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Chitin Primary Production

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Key Word Index—*Cyclotella*; *Thalassiosira*; phytoplankton; diatoms; primary production; photosynthesis; carbohydrates; protein; amino sugars; *N*-acetyl-D-glucosamine; chitin; chitan; biochemical fractionation.

Abstract—Previous perspectives of chitin's role in the biosphere have been deficient because the role of chitin in primary production has been overlooked. Reasons for this oversight include confusion of chitins with the putative protein precipitates forming upon 5% trichloroacetic acid treatment. The cellular biology of known phytoplankton chitin-producers is presented within the context of documented biochemistry and ultrastructure. Several biochemical strategies were used in substantiating the presence of chitin in primary products in *in situ* experiments. Alkali and sodium dodecylbenzenesulfonate solubilized proteins differentiate protein and chitin. The high values of chitin disclosed for primary production will require rethinking of analytical strategies previously used for biogeochemistry and physiological ecology especially with respect to carbohydrates. Primary production of chitin and chitan must now be considered within the phytoplankton taxa.

Introduction

A significant component of the aquatic ecological carbon and nitrogen budget has been previously overlooked in phytoplankton primary production. The methods previously used for biochemically characterizing phytoplankton primary production are summarized in Fig. 1. As a consequence of this oversight, this component of primary production has been overlooked within analytical schemes relevant for aquatic biochemical ecology. Chitan and related chitins are the subject of this deficit. This deficit has occurred even though MacLachlan *et al.* [1] thoroughly described the properties of chitan (homopolymer of β-1,4-linked *N*-acetyl-D-glucosamine) as

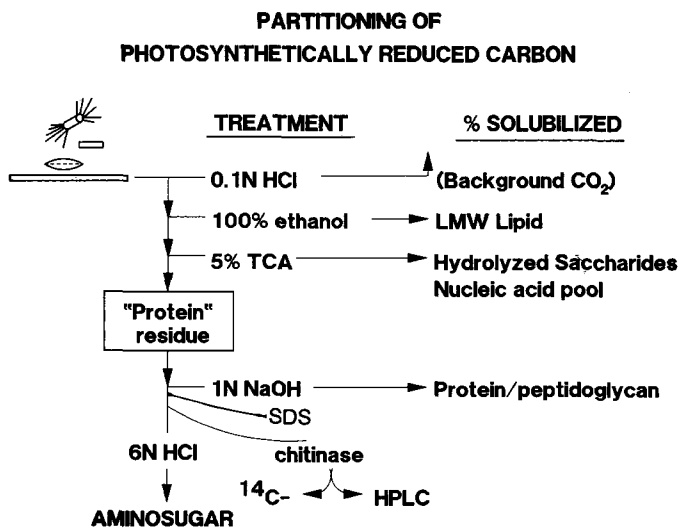

FIG. 1. SCHEMATIC DRAWING OF GENERALIZED BIOCHEMICAL PARTITIONING METHODS. The putative "protein residue" is the point of discussion of this paper. Subsequent fractionation steps are used in various combinations, depending upon the experimental requirements. Extractions and hydrolyses following the TCA precipitation step are required to identify chitin or chitan.

produced by *Thalassiosira fluviatilis* (*weissflogii*) and *Cyclotella cryptica* [2]. Both of these diatoms continue to produce chitan even under nitrogen limitation [3]. *Thalassiosira fluviatilis* chitan represents up to 34% of the total cell mass, including the silicate frustule [1]. Chitan described for these two organisms is a unique form of chitin. All animal and diatom sources of chitin are synthesized as linear repeating units of chitobiose, the disaccharide of β-1,4-linked *N*-acetyl-D-glucosamine. With the exception of diatom chitin, all other chitins are partially deacetylated after synthesis [2, 4]. Chitan is also unique among the chitans in that it is highly crystalline, not cross-linked with amino acids or other glycans, and is not complexed with calcium, magnesium, or other cations [1].

Herth *et al.* reported the detailed structure and site of synthesis for centric diatom chitan fibrils. Chitan fibrils are produced at invaginations of the cytoplasmic membrane which are located at the base of valve pores in centrate diatoms [5–7]. They found that when dyes such as calcofluor white were added to cultures, the fibrils were not extruded through the pores, but continued to be synthesized inside the frustules, outside the membrane [8].

Herth and Schnepf [9] reviewed chitin-fibril formation in algae. Chitin-fibril structure in *Thalassiosira fluviatilis* (centrate diatom alga) spines is highly crystalline in contrast to the lorica chitin of *Poterioochromonas stipitata* (chrysoflagellate alga) in which the chitin fibrils form an open mesh helix located outside the plasma membrane. The percentage of *N*-acetylation and other critical characteristics of the lorica chitin are not known. For the β-chitin of *Cyclotella meneghiniana* and *Thalassiosira fluviatilis* there are 2.5×10^5 and 7×10^5 GlcNAc glycosidic linkages formed per min per μm^2 of specialized synthesis membrane. Sites of chitin fibril synthesis for *Poterioochromonas stipitata* stalk produce 2×10^6 GlcNAc glycosidic linkages per min per μm^2 of specialized synthesis membrane.

Various algae have been reported to produce chitin or chitan. These include *Cyclotella socialis* [10], *Cyclotella cryptica* [3], *Thalassiosira fluviatilis* [1], *C. meneghiniana*, *Poterioochromonas stipitata* [9], *T. pseudonana* and natural populations [11]. Sharon [12] reviewed reports of chitin found in members of the Chlorophyceae (*Geosiphon*, *Cladophora glomerata*, *Oedogonium crassum*, *O. crassum amplum*, *Pithophora* spp., *Spirogyra*; *Mougeotia*). These findings of amino sugars (e.g. chitin) in lower heterotrophic forms is in distinct contrast to higher plants wherein only on occasion trace amounts of amino sugars are found.

At least one function of the centrate diatom exterior chitan spines is provision of buoyancy for the producing cell. When the spines are removed by gentle physical shearing action or digestion by chitinase [13] the otherwise intact diatom cells lose their normal buoyancy, settling 1.7 times faster after spine removal. Major steps in chitin biosynthesis are depicted in Fig. 2. The chitin synthesis inhibitor Polyoxin D, an antibiotic, effectively blocks synthesis of chitan fibers in *Thalassiosira weissflogii* and *Cyclotella cryptica* [14]. Polyoxin D is a competitive inhibitor of chitin synthetase (EC 2.4.1.16) [15]. Blocking chitan production with this antibiotic results in loss of cell buoyancy. However, the chitin synthesis inhibitor, Dimilin, a benzoyl urea pesticide, has no apparent affect on chitan fibril synthesis. Cell number and buoyancy, likewise, were unaffected. Surface ultrastructure of the Dimilin-treated cells, as evaluated by scanning electron microscopy was indistinguishable from control [14].

Lindsay and Gooday [16] showed that *Streptomyces* chitinase hydrolyzed only at the apices of purified chitan spines, causing shortening of the spines but not reduction of the width of the spines. This inference of processive hydrolysis was substantiated by HPLC analysis of products resulting from chitinase digestion of diatom spines which were purified from pure cultures of *Thalassiosira psuedonana* and from estuarine samples [11]. Only the disaccharide, chitobiose, was released from these two types of algal samples. In contrast, colloidal arthropod chitin was hydrolyzed by the same

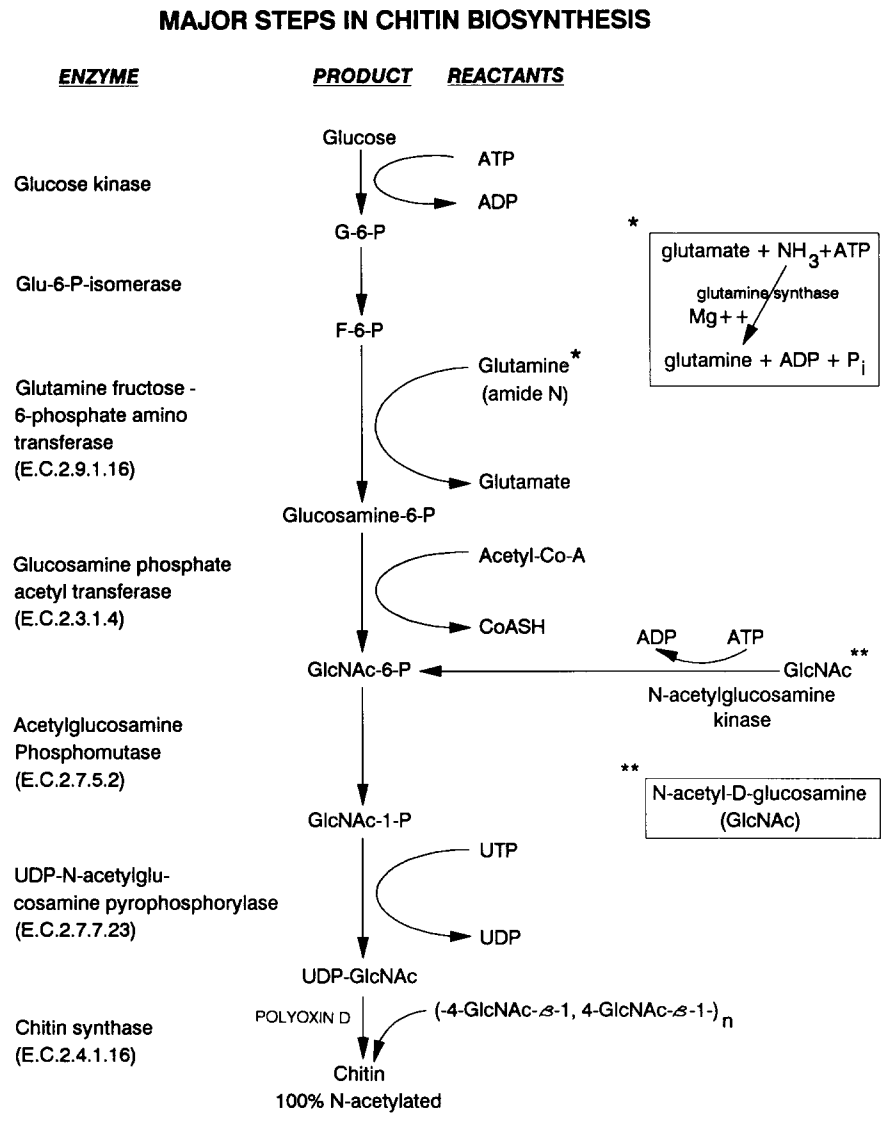

FIG. 2. MAJOR STEPS IN CHITIN BIOSYNTHESIS, INCLUDING THE LINKAGE BETWEEN AMINO ACID SYNTHESIS AND GLUCOSAMINE SYNTHESIS. Polyoxin D, a structural analogue of UDP-*N*-acetyl-D-glucosamine, is a competitive inhibitor of chitin synthetase. All producers of chitins other than chitan, articulate post-synthesis deacetylation and a plethora of subsequent organic crosslinking and calcifying steps.

Streptomyces griseus chitinase with an initial release of high order oligomers and subsequent formation of chitobiose [11].

Why, then, has not chitin or chitan been appropriately considered in the biochemistry of phytoplankton primary production? One of the reasons is that 5% trichloroacetic acid (TCA) at 100°C presumably hydrolyzed all phytoplankton polysaccharides [17, 18]. The biochemical frationation scheme in Fig. 1 depicts a generalized extraction series which conventionally terminates in the hot 5% TCA treatment which produces the putative protein precipitates. This generalized assumption was based upon radio-chromatography budgets of hydrolyzed carbohydrates for one alga, *Phaeodactylum*

tricornutum [18]. Subsequent work generally took the lead of this early work. One of the problems in the original work was the failure to realize that 4–6 N HCl hydrolysis at 100°C for 12–20 h is necessary to achieve complete chitin hydrolysis. Weaker acid hydrolysis such as 0.5–2.0 N HCl or 0.5 M H_2SO_4 are ineffectual in chitin hydrolysis, but are more than sufficient for most non-amino sugar hydrolyses [4, 19]. As a consequence, numerous investigations of primary production have evaluated dissolved and particulate carbohydrates by methods such as the phenol–sulfuric acid method expressing results as "total" carbohydrates [20]. In order for even free glucosamine (much less the *N*-acetylated form) to react with the conventional reducing sugar assays, the amine group must be removed. Once this is accomplished, then the free glucose reacts readily in the phenol–sulfuric acid system [21].

Smucker and Dawson [11] have shown that nearly all of a ^{3}H-labeled chitin (labeled at the C-1 position of *N*-acetate) remains an unfilterable precipitate after 30 min at 100°C in 5% TCA. The specific activity of the tritium label was used since it afforded over 10 times the sensitivity of a ^{14}C-1 label. A Whatman GF/F glass fiber filter was the carrier for cell components surviving the ethanol extraction. Therefore the glass fibers of the filter may have induced some non-enzymatic exchange of the ^{3}H. This non-enzymatic catalytic exchange phenomenon is widely known to occur in the presence of inorganics, often introducing problems of interpretation in environmental studies.

The present objective is to provide an overview of the current state of knowledge in primary production of chitin or chitan and present evidence of the possible ubiquity of the polymer in primary production. Several phytoplankton were screened for total amino sugars, setting the stage for future work in chitin analysis. Results of transmission electron microscopy of freeze-etch replicas of diatom chitan is discussed within the context of structure and function of chitan for centrate diatoms.

Materials and Methods

Phytoplankton cultures and growth media. Thalassiosira (TH 16) and *Cyclotella* (CY 2) cultures were obtained from Solar Energy Research Corporation; *Amphora*, from J. Weissman (Microbial Products Inc., Fairfield, CA) and *T. fluviatilis* from H. Millsaps (Chesapeake Biological Laboratory, CEES, The University of Maryland). Cell cultures used for analysis were received as grown in their respective silicate supplemented medium: TH 16, CY 2 and *Amphora* [22]; *T. fluviatilis*, F/2 medium [23]. Cells grown for radiolabeling were grown in F/2 medium.

Incorporation of [^{14}C]$NaHCO_3$ *into primary products.* Aliquots of culture fluids (49.5 ml) and 0.5 ml of 5 mM $NaHCO_3$ (specific activity 10 μCi/mmol) were dispensed into 50 ml borosilicate tubes stoppered with Teflon-lined caps. Following the appropriate incubation period, the contents of the tube were collected over a glass fiber filter (Whatman GF/F) and fractionated. Polyoxin D (25 ppm) (courtesy of H. Kuzuhara, Institute of Physical and Chemical Research, Sattaria, Japan) was added directly to sterile culture medium prior to initiation of the radiolabeling period. Labeled sodium bicarbonate was added to culture medium according to the same protocols as for the other fractionation studies (above) and the Polyoxin D control.

Fractionation of cell constituents. Labeled cells were fractionated using extensions of the methods described by Morris *et al.* [18]. The procedures, showing the methods used to fractionate the putative "protein" precipitate, are outlined in Fig. 1. One major change was made: addition of the sodium dodecylbenzene sulfonate (SDS) following the alkali extraction, in order to determine the efficacy of alkaline protein extraction. Specifics of filter manipulations and for liquid scintillation counting were in accordance with Smucker and Dawson [11].

Chitan purification. Thalassiosira fluviatilis was harvested from cells grown in 1-l volume in Doudoroff flasks of F/2 medium supplemented with sodium silicate and exposed to continuous fluorescent lighting (~4000 lux). At the start of incubation 5 ml of [^{14}C]$NaHCO_3$ stock solution was added to each litre to yield 12.5 μCi ^{14}C and 5 mM $NaHCO_3$. Aeration was achieved by once-daily swirling of the flasks. This minimal aeration was necessary in order to minimize fragmentation of the spines. Fifteen-day cultures were processed for harvesting the external segments of the chitan fibrils: 2–3 s homogenization of 500 ml per batch, 12 min centrifugation (8000 *g*) in 250 ml swinging buckets, pellets containing cells were discarded, supernatant containing the chitan fibers were collected over a 1-μm pore size Goretex PTFE membrane (only 600–700 ml of this supernatant would pass through the filter prior to causing flux rate failure). The result was a translucent gel. Immediately following collection, each filter was washed sequentially with 95% ethanol, water, 0.5 N HCl and water. With the vacuum off, the fibers were incubated for 5–10 min in the 0.5 N HCl prior to final water rinses. The individual chitan slurries were consolidated into a 40 ml Teflon centrifuge tube. At this point the concentrated gel of chitan fibers

was recoverable with a clinical centrifuge. Therefore, following each of the clean-up steps and washes, the chitan fibers were washed and collected by centrifugation. Even though the chitan fibers appeared quite clean they were sequentially extracted, in order to remove any significant contamination, with: 1 N NaOH at 100°C/30 min; washed once with water; 40 ml of 0.1 N HCl/25°C; 5% TCA/100°C/30 min; two water washes; 95% ethanol/25°C; 100% ethanol/100°C/30 min; three water washes. The resultant translucent gel was suspended in 0.02% sodium azide. Resultant particles had some fines. Using a strategy similar to that outlined for the chitinase assay it was determined that 20.7% of the diluted chitan preparation passed through Gelman A/E glass fiber filters (0.7–1 μm) and only 0.5% passed through 0.2 μm cellulose acetate filters. This means that colloids passing through the glass fiber filter are the source of background radioactivity.

Chitinase assay. Chitinase solution (500 μl) was placed into replicate 10×120 mm test tubes and equilibrated with 50 μl toluene for several minutes prior to addition of labeled substrate in order to inhibit microbial growth and uptake of enzyme hydrolysis products. Controls were heated or denatured with 100 μl 5% TCA prior to addition of labeled substrate. Hydrolysis products were determined by direct filtration into liquid scintillation cocktail and counted by liquid scintillation counting. In some cases, radiolabeled regenerated colloidal chitin (≥95% *N*-acetylated) was used for enzyme assays. Unlabeled regenerated colloidal chitin was used as controls for some of the hydrolyses.

Electron microscopy. Cultures of diatoms were collected via low pressure vacuum filtration at room temperature and transferred to sample holders. Cell paste was transferred to the gold specimen holders prior to quench freezing in liquid Freon 12. Samples were fractured at −190°C, etched and shadowed prior to carbon replication according to principles described in detail by Steere [24].

Results

Efficiency of the 1N NaOH protein extraction step for *T. fluviatilis* is demonstrated by the consistently low (0.3% total incorporated ^{14}C) residual SDS soluble fraction (Table 1). With respect to the biochemical partitions, there are only minor differences between the 6 h label and the 15 day labeling period. There is a slight increase in the final filter residue percentage of total incorporated label. The absolute increase in total incorporated label within each fraction is increased in the 15 day-labeled treatments; albeit this increase is not commensurate with the 14.5 day difference in total labeling period.

The chitin synthesis inhibitor, Polyoxin D (Table 2) induced only minor changes, a slight increase in the "protein" fraction. In this case, the TCA precipitate was not subfractionated to define the protein and chitin components. It was inappropriately assumed that the total fraction would be reduced with this inhibitor.

Using total amino sugar as a first index of possible chitin or chitan content of several algae (Table 3) it was observed that not only was there a wide difference between diatom types, but there was a significant difference between the methods of sample

TABLE 1. BIOCHEMICAL FRACTIONATION OF [^{14}C]$NaHCO_3$ LABELED *T. FLUVIATILIS**

			Distribution of ^{14}C-label†		
Label period	Ethanol*	TCA	NaOH	SDS	Filter
6 h	1,853,647	206,580	1,647,120	10,760	283,765
	(46.3%)	(5.2%)	(41.2%)	(0.3%)	(7.1%)
6 h	2,096,320	192,560	1,637,400	19,680	287,444
	(49.5%)	(4.5%)	(38.7%)	(0.5%)	(6.8%)
6 h	2,121,600	190,540	1,606,040	11,960	270,379
	(50.5%)	(4.5%)	(38.2%)	(0.3%)	(6.4%)
15 day	2,492,180	210,870	1,843,320	12,144	382,722
	(50.4%)	(4.3%)	(37.3%)	(0.2%)	(7.7%)
15 day	2,803,940	232,800	1,821,908	14,000	377,419
	(53.4%)	(4.4%)	(34.7%)	(0.3%)	(7.2%)

*Serial biochemical fractionation of ^{14}C-labeled components according to procedures discussed in the text and outlined in Fig. 1. All treatments were run at 100°C: 3 ml of 100% ethanol (EtOH)/20 min; 4 ml 5% trichloroacetic acid (TCA)/40 min; 4 ml of 1 N NaOH/60 min; 4 ml of 2% sodium docecylbenzene sulfonate (SDS)/30 min.

†Data is expressed as mean d.p.m. of ^{14}C solubilized by the stated treatment or in the final filter residue of triplicate measurements. Values in parentheses are percentage solubilization of the given extraction step.

TABLE 2. EFFECT OF POLYOXIN D ON CLASSICAL BIOCHEMICAL PARTITIONING OF *THALASSIOSIRA PSEUDONANA*

Sample I.D.	^{14}C Released by extraction treatments* 100% Ethanol	5% TCA	TCA residue
T. pseudonana			
Control 1	99,476	8,701	87,890
	(50.7)	(4.4)	(44.8)
2	102,765	18,238	96,044
	(47.3)	(8.4)	(44.2)
3	124,358	14,311	88,782
	(54.6)	(6.3)	(39.0)
Mean	108,866	13,750	90,905
	(51.0)	(6.4)	(42.6)
T. pseudonana			
Polyoxin D 1	89,622	9,856	91,826
	(46.8)	(5.2)	(45.0)
2	97,981	12,430	90,460
	(48.8)	(6.2)	(45.0)
3	93,496	11,209	86,335
	(48.9)	(5.9)	(45.2)
4	96,681	10,846	88,940
	(49.2)	(5.5)	(45.3)
Mean	94,445	11,085	89,390
	(48.5)	(5.7)	(45.9)

*d.p.m. of extracted label. Extraction/hydrolysis performed as described in the text. Numbers in parentheses indicate per cent of total incorporated label. Details of Polyoxin D application are described in Materials and Methods.

TABLE 3. AMINO SUGAR CONTENT OF SELECTED PHYTOPLANKTON

Alga	Treatment	Per cent GlcNAc equivalent*
Thalassiosira No. 16§¶	Filter press†	3.5
Thalassiosira No. 16§¶	Centrifuge‡	6.7
Thalassiosira weissflogii ‖	Air lift filter	1.8
Amphora No. 5§¶	Whole culture	2.4
Cyclotella No. 10§¶	Whole culture	10.3
Thalassiosira No. 2§	Whole culture	4.1
Chitin "Y"**	Regenerated chitin	98.7

**N*-Acetyl-D-glucosamine equivalents (35) expressed as mean per cent of sample total dry weight, including the ash weight for a minimum of three replicates per treatment. †Retentate of a conventional wine press. ‡Pellet of a continuous-flow basket centrifuge. §Raceway cultures with paddle wheel aeration, courtesy of Solar Energy Research Institute. ‖Culture grown in presence of air stone with resultant vigorous upwelling. Not the same strain of *Thalassiosira* or culture medium as for *Thalassiosira* No. 16. ¶Samples shipped in the presence of ethanol or methanol, therefore solvent affects on colorimetric response were considered. Alcohol levels used in these analyses presented no interference or enhancement of amino-sugar color response. **Regenerated colloidal chitin, ≥ 95% *N*-acetylated.

management prior to the dry weight and acid hydrolysis treatments. These samples were collected from culture fluids or shipping vials via filtration through pre-weighed glass fiber filters. Each of the diatoms tested expressed significant contents of amino sugars in comparison to background color of filter blanks.

The TEM images of freeze-fractured fibril networks which are sandwiched between the frustules and the plasma membrane are shown in Fig. 3. Fibril appearance (arrows in Fig. 3B) in the chitan ribbon emanating from the valve marginal pore is similar to the fibrils networked underneath the frustule. These fibrils innervate the dimpled surface which mirrors the micro-pores of the valve.

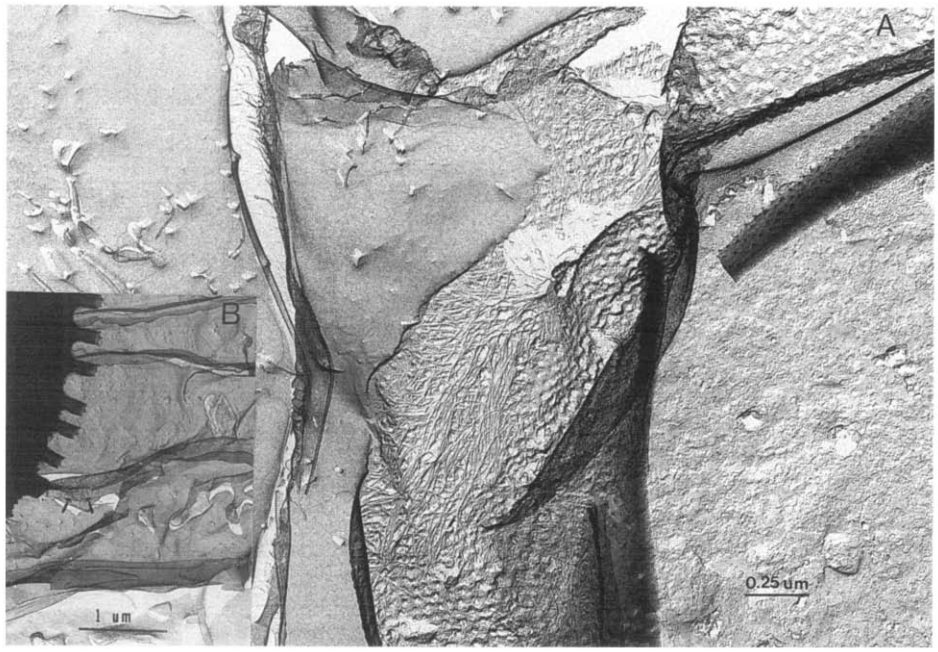

FIG. 3. TRANSMISSION ELECTRON MICROGRAPHS OF FREEZE-FRACTURED *THALASSIOSIRA WEISSFLOGGI* GROWN IN F/2 MEDIUM. (A) Low magnification of an intact frustule, revealing the marginal pores which are the exit sites of the chitan fiber bundles. (B) Higher magnification of a fracture plane occurring between the frustule and the plasma membrane, showing extensive networking of fibrils. These fibrils appear linked to the pore sites, having the same microstructure as the external ribbons of fibers. This region is the same area where chitan synthesis continues when polymer binding agents block "extrusion" of the fiber ribbons [5, 8]. Arrows show chitin fibrils which compose the diatom spines.

The *S. griseus* chitinase hydrolysis of chitan (Fig. 4) is consistent with the microscopic observations and model of Lindsay and Gooday [16] and the HPLC-defined end-product release patterns described by Smucker and Dawson [11]. After an initial equilibration, hydrolysis was linear for over 10 hours.

Discussion

Biochemical evidence presented in Table 1 confirms the results expected according to Smucker and Dawson [11] and Falk *et al.* [2]. However, there is a wide discrepancy between the total chitan values (31–34% total culture mass) reported by Falk *et al.* [2] and the average 7% incorporated ^{14}C into the filter residue (Table 1). One explanation is that the present work does not purport to demonstrate total chitan, only that there is additional biochemical evidence for chitin/chitan in the putative "protein" fraction often assumed in pelagic primary production. The hot alkali processing renders the glass fibers of the filters into an expanded mat, having lost its integrity. Even though this extracted filter is then transferred and processed over a new glass fiber filter, losses in this type of processing are yet unreported. From analysis of purified diatom chitan we know that up to 20% of the purified ^{14}C-chitan passes through Gelma A/E glass fiber filters which are similar in design and porosity to the Whatman GF/F filters used for the present biochemical fractionation. Furthermore, the *T. fluviatilis* (*weissflogii*) strains used [2] are not likely to have been the same as those used in the current work. Variances in culture conditions and differences in processing are additional explanations.

With the knowledge that chitan is in the classical 5% TCA "protein" precipitate, one would expect the chitin synthesis inhibitor, Polyoxin D, to perhaps reduce the final classic precipitate (Table 2). On the contrary, the antibiotic treatment resulted in a slightly increased classic "protein" component. The problem certainly could have been resolved had the protein been extracted as in Table 1. But, on the other hand, the similarity between treatments may simply reinforce the suggestion of Fig. 2 wherein there is a close link between amino acid synthesis and chitin synthesis. This finding merits further investigation in order to resolve the site of feedback inhibition of chitan synthesis.

The freeze-etch images of *T. fluviatilis* (Fig. 3) and the generally conceived fibrillar nature of chitan must not be limited to external fibers of these centrate diatoms. Herth *et al.* [8] have presented evidence that the flagellate *Poteriochromonas* produces chitin

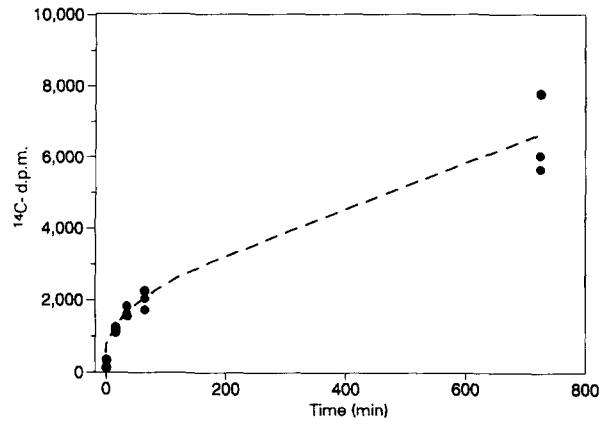

FIGURE 4. *STREPTOMYCES GRISEUS* CHITINASE DIGESTION OF PURIFIED CHITAN FIBERS *IN VIVO* RADIOLABELED AND ISOLATED FROM *THALASSIOSIRA WEISSFLOGII.*

fibrils in its lorica and that these fibrils are not exposed extracellularly as are the "spines" of centrate diatoms [1].

Long term hydrolysis of colloidal chitin [25, 26] reveals multiple stages of linearity. This is in contrast to the apparently single stage of product release rates observed by monitoring soluble radiolabeled product release. If there were a constant number of active hydrolysis sites, then one might expect linearity of hydrolysis of chitan spines. The linearity observed following the first level of substrate–enzyme equilibrium is consitent with the microscopic observations of Lindsay and Gooday [16] and the HPLC-monitored kinetics of *S. griseus* chitinase hydrolysis of *T. fluviatilis* chitan. What this means is that only the ends of the chitan fibrils are sites of hydrolysis and that endolytic effects are not seen.

Available evidence indicates that any measurement of total carbohydrates in primary production must include a new concept of what "total" means. Amino sugars must now be considered in ecological studies which focus on phytoplankton primary production. Additionally, detritus flux analysis must also include chitins from numerous invertebrates. Within the context of standard reducing sugar curves used for conventional phytoplankton, total carbohydrate assays, glucosamine, other free amino sugars such as mannosamine, *N*-acetyl-D-glucosamine (GlcNAc) and oligomers of GlcNAc give negligible response even when tested at 10 times the concentration of a neutral reducing sugar standard, D-glucose (Table 4). The amino sugar monomers must be deaminated in order for the glucose moiety to be readily reduced [21]. This resistance to the standard reducing sugar derivatization is not correlated with enzymatic digestion and transport. For example, Mayasich and Smucker [27] have shown that the digestive capacity for the chito-oligosaccharide is several times higher than for cellobiose and maltose in oyster digestive diverticulum.

The potential impact on oceanic carbon analysis deserves special mention since accountability of chitins (including chitan) may play a key role in deciding the efficacy of dissolved organic carbon (DOC) methods. Suzuki *et al.* [28] used several standard compounds in their determinations of per cent accountability by high temperature combustion. Although not chemically defined, a type of chitin was used which was "recovered" only at the upper edge of their instrument performance. The impact of cross-linking and complexation on this recovery is completely unknown. Furthermore,

TABLE 4. SUGAR RESPONSE TO THE PHENOL–SULFURIC ACID METHOD* OF DETERMINING REDUCING SUGARS

Sugar	% Expected response†
D-Glucose	100
Glycogen†	100
Acetic acid‡	0
Succinic acid‡	0
Ethanol‡	0
D-Glucitol‡	0
D-Gluconic acid‡	0
Bovine serum albumin‡	2.5
N-Acetyl-D-glucosamine†§	0
Glucosamine†	2.5

*Colorimetric response of individual sugars or polymers. Results from Dubois *et al.* [36], Handa [31], and Smucker and Pfister [19]. †Per cent of response based upon expected glucose content. ‡Per cent of response normalized only to weight and not to a fictionalized glucose content. §*N*-Acetyl-D-glucosamine did not yield a detectable response to the phenol–sulfuric acid method; even at 20 times the upper limit for the D-glucose standard curve.

the source and kind of chitin used by these authors was not even recognized as variable in chitin sources.

Using available primary production data, phytoplankton species abundance and species composition, chitin synthesis values were calculated for a site in the central mainstem of Chesapeake Bay: carbon production as a mean of four dates in May of 1972–73 at 1 m depth = 180 mg C m^{-3} h^{-1} [29]; from Kachur [30], 60% of this production is due to Bacillariophytes, i.e. 108 mg C m^{-3} h^{-1}; and assuming 10–30% of the bacillariophyte production is chitin [1, 11], then a conservative estimate of primary production of chitin (chitan) is 32.4 mg C m^{-3} h^{-1}.

It is common knowledge to producers of phytoplankton for bivalve rearing, that when the algae are transferred via pump, food quality realized by bivalves is reduced, in comparison to siphoned or drained phytoplankton cultures. We have observed significant quantities of chitan sheared from the cells of *T. fluviatilis* when large scale cultures were transferred to the Sharples centrifuge via centrifugal pump. The resultant "dissolved" phase was then ultrafiltered with ≥ 100,000 M_r hollow fiber filters (unpublished data).

The kind of interactions and circumstances in which chitin/chitan primary production must be considered are highlighted in Fig. 5. Present evidence strongly suggests that the high levels of chitin, chitan and other amino sugars associated with pelagic primary production will necessitate re-evaluation of biogeochemical data

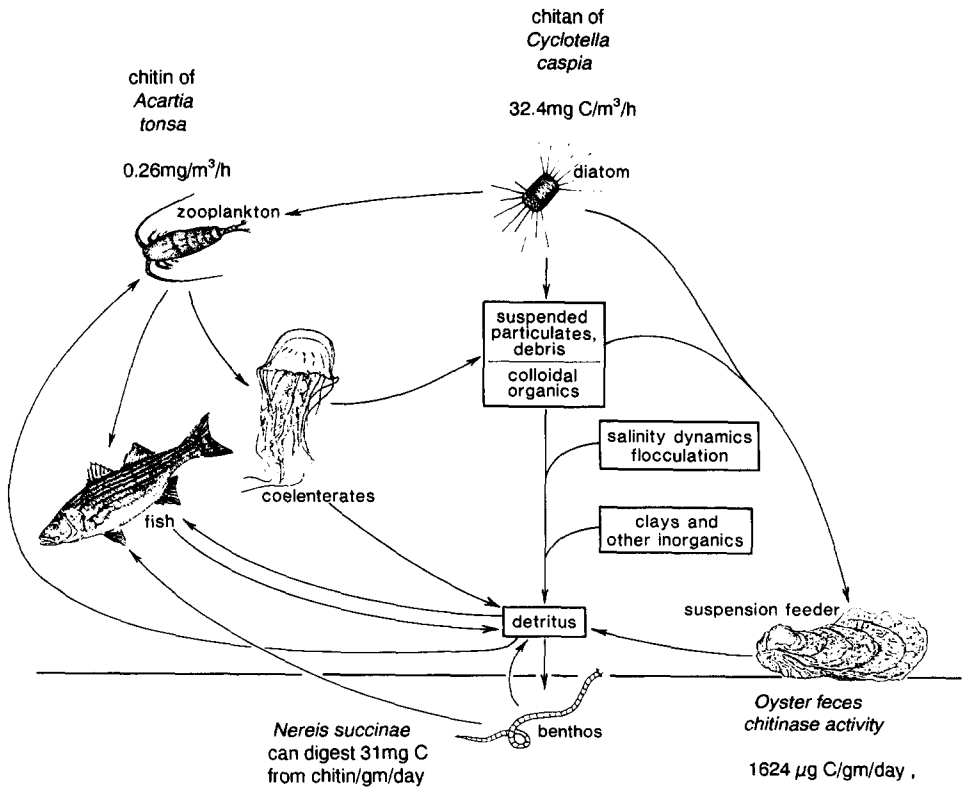

FIGURE 5. SCHEMATIC DRAWING OF MAJOR ESTUARINE AND MARINE COMPARTMENTS WHICH PARTICIPATE IN EITHER CHITIN PRODUCTION OR CONSUMPTION AND DIGESTION. Production values for *Cyclotella* are justified in the text and are shown as one indicator of phytoplankton primary production of chitin in the ecosphere. Production estimates for *Acartia tonsa* are derived from the population studies of Heinle [37]. Chitin digestion capacity was determined with radiolabeled colloidal chitin (details described in the text).

which purport to evaluate "total carbohydrates" [31, 32] in the dissolved phase of sea water and marine sediment humic and fulvic acids, respectively. Because chitin's role in the environment is usually conceptually limited to arthropod skeletons, the significance of animal alimentary digestive chitinases is sometimes limited to a can-opener function, enabling the host to access proteins [33, 34]. Many consumers (filter and suspension feeders) of phytoplankton have high digestive capacity for chitin (Fig. 5).

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