Gilmont, Susan

From: nobody@www.orst.edu
Sent: Monday, January 29, 2001 2:20 PM
To: Valley Circulation; Valley Circulation
Subject: Circulation Request

Below is the result of your feedback form. It was submitted by (on Mon Jan 29 14:20:28 PST 2001
---
recipient: valley.circ@orst.edu
subject: Circulation Request
Name: Eric Henry
Campus Phone: 737-3622
Second Phone: 757-8159
Univ Mailing Address: Dept. Chemical Engineering
Home Mailing Address: 1415 NW Garfield Ave
Corvallis, OR 97330
E-mail: henrye@bcc.orst.edu
Fax No.: 737-4600
Type of Request: Guin/HMSC Article
Book Author:
Title:
Call Number:
Journal Title: Botanica Marina
Article Author: Zhou, Z-G, and Lui, Z-L
Article Title: Effects of selenium on lipid peroxidation in Spirulina maxima
Journal Call Number: QK564 .B6
Volume No.: 40(2)
Date: 1997
Pages: 107-112
Comments:
submit.x: 71
submit.y: 16

Copy - Prospero

Effects of Selenium on Lipid Peroxidation in *Spirulina maxima*

Z.-G. Zhou[a,*,1] and Z.-L. Liu[b]

[a] Institute of Oceanology, Academia Sinica, Qingdao 266071, P. R. China
[b] Department of Biological Science and Technology, Nanjing University, Nanjing 210093, P. R. China

* Corresponding author

The oxidation-sensitive lipo-soluble materials of *Spirulina maxima* Setch. *et* Gardn. grown in the absence or presence of added selenium were analysed. Selenium could induce a decrease in the contents of total lipids, carotenoid and polyunsaturated fatty acids, such as linolenic acid, eicosadienoic acid, but brought about an increase in saturated fatty acids, such as capric acid and stearic acid. Lower concentrations of selenium enhanced chlorophyll *a* content, whereas concentrations higher than 20 mg L^{-1} lowered the chlorophyll *a*. On the basis of these results, there was no evidence for an *in vivo* functioning of selenium as an antioxidant. Instead, the observed decrease in content of oxidation-sensitive lipids of *Spirulina maxima* can best be explained as a selenite-induced oxidation effect. In order to confirm this conclusion, lipid peroxidation was measured in the alga cultured under various selenium concentrations. Based on an increase in the content of malondialdehyde (MDA) in the alga, a cause for the lipid decrease induced by selenium was that selenium *in vivo* might lead to lipid peroxidation. Hydroxyl radical (HO·) was detected directly using dimethyl sulfoxide (DMSO) as a molecular probe and quantified as the stable compound methanesulfinic acid. The HO· content of the alga grown in the presence of added selenite was lower than that of the control, and this provided direct evidence of selenium functioning as a scavenger for HO·. However, the change of HO· content did not correlate significantly with that of lipid peroxidation in the alga cultured under various selenium concentrations. This suggested that HO· was not responsible for the initiation of lipid peroxidation. According to the result detected by electron spin resonance (EPR), lipid peroxidation might be related to organic radicals.

Introduction

Possibly with the exception of accumulator plants, selenium has no effect on higher plants (Lauchli 1993), whereas microquantities of it could stimulate the growth or development of algae, such as the marine diatom *Thalassiosira pseudonana* (Hust.) Hasle *et* Heimdal (Price *et al.* 1987), the red alga *Porphyridium cruentum* (Ag.) Nag. (Wheeler *et al.* 1982), the blue-green alga *Phormidium luridum* var. *olivagea* Boresch (Sielicki and Burnham 1973), and the green alga *Chlamydomonas reinhardtii* Dangeard (Yokota *et al.* 1988). As a micronutrient for humans and animals, it might protect them against lipid peroxidation induced by cisplatin and alloxan (Wachowicz and Szwarocka 1994, Yadev *et al.* 1994). In contrast to this observation, it has been reported that selenium is able to induce a decrease of oxidation-sensitive lipids in the red alga *Porphyridium cruentum* (Gennity *et al.* 1985). It might cause lipid peroxidation in this red alga, but there is no direct evidence to prove this hypothesis. It has not been demonstrated that selenium correlates with lipid peroxidation in these algae.

In living organisms active oxygen radicals may attack biological macromolecules and produce biological free radicals, resulting in direct or indirect cellular damage (Halliwell and Gutteridge 1984a). Of the oxygen radical species, the hydroxyl radical (HO·) is the most reactive in chemical properties. Since Halliwell and Gutteridge (1984b) reported that HO· scavengers could inhibit the initiation of lipid peroxidation in several *in vitro* superoxide-generating systems, it has been suggested that HO· is responsible for the initiation of lipid peroxidation (Klotz *et al.* 1989).

Although a selenium concentration lower than 40 mg L^{-1} has an improvement effect on the growth of the filamentous blue-green alga, *Spirulina maxima* Setch. *et* Gardn., there is an inhibition effect on this alga if the concentration becomes higher (Zhou *et al.* 1995). On the basis of the facts that selenium has been shown to cause antioxidativity in animals but has the effect of a decrease in oxidation-sensitive lipids in a red alga (Gennity *et al.* 1985, Wachowicz and Szwarocka 1994, Yadav *et al.* 1994), we designed the present study to relate changes in malondialdehyde (MDA), an early product of lipid peroxidation, and changes in percentage of fatty acids, to the effect of selenium on lipid peroxidation in *Spirulina maxima*. Using dimethyl sulfoxide (DMSO) as a molecular

[1] Present address: Institute of Oceanology, Academia Sinica, 7 Nanhai Road, Qingdao 266071, P.R. China

probe, we utilize a newly reported method (Babbs *et al.* 1989) to detect HO· directly and quantify the formation of HO· *in vivo* in this alga. Relating the change in HO· to that of organic radicals assayed by an electron spin resonance (EPR), we investigate further the effect of selenium on the algal lipid peroxidation, in order to find out whether HO· is necessary for initiation of lipid peroxidation or not.

Materials and Methods

Algal species and culture conditions

Axenic cultures of *Spirulina maxima* Setch. *et* Gardn. were maintained in Zarrouk's liquid medium (Borowitzka 1988), which was prepared with deionized distilled water, in a growth chamber at 35 °C with a photoperiod of 16 : $\overline{8}$ h, L : $\overline{D}$ for 12 d. Illumination was provided by 40 W fluorescent lights and light intensity was 160 μmol $m^{-2} s^{-1}$. The cultures were continuously agitated by gentle bubbling with filtered air. When selenium was added, sodium selenite was present at various concentrations of mg L^{-1} selenium.

Lipid peroxidation

The method of Heath and Packer (1968) as modified by Dhindsa *et al.* (1981) was used to measure MDA, an early product of lipid peroxidation.

Algal cells were filtered using a 300-mesh silk net, and were washed with the above medium without additional sodium selenite. Some of the algal slurry was stored at −30 °C and freeze-dried by lyophilizer (Labconco). Another portion of the slurry was resuspended in a 0.2 mol L^{-1} citrate-phosphate buffer (pH 6.5) containing 0.5% Triton X-100, and disintegrated by an ultrasonic processor (Type JY-250) at 150 W for 2 min at an interval of 5 s in an ice bath. The homogenate was centrifuged at 20 000 *g* for 15 min, and the protein of the supernatant was determined by the procedure described by Bradford (1976) using bovine serum albumin (BSA) as a standard.

The supernatant (2 mL) was added to the same volume of 20% (w/v) trichloroacetic acid (TCA) containing 0.5% (w/v) 2-thiobarbituric acid. The MDA contents were determined according to the procedure described by Popham and Novacky (1991). The concentration of MDA was expressed as nmol mg^{-1} protein. Three replicates were performed for each treatment.

Free radicals

A known quantity of lyophilized algal cells were placed in quartz tubes, and the organic radicals in them were assayed by an electron spin resonance (EPR) (ER 200-D-SRC, Braker) at room temperature. The areas of EPR signal energy absorbance curves were analysed on the basis of the procedure described by Sheng *et al.* (1981).

The hydroxyl radical (HO·) was monitored with an assay similar to that developed by Babbs *et al.* (1989). Dimethyl sulfoxide was used as a molecular probe to trap HO·, because it is exceedingly non-toxic and can be tolerated by living systems at up to 1 mol L^{-1} concentration (Ashwood-Smith 1975) and because it is rapidly absorbed and distributed to all tissue compartments (Denko *et al.* 1967). Dimethyl sulfoxide is oxidized to form a single stable product, methane sulfinic acid (MSA), which was assayed by a color reaction with the diazonium salt, fast blue BB dye, after the removal of interfering lipophilic compounds by extraction and filtration.

Spirulina maxima was inoculated in test tubes containing 50 mL Zarrouk's medium, with various concentrations of selenium and 2 mL DMSO. In the control the alga was inoculated only with 2 mL DMSO in the medium. Axenic culture, collection, washing, lyophilization, and weighing were conducted according to the method described above.

Lyophilized samples were resuspended in 2 mL deionized distilled water, and pulverized with a mortar and pestle in liquid nitrogen. The supernatant was collected after centrifugation at 10 000 *g* for 20 min, and the volume was made up to 10 mL by adding deionized distilled water. The content of MSA of the supernatant was detected according to the procedure reported by Babbs *et al.* (1989). The concentration of sulfinic acid was calculated from an MSA standard curve, and MSA concentration of sample was expressed in nmol MSA g^{-1} dry weight (DW). Three replicates were performed for each treatment.

Lipid extraction and column chromatography

The lyophilized algal cells (1 g) were macerated with 100 mL isopropanol twice (24 h each time) at room temperature under N_2 (William 1982). The supernatant was collected after centrifugation at 2000 *g*, and the residue was shaken overnight with 100 mL chloroform ($CHCl_3$)-isopropanol (v/v, 1 : 1) under N_2. The combined extracts were evaporated almost to dryness under reduced pressure. The total lipid conglomerate was dissolved in a mixture of $CHCl_3$-methanol (MeOH)-H_2O before partition into organic and aqueous phases by the method reported by Folch *et al.* (1957). The organic phase containing lipid was removed. Then $CHCl_3$ and MeOH were added to the aqueous phase and the Folch partition was repeated once again to ensure quantitative lipid extraction. The combined organic phase was bubbled with N_2 at room temperature to remove the solvent, and was dissolved in 3 mL $CHCl_3$. To determine individual lipid fatty acid composition, the total lipids were separated into neutral and polar lipid fractions by silica column chromatography as described by Piorreck *et al.* (1984). A glass column (30 cm in height and 3.5

cm in inner diameter) was filled with a suspension of silica gel 60 in $CHCl_3$. The total lipid sample was transferred to the column and elution of the neutral fraction was first performed with $CHCl_3$. This fraction also contained all of the pigments. Subsequently, the polar fraction was eluted with MeOH. The major portion of the solvent of every eluted fraction was removed by evaporation under reduced pressure. Each fraction of lipid was then transferred in $CHCl_3$ to a preweighed test tube, concentrated in N_2 and dried to a constant weight in a vacuum desiccator.

Preparation of fatty acid methyl esters and gas chromatography

The fatty acid methyl esters (FAMEs) of the neutral and polar lipids were prepared by transmethylation with methanotic HCl under N_2 as described by Williams (1978). The FAMEs were quantified by GC-5A (Shimadzu). The flame ionization detector (FID) consisted of a stainless steel column (~3 m × 3 mm) packed with polyethylene glycol succinate (PEGS) (20% on Chromosorb W, 60~80 mesh). the column temperature was 160 °C and the flow rate, 40 mL N_2 min^{-1}. The injector and detector temperature were 260 °C

Determination of chlorophyll *a*, carotenoid and total protein

Chlorophyll *a* and carotenoid contents of the lyophilized algal cells were determined according to the method described by Jensen (1978). Total protein of the dried algal cells was estimated by the method of Kjeldahl (Zhang 1990).

Results

Spirulina maxima cultured in both the absence and presence of selenite was simultaneously harvested at the same stage of exponential growth (i. e. 12 d). The contents of total protein, chlorophyll *a*, and carotenoid of lyophilized algal cells were plotted against the selenium concentration (Fig. 1). In comparison with the control, the protein contents of the alga grown in additional selenite were lower, and decreased as selenium was increased. Likewise, similar changes in carotenoid occurred in the alga. However, the chlorophylll *a* of the alga cultured in the presence of selenium was higher than that in its absence, and it varied only slightly from 4 to 40 mg L^{-1} Se.

In order to study the effects of selenium on the algal oxidation-sensitive lipids, we analysed the lipo-soluble content (Table I) and fatty acid composition (Table II) of *Spirulina maxima* in the presence and absence of 8 mg L^{-1} added selenium as selenite. Compared with the control, 8 mg L^{-1} selenium induced a 15.75% decrease of total lipids in the alga. In this fraction, only neutral lipids decreased by 41.15%, whilst the polar lipids generally remained unaltered. Thus, selenium affected neutral lipids but not the polar ones. Although no significant difference of carotenoid was observed between the cases associated with the presence and absence of 8 mg L^{-1} added selenium, a descending tendency of carotenoid content

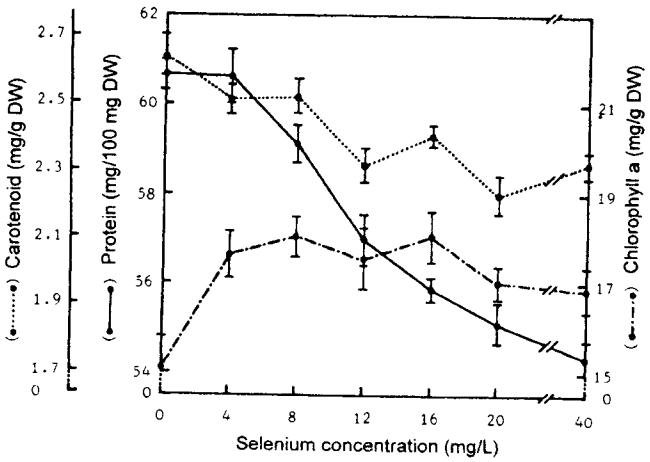

Fig. 1. Variations of carotenoid, chlorophyll *a* and protein contents in *Spirulina maxima* cultured in the absence or presence (e. g. from 4 to 40 mg L^{-1}) of selenium. The data are expressed as means ± SE (n = 3).

Table I. The contents of lipo-soluble materials from *Spirulina maxima* cultured in the absence or presence of additional 8 mg L^{-1} Se.

	Control	+ Se
Total lipids	132.54	111.66
Neutral	52.33	30.78
Polar	80.21	80.88
Chlorophyll *a*	15.08 ± 0.73[1]	18.04 ± 0.45[1]
Carotenoid	2.645 ± 0.061[1]	2.525 ± 0.051[1]

[1] The data are expressed as means ± SE (n = 3).

Table II. Fatty acid composition (% total fatty acids) of *Spirulina maxima* grown in the absence or presence of added 8 mg L^{-1} Se.

Fatty acid	Neutral lipids		Polar lipids	
	Control	+ Se	Control	+ Se
10 : 0	6.09	8.33	0.67	0.99
16 : 0	38.45	31.47	51.32	51.43
16 : 1	–[1]	–[1]	2.29	2.27
18 : 0	1.73	9.07	1.64	1.66
18 : 1	13.03	17.45	15.84	17.05
18 : 2ω6	7.27	12.44	10.49	9.93
18 : 3ω3	21.93	18.32	0.62	0.18
18 : 3ω6(γ)	8.87	2.92	14.69	15.13
20 : 0	–[1]	–[1]	0.70	0.43
20 : 2ω6	–[1]	–[1]	1.33	0.58

[1] Not detectable.

was found under the concentrations ranging from 4 to 40 mg L^{-1} (Fig. 1).

Compared with polar lipids (Table II), palmitoleic acid (16 : 1), arachidic acid (20 : 0) and eicosadienoic acid (20 : 2ω6) were not found in neutral lipids in the presence or absence of selenium, although selenium cannot alter fatty acid composition in *Spirulina maxima*. In neutral lipid the percentages of γ-linolenic acid (18 : 3ω6) and linolenic acid (18 : 3ω3) were reduced by 67.08% and 16.46%, respectively, but oleic acid (18 : 1) and linoleic acid (18 : 2ω6) were enhanced by 33.92% and 71.11%, respectively, by culturing in selenite-containing medium. The respective decreases of 70.97% and 56.39% of linolenic acid (18 : 3ω3) and eicosadienoic acid (20 : 2ω6) in polar lipid was observed in *Spirulina maxima* cultured in the presence of 8 mg L^{-1} selenium. All these changes led to an increase in the percentage of saturated fatty acids. For example, the percentage of capric acid (10 : 0) increased by 37.78% and 47.76%, respectively, in neutral and polar lipids when cultured in selenite-containing medium. The most marked increase in saturated fatty acid of the neutral lipids was stearic acid (18 : 0), which was 4 times more than that of the alga cultured without 8 mg L^{-1} selenium, whereas the contents of other saturated fatty acids in the presence or absence of 8 mg L^{-1} selenium, such as palmic acid (16 : 0), and arachidic acid (20 : 0), were relatively stable.

The MDA content of *Spirulina maxima* was estimated (Fig. 2) as a product of lipid peroxidation. In comparison with the control, the content of MDA of the alga cultured under the addition of selenium was higher, and it reached the highest at both 20 and 40 mg L^{-1} selenium. This provides direct evidence that selenium causes lipid peroxidation in *Spirulina maxima*.

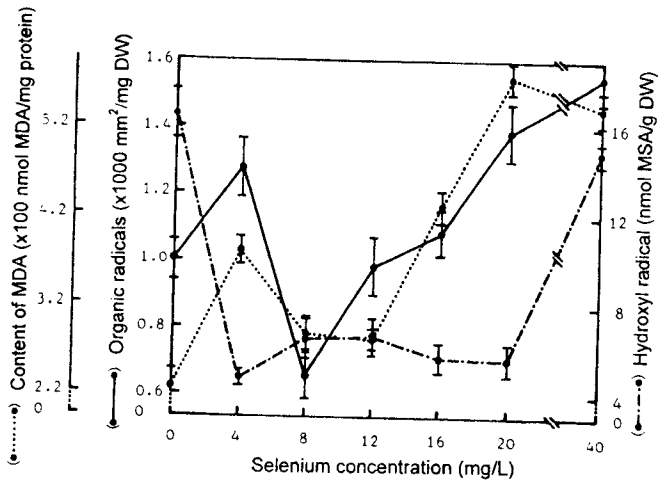

Fig. 2. Variations of malondialdehyde (MDA), hydroxyl and organic radical contents in *Spirulina maxima* grown in the absence or presence (e. g. from 4 to 40 mg L^{-1}) of selenium. Organic radical is assayed by an Electron Spin Resonance (EPR), and its content is expressed as mm^2 mg^{-1} dry weight based upon EPR signal energy absorbance curve. Hydroxyl radical and MDA contents are expressed as described in Materials and Methods. Error bars indicate the standard deviation of the mean of three replicates.

In order to explain the changes described above, especially those in polyunsaturated fatty acid and MDA, the organic radicals and hydroxyl radical of *Spirulina maxima* were subsequently evaluated (Fig. 2). It was found that the content of hydroxyl radical in *Spirulina maxima* grown in the presence of selenium was lower than that of the control, although it increased to 14.83 nmol MSA g^{-1} DW at 40 mg L^{-1} selenium. The hydroxyl radical remained stable at the lower level in selenium levels from 4 to 20 mg L^{-1}. This alteration differed from that of MDA. In contrast to the change of HO·, the organic radicals detected by an EPR had a similar trend of change to that of MDA, except at 8 and 12 mg L^{-1} selenium, the contents of organic radicals were higher than those of control. The pattern of changes in both organic radicals and hydroxyl radical suggested that selenium functioned as an eliminator for hydroxyl radical only, but it might result in either the production or the accumulation of organic radicals. Similar changes in both organic radicals and MDA also indicated that there the former is responsible for the production of the latter.

Discussion

The blue-green alga *Spirulina maxima* grows rapidly while being cultured under the concentrations ranging from 4 to 40 mg L^{-1} selenium as selenite, especially at 8 and 12 mg L^{-1} selenium (Zhou *et al.* 1995). A reason for this may be the higher activity of glutathione peroxidase, which is able to reduce H_2O_2 and hinder the generation of hydroxyl radical (Stadtman 1980, Zhou *et al.* 1995). It is observed that selenium can result indirectly in a decrease in MSA. However, the present observation that the oxidation-sensitive lipid content actually decreased, but the MDA content increased in *Spirulina maxima* grown in selenite, indicates that selenium is unable to enhance the antioxidant defences of the algal cells.

As the most reactive one in the oxygen species, the hydroxyl radical is generally considered to attack membrane lipids *in vitro* and initiate a chain reaction of lipid peroxidation (Halliwell and Gutteridge 1984a). From this, it can be deduced that selenium will improve the antioxidant defences of the algal cells if it leads to a decrease in the hydroxyl radical.

Selenium actually causes a decrease in neutral lipids and total lipids of this alga (Table I). Meanwhile, it induces a decrease of oxidation-sensitive lipids (carotenoid, linolenic acid, γ-linolenic acid, and eicosadienoic acid) (Table II), some of which, based upon the limited data available, appear to be accentuated in high concentrations of selenium (e. g. carotenoid, chlorophyll *a*) (Fig. 1).

There is other direct evidence to show that the peroxidation is induced by selenium and that is the in-

crease of MDA in the alga cultured in the presence of added selenite (Fig. 2). The decrease of oxidation-sensitive lipids and the increase of MDA strongly suggest that selenite exerts an oxidant effect. To a greater extent selenium affects this alga if the concentration is enhanced within the medium. Such an observation is also confirmed by earlier researchers who have reported that at both low and high light intensities, there is a decrease in oxidation-sensitive lipids of the red alga *Porphyridium cruentum* cultured in selenite (Gennity *et al.* 1985).

Selenium causes an increase of chlorophyll *a* in *Spirulina maxima* at low concentrations, but there is a decline when the selenium concentration is raised (Fig. 1). This observation is remarkably similar to the reported increase in chlorophyll *a* in the blue-green alga, *Phormidium luridum* var. *livagea* at low concentrations (10^{-6} mol L^{-1}) of selenium, and the decrease at higher concentrations (from 10^{-5} mol L^{-1}) (Sielicki and Burnham 1973). According to the reported data, the chlorophyll content of a green alga *Dunaliella primolecta* Butcher is not affected while growing in selenate (Gennity *et al.* 1985), and selenium inhibits porphobilinogen synthase activity and reduces the total chlorophyll content in light grown mung bean seedlings (Padmaja *et al.* 1989). These results and the present study show that selenium has a different effect on chlorophylls of organisms in response to variations in concentration; or that these organisms have a physiological difference while cultured in the presence of selenium. As one kind of oxidation-sensitive lipid, chlorophyll *a* tends to decrease while *Spirulina maxima* is cultivated at higher concentrations of selenium. This fact also shows that a high content of additional selenium will cause significant oxidation of the alga.

A decrease in the protein content of *Spirulina maxima* cultured in the presence of added selenium is a reminder that selenium is also able to oxidize sulfhydryl groups and that *in vitro*, it can replace the sulphur of cysteine at the iron-sulphur centre of ferredoxin (Stadtman 1979). An iron-sulphur protein is believed to be the primary electron acceptor and ferredoxin the next electron acceptor of photosystem I. When electron transport in PSI is blocked so that the primary electron acceptor becomes reduced, superoxide radical is produced (Bowler *et al.* 1992) to initiate a chain reaction. The resultant macromolecules, which are less stable and more reactive than normal ones, will easily lead to metabolic dysfunctions (Halliwell and Gutteridge 1984 a). If selenium has this effect on PSI, then active oxygen capable of oxidizing lipids will be generated so that selenium is able to lead to an increase in organic radicals assayed by an EPR.

Since the change in the hydroxyl radical differs from that of MDA in *Spirulina maxima* cultured under increasing concentrations of selenium, the hydroxyl radical is not necessary for the initiation of lipid peroxidation, although it is the most reactive of the oxygen species. This observation is also remarkably similar to that found in cucumber seedlings during bacteria-induced hypersensitive reaction (Popham and Novacky 1991). Meanwhile, Minotti and Aust (1987) have also demonstrated that HO· scavengers do not inhibit the *in vitro* lipid peroxidation initiated by iron and H_2O_2. Some investigators suggest that HO· will not migrate so rapidly from the site of its formation, because it is a highly reactive species (Svingen *et al.* 1978).

It is concluded that organic radicals estimated by an EPR might initiate lipid peroxidation from the similarity of the changes between them and MDA, as they are another type of oxygen radical. In the meantime, a decline in carotenoid is also responsible for lipid peroxidation (Fig. 1), because carotenoid can quench singlet oxygen extremely rapidly (Knox and Dodge 1985). So it is suggested that selenium might first cause superoxide radical by damaging PSI, and cause singlet oxygen by decreasing carotenoid, both of which would produce organic radicals consequently. Then the organic radicals would initiate lipid peroxidation, but not the hydroxyl radical.

References

Ashwood-Smith, M. J. 1975. Current concepts concerning radioprotective and cryoprotective properties of dimethyl sulfoxide in cellular systems. *Ann. N. Y. Acad. Sci. 243*: 246–256.

Babbs, C. F., J. A. Pham and R. C. Coolbaugh. 1989. Lethal hydroxyl radical production in paraquat-treated plants. *Plant Physiol. 90*: 1267–1270.

Borowitzka, M. A. 1988. Algal growth media and sources of algal cultures. *In*: (M. A. Borowitzka and L. J. Borowitzka, eds) *Micro-algal Biotechnology*. Cambridge University Press, Cambridge. pp. 456–465.

Bowler, C., M. V. Montagu and D. Inze. 1992. Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol. 43*: 83–116.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem. 717*: 1448–1454.

Denko, C. W., R. M. Goodman, R. Miller and T. Donovan. 1967. Distribution of dimethyl sulfoxide in the rat. *Ann. N. Y. Acad. Sci. 141*: 77–84.

Dhindsa, R. S., P. Plumb-Dhindsa and T. A. Thorpe. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalyse. *J. Exp. Bot. 32*: 93–101.

Folch, J., M. Lees and G. H. S. Slanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem. 226*: 497–509.

Gennity, T. M., N. R. Bottino, R. A. Zingaro, A. E. Wheeler and K. J. Irgolic. 1985. A elenite-induced de-

crease in the lipid content of a red alga. *Phytochemistry 24*: 2823–2830.

Halliwell, B. and J. M. C. Gutteridge. 1984a. Role of iron in oxygen radical reaction. *In*: (L. Packer, ed.) *Methods in Enzymology*. Academic Press, New York. pp. 47–56.

Halliwell, B. and J. M. C. Gutteridge. 1984b. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J. 219*: 1–14.

Heath, R. L., L. Packer. 1968. Photoperoxidation in isolated chloroplasts. 1. Kinetics and stoichiometry of fatty peroxidation. *Arch. Biochem. Biophys. 125*: 189–198.

Jensen, A. 1978. Chlorophylls and carotenoids. *In*: (J. A. Hellebust and J. S. Craigie, eds) *Handbook of Phycological Methods: Physiological and Biochemical Methods*. Cambridge University Press, Cambridge. pp. 59–70.

Klotz, M. G., R. Hoffman and A. Novacky. 1989. The critical role of the hydroxyl radical in microbial infection of plants. *In*: (J. Dainty, M. I. DeMichelis, E. Marre and F. Rasi-Caldogno, eds) *Plant Membrane Transport: The Current Position*. Elsevier, Amsterdam. pp. 657–662.

Knox, J. P. and A. D. Dodge. 1985. Singlet oxygen and plants. *Phytochemistry 24*: 889–896.

Lauchli, A. 1993. Selenium in plants: Uptake, function, and environmental toxicity. *Bot. Acta 106*: 455–468.

Mintti, G. and S. D. Aust. 1987. The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. *J. Biol. Chem. 262*: 1098–1104.

Padmaja, K., D. D. K. Prasad and A. R. K. Prasad. 1989. Effect of selenium on chlorophyll biosynthesis in mung bean seedlings. *Phytochemistry 28*: 3321–3324.

Piorreck, M. K-H. Baasch and P. Pohl. 1984. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry 23*: 207–216.

Popham, P. L. and A. Novacky. 1991. Use of dimethyl sulfoxide to detect hydroxyl radical during bacteria-induced hyper-sensitive reaction. *Plant Physiol. 96*: 1157–1160.

Price, N. M., P. A. Thompson and P. J. Harrison. 1987. Selenium: An essential element for growth of the coastal marine diatom *Thalassiosira pseudonona* (Bacillariophyceae). *J. Phycol. 23*: 1–9.

Sheng, P. G., Z. A. Shen and H. L. Wang. 1981. A simple assay of spin number by an EPR. *Anal. Chem. (China) 9*: 471–475. (In Chinese)

Sielicki, M. and J. C. Burnham. 1973. The effect of selenite on the physiological and morphological properties of the blue-green alga *Phormidium luridum* var. *olivagea*. *J. Phycol. 9*: 509–514.

Stadtman, T. C. 1979. Some selenium-dependent biochemical progresses. *Adv. Enzymol. 48*: 1–28.

Stadtman, T. C. 1980. Selenium-dependent enzymes. *Annu. Rev. Biochem. 49*: 93–110.

Svingen, B. A., F. O. O'Neal and S. D. Aust. 1978. The role of superoxide and singlet oxygen in lipid peroxidation. *Photochem. Photobiol. 28*: 803–809.

Wachowicz, B. and A. Szwarocka. 1994. Responses of pig flood platelets to cisplatin and sodium selenite: Lipid peroxidation and oxygen radical generation. *Biomed. Letters 49*: 147–152.

Wheeler, A. E., R. A. Zingaro and K. Irgolic. 1982. The effects of selenate, selenite, and sulfate on the growth of six unicellular marine algae. *J. Exp. Mar. Biol. Ecol. 57*: 181–194.

William, W. C. 1982. The isolation of lipids from tissues. *In*: (W. C. William, ed.) *Lipid Analysis*. 2nd Ed. Pergamon Press, Great Britain. pp. 17–23.

Williams, J. P. 1978. Glycerolipids and fatty acids of algae. *In*: (J. A. Hellebust and J. S. Craigie, eds) *Handbook of Phycological Methods: Physiological and Biochemical Methods*. Cambridge University Press, Cambridge. pp. 99–107.

Yadav, P., S. Sarkar and D. Bhatnagar. 1994. Protective effect of glutathione and selenium against alloxan induced lipid peroxidation and loss of antioxidant enzymes in erythrocytes. *J. Biosci. 19*: 19–25.

Yokota, A., S. Shigeoka, T. Onishi and S. Kitaoka. 1988. Selenium as inducer of glutathione peroxidase in low-CO_2-grown *Chlamydomonas reinhardtii*. *Plant Physiol. 86*: 649–651.

Zhang, Z. L. 1990. Assay for the content of protein. *In*: (Z. L. Zhang, ed.) *Laboratory Guide to Plant Physiology*. 2nd Ed. Higher Education Press, Beijing. pp. 175–185. (In Chinese)

Zhou, Z. G., Z. L. Liu and H. J. Chu. 1995. The influence of selenium on the antioxidation of *Spirulina maxima*. *J. Phycol.* (Suppl.) *31*: 17.