J. Phycol. 26, 299–306 (1990)

CHANGES IN CELL CHEMICAL COMPOSITION DURING THE LIFE CYCLE OF *SCRIPPSIELLA TROCHOIDEA* (DINOPHYCEAE)[1]

Thaithaworn Lirdwitayaprasit

Faculty of Agriculture, Kagawa University (United Graduate School), Miki-cho, Kita-gun, Kagawa-ken, 761-07 Japan

Tomotoshi Okaichi,[2] *Shigeru Montani, Tadashi Ochi*

Faculty of Agriculture, Kagawa University, Miki-cho, Kita-gun, Kagawa-ken, 761-07 Japan

and

Donald M. Anderson

Biology Department, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

ABSTRACT

The cellular content of carbon, nitrogen, amino acids, polysaccharides, phosphorus and adenosine triphosphate (ATP) was determined at several stages during the life cycle of the dinoflagellate Scrippsiella trochoidea *(Stein) Loeblich. Carbon per cell decreased slightly between exponential and stationary phase growth in vegetative cells whereas nitrogen per cell did not change. Both of these cellular components increased markedly on encystment and then decreased to vegetative cell levels during dormancy and germination. C/N ratios increased gradually during cyst dormancy and activation, reflecting a more rapid decrease in N than in C pools, even though both decreased through time. Amino acid composition was relatively constant during the vegetative cell stages; glutamic acid was the dominant component. Arginine was notably higher in cysts than in vegetative cells but decreased significantly during germination, suggesting a role in nitrogen storage. The ratio of neutral amino acids to total amino acids (NAA/TAA) decreased as cysts were formed and then gradually increased during storage and germination. The ratio of basic amino acids to total amino acids (BAA/TAA) changed in the opposite direction of NAA/TAA, whereas the ratio of acidic acids to total amino acids (AAA/TAA) was generally invariant. Amino acid pools were not static during the resting state in the cysts; there was degradation or biosynthesis of certain, but not all, classes of these compounds. The monosaccharide composition of cold and hot water extracted polysaccharides was quite different between cells and cysts. A high percentage of glucose in cysts suggests that the storage carbohydrate is probably in the form of glucan. Total cellular phosphorus was higher in all cyst stages than in vegetative cells. However, ATP·cell^{-1} decreased as vegetative cells entered stationary phase and encysted, and continued to decrease in cysts during dark cold storage. ATP increased only as the cysts were activated at warm temperatures in the light and began to germinate. The above data demonstrate that dormancy and quiescence are not periods of inactive metabolism but instead are times when numerous biochemical transformations are occurring that permit prolonged survival in a resting state.*

Key index words: adenosine triphosphate (ATP); amino acids; carbon; chemical composition; cyst; life cycle; nitrogen; phosphorus; polysaccharides; Scrippsiella trochoidea

Studies of the chemical composition of phytoplankton under laboratory conditions have been motivated by the importance of such information in general ecology and physiology (e.g. Sakshaug and Holm-Hansen 1977, Chan 1978, 1980, Hunter and Laws 1981), in assessing the nutritional value of species as food for higher trophic levels (e.g. Okaichi 1974, Hayashi et al. 1986) and in chemotaxonomy (Alam et al. 1984, Harvey et al. 1988). There are relatively few studies of chemical composition changes associated with a species' transition between life cycle stages. Most published data are for diatoms. For example, French and Hargraves (1980) showed that diatom resting spores held in darkness maintained low carbon specific respiration rates while continuing to accumulate or synthesize cellular carbon and chl *a*. Levels of carbon and chl *a* attained by the spores were higher than those of vegetative cells of the same species. Doucette and Fryxell (1983) showed that C/N and C/chl *a* were higher in *Thalassiosira antarctica* resting spores than in vegetative cells. Carbon content was lower in exponentially-growing vegetative cells and young resting spores relative to older spores that had been exposed to light, whereas nitrogen per cell was significantly higher in vegetative cells than in either young or old spores.

Dinoflagellate cell composition during encystment and excystment has only been examined in a few studies. In NO_3^- limited encystment medium, the C/N ratio of *Gyrodinium uncatenum* increased during exponential growth as cysts were formed, whereas in PO_4^{3-} limited encystment medium, the ratio was relatively invariant (Anderson et al. 1985). In a study complementary to the work reported here, Binder and Anderson (1990) examined the bio-

---

[1] Received 20 November 1989. Accepted 8 February 1990.

[2] Address for reprint requests.

chemical composition and metabolic activity of *Scrippsiella trochoidea* vegetative cells and cysts. Where that study focused on respiration rates and the changes in large macromolecular pools such as protein, lipid and carbohydrate, our study monitored cellular carbon, nitrogen, amino acids, polysaccharides, phosphorus and adenosine triphosphate (ATP) for the same species. Together, these two studies provide a detailed picture of the physiological changes that are associated with life cycle transformations in this important dinoflagellate species.

MATERIALS AND METHODS

Axenic cultures of *S.trochoidea* (Stein) Loeblich (isolated from the Seto Inland Sea by T. Yoshimatsu) were routinely grown in 500 mL flasks containing 300 mL autoclaved ESM-enriched Seto Inland seawater (31 ± 1%), without soil extract (Okaichi et al. 1983). Cultures were maintained at 21 ± 1° C under a 14:10 h L:D light cycle (cool white fluorescent illumination at approximately 4000 lux). One liter of stock culture was inoculated into each of five 10 L glass carboys containing 9 L of ESM enriched Pacific seawater (diluted to 31 ± 1‰ with distilled water) with HPO_4^{2-} at a reduced concentration of 0.28 μM. Cultures were maintained as described above and gently bubbled with sterile air.

The first samples were taken on the second day after inoculation during exponential growth (before the appearance of cysts and hereafter termed stage 1). The second sampling was on the ninth day when cells were in stationary phase and cyst production maximal. The motile vegetative cells were separated from sedimented cysts by siphon after air bubbling was stopped and the carboys allowed to stand undisturbed for 1 h. These vegetative cells were termed stage 2, and cysts collected at the same time were termed stage 3. Cysts at the bottom of the carboys were separated from remaining motile cells using Percoll-sorbitol seawater and density gradient separation (Price et al. 1978, Binder and Anderson 1987). The cyst samples were then divided into three groups. One was used immediately for chemical analyses (stage 3). The second group was kept in darkness at 5° C for 7 days before analysis (stage 4). The third group was stored in darkness at 5° C for 7 days, placed in the culture room (14:10 h L:D, 4000 lux, at 21° C) for 3 days and then analyzed (activated cysts; stage 5). Some stage 4 cysts were placed in 20 test tubes (15 × 10 mm) and centrifuged at 3000 rpm for 15 min. Old medium was removed and replaced with 1 mL of new enrichment medium. The cysts were sonicated with a Bransonic 220 for 5 min and incubated in the culture room as above. The newly germinated cells from those tubes were collected three days after incubation by removing the culture medium with a pipette (stage 6).

All samples obtained at each stage of the life cycle were filtered on ignited GF/C glass fiber filters and stored at −20° C prior to analysis, except samples used for cellular ATP and polysaccharides which were treated as described below. The samples on the GF/C filters were freeze dried, and carbon and nitrogen were measured using a Yanaco, MT-3 CHN analyzer. For amino acids, the filtered samples were hydrolyzed with 6 N HCl at 105° C for 22 h and then analyzed on a Hitachi, L-8500 high speed amino acid analyzer.

For cold water extraction of crude soluble carbohydrates, the filters were homogenized for 15 min in distilled water using a glass homogenizer and then centrifuged at 3000 rpm for 15 min. The supernatant was filtered and the dissolved polysaccharides precipitated with four volumes of ethanol. After centrifugation, the crude polysaccharide pellet was dissolved with distilled water and dialyzed for 2–3 days against running water. The solution was evaporated, freeze dried and hydrolyzed with 2 M trifluoroacetic acid (TFA) overnight at 100° C. Subsequent reduction, acetylation and analysis of alditol acetates of sugar by gas-liquid chromatography followed the method of Okutani (1984).

For hot water extraction of crude soluble carbohydrates, samples were first extracted with $CHCl_3$ to remove lipid and pigment. The defatted samples were extracted with hot 80% ethanol and centrifuged, the supernatant was discarded and the residue extracted three times with hot distilled water. The hot water soluble polysaccharides were then determined as described above.

Total cellular phosphorus was determined by the methods of Anderson et al. (1985) and Strickland and Parsons (1968). ATP extractions were done in triplicate. For each, 1 mL of sample was injected into 10 mL of boiling TRIS buffer (0.025 M, pH 7.7). Direct injections were used to avoid filtering artifacts (Hunter and Laws 1981). The extracted ATP was assayed in triplicate using an SAI integration photometer, Model 2000 (Science Applications, Inc.) (Holm-Hansen and Booth 1966).

RESULTS

Scrippsiella trochoidea grew exponentially in encystment medium at a rate of 0.37 divisions·day^{-1} (Fig. 1). Cysts first appeared on day 3 and reached a maximum on day 9, equivalent to approximately 10% of the vegetative cell number at stationary phase.

S. trochoidea contained 1.6 ng C·cell^{-1} in early log phase growth (Fig. 2A, stage 1) and 1.5 ng C·cell^{-1} in stationary phase (stage 2). Carbon content in cysts (stage 3) was 10.6 ng C·cyst$-^{1}$ and decreased gradually to 6.7 and 3.9 ng C·cyst^{-1} during the cold storage and activation periods, respectively.

Cellular nitrogen followed the same general pattern as carbon (Fig. 2B). Nitrogen per cell was 0.3 ng·cell^{-1} in vegetative cells (stage 1 and 2), sharply increased to 1.4 ng·cyst^{-1} and then gradually decreased to 0.8 and 0.4 ng·cyst^{-1} during the cold storage and activation periods, respectively. The C/N ratio (Fig. 2C) was 6.6 and 5.1 for vegetative cells at stages 1 and 2, respectively. Thereafter, the ratio increased to 8.0 on encystment (stage 3), to 9.1 after cold storage (stage 4) and to 10.7 (stage 5) during activation prior to germination.

The composition of amino acids from hydrolyzed vegetative cells and cysts was quite similar. In cells at stages 1, 2 and 6 (Fig. 3), glutamic acid was the dominant component followed by glycine, alanine and aspartic acid. Methionine and cystine could not be detected in vegetative cells. In cysts at stages 3, 4 and 5, glutamic acid was also dominant, followed by glycine, arginine, aspartic acid and alanine. Glutamic acid and arginine were always higher in resting stages than in vegetative cells.

Between stages 1 and 2, the ratio of neutral amino acids to total amino acids (NAA/TAA) decreased slightly in vegetative cells (Fig. 4). During cyst formation, NAA/TAA dropped about 20% (stage 2 to stage 3) and then increased gradually. After cyst germination, NAA/TAA in new cells (stage 6) was almost the same as in vegetative cells at stage 2. The ratio of basic amino acids to total amino acids (BAA/TAA) changed in an opposite pattern to NAA/TAA, whereas the ratio of acidic amino acids to total amino

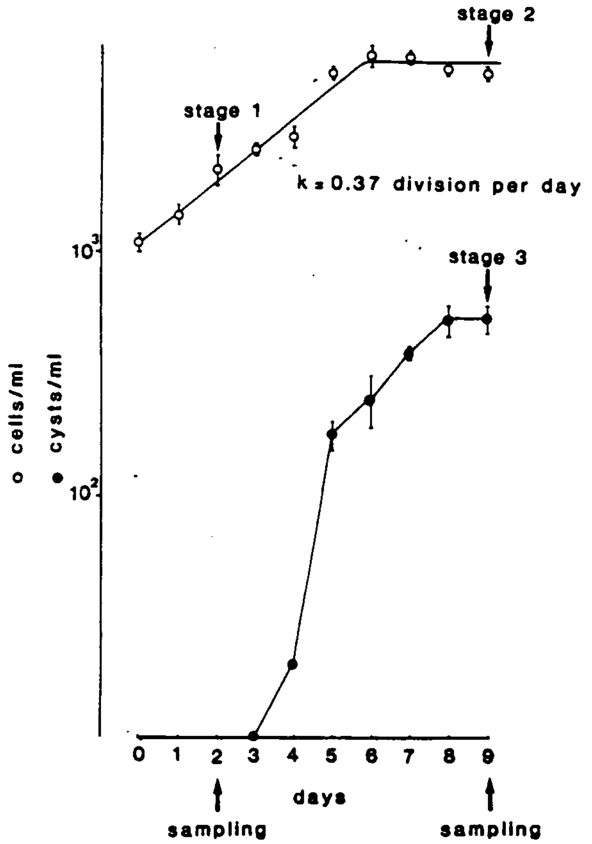

FIG. 1. Vegetative growth and cyst production dynamics of *S. trochoidea*.

acids (AAA/TAA) remained almost the same throughout the different stages.

Both cold and hot water extraction revealed that glucose was the dominant polysaccharide throughout the life cycle (Fig. 5, Table 1). During exponential growth, glucose, ribose and galactose were predominant in cold water extractable polysaccharides, whereas glucose, galactose and fucose were predominant with hot water extraction. In stationary phase cells following either cold or hot water extraction, glucose was higher than fucose and galactose (by factors of 1.7 and 2.7, respectively). In cysts, glucose was the main component with both extraction methods.

Cellular ATP (Fig. 6, Table 2) at stage 1 was 4.1 pg·cell^{-1} decreasing to 2.1 pg·cell^{-1} at stage 2. In new cysts (stage 3) harvested the same day as vegetative cells of stage 2, ATP was 3.4 pg·cyst^{-1}. This decreased to 2.4 pg·cyst^{-1} during cold storage (stage 4). During cyst activation in the culture room at warmer temperatures (stage 5), ATP content increased to 2.9 pg·cyst^{-1}. In newly germinated cells (stage 6), the ATP level returned to the same value as in vegetative cells in stage 1.

Total phosphorus in vegetative cells (Table 2) decreased about 42% between stage 1 and 2. In stage 3 cysts, total cellular phosphorus was over four times higher than that of stage 2 cells. After cysts were stored in the cold and transferred into warm culture room conditions for 3 days (activation, stage 5), P·cell^{-1} decreased slightly. The ratio of cellular ATP-phosphorus to total cellular phosphorus (ATP-P/TP) was essentially constant in motile stages 1 and 2 and about 3.4 times higher in resting stages 3, 4 and 5 (Table 2).

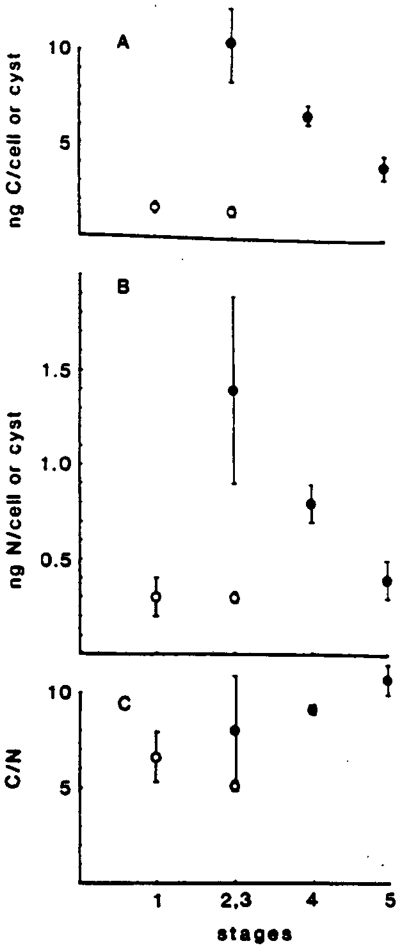

FIG. 2. C, N, and C/N of *S. trochoidea* at different stages. Vegetative cells (O); cysts (●); 1 = early log phase; 2 = stationary phase; 3 = new cysts; 4 = after cold storage for 7 days at 5° C (dormancy); 5 = after activation at 21° C for 3 days (activation); 6 = new vegetative cells (not shown).

The ratio of cell carbon to cell phosphorus gradually increased during the transition from vegetative cell to cyst and then gradually decreased during the cold storage and activation periods. This ratio was the same level in cysts before germination (stage 5) as it was in cells at stage 1.

DISCUSSION

Encystment and excystment of *Scrippsiella trochoidea* have been described by a number of authors (e.g. Wall et al. 1970, Watanabe et al. 1982, Binder and Anderson 1987). In contrast, little is known of the physiology of this or any other cyst-forming dinoflagellate during life cycle transformations.

In our experiments, physiological parameters were

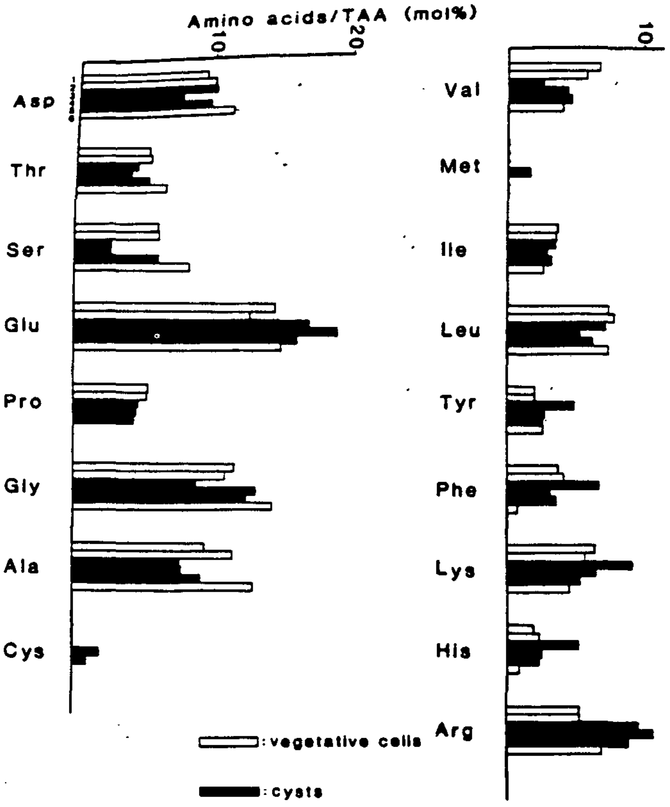

FIG. 3. Amino acid composition of *S. trochoidea* at different stages described in Figure 2.

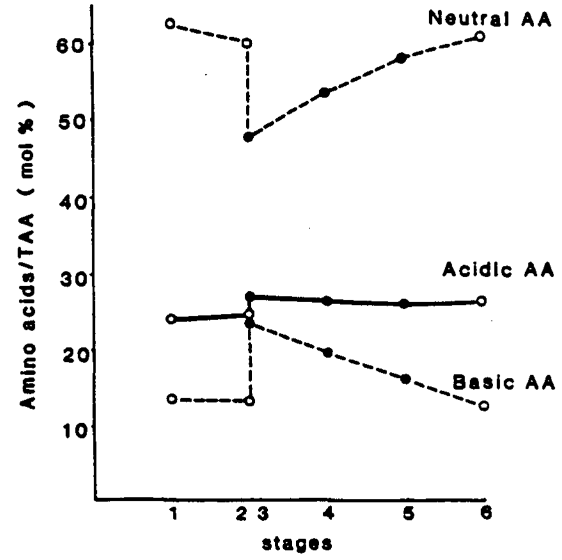

FIG. 4. Changes in amino acid composition in *S. trochoidea* vegetative cells (○) and cysts (●) at different life cycle stages described in Figure 2.

monitored at the different stages that occur during the transition from vegetative cell to cyst to vegetative cell. Stage 1 corresponds to rapid, exponential growth. Stage 2 represents stationary phase, and stage 3 represents newly-formed cysts. Stage 4 represents the period of maturation or true dormancy (Binder and Anderson 1987, Pfiester and Anderson 1987), stage 5 represents activation prior to germination and stage 6 the germling or newly-released vegetative cell. Only the first population of newly germinated cells was used for chemical analysis to avoid contamination from the other new cells which excysted on the day following incubation. About 80–90% of those activation cysts excysted within two weeks after incubation. Thereafter very few cells emerged from the remaining cysts (10–20%). The reasons why this cyst portion did not excyst are not clear. The physiological and environmental control of germination in this species was described by Binder and Anderson (1987).

Cellular carbon and nitrogen. Carbon per cell slightly decreased between stages 1 and 2 whereas nitrogen per cell did not change. Both of these components increased between 5 and 10 fold in stage 3 cysts (Fig. 2A, B). Since these increases were more than the factor of 2 expected from the fusion of two gametes, biosynthesis of storage products probably occurred prior to encystment. A similar accumulation was observed in carbohydrate, lipid, and protein in newly formed *S. trochoidea* cysts relative to vegetative cells in stationary phase (Binder and Anderson 1990). French and Hargraves (1980) showed

TABLE 1. *Monosaccharide composition of extracted polysaccharides from cells and cysts of* S. trochoidea. *Rha = rhamnose; Fuc = fucose; Rib = ribose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose.*

	Monosaccharide composition (mol %)							
	Rha	Fuc	Rib	Ara	Xyl	Man	Gal	Glc
Log phase cells (stage 1)								
Cold water extraction	2.2	3.4	21.2	ND[a]	2.4	7.8	13.9	49.2
Hot water extraction	1.1	8.1	10	1.5	3.0	3.8	8.6	63.9
Stationary phase cells (stage 2)								
Cold water extraction	4.8	23.6	5.0	2.3	1.0	6.3	14.3	42.9
Hot water extraction	1.4	23.4	9.5	1.3	3.9	6.6	15.2	38.6
Cysts (stage 4)								
Cold water extraction	ND	8.9	2.1	ND	ND	1.3	3.5	84.3
Hot water extraction	0.05	12.6	5.7	0.5	1.5	2.2	5.3	72.1

[a] Not detected.

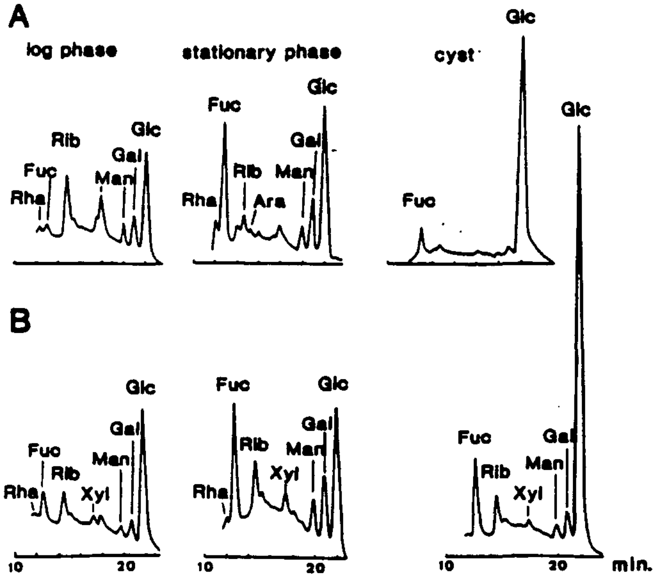

FIG. 5A, B. GLC separation of hydrolyzed cold water extracted (A) and hot water extracted (B) polysaccharides.

that the carbon content of spores of the diatom *Leptocylindrus danicus* Cleve was actually higher after storage in darkness than in vegetative cells and resting spores stored in the light. Such a carbon increase in the dark is difficult to explain. The authors speculate that it might have resulted from photosynthesis in response to undetected light leaks coupled with low respiration rates. Doucette and Fryxell (1983) showed higher carbon levels in *T. antarctica* spores than in vegetative cells, but those spores had been exposed to light prior to analysis. In our study, the increase in C of stage 3 cysts probably reflects photosynthesis and the accumulation of storage products by gametes or planozygotes in the light prior to encystment. Once the cysts were placed in darkness, $\mathrm{C \cdot cell^{-1}}$ decreased.

Since cell carbon and nitrogen decreased from stage 3 to 5 in *S. trochoidea*, it appears that C and N compounds were respired during dormancy and quiescence. Binder and Anderson (1990) demonstrated that carbohydrate is probably the major respiratory substrate in cysts during both quiescence and activation.

In stage 5, both cell carbon and nitrogen decreased, but the C/N ratio increased, suggesting that the respiration rate of stored nitrogen was higher than that of carbon during cyst activation. Doucette and Fryxell (1983) also showed that the C/N ratio of *T. antarctica* was higher in old spores than in both log phase vegetative cells and young resting spores; cellular nitrogen in vegetative cells was higher than in resting spores.

Amino acids. Chau et al. (1967) reported that combined amino acids in *Peridinium trochoidium* (= *S. trochoidea*) were predominantly glutamic acid, aspartic acid, leucine and alanine; cystine could not be detected. In general, these data agree with our results for vegetative cells, although we demonstrate that composition depends on the physiological state in each stage of the life cycle (Fig. 4). The ratio of neutral amino acids to total amino acids (NAA/TAA) decreased rapidly after cyst formation and then increased gradually during storage; the ratio of basic amino acids to total amino acids (BAA/TAA) changed in the opposite direction. These data are unexpected and difficult to explain. Clearly, amino acid pools are not static during the resting state in cysts; there is degradation or biosynthesis of certain, but not all, classes of these compounds. Upon germination, the new cell eventually reaches the same amino acid composition as the original vegetative cells prior to encystments.

Arginine was notably higher in all cyst stages than in all stages of vegetative cells (Fig. 3). Arginine is an important constituent of proteins but can also be a major nitrogen storage compound required for the biosynthesis of secondary products. Shargool et al. (1988) showed that the enzymes of arginine biosynthesis increased significantly during seed formation in pea plants, whereas the enzymes of arginine metabolism such as arginase and urease were most active during seed germination. This is entirely consistent with our data, which show that the rela-

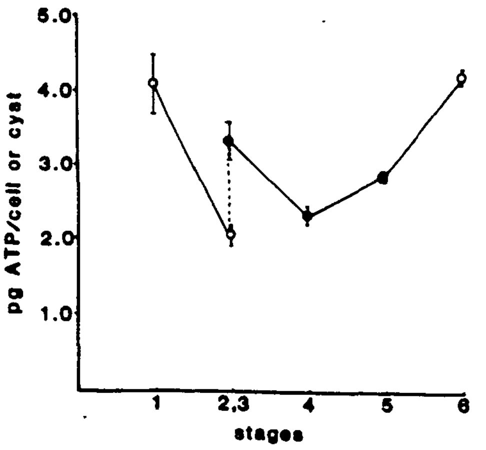

FIG. 6. ATP content of *S. trochoidea* cells (○) and cysts (●) at different cycle stages described in Figure 2.

TABLE 2. *Changes in cellular ATP, total phosphate (TP), ATP-phosphorus (ATP-P) and ratios of these variables at each life cycle stage. C = carbon; P = phosphorus; TP = total phosphorus.*

Stage	ATP ($\mathrm{pg \cdot cell^{-1}}$)	TP ($\mathrm{pg\ at \cdot P \cdot cell^{-1}}$)	ATP-P·cell^{-1} (by atom)	ATP-P/TP (by atom)	C/P (by atom)
1	4.14 ± 0.37	246.9	23.10	0.09	77.8
2	2.08 ± 0.14	142.3	11.61	0.08	126.5
3	3.35 ± 0.25	613.5	18.69	0.03	206.8
4	2.35 ± 0.13	622.4	13.11	0.02	129.2
5	2.89 ± 0.04	528.0	16.13	0.03	88.6
6	4.19 ± 0.07	—[a]	—	—	—

[a] No sample.

tive abundance of arginine increased during cyst formation (from stage 2 to stage 3) and decreased during cyst germination (from stage 5 to stage 6). It seems likely that nitrogen in cysts is stored in part as arginine that is then used as a source of nitrogen during germination.

Polysaccharides. Both cold and hot water extraction of polysaccharides revealed that glucose was the dominant sugar component. This result agrees with other observations on the distribution of sugars in natural populations of phytoplankton and in laboratory cultures. For example, Parsons et al. (1961) showed that the dominant sugar in hydrolysates of 11 species of marine phytoplankton was glucose; the minor sugars varied between species. Haug and Myklestad (1973) showed that glucose was the dominant monosaccharide in natural populations of marine phytoplankton. Our data are in agreement with these results but further demonstrate the major differences in the distribution of sugars between vegetative cells and cysts (Fig. 5, Table 1). The mol % glucose was notably higher in cysts than in vegetative cells, and ribose was correspondingly lower. Allan et al. (1972) found that polymers of the hot water extractable fraction were composed principally of glucose in five diatom species. The glucan polysaccharide is considered to be the most common food reserve material in algae (Boney 1966, Handa and Yanagi 1969). A water-extractable β-glucan, laminarin or leucosin appeared to be the main food reserve in the Phaeophyceae and Chrysophyceae, whereas a water extractable α-glucan, floridean starch, has been found to be the food reserve of the Rhodophyceae. In *Heterosigma akashiwo*, Raphidophyceae, the storage polysaccharide is also found as β-1,3-glucan (T. Ochi, unpubl.). The storage carbohydrate in *S. trochoidea* cysts is probably in the form of glucan, based on the predominance of glucose in this analysis.

Phosphorus and ATP. In PO_4^{3-} limited encystment cultures of *G. uncatenum*, Anderson et al. (1985) reported that cellular phosphorus gradually decreased from initial levels of 239 pg P·cell^{-1} to a subsistence quota of 29 pg P·cell^{-1} and stayed at that level in vegetative cells to the end of the experiment. Our study of *S. trochoidea* in similar medium showed the same initial trend, as cellular phosphorus decreased from stage 1 to stage 2 prior to cyst formation. In the cysts, however, it increased at stage 3 to about four times the level of stage 2 and remained high throughout cold storage. Here again, the increase in P per cell at stage 3 reflects in part the fusion of two cells to make one cyst. Since the observed increase was more than a factor of 2, our data indicated that the biosynthesis of storage phosphorus occurred during the encystment process. This observation is consistent with the speculation of Anderson et al. (1985) that nutrient uptake occurs during the planozygote stage.

In seeds of higher plants, stored phosphorus is in the form of the Ca, Mg or Mn salts of inositol hexaphosphate, such as phytin (Koller et al. 1962). Phytin constitutes up to 80% of the total phosphorus of seeds, the other 20% being organic phosphorus compounds such as nucleotides, nucleic acid, phospholipids, phosphorylated sugars and phosphoproteins. Inorganic phosphate appears to be present only in very small amounts. In the seed, an enzyme capable of hydrolyzing phytin appears during germination as the phytin disappears. This enzyme, phytase, is a phosphatase which apparently has a high affinity for phytin. The metabolism of phytin and phytase is clearly of great importance in seed germination. However, Mayer and Shain (1974) showed that the phytin-phytase system serves as a source for inorganic phosphorus during the later stage of germination when the phosphate supply may be limiting seed germination. For phytoplankton, it is clear that our level of knowledge is quite deficient in this area and that future efforts should investigate the chemical form of storage phosphorus in the cyst and its role in regulating cyst germination.

During cyst formation, cellular ATP decreased between stages 1 and 2, presumably due to the limiting concentration of phosphorus in the encystment medium. In newly-formed cysts (stage 3), cellular ATP was at an intermediate level between stage 1 and 2, again reflecting the fusion of two gametes. During cold storage, (dormancy; stage 4) ATP content decreased due to the probable use of ATP in general metabolism. Cysts in this stage were kept in the dark, and thus no new ATP was provided by photosynthesis; small amounts would be produced by respiratory catabolism in darkness. Even if light were present, Binder and Anderson (1990) demonstrated that photosynthetic capacity was unmeasurable in quiescent *S. trochoidea* cysts, although chlorophyll was present. ATP increased during activation (stage 5) in our study; in newly germinated cells (stage 6) it reached the same level as in the original vegetative cells. During germination of wheat seeds, ATP was shown to be necessary for organization of polyribosomes and the functioning of tRNA (Taylorson and Hendricks 1977). Germination may not depend on the synthesis of ATP since the small amount present in dry seeds can be sufficient for germination (Koller et al. 1962). In our study, the quiescent cysts of *S. trochoidea* maintained cellular ATP at a nearly constant level that is presumably sufficient for prolonged cyst survival in darkness in natural sediments. This level of ATP is probably sufficient for cyst germination as well since Binder and Anderson (1986) showed that this species can germinate in the dark after only a very brief pulse of light (sufficient to trigger photomorphogenesis but insufficient for photosynthesis). One way to monitor the metabolic condition of the cysts is to determine energy charge, EC (= [ATP + ½ ADP]/[AMP + ADP + ATP]), which is considered to be a good indicator of higher plant seed maturation and germination potential (Taylorson and Hendricks 1977). Further studies of biochemical processes in the life cycle of dinoflagellates or other classes of phytoplankton producing resting cells should investigate the value of EC as an indicator of metabolic condition.

In conclusion, our data provide a detailed picture of the complex physiological changes that occur as *S. trochoidea* alternates between vegetative and resting stages in its life history. Clearly, cysts and vegetative cells have different biochemical compositions. In particular, the newly-formed cysts contain elevated levels of carbon, nitrogen and phosphorus compared with cells at stationary phase. These levels reflect active accumulation during the encystment process, not just the doubling of elements that would occur as a result of gamete fusion. The biochemical composition of cysts changed during the cold storage and activation. Stored ATP is presumably important during excystment. Our results are consistent with the common view that dinoflagellate cysts represent life history stages formed with sufficient reserves of elemental and macromolecular compounds to survive extended periods under conditions unsuitable for vegetative metabolism.

The authors thank Dr. K. Okutani, Faculty of Agriculture, Kagawa University, for his advice in analysis of polysaccharides and for our use of his laboratory facilities, Miss A. Nakai, Faculty of Agriculture, Kagawa University, for her assistance and Mr. S. Yoshimatsu, Akashiwo Research Institute, Kagawa Prefecture, for providing his strain of *S. trochoidea*. This study was supported in part by the Scientific Research of Ministry of Education, Science and Culture (Monbusho) and by the National Sea Grant College Program Office, U.S. Department of Commerce, under grant No. NA86-AA-D-SG090, WHOI Sea Grant Project #R/B-92. Contribution No. 7259 from the Woods Hole Oceanographic Institution.

Alam, M., Randufa, R., Watson, D. A. & Loeblich III, A. R. 1984. Sterol distribution in the genus *Heterocapsa* (Pyrrhophyta) *J. Phycol.* 20:331-5.

Allan, G. G., Lewin, J. & Johnson, P. G. 1972. Marine polymers. IV. Diatom polysaccharides. *Bot. Mar.* 15:102-8.

Anderson, D. M., Coats, D. W. & Tyler, M. A. 1985. Encystment of the dinoflagellate *Gyrodinium uncatenum*: temperature and nutrient effects. *J. Phycol.* 21:200-6.

Binder, B. J. & Anderson, D. M. 1986. Green light-mediated photomorphogenesis in a dinoflagellate resting cyst. *Nature (Lond).* 322:659-61.

——— 1987. Physiological and environmental control of germination in *Scrippsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.* 23:99-107.

——— 1990. Biochemical composition and metabolic activity of *Scrippsiella trochoidea* (Dinophyceae) resting cysts. *J. Phycol.* 26:289-98.

Boney, A. D. 1966. *A Biology of Marine Algae.* Hutchinson Educational Ltd., London, 148 pp.

Chan, A. T. 1978. Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. I. Growth under continuous light. *J. Phycol.* 14:396-402.

——— 1980. Comparative physiological study of marine diatoms and dinoflagellates in relation to irradiance and cell size. II. Relationship between photosynthesis, growth, and carbon/chlorophyll *a* ratio. *J. Phycol.* 16:428-32.

Chau, Y. K., Chuecas, L. & Riley, J. P. 1967. The component combined amino acids of some marine phytoplankton species. *J. Mar. Biol. Assoc. U.K.* 47:543-54.

Doucette, G. J. & Fryxell, G. A. 1983. *Thalassiosira antarctica*: vegetative and resting stage chemical composition of an ice-related marine diatom. *Mar. Biol. (Berl.)* 78:1-6.

French, F. W. & Hargraves, P. E. 1980. Physiological characteristics of plankton diatom resting spores. *Mar. Biol. Lett.* 1:185-95.

Handa, N. & Yanagi, K. 1969. Studies on water-extractable carbohydrate of the particulate matter from the northwest Pacific Ocean. *Mar. Biol. (Berl.)* 4:197-207.

Harvey, H. R., Bradshaw, S. A., O'Hara, S. C. M., Eglinton, G. & Corner, E. D. S. 1988. Lipid composition of the marine dinoflagellate *Scrippsiella trochoidea. Phytochemistry* 27:1723-9.

Haug, A. & Myklestad, S. 1973. Studies on the phytoplankton ecology of the Trondheimsfjord. I. The chemical composition of phytoplankton populations. *J. Exp. Mar. Biol. Ecol.* 11:15-26.

Hayashi, T., Suitani, Y., Murakami, M., Yamaguchi, K., Konosu, S. & Noda, H. 1986. Protein and amino acid compositions of five species of marine phytoplankton. *Bull. Jap. Soc. Sci. Fish.* 52:337-43.

Holm-Hansen, O. & Booth, C. R. 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. *Limnol. Oceanogr.* 11:510-9.

Hunter, B. L. & Laws, E. A. 1981. ATP and chlorophyll *a* as estimators of phytoplankton carbon biomass. *Limnol. Oceanogr.* 26:944-56.

Koller, D., Mayer, M. A., Poljakoff-Mayber, A. & Klein, S. 1962. Seed germination. *Annu. Rev. Plant Physiol.* 13:437-65.

Mayer, A. M. & Shain, Y. 1974. Control of seed germination. *Annu. Rev. Plant Physiol.* 25:167-93.

Okaichi, T. 1974. Significance of amino acid composition of phytoplankton and suspensoid in marine biological production. *Bull. Jap. Soc. Sci. Fish.* 40:471-8.

Okaichi, T., Nishio, S. & Imatomi, Y. 1983. Mass culture of marine phytoflagellates, an approach to new sources of biologically active compounds. *In* Miyamoto, J. & Kearney, P. C. [Eds.] *IUPAC Pesticide Chemistry*, Vol. 2. Pergamon Press, New York, pp. 141-4.

Okutani, K. 1984. Structural investigation of the capsular polysaccharide produced by *Cryptococcus* isolated from seawater. *Bull Jap. Soc. Sci. Fish.* 50:1407-12.

Parsons, T. R., Stephens, K. & Strickland, J. D. H. 1961. On the chemical composition of eleven species of marine phytoplankton. *J. Fish. Res. Bd. Can.* 18:1001-16.

Pfiester, L. A. & Anderson, D. M. 1987. Dinoflagellate reproduction. *In* Taylor, F. J. R. [Ed.] *The Biology of Dinoflagellates.* Blackwell Sci., London, pp. 611-48.

Price, C. A., Readon, E. M. & Guillard, R. R. L. 1978. Collection of dinoflagellates and marine microalgae by centrifugation in density gradients of a modified silica sol. *Limnol. Oceanogr.* 23:548-53.

Sakshaug, E. & Holm-Hansen, O. 1977. Chemical composition of *Skeletonema costatum* (Grev.) Cleve and *Pavlova (Monochrysis) lutheri* (Droop) Green as a function of nitrate, phosphate and iron-limited growth. *J. Exp. Mar. Biol. Ecol.* 29:1-34.

Shargool, P. D., Jain, J. C. & Mckay, G. 1988. Ornithine biosynthesis and arginine biosynthesis and degradation in plant cells. *Phytochemistry* 27:1575-8.

Strickland, J. D. H. & Parsons, T. R. 1968. A practical handbook of seawater analysis. *Fish. Res. Board Can. Bull.* 167:1-311.

Taylorson, R. B. & Hendricks, E. S. B. 1977. Dormancy in seeds. *Annu. Rev. Plant Physiol.* 28:331-54.

Wall, D., Guillard, R. R. L., Dale, B., Swift, E. & Watabe, N.

1970. Calcitic resting cysts in *Peridinium trochoidium* (Stein) Lemmermann an autotrophic marine dinoflagellate. *Phycologia* 9:151–6.
Watanabe, M. M., Watanabe, M. & Fukuyo, Y. 1982. Encystment and excystment of red tide flagellates. I. Induction of encystment of *Scrippsiella trochoidea*. Nat. (Japan) Inst. Environ. Stud., Res. Rep. No. 30, Eutrophication and Red Tides in the Coastal Marine Environment, pp. 27–42.

J. Phycol. **26**, 306–311 (1990)

CARBON FIXATION IN CULTURED MARINE BENTHIC DIATOMS[1]

Paul V. Zimba,[2] *Michael J. Sullivan*

Department of Biological Sciences, P.O. Drawer GY, Mississippi State University
Mississippi State, Mississippi 39762

and

Hilary E. Glover

Bigelow Laboratory for the Ocean Sciences, McKown Point, West Boothbay Harbor, Maine 04575

ABSTRACT

The enzyme activity of ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBisCO) and phosphoenolpyruvate carboxylase (PEPC) was measured in four species of marine benthic diatoms isolated from subtidal sediments of Graveline Bayou, Mississippi. Enzyme activities were measured in cultures of Amphora micrometra *Giffen,* A. tenerrima *Aleem and Hustedt,* Nitzschia fontifuga *Cholnoky, and* Nitzschia vermicularis *Grunow that were grown at light levels supporting* μ_{max} *and at light-limiting irradiances. All four species exhibited similar RuBisCO : PEP ratios (range = 1–1.8) at* μ_{max}*; the lowest ratio (0.4) was observed in* A. micrometra. *Reduced light levels increased PEPC relative to that measured at* μ_{max} *in two species.*

Two-dimensional paper chromatography was used to determine the first products of carbon fixation in A. micrometra. *After a 15 s incorporation period, the first product of photosynthetic carbon fixation was 3-phosphoglycerate even though this alga had a PEPC activity that was three times higher than that of RuBisCO. After 30 s, over 50% of the recovered radioactivity was still in this compound. Stable carbon isotope analyses of a mixture of the four pennate diatoms also suggest the predominant carbon fixation pathway in these benthic diatoms was similar to C3 plants.*

Key index words: Amphora; *benthic diatoms; carbon fixation;* Nitzschia; *PEPC; photosynthetic pathways; RuBisCO; stable carbon isotope ratios*

Autotrophic carbon assimilation in plants is due primarily to two pathways: inorganic carbon fixation into C3 and C4 organic compounds. Both pathways require fixation of carbon dioxide by ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO, EC 4.1.1.39). In C-4 plants, this carboxylation is preceded by a decarboxylation reaction that liberates carbon dioxide from oxaloacetate, the formation of which was originally catalyzed by phosphoenol pyruvate carboxylase (PEPC, EC 4.1.1.31) or phosphoenol pyruvate carboxykinase (PEPCK, EC 4.1.1.49). PEPCK has been isolated from a variety of algae from the brown algal line including the Phaeophyceae (Weidner and Küppers 1973, Kerby and Evans 1983), Dinophyceae (Appleby et al. 1980), and Bacillariophyceae (Holdsworth and Bruck 1977, Kremer and Berks 1978) whereas PEPC is found in the Bacillariophyceae (Utter and Kolenbrander 1972, Davies 1979) and Chlorophyceae (Glover and Morris 1979). The principal difference between PEPC and PEPCK involves the phosphorylation of ADP to form ATP by PEPCK and a requirement of $MnCl_2$ or $MgCl_2$ for activation; $MgCl_2$ but not $MnCl_2$ is required for PEPC activation. These enzymes provide an energetically efficient mechanism for coupling anabolic (photosynthetic) reactions with catabolic ones associated with the TCA cycle (Kremer 1980, Kerby and Evans 1983).

Since the studies of Morris and coworkers on benthic diatoms (summarized in Morris 1980), little attention has been paid to benthic microalgal enzyme activity and the first products of carbon fixation. Morris (1980) reported much lower RuBisCO : PEPC activity ratios in several cultures of benthic diatoms as compared to planktonic diatoms and suggested C4 metabolism might be a dominant pathway of

---

[1] Received 27 December 1988. Accepted 8 February 1990.

[2] Current address and address for reprint requests: Department of Fisheries and Aquaculture, University of Florida, 7922 NW 71st St., Gainesville, Florida 32606.