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# THE BINDING OF SELENIUM TO THE LIPIDS OF TWO UNICELLULAR MARINE ALGAE

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SUMMARY: Axenic cultures of the green algae Dunaliella primolecta and red algae Porphyridium cruentum were grown in the presence of sublethal quantities of selenite. All purified lipids from both algae were found to contain bound selenium, except for saturated hydrocarbons. Of the lipids which contain selenium, carotenoid pigments contain the greatest concentrations. Lipid-associated selenium is not metabolically incorporated. The selenium is probably non-covalently bound to the lipids.

Selenium is incorporated by marine animals and plants and has been shown to be present in oils of marine fish (1-3) and marine invertebrates (1). The exact nature of the selenium-lipid interaction is not known. Higher plants grown in the presence of radioactive selenite also contain lipid-associated selenium (4-6). In two investigations (4,6) the suggestion was made that a seleno-lipid was synthesized by the plant and that the associated selenium was covalently bound, possibly replacing the alcohol linked oxygen of a fatty acid ester bond (R-CO-Se-R'). Nissen and Benson, (7) did not observe any incorporation of radioactive selenate into the lipids of green algae or higher plants.

In this investigation significant quantities of selenium are found to be associated with lipid fractions from unicellular marine algae grown in the presence of selenite (8). We report the results of investigations undertaken to determine whether the lipid-associated selenium is metabolically incorporated into the covalent structure of any of the algal lipid(s).

## METHODS

Dunaliella primolecta and Porphyridium cruentum were axenically cultured (9) and harvested by centrifugation during exponential growth ($10^6$ cell/ml). When added, selenite was present at a concentration of 10 ppm Se. Lipids of packed cells were

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extracted by the method of Folch, et al. (10). Total lipids were separated into neutral, glycolipid and phospholipid fractions by silicic acid chromatography (11). Further separation into individual lipids was effected by thin layer chromatography on silica gel H. The neutral lipid fraction was separated utilizing hexane, diethyl ether, acetic acid (80:20:1) v/v/v and the glycolipid and phospholipid fractions were separated utilizing chloroform, methanol, water (75,25,4) v/v/v as the developing solvents. Lipids unresolved in these systems were further purified by thin layer chromatography. Lipids were identified by comparison of migration on silica gel in three different solvent systems with that of standard lipids and by characteristic color reactions with specific chemical reagents. Pigment identification was based upon migration on silica gel and visible absorption spectra as compared with literature data. Following elution from silica gel and removal of solvent at reduced pressure, lipids and pigments were quantified by weighing. Pigments were also quantified by visible absorption spectroscopy using published extinction coefficients. Selenium was determined by atomic absorption spectroscopy (8).

## RESULTS AND DISCUSSION

Two marine algae incorporate selenium into total lipid extracts if selenite is added to the growth medium (8). Lipids from algae grown in the absence of added selenite do not have associated selenium. To determine which lipid(s) contained selenium, individual lipids were purified and their selenium concentrations determined. The results are presented in Table 1. Selenium appears to be incorporated into virtually all the lipids of both algae. The only exception is hydrocarbons, which are almost entirely saturated as determined by the presence of only 1% olefinic protons in the $^{1}$H-NMR spectra. In both algae the greatest selenium concentration is found in pigments, especially carotenoids.

To determine whether the Se is metabolically incorporated into algal lipids, the following experiment was conducted. Two cultures of P. cruentum were grown, one in the presence of added selenite (+Se) and another in the absence of selenite (-Se). Following harvest, the supernatant cell-free growth medium from algal cultures grown (+Se) was collected and saved. This cell-free growth medium contains non-utilized selenite as well as any unidentified selenium compounds metabolized and excreted by P. cruentum. During the extraction of lipids from P. cruentum (-Se), this selenium containing growth medium was substituted for the water otherwise added during lipid partition between aqueous and organic phases. Thus, selenium-free algal lipids came in contact with selenium compounds only during the period of lipid extraction.

The results of this experiment are presented in the last column of Table 1. The presence of selenium in lipids of P. cruentum grown in the absence of selenite, but extracted in the presence of selenium compounds, indicates that the element is bound

Table 1: The Selenium Content of D. primolecta and P. cruentum Lipids (ng Se/mg lipid).

| Lipid | D. primolecta + Se[a] | P. cruentum + Se[a] | P. cruentum -Se[b] |
|---|---|---|---|
| hydrocarbon | 0 | 96[c] | 0 |
| β-carotene | 1290 | | 0[d] |
| oxidized β-carotene | 404 | —[e] | —[e] |
| zeaxanthin | 148 | 1067 | 878[d] |
| pheophytin a | 69.3 | 480 | 220[d] |
| pheophytin b | 82.8 | —[e] | —[e] |
| lutein + sterol | 19.8[f] | 253[g] | 36[d] |
| unidentified xanthophyll | 109 | —[e] | —[e] |
| TG[h] | 5.60 | 212 | 28 |
| SE[h] | 8.67 | 173 | ND[i] |
| SQDG[h] | 28.9 | 311 | 72.7 |
| DGDG[h] | 17.3 | 249 | 54.0 |
| MGDG[h] | 13.5 | 127 | 36.4 |
| PE[h] | 17.0 | 19.6 | ND[i] |
| PG[h] | 32.3 | 189 | 120 |
| PI[h] | 44.0 | 256 | 200 |
| PC[h] | 9.1 | 149 | 74 |
| DGTS[h] | 23.9 | —[e] | —[e] |

[a]Algae grown in the presence of $10^{-2}$g Se/L as $SeO_3^{2-}$; [b]Algae grown in the absence of added Se, but lipids were extracted in the presence of cell free growth medium from exponential phase P. cruentum grown in $10^{-2}$g Se/L as $SeO_3^{2-}$; [c]β-carotene accounts for 6% of the fraction weight of the fraction; [d]Pigment degradation product; [e]Lipid not present in P. cruentum.; [f]Lutein accounts for 9% of the fractions weight; [g]Lutein accounts for 0.2% of the fractions weight; [h]TG=triglyceride; SE=sterol ester; SQDG=sulfoquinovosyl diglyceride; DGDG=diagalactosyl diglyceride; MGDG=monogalactosyl diglyceride; PE=phosphatidyl ethanolamine; PG=phosphatidyl glycerol; PI=phosphatidyl inositol; PC=phosphatidyl choline; DGTS=diacylglyceryl-O-4' (N,N,N-trimethyl) homoserine; [i]Not determined.

to lipids rather than metabolically incorporated. Again, the greatest concentration of selenium is in the pigments. All the pigments from these alga were found to be degraded, presumably oxidized. Cells grown in the absence of selenite have the normal content of unoxidized pigments, so it is possible that some compound in the cell

free medium used during extraction is responsible for the observed degradation. Selenium is found in all lipids from these algae except the saturated hydrocarbons and degraded β-carotene which contains a disrupted, conjugated system of double bonds (the UV absorption spectra indicates the presence of only conjugated di- and trienes).

If an analogous experiment is conducted, in which lipids from P. cruentum grown in the absence of selenium are extracted in the presence of a virgin culture medium containing 10 ppm Se as selenite, selenium is also found associated with both neutral and polar lipids. This indicates that selenite binds to the lipids of P. cruentum. There is no need to invoke the presence of unknown selenium compounds of metabolic origin to explain the binding of selenium to lipids. However, such selenium metabolites may also bind to lipids. It was observed that dimethyl diselenide, dimethyl selenide and elemental selenium (compounds believed to be present in both algae) bind in vitro to purified soy lecithin.

The possibility that Se is binding to double bonds in the lipids is suggested by the finding that the olefinic structure is the only feature common to all lipids which are found to contain Se. Pigments which contain most of the double bonds contain most of the selenium. All esterified lipids from both algae contain high proportions of unsaturated fatty acids. Lipids without double bonds (hydrocarbons) or with disruption of double bonds (degraded β-carotene) lack selenium. These observations suggest that selenium compounds bind to algal lipids non-metabolically and are not incorporated by cellular processes. The selenium associated with plant (4-6) and marine (1-3) lipids may also be the result of non-biosynthetic binding.

In an attempt to characterize the interaction between selenium and algal lipids, the experiments summarized in Table 2 were conducted. The lipid-associated selenium remains bound to lipids through repeated developments on and elution from silica gel. It cannot be transformed from one lipid to another upon co-incubation. Gaseous selenium compounds have been observed in algae (8). At least two of the selenium gases produced by plants, dimethyl selenide and dimethyl diselenide, are soluble in organic solvents and bind to lecithin in vitro. These gases may be responsible for the observed binding of selenium to algal lipids.

Table 2: Characterization of the Selenium Associated With Algal[a] Lipids

| Lipid Studied[d] | Treatment | Result |
|---|---|---|
| TG(+Se)[c], PC(+Se) | Prolonged purge with $N_2$ at 35°C. | No decrease in Se content. |
| DGDG(+Se)+MGDG(-Se) | Incubation together. | No transfer of Se between lipids. |
| TG(+Se) | Development on $AgNO_3$ impregnated Silica gel. | Selenium is removed from lipid and converted to $Se^0$. |
| PC(+Se) | Catalytic hydrogenation with $PtO_2$. | 95% decrease in Se content. |
| PC(+Se) | Incubation with $PtO_2$. | 45% decrease in Se content. |
| TG(+Se), PC(+Se), DGDG(-Se) | Acid or base catalyzed transesterification. | 80% of Se is extracted with organic solvent, but not associated with fatty acid methyl esters. |
| SQDG(+Se and -Se) DGDG(+Se and -Se) | Saponification followed by bromoacetophenone derivatization. | 3-4% of Se is associated with fatty acid-bromoacetophenone derivatives. |
| D. primolecta glycerolipids (+Se) | Transesterification followed by acid hydrolysis of glycerol backbone. | No Se is associated with purified fatty acid methyl esters, glycerol or polar head group. |
| TG(+Se) | Lipase hydrolysis. | 16% of Se is associated with lipid following hydrolysis. Selenium content of unhydrolized TG is unaltered. |

[a]Unless otherwise indicated, all lipids studied were extrated from P. cruentum; [b]Abbreviations are the same as those used in Table 1; [c](+Se) indicates that the lipid studied was isolated from algae grown in the presence of $10^{-2}$g Se/L as $SeO_3^{2-}$. (-Se) indicates that the lipid studied was isolated from algae grown in the absence of added Se, but lipids were extracted in the presence of cell free growth medium from exponential phase P. cruentum grown in $10^{-2}$g Se/L as $SeO_3^{2-}$.

An attempt to remove bound selenium from triglycerides and phosphatidyl choline by maintaining the lipid at 35°C under a stream of $N_2$ gas for 5 hours was unsuccessful. Selenium attached to purified algal lipids is tightly bound as estimated by its retention during repeated chromatography for purification and the inability of bound selenium to be transferred among the various lipids. Lipid bound selenium is also non-volatile.

In an attempt to show a preference of selenium for the more unsaturated fatty acids, triglycerides were separated on $AgNO_3$-impregnated silica gel into nine different fractions varying in their degrees of fatty acid unsaturation. No selenium was

detected in any of the fractions. Apparently the selenium precipitates with the $Ag^+$ or is oxidized to elemental Se by $Ag^+$ since the formation of (red) elemental selenium was noted. Hydrogenation of phosphatidyl choline removes 95% of the bound selenium, but a 45% decrease is selenium content is effected by simply agitating the lipid with the platinum oxide used as hydrogenation catalyst. Similar results were obtained by Elson and Ackman (2) who were able to remove 94%. of Se from fish oil by partial hydrogenation. The bound selenium is labile to gentle chemical treatments.

A more drastic chemical hydrolysis of Se- containing algal lipids was carried out in an attempt to localize the portion of the lipid molecule to which selenium was bound. This resulted in extensive selenium labilization. Following either acid or base catalyzed transesterification of glycerolipids, 80% of the Se was found in the organic-soluble fraction which contained the fatty acid methyl esters. The remaining Se was in the aqueous phase. Following purification, no Se remained in the fatty acid methyl esters. Saponification of glycolipids, followed by derivatization of fatty acids with bromoacetophenone (12) results in loss of 75% of the Se originally present in the lipid. Of the Se which is extracted by organic solvents following saponification and derivatization, 15% is found associated with the fatty acid-bromoacetophenone derivative while 75-95% remains at the origin of the chromatographic plate used to purify the derivatives. When D. primolecta glycerolipids are transesterified and their polar backbones hydrolyzed into their component glycerol and head-group, no Se is detected in any of the purified hydrolysis product.

Most of the lipid-associated Se is labilized by enzymatic hydrolysis of the lipid. Treatment of triglycerides with pancreatic lipase resulted in hydrolysis of 93% of the triglyceride. Only 16% of the Se originally present in the triglyceride is recovered in purified hydrolysis products (glycerol was not isolated). Of that amount 54% is bound to unhydrolyzed triglyceride, 17% to free fatty acids and the remainder (28%) remains with the monoglycerides. Since the unhydrolyzed triglyceride recovered following lipase treatment had the same Se content (192 ng/mg lipid) as the starting triglyceride, it would appear that loss of selenium from the lipid molecule is coincidental with fatty acid hydrolysis. This tends to implicate the fatty acid ester bond in selenium binding.

The ease with which the lipid associated selenium is removed by gentle chemical ($Ag^+$, $PtO_2$) or enzymatic means implies that it is non-covalently bound to the lipid, but the possibility of a labile covalent interaction cannot be excluded. The site of selenium binding on the intact lipid is uncertain. Removal of selenium by hydrogenation suggests an association with double bonds while lipase-induced selenium loss implicates the ester linkage.

Because selenium binding to algal lipids occurs during lipid extraction, there is no proof that it occurs *in vivo*. Both alga grow normally in selenite and exhibit unaltered morphology. It should be noted that *P. cruentum*, which has the greater lipid associated selenium content, exhibits selenite induced decreases in pigment, glycerolipid and biloprotein content (13).

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