (^{14}C and ^{15}N) and enzymatic studies are in progress and should give more information on the effect of UV-B irradiance on microorganisms.

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