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SELENIUM CONTAINING AMINO ACIDS AND PROTEINS IN MARINE ALGAE

NESTOR R BOTTINO*‡, CATHERINE H BANKS, KURT J IRGOLIC, PAT MICKS, ANDREW E WHEELER and RALPH A ZINGARO†

Department of Biochemistry and Biophysics* and Department of Chemistry†, Texas A&M University, College Station, TX 77843, USA

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Key Word Index—*Dunaliella primolecta*, *Porphyridium cruentum*, marine algae, seleno amino acids, selenium metabolism

Abstract—The unicellular marine algae, *Dunaliella primolecta* Butcher, *Chlorella* sp and *Porphyridium cruentum* (S F Grey) were grown in artificial sea water containing a sublethal concentration of selenite, 10^{-2} g Se/l Both free- and protein-bound seleno-amino acids were identified The initial steps of selenium incorporation seem to involve the use of the sulfur enzymatic machinery resulting in the replacement of some of the sulfur by selenium in both free amino acids and proteins At relatively low selenium concentrations, selenium-specific enzymes seem to be in operation

INTRODUCTION

Oxoselenium anions in the environment affect the growth of marine algae in a number of ways, depending on the oxidation state of the element, its concentration, the concentration of sulfate, and the species of algae [1] Thus, relatively low concentrations of either selenate or selenite are able to promote algal cell growth, while relatively high levels of selenate (10^{-2} g/l) are lethal Equally high levels of selenite increase growth in some algae and have no deleterious effects in others at higher levels [1] In seeking an explanation for these observations, experiments have been undertaken to determine whether inorganic selenium dissolved in the marine growth medium was incorporated by the algae and, if so, into what biochemical compounds it was transformed This investigation of the selenium-containing molecules in algae is helpful in tracing the transfer of selenium through aquatic ecosystems Furthermore, it is possible that the phenomena evident in algae may also be representative of those occurring in higher organisms

Dunaliella primolecta and *Chlorella* sp, two marine species of marine algae which show intrinsically different growth patterns as has been previously reported [1], were used throughout the experiments In some experiments, an alga which displays yet another behavior, *Porphyridium cruentum*, was also studied These algae were grown in the presence of sublethal concentrations of selenite The selenium present in the cells and in their biochemical components was assayed by atomic absorption spectroscopy (AAS) This method differs from that of a previous study [2] in that it does not require the use of radioisotopes

Although selenium was found to be present in several biochemical components, including proteins, amino acids and perhaps lipids and carbohydrates [3], this report is concerned primarily with the chemical characteristics of the selenium-containing proteins and amino acids

RESULTS

The results of the fractionation of the free amino acid extracts and protein hydrolysates by ion-exchange chromatography are discussed below Since the eluates were analysed for Se, only seleno-amino acids appear in the chromatogram peaks illustrated in Figs 1–4

Isolation and identification of free seleno-amino acids

(a) Dunaliella primolecta The first amino acid peak (Fig 1, A) was eluted with 20 ml of 1 5 M HCl and corresponded to the Se analogues of cysteic acid or taurine Analyses of the sample by TLC using solvents 1 or 2 indicated that the peak corresponded to 'selenocysteic acid' Corroboration was indicated by conversion to the *O*-methyl ester (BF_3–MeOH) and TLC mobility of the derivative

The second peak (Fig 1, B) was eluted with 50 ml of 1 5 M HCl Its mobility coincided with that of *S*-methyl cysteine and presumably with that of its Se analogue, other possible components of this fraction were the selenoxide and selenone of *Se*-methyl selenocysteine TLC of this fraction using solvents 1 and 2 indicated the presence of *Se*-methyl selenocysteine and *Se*-methyl selenocysteine selenoxide A 2-D preparative TLC using the same solvents revealed three spots, all of which contained Se These results suggested that *Se*-methyl selenocysteine, its selenoxide and its selenone were all present

The third peak (Fig 1, C) was eluted with 20 ml of 4 M HCl Analysis of this fraction by TLC with solvents 1 and 2 independently pointed to the presence of Se analogues of cysteine, cystine and methionine (comparison with authentic samples) Treatment with H_2O_2 caused two of the spots, selenocysteine and selenocystine, to remain at the origin when either solvent was used The 2-D TLC preparative run of oxidized sample using solvent 1 in the first direction and solvent 2 in the second, resulted in the

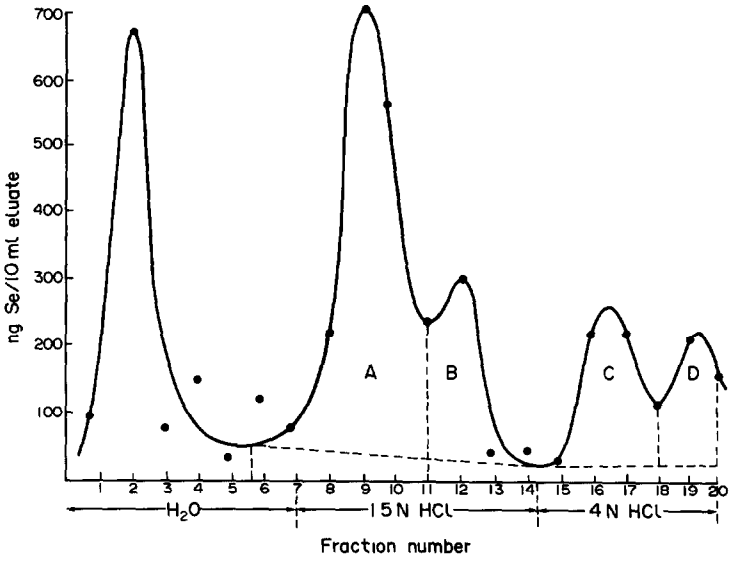

Fig 1 Fractionation by Dowex 50W-X8 ion-exchange chromatography of the selenium containing free amino acids of *Dunaliella* Amino acids were eluted by the solvents indicated in the abscissa Broken lines delineate the areas of the peaks for planimetry

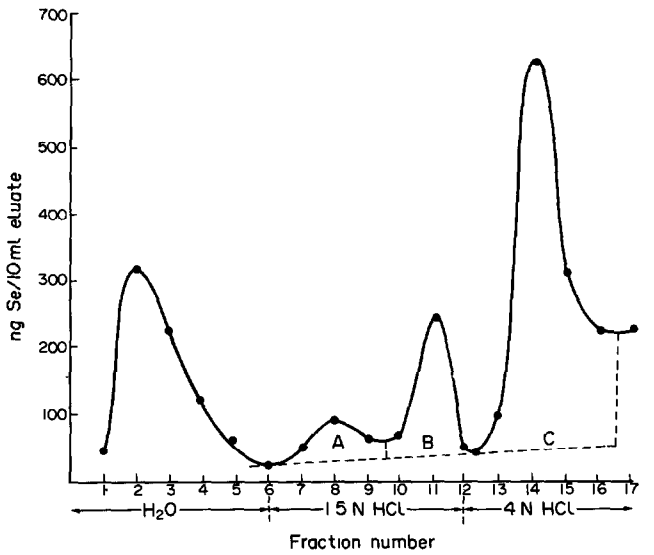

Fig 2 Fractionation by Dowex 50W-X8 ion-exchange chromatography of the selenium-containing free amino acids of *Chlorella*

separation of three spots which were found to contain Se when analysed by AAS

The fourth peak (Fig 1, D) was eluted with 50 ml of 4 M HCl Using TLC with solvent 1, this fraction showed a single ninhydrin positive spot which contained Se Tentative identification of the sample as *Se*-methyl selenomethionine was confirmed in the following way Treating the sample with alkali changed the mobility of the spot in solvent 1 to that of methionine and also to that of alkali-treated *S*-methyl methionine or *Se*-methyl selenomethionine

(b) Chlorella *sp* The seleno amino acid profile of *Chlorella* is shown in Fig 2 Three major selenium-containing amino acid peaks were obtained which corresponded well with those identified for *Dunaliella* 'selenocysteic acid' in the fraction eluted with 20 ml of 1 5 M HCl (peak A), *Se*-methyl selenocysteine, its selenoxide and its selenone in the fraction eluted with 50 ml of 1 5 M HCl (peak B), and selenocysteine, selenocystine and selenomethionine in the fraction eluted with 20 ml of 4 M HCl (peak C)

'Selenocysteic acid' co-chromatographed with the product formed by peroxide oxidation of selenocystine on TLC plates developed with solvents 1, 2, 3 or 4 A 2-D

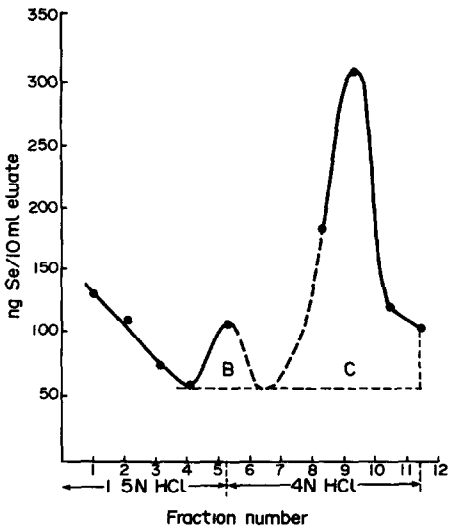

Fig 3 Fractionation by Dowex 50W-X8 ion-exchange chromatography of the seleno amino acids hydrolysed from the proteins of *Dunaliella* Points corresponding to fractions 6 and 7 are not plotted because of the values obtained were considered to be unreliable because of instrument malfunction

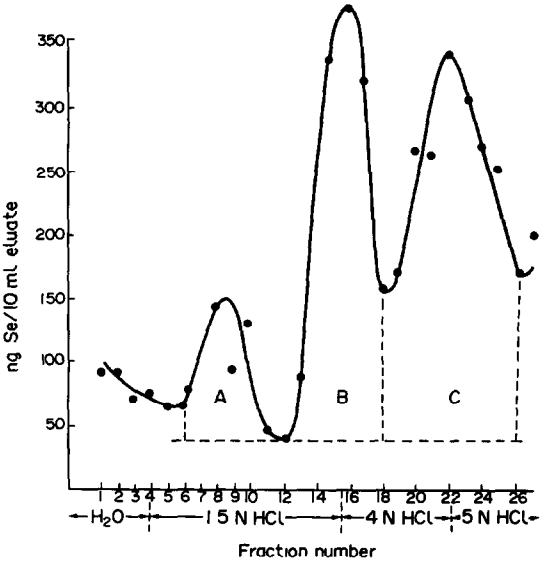

Fig 4 Fractionation by Dowex 50W-X8 ion-exchange chromatography of the seleno amino acids hydrolysed from the proteins of *Chlorella*

preparative TLC run with solvents 1 and 2, yielded three spots, of which only one contained Se and occupied the position expected for 'selenocysteic acid' Methylation of this material was accompanied by the corresponding change in mobility due to formation of the *O*-methyl ester of 'selenocysteic acid'

The presence of *Se*-methyl selenocysteine (peak B) in *Chlorella* extracts was confirmed by its TLC mobility using solvent 1, which coincided with that of *S*-methyl cysteine Upon oxidation, the amino acid co-chromatographed with *S*-methyl cysteine sulfoxide A 2-D TLC using solvents 1 and 2 yielded two spots which contained Se and corresponded to *Se*-methyl selenocysteine selenone and other unidentified components

The third amino acid mixture obtained from the Dowex column (peak C) consisted of the Se analogues of cysteine, cystine and methionine as identified by TLC using solvent 1 After treatment with H_2O_2 the two faster moving spots, selenocysteine and selenocystine, remained at the origin The mixture was also subjected to mass spectral analysis Molecular ion peaks corresponding to selenomethionine and selenocystine were detected To confirm further the finding of selenomethionine, the amino acid mixture from the Dowex column was fractionated by TLC using solvent 1 Three bands were obtained, all of which contained Se

A comparison of the seleno amino acids of *Dunaliella* with those of *Chlorella* showed drastic quantitative differences (Table 2) Whereas both algae contained 'selenocysteic acid,' *Se*-methyl selenocysteine, selenocystine and selenomethionine, these components were present in different proportions 'Selenocysteic acid' predominated in *Dunaliella*, whereas the fraction containing selenocysteine, 'selenocysteic acid,' and selenomethionine was most abundant in *Chlorella* Furthermore, *Dunaliella* contained significant amounts of *Se*-methyl selenomethionine, whereas this amino acid was not found in *Chlorella* At this point, it was not clear whether these differences were due to variations in the biochemical compositions of the algae, or to the fact that the algae were analysed at different stages of growth The fact that both algae incorporate *ca* equal amounts of Se (Table 1) seems to rule out the Se concentration of the growth medium as the cause of the biochemical differences between the algae

Isolation and identification of the seleno amino acids present in algal proteins

(a) Dunaliella primolecta Figure 3 shows the ion-exchange chromatogram of the *Dunaliella* protein hydrolysate Most of the Se which was recovered (91%) belonged to only one peak (Fig 3, C) corresponding to a

Table 1 Selenium concentrations in biochemical components of various algae*

	Dunaliella primolecta		*Chlorella* sp		*Porphyridium cruentum*	
	μg Se/100 mg	per cent	μg Se/100 mg	per cent	μg Se/100 mg	per cent
Total	39 1		52 2		24 5	
Proteins and polysaccharides	6 1	15 6	0 7	1 3	2 0	8
Lipids	4 6	11 8	0 5	1 0	19 6	80
Amino acids and soluble carbohydrates	28 4	72 6	51 0	97 7	2 9	12

*Algae were exposed to 10^{-2} g/l Se as selenite Biochemical components were isolated by the TCA extraction procedure (see Experimental) Se concentration is expressed in μg Se/100 mg dry wt

Table 2 Relative proportions of Selenium in amino acid fractions separated by ion-exchange chromatography (Figs 1–4) (Se %)*

	Peak A†	Peak B†	Peak C†	Peak D†
A Free amino acids				
Dunaliella	56 8	16 9	17 1	9 2
Chlorella	7 5	17 7	74 8	—
B Protein hydrolysate				
Dunaliella	—	9 1	90 9	—
Chlorella	10 5	35 9	53 6	—

*Relative proportions of the fractions were determined by planimetry of the areas under the chromatographic peaks

†Peak A, 'selenocysteic acid', Peak B, *Se*-methyl selenocysteine, its selenone and its selenoxide, Peak C, selenocysteine, selenocystine and selenomethionine, Peak D, *Se*-methyl selenomethionine

mixture of selenocysteine, selenocystine and selenomethionine This was verified by TLC using solvent 1, which yielded three spots Upon treatment with H_2O_2 the two previously faster moving spots, selenocysteine and selenocystine, remained at the origin Furthermore, a 2-D TLC run using solvents 1 and 2, resulted in the separation of three spots, all of which contained Se

(b) Chlorella *sp* Three major seleno amino acids were found in the protein hydrolysates of *Chlorella* (Fig 4) Peak A was identified as 'selenocysteic acid' as indicated previously Peak B, which eluted with 50 ml of 1 5 M HCl, corresponded to *Se*-methyl selenocysteine and *Se*-methyl selenocysteine selenoxide These compounds co-chromatographed with *S*-methyl cysteine and *S*-methyl cysteine sulfoxide with TLC using solvent 1 Further verification was obtained by 2-D preparative TLC using solvents 1 and 2 Of the three spots that were obtained, only two contained Se They were identified as *Se*-methyl selenocysteine and *Se*-methyl selenocysteine selenoxide by co-chromatographing with the appropriate standards

The third peak in the *Chlorella* protein hydrolysate (Fig 4, C) consisted of selenocystine as shown by TLC using solvent 1 A 2-D preparative TLC run using solvents 1 and 2, yielded four spots of which only three contained Se The identity of the samples was confirmed by their TLC behavior against known standards, before and after oxidation

Changes in Se-containing amino acids with time

The results of the experiments just reported suggest that the seleno-amino acid pattern of an alga exposed to sublethal Se concentrations depends on the stage of algal growth To test this hypothesis, *Dunaliella* was grown for varying periods of time, from 9 to 32 days after inoculation, i e from the middle of the exponential growth phase to well into the stationary growth phase [1] The results are summarized in Table 3 It is observed that the seleno-amino acid pattern of algal cells exposed to a relatively large amount of Se does change with the period of exposure and affects both the free amino acid pool and proteins, although in a different manner

DISCUSSION

The experiments described demonstrate that when algae are exposed to environmental Se in the form of selenite, they are able to incorporate the element to different degrees, depending on the algal species (Table 1) It must be borne in mind, however, that although the algae were exposed to the same concentration of Se in their growth medium (10^{-2} g/l Se as selenite), they were maintained in the medium for periods of time which varied with the growth rate of the individual species

The distribution of Se among biochemical components of algae shown in Table 1 differs markedly from that reported by Wrench [2] Most of the Se was associated with the fraction containing amino acids and soluble carbohydrates of *Dunaliella primolecta* and *Chlorella* sp, and with the lipid fraction of *Porphyridium cruentum* Wrench [2] found most of the radioactive Se in the protein fraction of *Tetraselmis tetrathaele* and *Dunaliella minuta* These differences are probably due to the nature of the algal species, or the extraction procedures and method of detection

The amino acids, both free and protein-derived, which we found associated with selenium, are all sulfur analogs, suggesting that when exposed to sub-lethal, but higher than trace concentrations of Se, the algal cells tend to substitute Se for part of their sulfur Thus, under overloading conditions, Se appears to use the sulfur enzymatic

Table 3 Free and protein-derived seleno-amino acids in *Dunaliella primolecta* exposed to selenite for various periods of time

Time of exposure in days (harvest time)	Free seleno amino acids					Protein derived seleno amino acids				
	Peak A*	Peak B	Peak C	Peak D	Unknowns	Peak A*	Peak B	Peak C	Peak D	Unknowns
9	29 7	3 9	5 4	32 9	28 1			37 9	62 1	
12	20 7	20 5	28 0	28 4	2 4	11 2	18 6		70 2	
15	13 8	27 0	30 2	22 0	7 0	30 7	12 5	43 8		13 0
20			64 9	35 1		50 5	2 1	18 6	28 8	
25	14 3	45 9		13 3	26 5			61 2	12 6	26 6†
32	5 1	6 0	44 8	10 5	33 6	24 7	53 3	22 0		

*Peak A, 'selenocysteic acid', Peak B, *Se*-methyl selenocysteine, its selenoxide and its selenone, Peak C, selenocysteine, selenomethionine and selenocystine, Peak D, *Se*-methyl selenomethionine Amino acid fractions were obtained by ion-exchange chromatography Selenium was determined in each 10 ml eluate by atomic absorption spectrometry The area under each peak was determined by planimetry of the chromatograms

†Sums of three unknowns

system, while under normal, lower levels, Se-specific enzyme systems seem to be in operation, at least in bacterial systems [4]

The amino acids found to contain Se in the present work are qualitatively the same as those reported previously by Wrench [2] However, his identification of the amino acids was based solely on their column chromatographic behavior Our identification was supplemented by separation using various TLC systems, in several cases before and after chemical modification In this sense, our observations should be more definitive *Se*-methyl selenomethionine that we have characterized is probably one of his unknowns Table 2 shows that most of the Se in free amino acids derived from *Dunaliella* was present as 'selenocysteic acid' On the other hand, the prevailing fraction of Se analogues in the free amino acids of *Chlorella* and in the proteins of both algae, was a mixture containing selenocysteine, selenocystine and selenomethionine

During the initial stages of this study, the algae were analysed at undetermined stages of growth Some of the differences in individual seleno-amino acid content could be due to the duration of the exposure to Se or to the varying concentrations of Se in the growth medium This possibility was explored in the time study (Table 3) which shows that the pattern of Se incorporation into algal amino acids and protein depends on the duration of exposure The data in Table 3 show also that the time pattern found in free seleno-amino acids does not coincide with that found in protein-bound seleno-amino acids, even though the seleno-amino acids detected in both pools are essentially the same Thus, from these data it is very difficult to establish the existence of any possible relationship between two pools Table 1 shows, however, that more of the Se associates with the amino acid fraction than the protein fraction

The information about free and protein-bound seleno amino acids provided by the two major experiments described herein can be tentatively explained by a common scheme such as the one shown in Fig 5 In this proposal, it is assumed that selenocysteic acid is the first amino acid formed after the cell invasion by Se Selenocysteic acid is then methylated to *Se*-methyl selenocysteine selenone This product undergoes reduction

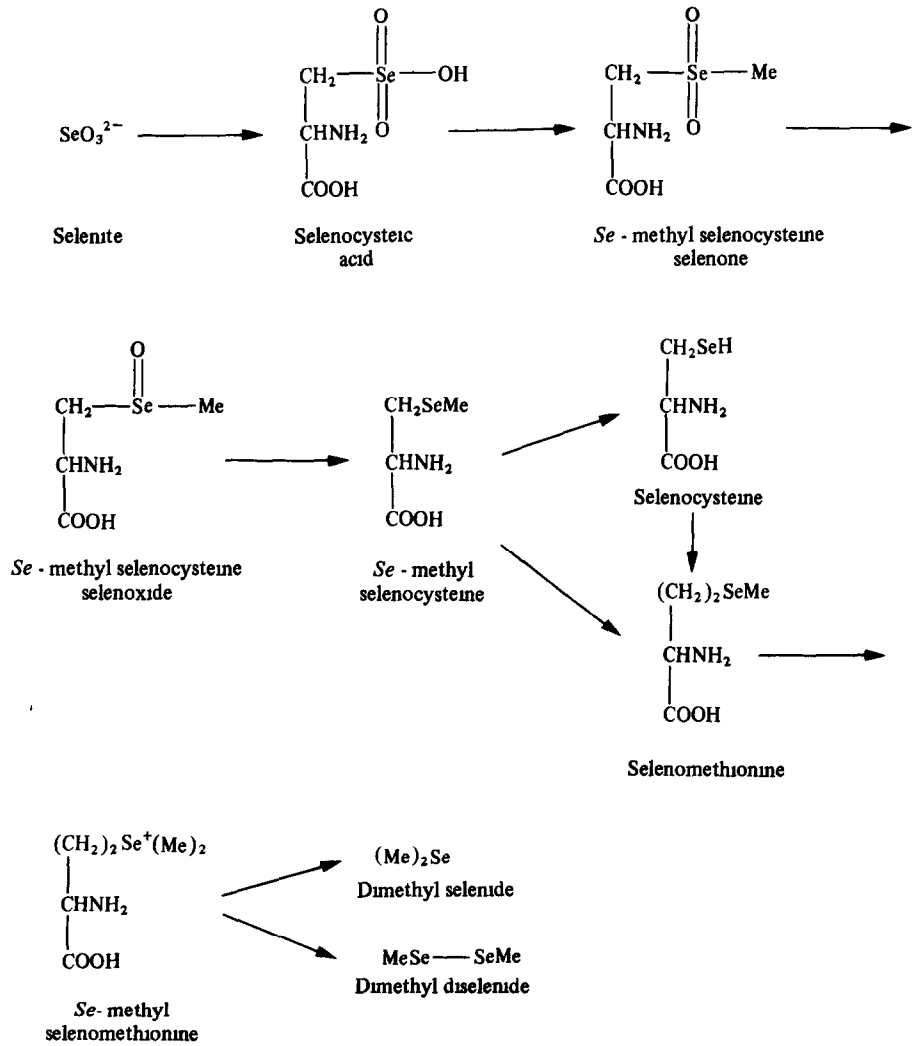

Fig 5 Proposed metabolic events that would relate the seleno amino acids found in the present report in both the free and protein-bound fractions All compounds listed have been identified

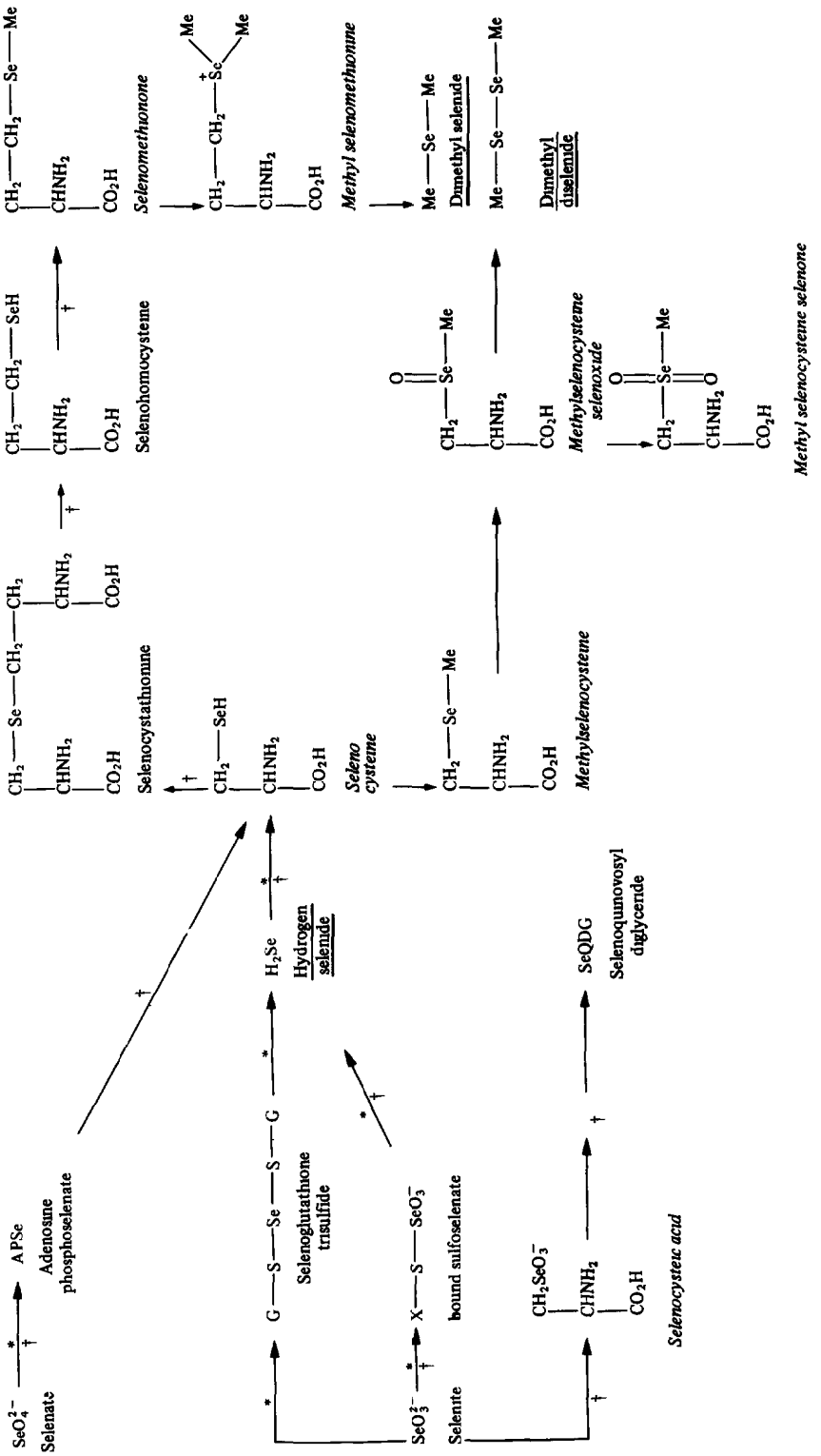

Fig 6 Hypothetical metabolism of selenium in plants All selenium compounds, except selenoquinovosyl diglyceride, have been detected in plants Those in italics have been detected in *Dunaliella* and *Chlorella*, whereas the presence of those underlined is suspected *Reactions occurring *in vitro* with selenium compounds †Normal paths of sulfur metabolism in plants

to the respective selenoxide and finally to *Se*-methyl selenocysteine Somewhere in this area the formation of selenocysteine occurs Selenocysteine, *Se*-methyl selenocysteine or both, may conceivably be converted into selenomethionine and this compound is methylated further to *Se*-methyl selenomethionine This compound may be a precursor of methylated selenides in higher plants [5, 6] and has presumably the same role in algae We were able to detect the presence of methylated selenides from cultures supplemented with selenite by their smell, and AAS analyses of the gases evolved from the algal cultures confirmed the presence of Se However, in our hands, GC and GC/MS analyses have so far given uncertain results about the exact chemical nature of the compounds evolved

Thus, our proposal constitutes a series of methylation–reduction and reduction steps that would provide a way of eliminating toxic Se from the cell It is interesting to note that a series of methylation–reduction reactions appear to be operational also in the detoxification of arsenic in living cells [5] It is conceivable that the reduction of organoselenium and organoarsenic compounds does not occur in living cells without the involvement of a concurrent methylation process

Recently, an alternative proposal has been put forward by Gennity [3] to explain Se metabolism in plants The scheme, shown in Fig 6, is based on two assumptions First, the biochemical mechanisms occurring in higher plant cells are basically analogous to those in algal cells Second, Se follows sulfur metabolism when it is being incorporated into amino acids and proteins In this scheme, selenite is either converted to selenocysteic acid or to hydrogen selenide and selenocysteine This compound is further metabolized by alternate pathways In one of these, selenocysteine is converted to selenocystathionine which is probably one of the unknown peaks in our ion-exchange chromatograms, and selenohomocysteine Methylation of this compound produces selenomethionine and methyl selenomethionine, which gives rise to gaseous dimethyl selenide In the second pathway, selenocysteine is methylated to methyl selenocysteine, which is then oxidized to its selenoxide and selenone The selenoxide gives rise to dimethyl diselenide

EXPERIMENTAL

Axenic cultures of *Dunaliella primolecta* Butcher, *Chlorella* sp and *Porphyridium cruentum* (S F Grey) were grown in Mueller's artificial sea water medium (MASW) in the presence of Se at 10^{-2} g/l in the form of selenite as described [1] In the initial experiments, the algae were grown in 2 l batches of medium in 6 l flasks to the late exponential stage (10^6 cells/ml), which involved different periods of growing time for the different species These experiments were repeated until the results were quantitatively reproducible The cultures were harvested by centrifugation or filtration, and repeatedly washed with MASW with no added Se yielding *ca* 0 5–2 0 g (dry wt, lyophilized) of cells The same procedure was followed in the time study experiment in which only *Dunaliella* was used Replicates (10, 200 ml each) were combined at the respective times In this manner, a statistical average was built into the experiment The time periods selected for harvesting *Dunaliella* were 9, 12, 15, 20, 25 and 32 days In all cases, growth was determined by measuring turbidity with a Bausch and Lomb Spectronic 20 spectrophotometer equipped with a far red lamp, a 660 nm filter, and adjusted to a wavelength of 680 nm [1]

Extraction of algae Algal amino acids, proteins, carbohydrates, lipids and nucleic acids were extracted by two different procedures. The first involved treatment of 1–2 g of washed cells with *ca* 25 ml of a 5% trichloroacetic acid (TCA soln) [7] The slurry of cells was homogenized using a Potter Elvehjem tissue grinder, filtered under red pres through a sintered glass funnel of fine porosity, and the residue washed several times with 5 ml aliquots of the cold TCA soln The residue contained the proteins, insoluble polysaccharides and nucleic acids The TCA soln was then extracted with $CHCl_3$–MeOH (2 1) to isolate the lipid fraction The resultant TCA soln contained free amino acids and soluble carbohydrates These two fractions were analysed for Se by AAS

The second extraction procedure was based on that previously described [8] The cells were homogenized as described and extracted first with 80% and then 95% hot EtOH (3 × 50 ml) The resulting insoluble material containing proteins, nucleic acids and insoluble polysaccharides was separated by filtration from the hot EtOH and set aside After evaporation of the EtOH extract, the residue was dissolved in 40 ml H_2O, and this soln was extracted with petrol to obtain a soln of lipids The aq layer was evaporated to yield free amino acids and soluble carbohydrates Evaporations were performed on the liquids contained in a round-bottomed flask connected to a vacuum pump Heat furnished from an external steam bath was occasionally applied

Both the TCA and EtOH methods of extraction were compared and found to give identical results The TCA extraction procedure was used in ref [2] and, therefore, initially used by us to provide a basis for comparison of those results with ours However, this method was so tedious that we changed to the EtOH method which greatly reduced the time necessary to perform the extractions

Protein hydrolysis The insoluble material containing proteins was subjected to the enzymatic hydrolysis procedure of ref [2] The protein-containing material from both extraction procedures was slurried in 15 ml of 0 01 M KPi buffer, pH 7 6, containing a small amount of chloramphenicol to prevent bacterial contamination Pronase (*ca* 70 mg) (Protease type V, Sigma) was then added, and the resulting suspension was stirred for 24 hr The suspension was filtered and the residue was again suspended and treated with pronase for an additional 24 hr The material was then filtered and the filtrates were combined The enzyme was precipitated by the addition of *ca* 5 ml of Me_2CO and removed The solns were evaporated by placing the sample in a vacuum desiccator over NaOH pellets and connecting the desiccator to a vacuum pump This yielded a residue of amino acids The residue coming from the above filtration (polysaccharide and nucleic acid fraction) was washed and stored for subsequent Se analysis

Fractionation of amino acids by ion-exchange chromatography The free amino acids, as well as the amino acids resulting from protein hydrolysis, were chromatographed using a column of Dowex 50W-X8-400 (i d 1 5 cm, length 15 cm, sample wt 0 015–0 3 g) The resin was activated by treatment with HCl, and then washed to neutrality The columns were eluted routinely, except when otherwise indicated, with 60 ml H_2O, 60 ml 1 5 M HCl, and 60 ml 4 M HCl Eluates of 10 ml each were collected and analysed further by TLC on Silica Gel 60 H (Merck) using four solvent systems (1) Me_2CO–H_2O–pyridine–HOAc, 75 20 5 1, (2) *n*-BuOH–HOAc–H_2O, 12 3 5, (3) *n*-BuOH–HOAc–H_2O, 25 6 25, (4) *n*-BuOH–pyridine–H_2O, 1 1 1

Relative concentrations of the various selenium-containing fractions from the Dowex 50W column were measured by planimetry of the chromatographic bands (Figs 1–4)

To be sure that selenite was not present in the amino acid

eluates a soln containing a known concn of H_2SeO_3 and cystine was passed through the Dowex 50 column used for the amino acid separations Elution with H_2O yielded the selenite quantitatively and elution with 60–70 ml of 4 M HCl did not yield any detectable Se in any of the fractions Also, to prove that the selenite could be separated from the carbohydrates, a known amount of selenite was passed through a Dowex 1 column and washed with H_2O No Se was detected in the H_2O No Se was detected in the H_2O eluate

Authentic samples of the following seleno-amino acids were available and used to confirm the identities of the seleno-amino acids selenocysteine, selenocystine, selenocystamine, selenoethionine and selenomethionine These and the sulfur amino acids were purchased from Sigma

'Selenocysteic acid' is reported as a metabolic product in this paper, but authentic samples of this compound were not available The term 'selenocysteic acid' is used in this paper for the labeling of that compound (or compounds) which contained Se and eluted with the amino acids Furthermore, this Se compound displayed the same elution characteristics, on the column and co-chromatographed with the product formed by the hydroperoxide oxidation of selenocystine It was suggested [9] that the compound formed by hydroperoxide oxidation of selenocystine is the seleninic acid of selenocystine Interestingly, the 'selenocysteic acid' report in this work co-eluted with cysteic acid It has also been stated [9] that "the mobility of a sulfur compound and its selenium analog was found to be essentially the same in each solvent system studied " The compound identified in this paper as *O*-methylselenocysteic acid showed identical chromatographic properties following *O*-methylation (BF_3–MeOH) as that obtained by BF_3–MeOH treatment of the product obtained by the hydroperoxide oxidation of selenocystine Hence, the absolute identification of the compound identified as 'selenocysteic acid' is not certain It is quite certain that it is an amino acid containing Se in a higher oxidation state The need for a better understanding of selenonic acids, especially selenocysteic acid, has been mentioned by several authors [10, 11]

Se *analysis* Amino acid samples from both free amino acid and protein hydrolysate fractions were evaporated to dryness in a vacuum desiccator over NaOH To each sample was added 3 ml of a 0 1 N HNO_3 soln containing 400 ppm $Ni(NO_3)_2$ and sonicated for 3 min at room temp Aliquots (50 μl) of the samples were injected into a Perkin–Elmer 272 Atomic Absorption Spectrometer equipped with a 2200 HGA power unit, using pyrolytically coated graphite cuvettes Argon was used as the carrier gas The AA spectrometer employed the following program drying temp, 95° for 70 sec, ashing temp, 600° for 10 sec, and atomizing temp, 2700° for 4 sec Se was determined by comparison of *A* to peak height Peak heights were calibrated using known concs of selenious acid as standards

Isolation and identification of Se-*containing gas* Axenic cultures of *Dunaliella primolecta*, which had been previously grown to the late exponential phase, were inoculated into gas bottles containing 1 5 l of sterile MASW to which 10^{-2} g/l Se as selenite had been added A stream of air filtered through a 0 45 μm pore size Millipore filter was allowed to continuously bubble through the cultures After the cultures had reached the exponential phase of growth and gas production was evident, as indicated by the garlic-like odor, the effluent was bubbled through *ca* 50 ml of CH_2Cl_2 maintained at $-77°$ for 7 days Br_2 was added to the gas-CH_2Cl_2 soln which was allowed to return to room temp The brominated soln was evaporated under red pres yielding a small residue of a pale yellow oil which was found by AAS to contain Se

A series of unsuccessful attempts were made to trap sufficient quantities of the gas(es) to allow for GC/MS separation and identification of the selenium-containing gases The attempts were severely limited by the difficulties involved in obtaining a large enough mass of cells per vol of culture that would produce the desired quantity of these Se-containing gases

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