International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 3 (1): 109-113, 2007
ISSN 1811-9700

Ultraviolet-B Irradiation Alters Amino Acids, Proteins, Fatty Acids Contents and Enzyme Activities of *Synechococcus leopoliensis*

Nadia H. Noaman
Department of Botany, Faculty of Science,
Alexandria University, Alexandria, Egypt

Abstract: In a previous study, four mutants (M_1, M_2, M_3 and M_4) were isolated by exposure of *Synechococcus leopoliensis* to ultraviolet-B irradiation for 5, 10, 15 and 20 min, respectively. Analyses were performed to make a comparison to the effect of UV on the contents of amino acids, proteins, fatty acids and some enzyme activities of the parent type and mutants. The study shows that total proteins and some amino acids in *S. leopoliensis* as lysine and arginine decreased by the exposure to UV irradiation, while aspartic increased. Cysteine, alanine and valine completely disappeared from M_1, M_2 and M_3, respectively, while proline disappeared from M_1 and M_4. A drop in the contents of the aromatic amino acids, phenylalanine and tyrosine occurred in the four mutants. *S. leopoliensis* showed the disappearance of four fatty acids by the exposure to UV for 10 min, While C17: 1, C 8: 0 and C 15: 0 disappeared from M_1, M_3 and M_4, respectively. C14: 0, which was absent at the parent type, appeared at all the mutants and it was found to increase with increasing the exposure time. All fatty acids of *S. leopoliensis,* which were present at the parent type, decreased after exposure to UV for 5 min except C15: 0. 10 min and above caused all the fatty acids to decrease with increasing the exposure time except C6: 0, C10: 0, C11: 0, C14: 0, C15: 0 and C18: 3. There was a drop in C18:3 in *S. leopoliensis* by its exposure to UV for 5 min, which was followed by the increase of that fatty acid with increasing the exposure time. Total saturated, mono, polyunsaturated fatty acids and proteins showed a decrease by increasing the exposure to UV. Enzymes namely μ-esterase, peroxidase and glutamate dehydrogenase were studied for *S. leopoliensis* and the four mutants. Five minutes exposure to UV increased the activity of peroxidase and glutamate dehydrogenase, but lowered the activity of α-esterase by 93%. More than 5 min exposure to UV caused glutamate dehydrogenase activity to decrease with increasing the exposure time, while peroxidase activity increased. The results of decreased UV-absorbers amino acids in the four mutants suggests that the mutants have a defect in the ability to synthesize them to protect the mutants against the damaging affect of UV radiation. The findings also suggests that the increase of exposure to UV-B irradiance not only affect the contents of amino acids, fatty acids and proteins but also alters enzyme activities in *S. leopoliensis*.

Key words: Algae, ultraviolet, amino acids, proteins, fatty acids, enzyme

INTRODUCTION

The ozone layer shields the earth from harmful UV radiation which has shown to adversely affect a number of photochemical and photobiological processes in a wide variety of aquatic organisms. The effects of ultraviolet irradiation on algae have become an important issue as a man-made depletion of the protective ozone layer has been reported. Enhanced ultraviolet irradiation is considered one of the global environmental problems.

There have been many reports concerning the effects of the ultraviolet irradiation to the growth (Xue *et al*., 2005), photosynthesis (Huovinen *et al*., 2006), morphology (Wu *et al*., 2005), ultrastructure (Holzinger and Lutz, 2006), respiration (Kashian *et al*., 2004) and enzyme activities (Shiu and Lee, 2005) of algae.

UV irradiation was also found to increase the levels of DNA damage (De Bakker *et al*., 2005; Roleda *et al*., 2006) and change the contents of sugars (Bhandari and Sharma, 2006), pigments (Kim and Lee, 2005), proteins, amino acids (Korbee *et al*., 2005) and fatty acids (Meireles *et al*., 2003) of algae.

Analyses were conducted to assess the impact of UV-B doses upon amino acids, proteins, fatty acids contents and some enzyme activities of *Synechococcus leopoliensis*.

MATERIALS AND METHODS

Strain and culturing: The parent strain *Synechococcus leopoliensis* Utex 625 was obtained from Utex algal collection, Austen University, USA. Axenic cultures

of *S. leopolienses* were conducted in a culture cabinet and light intensity was 3000 Lux. Culture were grown in GB-11 medium (Vonshak, 1986) under a regime of 16 h light 8 h dark cycles at 35°C and pH 8.

UV-B Irradiation process: Four mutants (M_1, M_2, M_3 and M_4) were previously isolated from *S. leopoliensis* by Noaman *et al.* (2005) by its exposure to UV for 5, 10, 15 and 20 min, respectively. UV lamp was of a low pressure germicidal one (Uvp, Inc. UVGL-25 San Gabriel, CA, USA).

Amino acids determination: Amino acids determination was performed according to the method of Winder and Eggom (1966). The System used for the analysis was high performance, amino acid analyzer (Sykam, GMBH amino acid analyzer, Germany).

Protein determination: Total proteins were determined according to Hartee (1972).

Fatty acids determination: It was performed according to Radwan (1978).

Enzyme activities estimation: Esterase assay was performed according to Ayene *et al.* (1988), peroxidase according to Smith *et al.* (1990), while glutamate dehydrogenase activity was measured as described by Kieronczyk *et al.* (2003).

RESULTS

Table 1 shows the contents of amino acids of the parent type *Synechococcus leopoliensis* and its mutants (M_1, M_2, M_3 and M_4) which were previously isolated by Noaman *et al.* (2005) by exposure of *S. leopoliensis* to UV-B irradiation for 5, 10, 15 and 20 min, respectively.

Some amino acids in *S. leopoliensis* as lysine and arginine decreased by the exposure to UV irradiation, while aspartic increased. It must be mentioned that cysteine, alanine and valine completely disappeared from M_1, M_2 and M_3, respectively, while proline disappeared from M_1 and M_4. A drop in the contents of the aromatic amino acids phenylalanine and tyrosine was noticed in the four mutants especially in M_1 and M_4. Total proteins decreased by increasing the exposure time of *S. leopoliensis* to UV irradiation (Table 1).

S. leopoliensis showed the disappearance of four fatty acids by the exposure to UV for 10 min, while C 17: 1, C 8: 0 and C 15: 0 disappeared from M_1, M_3 and M_4, respectively. C 14: 0, which was absent at the parent type, appeared at all the mutants and it was found to be increase with increasing the exposure time to UV (Table 2).

Table 1: Amino acids contents of the parent type (P) *Synechococcus leopoliensis* grown for 21 days and its mutants M_1, M_2, M_3 and produced by exposure to UV-B irradiation

	Amino acid (μg mL^{-1}) and protein (μg g^{-1} fresh weight) contents				
Compound	P	M_1	M_2	M_3	M_4
Aspartic	24.23	39.04	76.28	70.82	44.58
Glutamic	25.45	13.24	62.62	47.06	21.04
Threonine	52.58	49.24	74.97	68.06	41.72
Serine	37.53	23.55	56.68	60.52	30.94
Glycine	48.96	24.53	110.14	74.00	32.73
Alanine	36.65	12.79	-	34.29	19.01
Leucine	63.02	12.10	91.87	123.22	32.06
Isoleucine	62.35	271.29	92.62	81.67	35.88
Valine	10.23	14.60	45.95	-	14.28
Cysteine	40.86	-	88.77	80.48	26.31
Methionine	41.38	18.81	38.66	37.91	24.11
Histidine	150.67	101.38	157.87	155.46	127.49
Lysine	60.81	21.94	56.66	46.84	31.37
Arginine	81.25	11.31	70.18	45.88	34.95
Phenylalanine	106.11	16.67	93.98	93.39	45.25
Tyrosine	80.51	18.29	74.54	67.87	28.04
Proline	0.55	-	40.81	30.18	-
Total Protein	69.00	68.00	67.72	53.05	28.13

Table 2: Fatty acids contents of the parent type (P) *Synechococcus leopoliensis* grown for 21 days and its mutants produced by exposure to UV-B irradiation

		Fatty acid content (μg g^{-1} fresh weight)				
Fatty acid		P	M_1	M_2	M_3	M_4
Saturated						
	C6:0	0.742	0.151	-	0.207	0.212
	C8:0	0.314	0.115	0.074	-	0.061
	C10:0	0.701	0.078	0.119	0.137	0.263
	C11:0	0.293	0.119	-	0.227	0.154
	C12:0	0.328	0.119	0.118	0.086	0.057
	C13:0	1.553	0.727	0.687	0.567	0.439
	C14:0	-	0.111	0.233	0.487	0.488
	C15:0	1.036	1.847	-	0.254	-
	C16:0	13.822	2.293	2.281	1.657	0.942
	C17:0	0.529	-	0.156	0.112	0.108
	C18:0	1.353	0.252	0.171	0.139	0.138
	C21:0	0.980	0.571	0.558	0.430	0.334
	C22:0	0.546	0.321	0.315	0.232	0.186
Total		22.197	6.704	4.712	4.535	3.38
Mono unsaturated						
	C14: 1	1.061	0.637	0.110	0.109	0.108
	C15: 1	2.703	1.370	1.368	1.071	0.842
	C16: 1	1.773	0.526	0.525	0.520	0.519
	C17: 1	1.775	1.000	0.901	0.751	0.614
	C18: 1	2.729	0.332	0.288	0.287	0.281
Total		10.041	3.865	3.192	2.738	2.364
Poly unsaturated						
	C18: 2	2.347	0.183	_	0.115	0.114
	C18: 3	8.101	1.266	1.308	1.438	1.481
	C22:6	15.552	8.190	8.167	6.074	5.553
Total		26.000	9.639	9.475	7.627	7.148

All fatty acids of *S. leopoliensis*, which were present at the parent type, decreased after exposure to UV for 5 min except C 15: 0. 10 min and above caused all the fatty acids to decrease with increasing the exposure time except C6: 0, C10: 0, C11: 0, C14: 0, C15: 0 and C18: 3. There was a drop in C18:3 in *S. leopoliensis* by its exposure to UV for 5 min, which was followed by the increase of that fatty

Table 3: Enzyme activity of the parent type (P) *Synechococcus leopoliensis* grown for 21 days and its mutants M_1, M_2, M_3 and M_4 produced by exposure to UV-B irradiation

	Enzyme activity (μmole min^{-1})		
Type	Esterase	Peroxidase	Dehydrogenase
P	44	15	3
M_1	2	16	11
M_2	2	17	8
M_3	2	20	8
M_4	2	21	4

acid with increasing the exposure time. Polyunsaturated fatty acid of 22 carbon atoms decreased by increasing the exposure time. Total saturated, mono and polyunsaturated fatty acids of *S. leopoliensis* showed a decrease by increasing the exposure to UV.

Table 3 showed that 5 min exposure to UV was enough to produce stress to *S. leopoliensis* causing esterase activity to be lowered by 93%. Activity of peroxidase enzyme increased with increasing the exposure time of *S. leopoliensis* to UV irradiation, while glutamate dehydrogenase activity decreased with increasing the exposure time except after the first 5 min, which increased the activity nearly four times.

DISCUSSION

Plants produce secondary metabolites that absorb UV and prevent it from penetrating. UV causes oxidative stress as a result of secondary free radical formation (Foyer *et al*., 1994). Cellular UV absorbers as aromatic amino acids can be activated by UV irradiation and react with molecular oxygen and superoxide radicals (Peak and Peak, 1987).

UV- B radiation decreased or increased the contents of UV- absorbing compounds in many algae (Xue *et al*., 2005). Wu *et al*. (2005) observed that there was no significant change in UV-absorbing compounds by exposure of the cyanophyte *Arthrospira platensis*, suggesting that these compounds were not effectively used as protection against UV radiation and Xuo *et al*. (2005) stated that the measurements of UV-B absorbing compounds did not necessarily provide a good indicator of tolerance to UV-B.

The increase of some amino acids and decrease of others in *S. leopoliensis* by exposure to UV-B irradiation were noticed. Alanine decreased at all mutants, a phenomenon which occurred at *Phaeocystis pouchetii* by its exposure to UV-B irradiation, which was discussed by the damaging effect on the uptake of inorganic nitrogen and nitrogen metabolism (Dohler, 1992).

Aspartic acid in *S. leopoliensis*, increased by exposure to UV-B irradiation in contrast to Dohler (1984), who found that aspartic acid decreased in *Lauderia annulata* as a response to UV-B irradiation which was discussed in relation to the impact of UV-B upon carbon and nitrogen metabolism.

Proline showed very high increase in M_2 and M_3, while it disappeared in M_1 and M_4. i.e., they lossed the ability to synthesize proline. A three-fold increase in proline occurred in *Chlamydomonas nivalis* by exposure to UV (Duval *et al*., 1999), which was accounted by stimulation of UV to the biochemical pathways related to proline metabolism. M_2 and M_3 were found to accumulate proline, phenomenon that was detected for the first time by Saradhi *et al*. (1995) who proved that UV-radiation accumulate proline that can protect plant cells against UV-radiation induced peroxidative processes. Although phenylalanine and tyrosine are aromatic amino acids, which can absorb UV-B irradiation (Martin *et al*., 1985), a drop in their contents in the four mutants was noticed i.e., the mutants were found to have a defect in the ability to synthesize UV-B absorbing amino acids.

S. leopoliensis showed the decrease of total proteins by increasing the exposure time. Damage and degradation of protein by UV is proved in algae (Xue *et al*., 2005). Kumar *et al*. (2003) proved the inhibition of nitrogenase enzyme by UV which may be the cause for inhibition of protein synthesis in *S. leopoliensis* or the damage may be due to the ability of protein to absorb UV which was proved by Ziska and Teramura (1992).

In contrast to Bhandari and Sharma (2006) who found that fatty acid profile of *Phormidium corium* did not show any qualitative changes due to exposure to UV-B irradiation, *S. leopoliensis* showed the disappearance of four fatty acids by the exposure to UV for 10 min, While C 17:1, C 8:0 and C 15:0 disappeared from M_1, M_3 and M_4, respectively. C 14: 0, which was absent at the parent type, appeared at all the mutants.

The drop in C 18: 3 in *S. leopoliensis* by its exposure to UV for 5 min was followed by the increase of that fatty acid of 18 carbon atoms with increasing the exposure time, while polyunsaturated fatty acid of 22 carbon atoms decreased by increasing the exposure time. Kobayashi (1998) found an increase in fatty acids of 18 carbon atoms by UV irradiation but that of 20-22 carbon atoms was not affected by the exposure time.

Ultraviolet irradiation increases fatty acids of *Chaetoceros simplex* (Boutry *et al*., 1976) and *Pavalvo lutheri* (Meireles *et al*., 2003). UV irradiation resulted in an increase of polyunsaturated fatty acids and a reduction of saturated fatty acids in *Phaeodactylum tricornutum* and *Chaetoceros muellrei* (Liang *et al*., 2006). Total saturated, mono and polyunsaturated fatty acids of *S. leopoliensis* showed a decrease by increasing the exposure time to UV, which can be discussed by the suggestion of Kobayashi

(1998) that UV causes the splitting of fatty acids and the finding of He *et al*. (2002) that photooxidative damage by UV-radiation, including lipid peroxidation was determined in cyanobacteria.

UV radiation was found to reduce the activity of α-esterase enzyme in *S. leopoliensis* by 93% after 5 min, which proved that UV had more stress effect on esterase enzyme than peroxidase and glutamate dehydrogenase i.e., the most affected enzyme by UV was esterase, which indicated that it was the most sensitive one. α-esterase was proved by Elsalhin (2004) to be the most affected enzyme in *Dunaliella bardawil* by the environmental stress.

Exposure of some marine macroalgae to UV radiation resulted in no significant change in the activity of peroxidase (Aguilera *et al*., 2002). UV radiation was found to increase the activity of peroxidase in *S. leopoliensis* with increasing the exposure time to UV. Peroxidases are mediators of O_2 toxicity and were proved to contribute protection of plants from UV radiation stress (Jansen *et al*., 2001).

S. leopoliensis exposed 10, 15, or 20 min to UV irradiation have lower activities in glutamate dehydrogenase than those exposed for 5 min. UV was found to affect the process of nitrogen assimilation and its key enzymes like glutamate dehydrogenase (Xu and Zhou, 2004), which in turn affect amino acids synthesis, which appeared highly, injured in M_4 (highest exposure time to UV) compared to M_2 and M_3.

REFERENCES

Aguilera, J., A. Dummermuth and U. Karsten, 2002. Enzymatic defences against photooxidation tress induced by ultraviolet radiation in Arctic marine macroalgae. Polar Biol., 25: 432-441.

Ayene, S.I., R.K. Kaleand P.N. Srivastava, 1988. Radioprotective effect of 2-mercaptopropionyl glycine on radiation induced lipid peroxidation an denzyme release in erythrocyte. Int. J. Radiat. Biol., 51: 629-639.

Bhandari, R. and P.K. Sharma, 2006. Effect of UV-B on photosynthesis, membrane lipids and MAAs in marine Cyanobacterium *Phormidium corium* (Agardh) Gomont. Indian. J. Exp. Biol., 44: 330-335.

Boutry, J.L., M. Barbier and M. Ricard, 1976. The marine diatom *Chaetoceros simplex* calcitrans Paulsen and its enviroment. Effects of light and Ultraviolet Irradiation on the biosynthesis of fatty acids. CR Acad. Sci. D., 282:239-242.

De Bakker, N.V., P.M. van Bodegom, and W.H. von de Poll *et al*., 2005. Is UV radiation affecting Charophycean algae in shallow fresh water systems?. New Phytol., 166: 957-966.

Dohler, G., 1984. Effects of UV-B radaition on the marine diatoms *Lauderia annulata* and *Thalassiorsira rotula* grown in different salinities. Mar. Biol., 83: 247-253.

Dohler, G., 1992. Impact of UV-B radiation on uptake of ^{15}N-ammonia and ^{15}N-nitrate by phytoplankton of the Wadden Sea. Mar. Biol., 112: 485-489.

Duval, B., K. Shetty and W. Thomas, 1999. Phenolic compounds and antioxidants properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. J. Applied Phycol., 11: 559-566.

Elsalhin, H.E., 2004. Effect of crude petroleum oil on growth and synthesis of some metabolites in algae M.Sc. Thesis Alex. Univ., pp: 204.

Foyer, C.H., M. Lelandais and K.J. Konert, 1994. Photooxidative stress in plants. Physiol. Plant, 91: 696-717.

Hartee, E.F., 1972. A modification of Lawry method that gives a linear photometric response. Anal. Biochem., 41: 422-430.

He, Y.Y., M. Klisch and D.P. Hader, 2002. Adaptation of cyanobacteria to UV-B stress correlated with oxidative stress and oxidative damage. Photochem. Photobiol., 76: 188-196.

Holzinger, A. and C. Lutz, 2006. Algae and UV irradiation: Effects on ultrastructure and related metabolic functions. Micron., 37: 109-207.

Huovinen, P., I. Gomez and C. Lovengreen, 2006. A five-year study of solar ultraviolet radiation southern Chile (39 degrees S) Potential impact of physiology of coastal marine algae?. Photochem. Photobiol., 82: 515-522.

Jansen, M.A.K., R.E. Noort and M.Y.A. Lagrimini *et al*., 2001. Phenol-oxidizing peroxidases contribute protection of plants from ultraviolet radiation. Plant Physiol., 26: 1012-1023.

Kashian, D.R., B.A. Prusha and W.H. Clemenrs, 2004. Influence of total organic carbon and UV-B radiation on zinc toxicity and bioaccumulation in aquatic communities. Environ. Sci. Technol., 38: 6371-6376.

Kieronczyk, A., S. Skeie and T. Langsrud *et al*., 2003. Cooperation between *Lactococcus lactis* and non-starter lactobacilli in the formation of cheese aroma from amino acids. Applied Environ. Microbiol., 61: 734-739.

Kim, S.C. and D.K. Lee, 2005. Inactivation of algal blooms in eutrophic water of drinking water supplies with the photocatalysis of TiO_2 thin film on hollow glass beads. Water Sci. Technol., 52: 145-152.

Kobayashi, Y., 1998. Change of hydroperoxy fatty acids formed by ultraviolet irradiation of bovien retinas-determination of chemiluminescence assay. Nippon Ganka Gakkai Zassi, 102: 15-21.

Korbee, N., F.I. Figueroa and J. Aguilera, 2005. Effect of light quality on the accumulation of photosynthetic pigments, proteins and mycosporine-like amino acids in the red *Porphyra leucosticta* (Bangiales, Rhodophyta). J. Photochem. Photobiol. B, 80: 71-78.

Kumar, A., M.B. Tyagi and P.N. Jha *et al*., 2003. Inactivation of cyanobacterial nitrogenase after exposure to ultraviolet-B radiation. Curr. Microbiol., 46: 380-384.

Liang, Y., J. Beardall and P. Heaud, 2006. Effects of nitrogen source and UV radiation on the growth, chlorophyll fluorescence and fatty acid composition of Phaeodactylum tricornutum and Chaetoceros muelleri (Bacillariophyceae). J. Photochem. Pholobiol. B, 82: 161-172.

Martin, D.W., P.A. Mayes and V.W. Rodwell *et al*., 1985. Amino Acids and Peptides in: Harper's review of Biochemistry. 20th Edn., Lange Medical Publications, Los Altos California, pp: 21-31.

Meireles, L.A., A.C. Guedes and F.X. Malcata, 2003. Increase of yields of eicosapentaenoic and docosahexaenoic acids by the microalga *Pavalvo lutheri* following random mutagensis. Biotechnol. Bioeng., 81: 50-55.

Noaman, N.H., M.A. Osman and A.M. Khaleafa *et al*., 2005. The application of PCR-RFLP-DNA and protein profiles for the differentiation of *Synchococcus leopoliensis* and its mutants. Egypt J. Biotechnol., 11: 31-40.

Peak, M. and J.G. Peak, 1987. Photosensitized DNA damages. Photochem. Photobiol., 41: 575-582.

Radwan, S.S., 1978. Coupling of two dimensional thin layer chromatography with GC for the quantitative analysis of lipid classes and their constituents fatty acids. J. Chromatograph Sci., 11: 538-542.

Roleda, M.Y., C. Wiencke and D. Hanelt, 2006. Thallus morphology and optical Characteristics affect growth and DNA damage by radiation in Juvenile *Arctic lamenaria* sporophytes. Planta, 223: 407-417.

Saradhi, P.P., S. AliaArora and K.V.S.K. Prasad, 1995. Proline accumulates in plants exposed to uv radiation and protects them against uv-induced peroxidation. Biochem. Biophys. Res. Commun., 209: 1-5.

Shiu, C.T. and T.M. Lee, 2005. Ultraviolet-B-induced oxidative stress and responses of the ascorbate-glutathione cycle in the marine macroalga *Ulva fasciata*. J. Exp., 56: 2851-2865.

Smith, A.T., N. Santama and S. Dacey *et al*., 1990. Expression of a synthetic gene for horse radish peroxidase C in *Escherichia coli* and folding and activation of the recombinant enzyme with Ca^{2+} and heme. J. Biol. Chem., 261: 13335-13343.

Vonshak, A., 1986. Laboratory Techniques for the Cultivation of Microalgae. In: CRC Handbook of Microalgae Mass Culture. Richmond, A. (Ed.), CRC Press Inc. Boca Raton, Florida, pp: 117-145.

Winder, K. and O.B. Eggum, 1966. Protein hydrolysis Adescription method used at the department of animal physiology in Copenhagen. Acta Agric. Scandinavia, 11: 115-123.

Wu, H., K. Gao and V.J.E. Villafane *et al*., 2005. Effects of solar UV radiation on morphology and photosynthesis of filamentous cyanobacterium *Anthrospira plantensis*. Applied Environ. Microbiol., 71: 5004-5013.

Xu, Z. and G. Zhou, 2004. Research advance in nitrogen metabolism of plant and its environmental regulation. Ying Yong Sheng Tai Xue Bao, 1: 511-516.

Xue, L., Y. Zhang and T. Zhang *et al*., 2005. Effects of enhanced ultraviolet-B radiation on algae and cyanobacteria. Crit. Rev. Microbiol., 31: 79-89.

Xuo, Y., Q. Liu and B. Lin *et al*., 2005. Physiological responses of 2 year-old *Acer davidil* seedlings to short-term enhanced UV-B radiation. Ying Yong Sheng Tai Xue Bao, 1: 1682-1986.

Ziska, L.H. and A.H. Teramura, 1992. CO_2 enhancement of growth and photosynthesis in Rice (*Oryza sativa*): Modification by increased ultraviolet-B radiation. Plant Physiol., 99: 473-481.