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A SELENITE-INDUCED DECREASE IN THE LIPID CONTENT OF A RED ALGA

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Key Word Index—*Dunaliella primolecta*; *Porphyridium cruentum*; unicellular marine red algae; lipid oxidation; selenium.

Abstract—Two unicellular marine algae (*Dunaliella primolecta* and *Porphyridium cruentum*) have been found to contain a selenium-inducible, non-enzymatic glutathione peroxidase activity when cultured in the presence of selenite. To test the possibility that selenium functions *in vivo* as an antioxidant in these algae, a detailed examination of the lipid content of algae cultured in the presence or absence of selenite was conducted. If selenium augments the antioxidant defenses of algal cells, an increase in the content of oxidation-sensitive lipids would be expected. The fatty acid, chlorophyll, phospholipid and glycolipid content of the green alga *D. primolecta* was not affected by growth in selenite. At low light intensity there was a moderate decrease in the chlorophyll and polyunsaturated fatty acid content of the red alga *P. cruentum* when cultured in selenite. At higher light intensity the content of all fatty acids, phospholipid, glycolipid, chlorophyll, carotenoid and phycoerythrin decreased in *P. cruentum* grown in selenite. Since growth in selenite did not increase the quantity of oxidation-sensitive lipids in either alga, there is no evidence for an *in vivo* functioning of selenium as an antioxidant. Instead, the observed decrease in lipids of the red alga *P. cruentum* can best be explained as a selenite-induced oxidative effect.

INTRODUCTION

The presence of selenite in the culture medium has been shown to improve the growth of a wide variety of unicellular marine algae [1]. Growth in the presence of added selenite was able to double a non-enzymatic, glutathione peroxidation activity in cell free extracts of two of these algae [2]. This selenite-induced enhancement of GSH reduction of H_2O_2 and organic hydroperoxides is probably due to the presence of selenium compounds within the algal cell. Algae grown in selenite contain a variety of selenium containing molecules such as selenocystine and selenomethionine [3] which are able to enhance the rate of GSH-mediated hydroperoxide reduction [2, 4]. These observations suggest that selenium may exert a glutathione peroxidase-independent antioxidant effect in marine algae which results in improved growth.

To test this hypothesis, experiments were conducted utilizing the two algae, *Dunaliella primolecta* Butcher and *Porphyridium cruentum*, which are known to have selenium-induced non-enzymatic, apparent glutathione peroxidase activity [2]. Chloroform–methanol extracts from algae cultured in the presence and absence of selenite were examined for quantitative changes in the level of oxidation-sensitive lipids (chlorophyll, carotenoid, polyunsaturated fatty acids). No qualitative variation in the glycerolipid, pigment or fatty acid content of algae cultured in selenite has been observed [5]. If selenium is acting as an antioxidant *in vivo*, an increase in the cellular content of oxidation-sensitive lipids would be anticipated in algae cultured in selenite containing medium. Analogous experiments have revealed decreases in the polyunsaturated fatty acid content of vitamin E and selenium-deficient animals [6]. This approach should also reveal any selenium induced inhibition of fatty acid synthesis. The interaction of selenite with liver fatty acid synthetase sulphydryl groups is responsible for the *in vivo* loss of enzyme activity in selenium-injected chicks [7].

RESULTS

In the first experiment, logarithmic *D. primolecta* or *P. cruentum* were grown simultaneously in either the absence or presence of selenite under constant illumination from fluorescent bulbs (115 μ einstein/m^2/sec). The cellular content of *D. primolecta* total lipid, neutral lipid (sterol esters, triacylglycerols and unesterified fatty acids) and polar lipid (phospholipid and glycolipid) fatty acids is shown in Table 1. No selenite-induced changes were observed. Likewise, no selenium-induced alteration in the chlorophyll, phospholipid or glycolipid content of *D. primolecta* was observed (Table 2).

When the fatty acid content of the red alga *P. cruentum* was examined (Table 3) a 30% decrease in the total lipid content of eicosapentaenoic acid (20:5ω3) from selenite-treated alga was observed. Upon separation of the total lipid into neutral and polar lipid fractions, it became evident that the selenite-induced decrease in 20:5ω3 content was entirely in the polar lipid fraction. A more moderate 25% decrease in the hexadecenoic (16:1) and arachidonic (20:4ω6) acid content of polar lipids was observed in selenite-treated algae. These decreases were

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✠ Deceased.

Table 1. Fatty acid composition of *D. primolecta* grown in the absence and presence of added selenite ($\mu g/10^8$ cell)

	Total lipids		Neutral lipids		Polar lipids	
Fatty acid*	−Se	+Se	−Se	+Se	−Se	+Se
14:0	3.3±0.4†	3.4±0.4	4.4±0.9	4.7±0.8	1.6±0.6	1.3±0.2
14:1	0.44±0.2	0.42±0.1	0.49±0.6	0.33±0.2	0.57±0.2	0.41±0.1
14:2	7.0±0.9	7.3±0.6	4.3±1.3	4.8±0.8	4.8±0.4	5.2±0.4
16:0	100±8.1	90±7.3	27±6.5	23±2.7	95±3.3	96±7.1
14:3+16:1‡	11±0.4	11±1.1	1.4±0.9	1.5±0.3	12±1.0	13±1.1
16:2+17:1	3.4±0.4	5.4±0.7	2.4±2.0	2.7±0.8	3.9±0.7	6.5±0.6
16:3±18:0	12±0.8	14±1.4	2.7±1.2	2.0±0.4	12±2.3	16±2.4
16:3+18:1‡	26±2.4	23±1.8	11±3.1	9.8±2.3	25±6.3	23±2.3
16:4	51±2.0	49±3.4	2.8±1.0	2.5±0.4	48±2.3	57±4.3
18:2ω6+ω9	39±1.8	38±3.3	12±3.7	11±1.7	40±8.8	41±5.6
18:3ω6	21±0.8	20±1.2	7.8±2.1	6.7±0.7	18±1.1	20±1.8
18:3ω3	140±6.4	140±8.7	20±6.5	16±2.5	140±1.7	150±15
18:4	1.8±0.1	1.6±0.1	1.0±0.7	0.73±0.3	1.8±0.1	1.7±0.2

*The number before the colon indicates the number of carbons in the fatty acid while the number following the colon indicates the number of double bonds.

†Standard deviation of the mean of three replicates.

‡More than one isomer may be present.

Table 2. Chlorophyll, lipid phosphorus and lipid carbohydrate content of *D. primolecta* and *P. cruentum* grown in the absence and presence of added selenite ($\mu g/10^8$ cell)

	D. primolecta		*P. cruentum*	
	−Se	+Se	−Se	+Se
Chlorophyll *a*	71.6±9.9*	83.1±8.2	6.8±0.45	5.2±0.82†
Chlorophyll *b*	47.9±6.8	53.6±5.0	—	—
Total chlorophyll	120±16.7	137±6.1	—	—
Phosphorus	4.30±0.14	4.16±0.22	4.8±0.21	4.4±0.72
Hexose	91.4±16.4	95.7±9.02	—	—

*Standard deviation of the mean of three replicates.

†Significantly lower than algae grown in the absence of added selenite as determined by the one-tailed Students *t*-test at the $p < 0.05$ level.

Table 3. Fatty acid composition of *P. cruentum* grown in the absence and presence of added selenite ($\mu g/10^8$ cell)

	Total lipids		Neutral lipids		Polar lipids	
Fatty acid*	−Se	+Se	−Se	+Se	−Se	+Se
16:0	92±9.8†	90±7.8	17±2.3	18±3.5	94±4.5	89±15
16:1‡	9.2±0.8	6.7±1.4	0.52±0.2	0.62±0.3	9.7±0.5	7.0±1.0§
18:0	2.3±1.1	3.4±0.4	1.3±0.2	1.7±0.4	5.7±1.5	4.4±0.3
18:1‡	6.0±0.8	6.2±0.5	2.3±0.1	2.5±0.5	0.11±0.1	0.13±0.0
18:2ω6	23±2.0	26±3.8	10±1.3	11±2.6	18±2.1	18±2.9
20:2ω6	5.7±1.2	7.3±1.4	0.38±0.4	0.90±0.1	8.3±0.5	9.0±1.7
20:4ω6	77±11	74±12	22±3.0	19±3.6	75±4.4	61±6.7§
20:5ω3	32±5.3	22±2.1	6.8±3.1	5.2±0.9	36±4.7	21±1.2‖

*The number before the colon indicates the number of carbons in the fatty acid while the number following the colon indicates the number of double bonds.

†Standard deviation of the mean of three replicates.

‡More than one isomer may be present.

§‖Significantly lower than algae grown in the absence of added selenite as determined by the one-tailed Students *t*-test at the $p < 0.05$ (§) and $p < 0.01$ (‖) level.

also observed following the HPLC analysis of fatty acid bromoacetophenone derivatives. The decreases in 16:1 and 20:4ω6 content are not apparent when total lipid fatty acids are examined due to the presence of neutral lipid fatty acids which exhibit no selenite induced changes. A 25% decrease in the chlorophyll *a* content, but no change in the phospholipid content of *P. cruentum* grown in selenite was observed (Table 2). The glycolipid content of this red alga could not be determined due to the presence of large quantities of non-lipid carbohydrate (presumably from the polysaccharide sheath surrounding the algae) contaminating the chloroform–methanol extracts.

In the experiments just described, *P. cruentum* grown in the presence or absence of selenite were both harvested after 27 days of growth. Since selenite improves the growth of *P. cruentum*, the selenite-treated cells had progressed further into the exponential phase ($0.61 \pm 0.15 \times 10^6$ cell/ml) than algae grown in the absence of selenite ($0.34 \pm 0.05 \times 10^6$ cell/ml). It therefore seemed possible that the selenite-induced alteration in *P. cruentum* lipid content was actually a growth related change. This possibility was tested by the following experiment.

Porphyridium cruentum was grown in the absence or presence of 10 ppm Se as selenite under constant illumination (195 μ einstein/m^2/sec). For technical reasons the light intensity used in this and the following experiment was greater than that utilized in the first experiment described. Algae cultured both in the absence and presence of selenite were simultaneously harvested at the same stage of exponential growth and their lipids extracted and analysed concurrently. Furthermore, three different periods during exponential growth were examined. Cells were in early exponential growth after 20 days of culture ($0.79 \pm 0.12 \times 10^6$ cell/ml), mid-exponential growth by 28 days ($1.8 \pm 0.15 \times 10^6$ cell/ml) and in the late exponential phase by 35 days ($2.1 \pm 0.12 \times 10^6$ cell/ml).

The cellular content of total fatty acid and major individual fatty acids of total lipids from *P. cruentum* grown in the absence and presence of selenite is shown in Fig. 1. Total lipids were not separated into neutral and polar lipid fractions in this experiment. There is a selenite induced decrease in cellular total fatty acids of roughly 25% at each time period examined during exponential growth at high light intensity. All of the individual fatty acids present in the selenite-treated algae were lowered to some extent. The most pronounced decrease was in the 20:5ω3 content which at day 20 was reduced by 55%. The content of other major fatty acids; palmitate (16:0), hexadecenoate (16:1), and arachidonate (20:4ω6) were reduced 25% to 40% by culture in selenite-containing medium. Linoleate (18:2ω6) and eicosadienoate (20:2ω6) (data not shown) content was only marginally reduced (0–20%) in the presence of selenite.

Both the chlorophyll *a* and carotenoid content of selenite-treated cells grown at high light intensity was reduced 40% by day 20 (Fig. 2). This decrease became even more marked with time, reaching 60% by day 35. The selenite-induced 50% decline in the absorption at 668 nm of sonicated algae (Table 4) confirms the observation that the extracted chlorophyll *a* content is reduced 60% during late exponential growth. The phospholipid content was reduced 20% to 30% by the presence of selenite during growth (Fig. 2).

During harvest of *P. cruentum* at day 28, a noticeable bleaching of the red coloration of selenite treated cells was observed. In order to quantify this apparent decrease in biliprotein, the cellular content of protein, phycoerythrin and chlorophyll *a* was estimated at the next time period (day 35). These data are shown in Table 4. The cellular content of protein was unaltered by growth in the

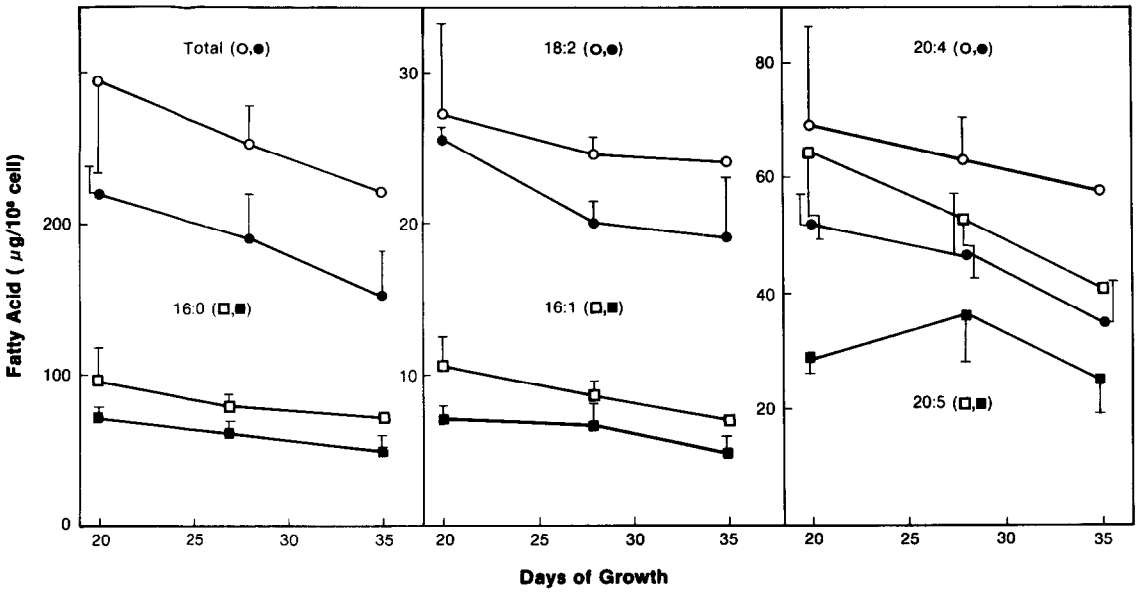

Fig. 1. The fatty acid content of *P. cruentum* cultured in the absence (open symbols) or presence (closed symbols) of 10 ppm selenium as added selenite. The cellular content of total fatty acid, palmitate (16:0), linoleate (18:2), hexadecenoate (16:1), arachidonate (20:4) and eicosapentaenoate (20:5) are shown. Error bars indicate the standard deviation of the mean of three replicates.

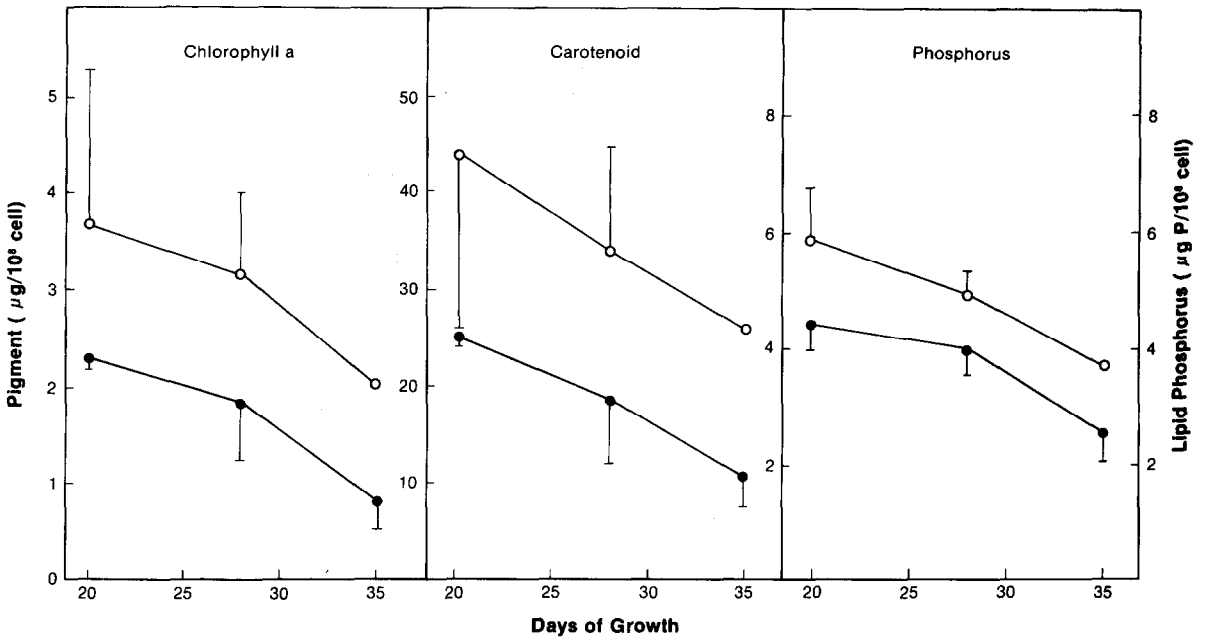

Fig. 2. The pigment and phospholipid content of *P. cruentum* cultured in the absence (open squares) or presence (closed squares) of 10 ppm selenium as selenite. Error bars indicate the standard deviation of the mean of three replicates.

Table 4. Pigment and protein content of *P. cruentum* cultured for 35 days in the presence or absence of added selenite

	−Se	+Se
B-phycoerythrin (μg/mg protein)	538 ± 65.1*	357 ± 106†
Chlorophyll *a* (absorption at 668 nm/mg protein)	0.067 ± 0.016	0.032 ± 0.021†
Protein (mg/10^8 cell)	4.34 ± 0.64	4.11 ± 1.02

*Standard deviation of the mean of three replicates.

†Significantly lower than value from algae grown in the absence of added selenite as determined by the one-tailed Students *t*-test at the $p < 0.10$ level.

presence of selenite, but B-phycoerythrin content was reduced by 35% and chlorophyll *a* was reduced by 50%.

To determine whether the selenite induced fatty acid decrease is specific to a particular individual lipid, the following experiment was conducted. *P. cruentum* was cultured in either the presence or absence of 10 ppm Se as selenite under constant illumination at high light intensity (195 μ einstein/m^2/sec). Large quantities of algae were harvested during early exponential growth (30 days). Lipids were extracted, individual polar lipids were isolated and their fatty acid composition was determined. Since the selenite-induced decrease in 20:5ω3 content is proportionally greater than decreases in other fatty acids during early exponential growth (Fig. 1), those lipid classes exhibiting this marked decline in 20:5ω3 content should be apparent when a comparison of their fatty acid compositions is made.

The selenite-induced decrease in polar lipid 20:5ω3 content is apparent in all phospholipids (Table 5) and glycolipids (Table 6) present in *P. cruentum*. The decrease is not restricted to a particular lipid class. The decrease in 20:5ω3 content is most noticeable in the glycolipids which normally contain the greatest proportion of this fatty acid. The apparent increases in 16:0 and 20:4ω6 in Tables 5 and 6 are an artifact of the method utilized to express the data. The values in a column represent a percentage of the weight of all the fatty acids in that lipid. A quantitative decrease in the percentage of 20:5ω3 necessitates an increase in the other fatty acid percentages and is most noticeable in those fatty acids (16:0 and 20:4ω6) which comprise the greatest proportion of the total.

The observation that most cellular 20:5ω3 is present in glycolipids of *P. cruentum* provides indirect evidence that selenite also induces a decrease in the cellular content of glycolipid. Since the content of 20:5ω3 is reduced by selenite (Table 3 and Fig. 1) and most 20:5ω3 is present in glycolipids (Table 6) which make up 50% by weight of the polar lipid fraction (the other 50% being phospholipid), it follows that the cellular content of glycolipid must also be reduced by growth in the presence of selenite. Growth in selenite does not induce formation of lyso-glycolipids.

Table 5. Effect of selenium on the fatty acids of *P. cruentum* phospholipids (weight percent)

	PC†		PE†		PG†		PI†	
Fatty acid*	−Se	+Se	−Se	+Se	−Se	+Se	−Se	+Se
14:0	tr‡	0.1	0.4	0.4	0.3	0.4	0.3	0.8
16:0	18.4	23.3	38.6	42.4	37.6	42.7	44.3	44.6
16:1§	tr	0.3	4.7	0.3	37.1	35.5	1.4	2.1
18:0	1.3	0.2	3.1	0.6	1.5	2.6	2.3	0.5
18:1§	1.4	2.4	0.7	1.1	0.4	0.7	2.1	2.9
18:2ω6	8.5	12.8	3.2	6.2	0.8	1.5	40.5	39.6
18:3ω6	0.4	0.6	0.3	0.4	tr	tr	0.1	tr
18:3ω3	—	—	tr	tr	tr	—	tr	tr
20:2ω6	0.9	1.1	0.1	0.2	0.3	0.4	0.8	0.8
20:3ω6	1.3	1.4	0.4	1.4	—	—	0.3	0.4
20:4ω6	53.4	50.1	36.6	39.6	4.8	8.5	4.6	3.5
20:5ω3	13.4	5.4	11.0	7.0	13.2	6.3	1.6	0.8
Unknowns	1.4	2.3	0.9	0.4	4.0	1.4	1.7	4.0

*The number before the colon indicates the number of carbons in the fatty acid while the number following the colon indicates the number of double bonds.

†PC = phosphatidyl choline, PE = phosphatidyl ethanolamine; PG = phosphatidyl glycerol; PI = phosphatidyl inositol.

‡Trace.

§More than one isomer may be present.

Table 6. Effect of selenium on the fatty acids of *P. cruentum* glycolipids (weight percent)

	MGDG†		DGDG†		SQDG†	
Fatty acid*	−Se	+Se	−Se	+Se	−Se	+Se
14:0	0.3	0.7	0.3	0.5	0.1	0.3
16:0	26.6	32.4	44.8	39.6	49.5	50.6
16:1‡	0.1	0.4	0.5	0.4	0.1	0.5
18:0	2.8	0.5	1.1	0.4	3.7	0.6
18:1‡	0.8	1.1	0.6	1.0	1.9	3.6
18:2ω6	21.9	26.1	11.9	17.7	1.2	2.4
18:3ω3	tr§	0.3	—	tr	1.0	1.1
20:2ω6	0.3	—	0.3	0.5	19.8	24.0
20:3ω6	0.3	0.4	0.1	0.3	0.1	0.4
20:4ω6	9.2	16.7	7.3	17.8	4.0	8.9
20:5ω3	37.9	20.2	32.8	20.7	18.4	7.3
Unknowns	0.4	1.2	0.3	1.1	0.2	0.3

*The number before the colon indicates the number of carbons in the fatty acid while the number following the colon indicates the number of double bonds.

†MGDG = monogalactosyl diglyceride; DGDG = digalactosyl diglyceride; SQDG = sulfoquinovosyl diglyceride.

‡More than one isomer may be present.

§Trace.

DISCUSSION

The green alga *Dunaliella primolecta* and red alga *Porphyridium cruentum* grow more rapidly when cultured in the presence of added selenite [1]. Growth in selenite induces a non-enzymatic hydroperoxide reductase activity in both algae [2] which is probably attributable to selenium compounds such as selenite or selenocystine, selenomethionine and dimethyl diselenide which are produced metabolically by the algae [3]. The present observation that the content of oxidation-sensitive lipids is unaltered in *D. primolecta* and actually decreased in *P. cruentum* grown in selenite, indicates that *in vivo* selenium does not enhance the antioxidant defenses of the algal cell. There is no evidence to indicate that selenium has an antioxidant function in marine algae or that glutathione peroxidase is present [2].

Instead, growth of *P. cruentum* in selenite induces a

decrease in the cellular content of oxidation-sensitive lipids (chlorophyll, cartenoid, arachidonate and eicosapentaenoate) which, based upon the limited data available, appears to be accentuated at higher light intensities. While any explanation of the selenite induced lipid depletion remains speculative, the present observation, when considered in light of previous findings, strongly suggest that selenite exerts an oxidant effect. Selenite has been observed to cause the bleaching of chlorophyll, inhibition of photosynthesis and cellular breakdown of a blue-green algae [8]. Increased fatty acid peroxidation has been reported to occur in vitamin E and selenium deficient rats following injection with selenite [9].

During early exponential growth at low light intensity, the chlorophyll and eicosapentaenoate content of *P. cruentum* is reduced by 25% and 30%, respectively (Tables 2 and 3). At higher light intensity chlorophyll and carotenoids are reduced by 40% (Fig. 2) while eicosapentaenoate content has decreased by 55% (Fig. 1). At late exponential growth the pigment content of selenite grown cells had been further depleted to 40% of control values (Fig. 2). The observed decrease in extracted chlorophyll *a* was confirmed by measuring the selenite-induced reduction of absorption at 668 nm in crude cell homogenates (Table 4). Those lipids most sensitive to oxidation (chlorophyll, carotenoid, eicosapentaenoate) are decreased preferentially by selenite and the extent of depletion is enhanced under conditions (high light intensity) which favour oxidative damage to lipids.

At low light intensity, hexadecenoate was the only fatty acid other than the polyunsaturated arachidonate and eicosapentaenoate which exhibited a selenite-induced decline (Table 3). This observation is remarkably similar to the reported decrease, attributed to lipid oxidation, of only hexadecenoate and polyunsaturated (trienoic) fatty acids in illuminated isolated chloroplasts [10].

At high light intensity, selenite induces a decrease in the phospholipid, glycolipid and overall fatty acid content of *P. cruentum* (Figs 1 and 2). This observation is reminiscent of decreases in the glycolipid content of higher plants which occurs shortly following illumination of etiolated or green tissue [11–13]. Light-induced glycolipid and phospholipid depletion has been observed to occur concomitantly with fatty acid oxidation in isolated chloroplasts [14, 15]. Chlorophyll is capable of photosensitizing monogalactosyl diglyceride to oxidation in isolated chloroplasts and *in vitro* [15]. The selenite-induced depletion of *P. cruentum* glycolipids, phospholipids and fatty acids is therefore consistent with a selenium promoted lipid oxidation.

By the middle of exponential growth at high light intensity a visible bleaching of the red coloration of *P. cruentum* cultured in selenite was observed. This was confirmed, during late exponential growth, to amount to a 35% decrease in the cellular content of phycoerythrin (Table 4) the major biliprotein in *P. cruentum* [16]. This finding is also consistent with a selenite-induced oxidation, since algal biliproteins have been shown to be sensitive to light-induced peroxidation *in vivo* [17]. This decline in phycoerythrin content is not due to a general selenite-induced inhibition of protein synthesis since the cellular protein content does not differ from that of controls (Table 4) and selenite treated algae are growing faster than controls.

While all of the present observations are consistent with a selenite-induced oxidative effect in *P. cruentum*, any mechanism to explain such an effect remains speculative. It is possible that selenite interferes with the function of antioxidants already present in the algae. *In vitro*, selenite is able to block the protective effect of α-tocopherol against the chemically induced peroxidation of vitamin E deficient erythrocytes [18]. Vitamin E is present in both red and green algae [19] and is located in the chloroplasts of plants in fairly high concentrations [20]. Selenite is able to cause the oxidation of GSH [21]. Since reduced GSH and ascorbate are important antioxidants found in high concentration within plant chloroplasts [22], selenite could conceivably sensitize algal lipids to oxidative damage by abetting the oxidation of these endogenous soluble antioxidants.

Selenite is also capable of oxidizing sulphydryl groups [21] and *in vitro*, selenium can replace the sulphur at the iron–sulphur centres of ferredoxin [23]. An iron–sulphur protein is believed to be the primary electron acceptor and ferredoxin the next electron acceptor of chloroplast photosystem I [22]. When electron transport in photosystem I is blocked such that the primary electron acceptor becomes reduced, superoxide radical is produced [22]. If selenium had this effect upon photosystem I, active oxygen species capable of oxidizing lipids would be generated.

While the lipid content of the red alga *P. cruentum* is decreased by selenite, the lipid content of the green alga *D. primolecta* is unaffected. This differential response to selenite may reflect a physiological difference among algae, or the lack in *D. primolecta* of those fatty acids (20:4 and 20:5) which are most influenced by selenite at low light intensity. Alternately, some selenium compound metabolically produced by the alga, instead of selenite *per se*, may be responsible for the observed effects. Evidence suggests the two algae differ in the quantity and variety of selenium compounds present within the cell [2, 3, 5].

EXPERIMENTAL

Algal culture. Axenic cultures of the green alga *Dunaliella primolecta* and the red alga *Porphyridium cruentum* were grown photoautotrophically at 22° in Muller's artificial sea water (MASW) as described previously [1, 2]. Sulphur at 850 ppm as Na_2SO_4 was present in all growth media. When added, Se was present at 10 ppm as Na_2SeO_3. It should be noted that algae grown in the absence of added SeO_3^{2-} are not necessarily devoid of Se since traces may contaminate media salts. At 10 ppm Se all algae exhibit slightly greater rates of growth as compared to algae cultured in the absence of SeO_3^{2-} [1]. No Se-induced morphological alteration in algae is observed under these conditions.

For studies in which the quantitative determination of cellular fatty acid, pigment, lipid phosphorus and lipid carbohydrate was made, algae were grown simultaneously, either in the presence or absence of added SeO_3^{2-}, in 250 ml with care being taken to provide a uniform inoculum to each flask. Cells in these studies were harvested and analysed concurrently.

For studies in which individual lipids of *P. cruentum* were isolated and analysed for fatty acid composition, algae were grown in 250 ml and pooled at harvest. Algae cultured in the absence of SeO_3^{2-} and those grown in its presence were grown at different times. All algae were harvested during exponential growth. Cell growth was monitored by measuring the increase in turbidity at 680 nm. Following harvest, algae were resuspended and cells were counted using a hemocytometer.

Lipid analysis. Cells were harvested by centrifugation at 1500 *g*

for 10 min. They were resuspended once in MASW lacking SeO_3^{2-} and recentrifuged. Packed cells were resuspended and sonicated in mixtures of $CHCl_3$–MeOH–H_2O before partition into organic and aq. phases by the method of ref. [24]. The organic phase which contains lipid was then removed. $CHCl_3$ and MeOH were added to the aq. phase and the cell sonication and Folch partition were repeated twice to ensure quantitative lipid extraction. For the determination of individual lipid fatty acid composition, total lipids were separated into neutral lipid, glycolipid and phospholipid fractions by silicic acid column chromatography as described in ref. [25]. When the quantitative fatty acid content of algae was determined, total lipids were fractionated by silicic acid chromatography into neutral and polar (phospho and glycolipid) lipid fractions [26]. Further separation into individual lipids was effected by prep. TLC on silica gel H (EM Reagents) of 0.5 mm thickness. The glycolipid and phospholipid fractions were separated utilizing $CHCl_3$–MeOH–H_2O (75:25:4) as the developing solvent.

Lipids were identified by comparison of TLC migration in three different solvent systems with the migration of standard lipids when available and by characteristic colour reactions with specific chemical reagents (Dragendorff stain for choline, ninhydrin stain for amino groups, α-naphthol stain for carbohydrate, $KMnO_4$ for vicinal hydroxyls, and Dittmer and Lester stain for phosphate [27]). The lipids isolated from *P. cruentum* observed in this investigation corresponded to those reported in ref. [28].

When the fatty acid composition of individual lipids was determined, fatty acid methyl esters (FAME) were prepared by transesterification using BF_3 as a catalyst [29]. When the quantitative content of fatty acids was to be determined, transesterification using H_2SO_4 as catalyst [30] following addition of a fatty acid internal standard (docosanoic acid for *D. primolecta* and heptadecanoic acid for *P. cruentum*) was the method utilized. Both transesterification methods yield the same results with lipids from these algae. On occasion, fatty acid bromoacetophenone (BAP) derivatives were prepared following lipid saponification in 70% EtOH and analysed by HPLC as described in ref. [31] to verify results obtained by GC analysis of FAME. Before analysis, FAME and fatty acid BAP derivatives were purified by TLC in hexane–Et_2O (9:1) or hexane–Et_2O–HOAc (90:10:1).

FAME were quantified by a digital integrator following GC separation and FID detection. *Dunaliella primolecta* fatty acids were identified by co-migration with standard lipids in two different packed columns [10% EGSSX (Applied Science Laboratories, Inc.) run isothermally at 175° and 10% SP-2330 (Supelco, Inc.) temp. programmed from 140° to 210° at 3°/min] and a 100 m glass capillary column coated with SP-2340 (Quadrex, Inc.). GC analysis of FAME separated by argentation TLC [32] into saturated, monoenoic, dienoic, trienoic and tetraenoic fractions, before and after hydrogenation was conducted to confirm FAME identification. The fatty acids of *D. primolecta* identified in this way were identical to those reported to be present in this alga in ref. [33], except that no fatty acids with chain lengths greater than 18 carbons were observed in the present investigation. Both the absence [34] and presence [33] of 20- and 22-carbon fatty acids in *D. tertiolecta* has been reported. The present discrepancy is therefore not unprecedented.

Porphyridium cruentum FAME were identified by co-migration with authentic lipids on the two packed columns utilized in the separation of *D. primolecta* FAME and by the HPLC migration of fatty acid BAP derivatives in an IBM 250 × 4.5 mm column packed with 5 μm octadecyl-bonded spherical silica. Hydrogenation was also used to confirm the identification of certain FAME. The double bond positions of arachidonic acid (20:4ω6) and eicosapentaenoic acid (20:5ω3) have previously been ascertained by oxidative methods [28, 35]. The observed fatty acid composition of *P. cruentum* was in excellent agreement with published results [28].

Lipid phosphorus was determined by the Bartlett procedure as reported in ref. [36]. Lipid carbohydrate was determined by the method reported in ref. [37]. Total chlorophyll, chlorophyll *a* and *b* and carotenoid content were determined by the method of ref. [38].

For the determination of biliprotein and chlorophyll *a* content of *P. cruentum*, harvested cells were sonicated after being frozen and thawed once. The visible spectrum of the resulting whole cell homogenate was then measured between 400 and 730 nm. After correcting for light scattering by subtracting the absorption at 730 nm, the B-phycoerythrin content was determined at 560 nm assuming an extinction coefficient of 82 [39]. The chlorophyll *a* content was estimated from the absorption at 668 nm after correcting for light scattering. Protein was determined by the method of ref. [40] at 725 nm using BSA as the standard.

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REFERENCES

1. Wheeler, A. E., Zingaro, R. A., Irgolic, K. J. and Bottino, N. R. (1982) *J. Exp. Mar. Biol. Ecol.* **57**, 181.
2. Gennity, J. M., Bottino, N. R., Zingaro, R. A., Wheeler, A. E. and Irgolic, K. J. (1985) *Phytochemistry* **24**, 2817.
3. Bottino, N. R., Banks, C., Irgolic, K., Micks, P., Wheeler, A. E. and Zingaro, R. A. (1984) *Phytochemistry* **23**, 2445.
4. Caldwell, K. A. and Tappel, A. L. (1965) *Arch. Biochem. Biophys.* **112**, 196.
5. Gennity, J. M., Bottino, N. R., Zingaro, R. A., Wheeler, A. E. and Irgolic, K. J. (1984) *Biochem. Biophys. Res. Commun.* **118**, 176.
6. Farnsworth, C. C., Stone, W. L. and Dratz, E. A. (1979) *Biochim. Biophys. Acta* **552**, 281.
7. Donaldson, W. E. (1977) *Chem. Biol. Interact.* **17**, 313.
8. Sielicki, M. and Burnham, J. C. (1973) *J. Phycol.* **9**, 509.
9. Dougherty, J. J. and Hoekstra, W. G. (1982) *Proc. Soc. Exp. Biol. Med.* **169**, 209.
10. Heath, R. L. and Packer, L. (1968) *Arch. Biochem. Biophys.* **125**, 189.
11. Sellden, G. and Selstam, E. (1976) *Physiol. Plant.* **37**, 35.
12. De Kok, L. J. and Kuiper, P. J. C. (1977) *Physiol. Plant.* **39**, 123.
13. Tevini, M. (1977) in *Lipid and Lipid Polymers in Higher Plants* (Tevini, M. and Lichtenthaler, H. K., eds) pp. 121–145. Springer, Berlin.
14. Hoshina, S., Tetsuo, K. and Nishida, K. (1975) *Plant Cell. Physiol.* **16**, 465.
15. Selstam, E. (1978) *Physiol. Plant.* **44**, 26.
16. Haxo, F. T., Oheocha, C. and Norris, P. (1955) *Arch. Biochem. Biophys.* **54**, 162.
17. O'Carra, P. and Oheocha, C. (1976) in *Chemistry and Biochemistry of Plant Pigments* (Goodwin, T. W., ed.) pp. 328–376. Academic Press, New York.
18. Bunyan, J., Green, J., Edwin, E. E. and Kiplock, A. T. (1960) *Biochem. J.* **77**, 47.
19. Draper, H. H. (1980) in *Vitamin E: A Comprehensive Treatise* (Machlin, L. J., ed.) p. 391. Marcel Dekker, New York.
20. Goodwin, T. W. (1977) in *Lipids and Lipid Polymers in Higher Plants* (Tevini, M. and Lichtenthaler, H. K., eds) pp. 29–47. Springer, Berlin.
21. Tsen, C. C. and Tappel, A. L. (1958) *J. Biol. Chem.* **233**, 1230.

22. Halliwell, B. (1978) *Prog. Biophys. Molec. Biol.* **33**, 1.
23. Fee, J. A. and Palmer, G. (1971) *Biochim. Biophys. Acta* **245**, 175.
24. Folch, J., Lee, M. and Stanley, G. H. S. (1957) *J. Biol. Chem.* **226**, 497.
25. Rouser, G., Kritchevsky, G. and Yamamoto, A. (1967) in *Lipid Chromatographic Analysis* (Marinetti, G. G., ed.) pp. 99–162. Marcel Dekker, New York.
26. Bergstrom, B. (1952) *Acta Physiol. Scand.* **25**, 101.
27. Kates, M. (1972) *Techniques of Lipidology*, pp. 415–424. American Elsevier Publishing, New York.
28. Nichols, B. W. and Appleby, R. J. (1969) *Phytochemistry* **8**, 1907.
29. Bottino, N. R. (1975) *Comp. Biochem. Physiol.* **50B**, 479.
30. Wood, R. (1972) *Lipids* **7**, 596.
31. Wood, R. and Lee, T. (1983) *J. Chromatogr.* **254**, 237.
32. Lilly, M. L. and Bottino, N. R. (1981) *Lipids* **16**, 871.
33. Chuecos, L. and Riley, J. P. (1969) *J. Mar. Biol. Assoc. U.K.* **49**, 97.
34. Ackman, R. G., Tocher, C. S. and McLachlan, J. (1968) *J. Fish. Res. Bd Can.* **25**, 1603.
35. Pohl, P., Wagner, H., and Passig, T. (1968) *Phytochemistry* **7**, 1565.
36. Kates, M. (1972) *Techniques of Lipidology*, pp. 355–356. American Elsevier Publishing, New York.
37. Kates, M. (1972) *Techniques of Lipidology*, p. 359. American Elsevier Publishing, New York.
38. Jensen, A. (1978) in *Handbook of Phycological Methods* (Hellebust, J. A. and Craigie, J. S., eds) pp. 59–70. Cambridge University Press, Cambridge.
39. Siegelman, H. W. and Kyeia, J. H. (1978) in *Handbook of Phycological Methods* (Hellebust, J. A. and Craigie, J. S., eds) pp. 71–79. Cambridge University Press, Cambridge.
40. Layne, E. (1957) *Methods Enzymol.* **3**, 447.