J. Phycol. **26,** 289–298 (1990)

BIOCHEMICAL COMPOSITION AND METABOLIC ACTIVITY OF *SCRIPPSIELLA TROCHOIDEA* (DINOPHYCEAE) RESTING CYSTS[1]

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ABSTRACT

The composition and metabolic activity of cysts of the marine dinoflagellate Scrippsiella trochoidea *(Stein) Loeblich were examined during dormancy, quiescence, and germination. On a per cell basis, newly formed cysts contained an order of magnitude more carbohydrate but significantly less protein and chlorophyll a than did exponentially growing vegetative cells. Loss of lipid and carbohydrate from cysts during the initial dormancy period reflected a respiration rate estimated to be 10% of the respiratory activity in vegetative cells. Among older, quiescent cysts the calculated respiration rate decreased further to approximately 1.5% of the vegetative rate and appeared to proceed largely at the expense of carbohydrate reserves. These estimated rates of respiration were in good agreement with direct measurements of cyst oxygen consumption. The transfer of quiescent cysts to conditions permissive for germination resulted in a rapid increase in respiration rate, as evidenced by carbohydrate loss and O_2 consumption. The increased respiratory activity was followed by an increase in protein content and, later, by an increase in chlorophyll a content and photosynthetic capacity. Just prior to germination the P/R ratio became greater than 1, and the estimated chlorophyll-specific photosynthetic activity reached 75% of the rate in vegetative cells. Complete restoration of photosynthetic and respiratory capacity apparently was not achieved until after excystment. These data confirm the common assumption that dinoflagellate cysts represent true "resting" cells, containing extensive energy reserves and displaying greatly reduced metabolic activity.*

Key index words: cyst; dinoflagellate; dormancy; germination; Scrippsiella

Many species of dinoflagellates produce resting cysts as part of their life history (Dale 1983, Pfiester and Anderson 1987). These cysts are the result of sexual fusion and are generally assumed to represent inactive stages capable of surviving conditions that do not support vegetative growth. Although the potential importance of cyst production, dormancy, and germination in controlling natural dinoflagellate population dynamics has been recognized for some time, the physiological characteristics of these cysts remain largely unknown. In fact, the common assertion that dinoflagellate cysts represent "resting" stages has not yet been supported with direct physiological measurements. We previously described the environmental and endogenous control of germination in cysts of the marine dinoflagellate *Scrippsiella trochoidea* (Binder and Anderson 1986, 1987); in the present paper we directly examine the gross biochemical and metabolic characteristics of these cysts.

Our present knowledge regarding the metabolism of dinoflagellate cysts is derived exclusively from light-microscope and ultrastructural studies. The widely reported inclusion of lipid globules or starch grains in cysts suggests that the production of storage compounds accompanies cyst formation (Wall and Dale 1969, von Stosch 1973, Anderson 1980, Chapman et al. 1982). Studies in which the planozygote stage was observed indicate that this production occurs prior to the actual encystment of the cell (von Stosch 1973, Chapman et al. 1982).

The extensive ultrastructural rearrangement (compared to that of vegetative cells) reported in cysts of *Woloszynskia tylota* attests to the magnitude of the metabolic changes which most likely accompany cyst formation (Bibby and Dodge 1972). In particular, the loss of membranous components from the cytoplasm, the aggregation of thylakoids and presence of lipid bodies within the chloroplasts, and the altered appearance of the chromosomes were taken by the authors to suggest a general reduction in metabolic activity.

These observations are consistent with the view that dinoflagellate cysts represent resting stages in which decreased metabolic activity is combined with accumulation of storage compounds to enhance longevity under conditions which preclude a normal photoautotrophic existence. The results presented in this paper provide the first direct support for this hypothesis.

MATERIALS AND METHODS

Cyst production, storage, and germination. Isolation of the axenic clone of *Scrippsiella trochoidea* (Stein) Loeblich, designated SA10, and maintenance of cultures was as described previously (Binder and Anderson 1987).

S. trochoidea cultures produce cysts as they enter stationary phase (Binder and Anderson 1987). The cysts used in this study were all from batch cultures of SA10 grown in Vineyard Sound seawater supplemented with 1/10 f/2 nutrients (with 50 μM NH_4Cl instead of $NaNO_3$) (Guillard and Ryther 1962) at 18° C under a 14:10 h LD cycle (cool white fluorescent lamps, approximately 450 $\mu E \cdot m^{-2} \cdot s^{-1}$). For the composition time course, 140 25-mL cultures (in 25 × 150 mm borosilicate tubes) were inoculated from a single exponentially growing culture and incubated as above. Culture growth and encystment were monitored daily in

[1] Received 11 September 1989. Accepted 25 January 1990.

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three of those tubes. Within 12 days of the first appearance of cysts, the cultures were enriched with 1/10 f/2 nutrients and placed in darkness at either 3° C or 18° C. No germination occurs under these storage conditions (Binder and Anderson 1986). Germination was initiated by incubation at 18° C under a 14:10 h LD cycle.

Sampling. At each sampling time point, three culture tubes from each storage treatment were harvested; these represented the three replicates for that treatment in all subsequent analyses. Three aliquots of GF/F-filtered Vineyard Sound seawater (= "VSSW") were carried through the entire harvesting procedure on each sampling day and later served as blanks for all analyses.

Cysts were separated from vegetative cells with a Percoll-sorbitol step gradient (Price et al. 1978, Binder and Anderson 1987). After centrifugation (600 × *g*), vegetative *S. trochoidea* cells are retained at the Percoll-seawater interface, whereas cysts are pelleted at the bottom of the tube. Both fractions were recovered, washed, and resuspended in 2 mL VSSW. Small aliquots were removed from each suspension for cell counts and photography (for sizing), and the remainder was filtered at low vacuum (<2.5 cm Hg) onto ashed GF/C glass fiber filters. The filters were stored in 2:1 (v:v) chloroform/methanol at −20° C in Teflon®-capped borosilicate vials, the head spaces of which were flushed with N_2 prior to sealing. Samples for cell counts were preserved in 5% Utermöhl's solution (Utermöhl 1958). Cell volumes were calculated from length and width measurements (at least 40 per sample) in photographs of unpreserved cells, assuming an ellipsoidal cell shape.

Biochemical extraction. Samples were analyzed for chlorophyll *a*, lipid, carbohydrate, and protein following a sequential extraction scheme adapted from Li et al. (1980; see also Roberts et al. 1955, Sutherland and Wilkinson 1971, Hitchcock 1983).

Sample filters were carefully transferred to 12 mL graduated Teflon®-capped centrifuge tube tissue grinders (Bellco Glass, Inc., Vineland, NJ) and ground with glass pestles by hand for 2 min. Microscopic examination revealed that this treatment disrupted 98% of the cysts originally present. After grinding, the solvent in which the sample filters had been stored was added to the corresponding tube, and the samples were centrifuged at 2000 × *g* for 20 min at room temperature in a horizontal rotor. The pellet was resuspended in chloroform/methanol and centrifuged again. The combined supernatants from these two spins represented the "C/M" fraction containing lipids, chlorophyll *a*, and low molecular weight compounds.

The extracted pellets were resuspended in 5% (w/v) trichloroacetic acid (TCA), incubated at 90° C for 20 min, and centrifuged as above. The supernatant was set aside as the "TCA fraction," containing carbohydrates. In contrast to the results of Hitchcock (1983), who reported that only a small proportion of the total carbohydrate in *Gonyaulax tamarensis* was solubilized in hot TCA, we found that recovery of particulate carbohydrate in this fraction was approximately 78% of the total carbohydrate present in vegetative cells and 90% of that in cysts of *S. trochoidea* (Binder 1986). Nevertheless, the carbohydrate values reported in this study are the sum of carbohydrate measured in both the TCA and NaOH fractions (see below).

Finally, the TCA-extracted pellets were resuspended in 0.1 N NaOH and digested for 20 min at 90° C. Neither increased NaOH concentration nor increased digestion time resulted in increased protein liberation (Binder 1986). The digested samples were centrifuged, and the supernatant was removed and saved as the "NaOH fraction," containing protein and some residual carbohydrate. This fraction was neutralized with 1 N HCl prior to analysis.

Biochemical analyses. Chlorophyll *a* and phaeophytin were measured in the C/M fraction fluorometrically (Model 10 fluorometer, excitation filter #10-250, emission filter #10-051; Turner Designs, Mountain View, CA) (Strickland and Parsons 1972). The assay was calibrated with extracts from exponentially growing *S. trochoidea* cells, the chlorophyll *a* content of which was determined spectrophotometrically using the equations given by Parsons and Strickland (1963). The recovery of chlorophyll *a* in chloroform/methanol was not significantly different than that in 90% acetone. Prior to determining that acidification had no detectable effect on the subsequent recovery of lipid from the fraction, we did not measure phaeophytin in addition to chlorophyll *a* (this is the case for all vegetative cell samples and for cyst samples from days 21–78). Therefore, for Figure 1 no correction for phaeophytin was employed. Note that because exponentially growing cells have negligible phaeophytin (unpubl. data), the differences between exponential-phase cells and stationary-phase cells or cysts reported here (Fig. 1) represent conservative estimates. For comparisons among cysts, a constant phaeophytin/chlorophyll *a* ratio was assumed for days 21–78 (0.43 and 0.24 for cysts stored at 18° C and at 3° C, respectively), based on the measured ratios on days 101 and 141 of the time course and the initial days of the cyst germination experiment. The alternate assumption of equal phaeophytin/chlorophyll *a* ratios at both temperatures does not qualitatively change the relationships shown in Figure 2 and Table 1.

Lipids were assayed gravimetrically after two Bligh and Dyer (1959) extractions of the C/M fraction as follows. To the C/M samples was added 0.25 volumes of 0.9 $g \cdot L^{-1}$ $MgCl_2 \cdot 6H_2O$. After standing for 60 min at 5° C, samples were centrifuged (20 min, 2000 × *g*), and the upper phase was discarded. The samples were evaporated to dryness under N_2 at 40° C, redissolved in 1.6 mL chloroform/methanol, and extracted again, as above. The lower (chloroform) phase from this second extraction was loaded into pre-weighed aluminum weigh-boats, evaporated on a warm hotplate, and weighed to the nearest 0.1 μg (on a Perkin Elmer AD-2 Autobalance). VSSW blanks, carried through the entire sampling and extraction procedure, were subtracted from the sample values.

Protein was measured in the NaOH fraction with an adaptation of Bradford's (1976) dye-binding assay (Spector 1978). One milliliter of Coomassie Brilliant Blue solution (Biorad Laboratories, Richmond, CA) was added to duplicate 0.2 mL aliquots of the neutralized NaOH fraction, vortexed, and the absorbance at 595 nm read within 15 min. Bovine serum albumin was routinely employed as the standard, but the protein values reported were normalized to their bovine gamma globulin equivalents, as the response to this protein by the assay is considered more generally representative.

Carbohydrate was measured in the TCA and NaOH fractions using the phenol-H_2SO_4 assay of Dubois et al. (1956). Glucose standards in the appropriate blank solutions were used to calibrate the assay.

Metabolic rates. Photosynthesis and respiration were measured as oxygen production or consumption in a water-jacketed Clark-electrode incubation chamber (Rank Bros., Cambridge, England) similar to the one described by Delieu and Walker (1972). Illumination was provided by a 150 W incandescent flood lamp; light intensity was controlled with layers of black nylon screening and was measured with a scalar irradiance meter (Biospherical Instruments, Inc., San Diego, CA). The electrode was calibrated using N_2-flushed and air-saturated seawater as end points. Oxygen production or consumption rates were derived graphically from the chart recorder output and were corrected for oxygen consumption by the electrode. Preliminary experiments with vegetative *S. trochoidea* cells showed that O_2 consumption or production remained linear for at least 2 h under the assay conditions we employed.

For the germination experiment, cysts (which had been stored at 3° C in the dark) from 20 culture tubes were aseptically harvested, combined in a single 2.5 mL suspension in f/2 medium (Guillard and Ryther 1962), and incubated at 18° C under a 14:10 h LD cycle. Photosynthesis and respiration in this suspension were measured at the same time (mid-day) on each day. Imme-

diately prior to these measurements, the suspension was freed of any newly germinated vegetative cells with a Percoll step gradient (see above) and resuspended in fresh medium. Thus, the measured rates reflect the metabolic activity of encysted cells only. After this separation, the cysts were loaded into the electrode chamber and incubated at 18° C for 30 min in darkness, followed by sequential 30 min periods of light (260 $\mu E \cdot m^{-2} \cdot s^{-1}$), dark, and light. Oxygen consumption or production became linear within the first 5 min of each incubation period and remained constant for the remainder of that period. Mean respiration and photosynthetic rates were calculated using the two measurements made during the dark and light incubations on each day, respectively. Incubation of cysts at higher light intensities did not result in an increase in oxygen production, indicating that the measured photosynthetic rates were light-saturated. Following these measurements, the cyst suspension was recovered from the electrode, and its incubation under the LD cycle continued. Examination of the cyst suspension at the close of the experiment using epifluorescent microscopy (DAPI-stained samples; Porter and Feig 1980) failed to reveal significant numbers of bacteria relative to sterile controls.

Statistical analysis. Two-way ANOVA was employed to analyze the long term trends in cyst composition, taking time and temperature as the two treatment variables. As this approach necessitates a balanced design, the initial point (day 21) is excluded from this analysis. The F-max and Kolmogorov-Smirnov tests confirmed that sample variances were homogeneous and that sample values were normally distributed (Sokal and Rohlf 1981). Rates of carbohydrate loss were estimated with least squares linear regression, and the significance of the slope was tested within the regression ANOVA.

Among the measured rates of respiration and photosynthesis, significance was tested within the appropriate ANOVA, employing a priori pair-wise daily comparisons of O_2 consumption in the dark vs. the electrode blank, and of O_2 consumption in the dark vs. net production in the light, respectively. Standard errors for calculated gross photosynthesis and P/R values were derived from error propagation calculations, for which the error-MS term from the ANOVA was used as the within-sample variance, and its degrees of freedom (10) were used as the degrees of freedom associated with the SE estimate.

For estimating respiration rates from carbohydrate and lipid data, complete oxidation of substrate via standard respiratory pathways was assumed (Lehninger 1975). Thus, the ratio of μg substrate consumed to μmol O_2 produced was taken as 30 and 11 for carbohydrate and lipid, respectively.

RESULTS

Cysts and vegetative cells of *S. trochoidea* differed significantly in their biochemical composition (Fig. 1). In particular, late exponential-phase cells contained three times more protein and four times more chlorophyll *a* per cell than did newly formed cysts. On the other hand, the carbohydrate content of these cells was an order of magnitude less than that of cysts. Lipid concentrations in both stages were about equal.

Stationary-phase vegetative cells of *S. trochoidea* were considerably smaller than exponential cells (cell volumes = 2300 and 5900 μm^3, respectively); however, the per-cell composition differences between these two cell types was not solely the result of cell volume differences. The composition of stationary-phase cells, when normalized to cell volume, appeared to be intermediate between exponential-phase cells and cysts with regard to protein, carbohydrate, and chlorophyll *a* (Fig. 1). The lipid content of all three cell types was comparable on a per volume basis. For comparative purposes, twice the (per cell) stationary-phase composition is indicated in Figure 1, since each cyst is assumed to be formed by the fusion of two gametic cells.

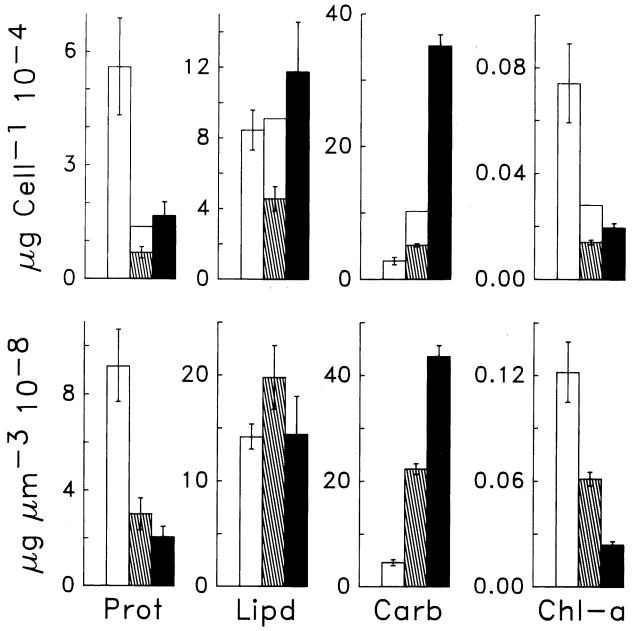

FIG. 1. Biochemical composition of late exponential-phase cells (open bars), stationary-phase cells (hatched bars), and newly formed cysts (dark bars). Top row: Composition on per cell basis; open bars above hatched bars indicate twice the stationary-phase cell composition (see text). Bottom row: Composition normalized to cell volume. Means ± SE (n = 3).

Changes in the biochemical composition of cysts stored in the dark at 3° C and at 18° C were monitored over time (Fig. 2). Cysts stored at 18° C contained significantly less lipid and chl *a* and significantly more carbohydrate over the course of the experiment than those stored at 3° C (Table 1). Protein content was not affected by storage temperature. All cysts for this experiment were produced in the same cultures at the same time and therefore must have had identical biochemical composition initially.

Storage time exerted a statistically significant effect on all four parameters (Table 1). Since the initial composition sample (day 21) was excluded from this analysis (see Methods), this time effect is independent of those changes which occurred within the first 28 days of storage. The significance of the effect of storage time on protein is clearly the result of the single low protein values from day 141 (Fig. 2B) and must therefore remain suspect. However, for lipid and chl *a*, the time effects are reflected as monotonic upward or downward trends, respectively. Only in the case of carbohydrate is the effect of storage time different at different temperatures (i.e. the interaction term for this parameter is significant [Table

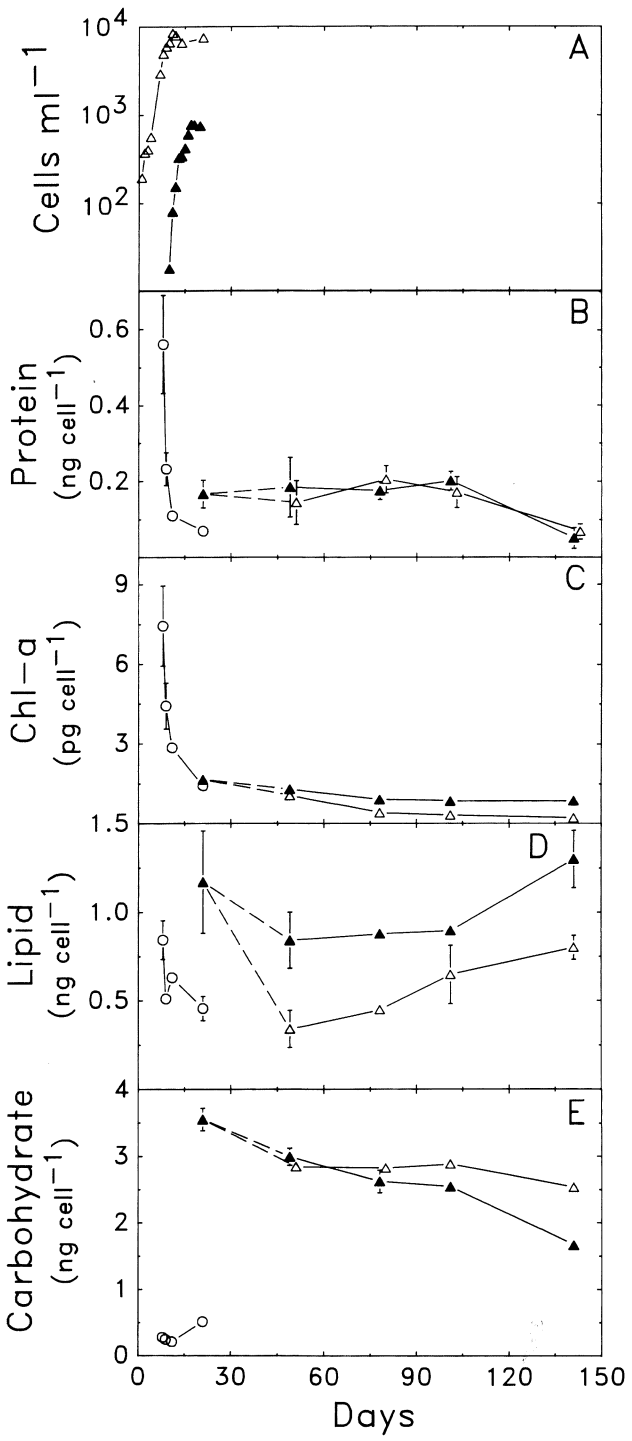

FIG. 2A–E. Changes in biochemical composition in vegetative cells during cyst formation and in cysts during dormancy and quiescence. A) Culture growth and cyst formation: vegetative cells (△) and cysts (▲); B) protein; C) chlorophyll *a*; D) lipid; E) carbohydrate per cell over time: vegetative cells (○), cysts stored at 18° C (△), cysts stored at 3° C (▲). Cyst age was taken as 0 on day 15; storage treatments were initiated on day 21. Means ± SE (n = 3).

TABLE 1. *Statistical analysis of changes in biochemical composition of quiescent cysts over time. Cysts stored at 3°C or 18°C in the dark. Two-way ANOVA of data from Fig. 2, days 49–141. NS = P > 0.05, * = P < 0.05, *** = P < 0.001.*

Constituent	Factor		
	Time	Temperature	Interaction
Protein	*	NS	NS
Lipid	*	***	NS
Carbohydrate	***	***	*
Chl *a*	***	***	NS

1]). Thus, from day 49 through 141, carbohydrate in 3° C-stored cysts decreased steadily, while that in 18° C-stored cysts dropped only very slowly, if at all (Fig. 2E).

Germination was initiated by incubating cysts (stored at 3° C in the dark) at 18° C under a 14:10 h LD cycle (Fig. 3). Upon such activation, the rate of carbohydrate loss increased by greater than 10-fold relative to that in unactivated cysts. This change in rate occurred within 12 h of activation; the rate of carbohydrate loss remained high thereafter, through at least 48 h. Within the first 24 h, cyst protein content began to increase; it more than doubled by 72 h. After a lag of 24 to 36 h, chl *a* content increased dramatically, reaching a maximum at 72 h of five times its initial level, and then declined to near initial levels by 120 h. Lipid content did not change significantly over the course of the experiment. Note that by 120 h, 70% of the cysts had germinated (Fig. 3C). The maximum germination frequency for this batch of cysts (measured on a subsample just prior to the experiment) was 85%. Protein and lipid measurements do not extend beyond 72 h because the decrease in sample size after this time resulting from germination reduced these parameters to concentrations near or below their limits of detection.

The compositional changes observed in activated cysts were paralleled by changes in metabolic activity (Fig. 4). Cyst respiration rate increased by an order of magnitude during the first 3 days of incubation. Gross photosynthetic capacity ("P-gross") was unmeasurable initially and on day 1 but increased sharply thereafter. As a result of the large increase in respiration rate over the same period, however, net photosynthesis was not greater than zero until day 4, the first day that germination was observed. At that time the P-gross/R ratio reached approximately 1.4 (Table 2).

Despite the dramatic increases in respiration and photosynthetic capacity in activated cysts, the maximum observed rates were still considerably below the rates for exponentially growing *S. trochoidea* vegetative cells (Table 2). On a per cell basis, respiration rate and P-gross in fully activated cysts were approximately 50% and 30%, respectively, of the values for vegetative cells. Note, however, that the estimated photosynthetic rate normalized to chl *a* was

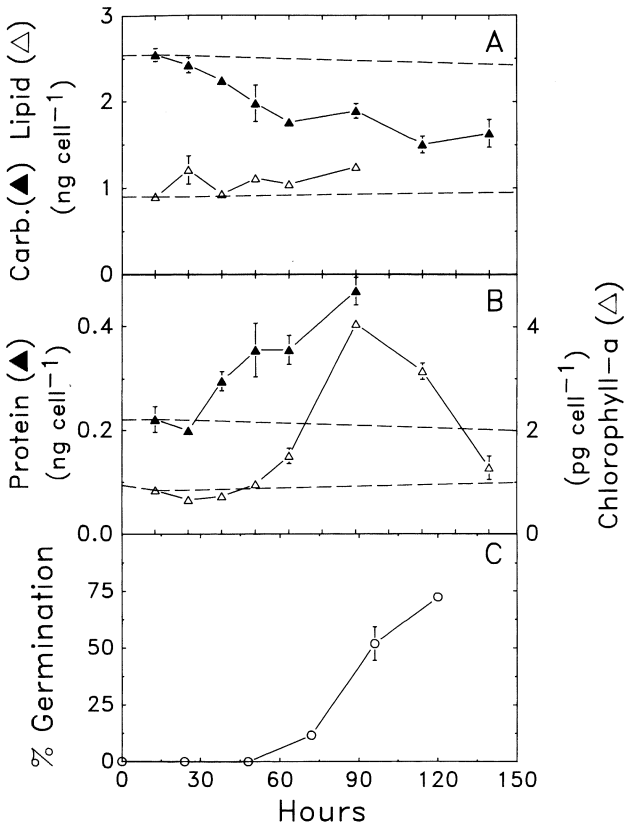

FIG. 3A–C. Changes in cyst composition during germination. Cysts stored in the dark at 3° C and transferred to a LD cycle at 18° C at time 0. A) Carbohydrate (▲) and lipid (△) per cyst. B) Protein (▲) and chlorophyll *a* (△) per cyst. C) Germination frequency. Dashed lines indicate the trends in composition in quiescent cysts over the same time period (from Fig. 2). Means ± SE (n = 3).

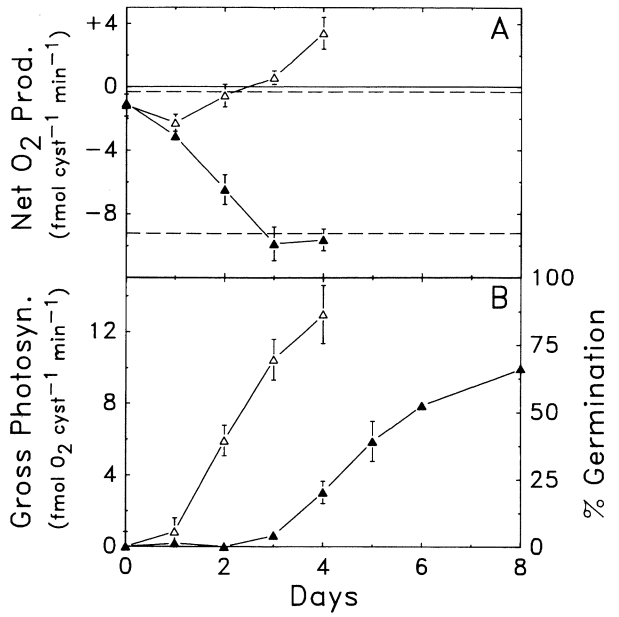

FIG. 4A, B. Metabolic activity of cysts during germination. Cysts stored in the dark at 3° C and transferred to a LD cycle at 18° C at time 0. A) Net oxygen consumption in the dark (▲) and production under saturating light intensity (△). Broken lines indicate respiration rates of quiescent and germinating cysts calculated from carbohydrate loss (Figs. 2, 3). Means ± SE (n = 2). B) Gross photosynthesis calculated from (A) (△); bars indicate SE (df = 10, see Methods); germination frequency (▲), means ± SE (n = 3).

reduced by only 25% in these activated cysts relative to vegetative cells (Table 2).

DISCUSSION

Our data show for the first time the biochemical changes that accompany encystment, dormancy, and germination in dinoflagellates. They support the idea that cysts and vegetative cells of *S. trochoidea* differ significantly in gross composition and in metabolic activity. Relative to healthy vegetative cells, the respiration rate in quiescent cysts is reduced an estimated 60-fold. Although these cysts do contain chlorophyll, they show no measurable photosynthetic capacity. Cysts contain relatively large amounts of carbohydrate which serve as their major energy source during quiescence and germination. These results are consistent with the view that dinoflagellate cysts represent inactive life history stages equipped to survive extended periods under conditions unsuitable for photoautotrophic vegetative growth.

Encystment. In considering the transition from exponentially growing cells to resting cysts, the changes that occur in vegetative cells in response to nutrient limitation must be distinguished from those that are specific to cyst formation. Since encystment in dinoflagellates is often induced by nutrient limitation (Pfiester and Anderson 1987), this distinction may seem somewhat arbitrary. However, the fact that 80% of the vegetative population does not encyst in our nutrient-limited experimental cultures (Binder and Anderson 1987) argues strongly that the response to nutrient depletion and the process of encystment are indeed separate, though perhaps not independent, phenomena.

The reduction in protein and chl *a* and the increase in carbohydrate we observed in vegetative *S. trochoidea* cells as they reached stationary phase are consistent with the changes generally observed in nitrogen-starved algal cultures (Fogg 1959, Myklestad 1974, Sakshaug and Holm-Hansen 1977, Morris 1981). Although lipid is accumulated in nitrogen-starved cells of some algae (Fogg 1959, Shifrin and Chisholm 1981), carbohydrate is clearly the predominant storage product in *S. trochoidea*: it increased approximately 500% in stationary-phase cells, whereas lipid increased by only 40% (Fig. 1).

The changes that accompanied cyst formation in *S. trochoidea* were qualitatively similar to those observed for nitrogen starvation in vegetative cells. In fact, if we assume that one cyst is formed by the sexual fusion of two stationary-phase cells (Wata-

TABLE 2. *Comparison of the respiratory and photosynthetic activity in cysts and vegetative cells. Except as noted, rates based on direct measurements of O_2 consumption or production. Values in parentheses not significantly different than zero but included for comparison. Numbers in brackets refer to the degrees of freedom associated with the standard error estimate. Data for activated cysts are from Figure 4. ND = no data.*

		fmol $O_2 \cdot cell^{-1} \cdot min^{-1}$ (mean ± SE)	[df]	nmol $O_2 \cdot \mu g$ chl $a^{-1} \cdot min^{-1}$ (mean ± SE)	P/R ± SE
Respiration					
Cysts					
Quiescent	(3° C)	0.34 ± 0.034[a]	[3]	—[a,b]	ND
Activated (day 0)	(18° C)	(1.1 ± 0.59)	[10]	(1.4 ± 0.79)[c]	(−0.12 ± 0.79)
(day 4)	(18° C)	9.6 ± 1.16	[10]	2.4 ± 0.28[c]	1.35 ± 0.23
Vegetative cells	(18° C)	22.8 ± 1.95	[7]	1.9 ± 0.16	2.20 ± 0.22
Gross photosynthesis					
Cysts					
Quiescent		ND		ND	
Activated (day 0)	(18° C)	(−0.12 ± 0.83)	[10]	(−0.2 ± 1.10)[c]	
(day 4)	(18° C)	13.0 ± 1.63	[10]	3.2 ± 0.40	
Vegetative cells	(18° C)	50.3 ± 2.58	[7]	4.1 ± 0.21	

[a] Based on loss of carbohydrate, Figure 2.
[b] Not calculated: chl $a \cdot cyst^{-1}$ changed over time period of measurement.
[c] Based on day 0 or day 3 chl $a \cdot cyst^{-1}$ values in Figure 3.

nabe et al. 1982), the protein, lipid, and chl *a* contributed by both of these cells could account for the level of these constituents in cysts without any further synthesis or degradation (Fig. 1). However, since the small population of vegetative cells that fuse and ultimately form cysts may not have the same composition as the "mean" stationary-phase cell, the possibility of net synthesis or degradation of these constituents during encystment cannot be discounted at present.

In contrast to protein, lipid, and chl *a*, carbohydrate was clearly accumulated in cysts at levels far exceeding those in nitrogen-starved cells. The contribution of carbohydrate to a cyst by a pair of stationary-phase cells would account for less than a third of the total cyst carbohydrate content (Fig. 1). Thus, active carbohydrate synthesis and accumulation represents a major biochemical change specifically accompanying encystment. This finding is consistent with the common observation of increases in starch granules in various dinoflagellate cysts, relative to vegetative cells (Wall and Dale 1969, von Stosch 1973, Anderson 1980, Chapman et al. 1982). The time period during which the synthesis of excess carbohydrate occurs cannot be discerned in the present study. However, microscopic studies of dinoflagellate encystment suggest that this storage product accumulates in the planozygote stage (von Stosch 1973, Chapman et al. 1982). This conclusion is consistent with the presence of active photosynthesis in *G. tamarensis* planozygotes (Glibert et al. 1988).

The high carbohydrate/lipid ratio in cysts (3.0) relative to that in exponential- and stationary-phase vegetative cells (0.3 and 1.1, respectively) emphasizes the increased importance of carbohydrate as a storage compound in cysts. Still, due to the high theoretical energy yield of lipid compared to carbohydrate (9.46 vs. 4.18 $kcal \cdot g^{-1}$ [Lehninger 1975]), the "energy" stored in cyst carbohydrate is only marginally greater than that in lipid. (These calculations assume that all carbohydrate and lipid is available for respiration.) It is noteworthy that carbohydrate, rather than lipid, is the appropriate storage product in an organism that must metabolize under anoxic conditions (Hochachka 1980).

Although no dramatic accumulation of lipid was apparent in *S. trochoidea* cysts (compare the per-volume lipid content in exponential cells and cysts, Fig. 1), this does not preclude changes in relative lipid class composition, as has been observed in other algal resting stages (Berkaloff and Kader 1975, Lichtlé and Dubacq 1984). In particular, the decrease in membranous structures and increase in lipid droplets observed in the dinoflagellate *Woloszynskia tylota* during encystment (Bibby and Dodge 1972) could have reflected an increase in triacylglycerides (storage lipids) at the expense of phospholipids (membrane components).

Dormancy and quiescence. Scrippsiella trochoidea cysts undergo a period of dormancy lasting approximately 25 days, during which germination cannot occur (Binder and Anderson 1987). After this period, cysts incubated under permissive conditions germinate, whereas those kept under non-permissive conditions remain quiescent. It is appropriate, therefore, to separately consider changes that occur during the dormancy period (between day 21 and day 49 in the present study) and those that occur subsequently, in quiescent cysts.

The loss of both carbohydrate and lipid between days 21 and 49, at both 3° C and 18° C, suggests significant metabolic activity during the dormancy period in *S. trochoidea* cysts (Fig. 2, broken lines). The rate of carbohydrate loss at both temperatures in this time interval was comparable to the long term

rate of loss in 3° C cysts and greater than the long term rate at 18° C (see below). Lipid degradation was significantly greater at 18° C than at 3° C during dormancy, presumably reflecting a higher rate of respiration at the higher temperature. Although variability in the data makes quantification of lipid disappearance tenuous, the calculated energy yield from lipid during dormancy is comparable to that from carbohydrate in cysts incubated at 3° C and is three times that of carbohydrate for cysts at 18° C. The estimated overall respiration rate at 18° C (2.5 fmol $O_2 \cdot cyst^{-1} \cdot min^{-1}$) is still only 10% of the rate measured in vegetative cells (Table 2).

The patterns of carbohydrate and lipid utilization established during dormancy were greatly altered as cysts entered the quiescent state. Although the rate of loss of carbohydrate remained approximately constant in 3° C cysts over the entire experiment, it was greatly reduced in 18° C cysts after day 49. Thus, the calculated respiration rate was four times greater in cysts stored at 3° C than in those stored at 18° C. The explanation for this difference is not clear. Respiration rate is expected a priori to decrease or at best (in the case of some eurythermic algae) to remain constant as temperature decreases (Ryther and Guillard 1962, Soeder and Stengel 1974). Increased respiratory rates in *S. trochoidea* at lower temperatures might reflect a decrease in respiratory efficiency or an increase in maintenance cost at these temperatures. More data are obviously required before the influence of temperature on respiration in quiescent cysts can be firmly established.

Assuming the rate of carbohydrate loss in quiescent cysts remains constant over time, cysts stored at 3° C would be expected to deplete their carbohydrate reserves in approximately 240 days. The observation of sustained viability in *S. trochoidea* cysts for at least 350 days (Binder and Anderson 1987) therefore suggests that either respiration rate decreases as cysts age, or that lipid or protein become important respiratory substrates.

The gradual decrease in carbohydrate in quiescent cysts was accompanied by a gradual increase in lipid. Although the variability in the lipid data is high with respect to the changes observed, statistical analysis (Table 1) suggests that the increase in lipid over time is real. Again, these results are unexpected and difficult to explain. That lipid can be synthesized at the expense of endogenous reserves in the dark is not surprising (Miller 1962). However, such synthesis is quite expensive in terms of ATP and reducing power, and its possible benefit to quiescent cysts is not immediately apparent. Interestingly, net synthesis of lipids in diatom resting cells at low temperature in the dark was reported by Anderson (1975). Furthermore, French and Hargraves (1980) observed an increase in cellular carbon in diatom resting spores stored in the dark. These observations imply the presence of a significant anabolic component in the overall metabolism of resting cells which may also be reflected in the increased lipid content of *S. trochoidea* cysts observed in this study.

The lack of measurable photosynthetic activity immediately subsequent to activation (Fig. 4) indicates that quiescent cysts retain little, if any, photosynthetic capacity, despite the presence of chlorophyll *a*. Had the chlorophyll-specific photosynthetic rate been equal to that in vegetative cells, calculations indicate that we should have been able to detect gross photosynthesis in cysts at that time. Therefore, the reduced photosynthetic capacity among quiescent cysts appears to result from a disruption of the cellular photosynthetic machinery beyond simple reduction in chl *a* content.

Overall, these data confirm the common assumption of reduced metabolic activity in dinoflagellate cysts. The highest estimate of respiratory activity among non-activated cysts occurred during the dormancy period in 18° C-stored cysts, and this was still an order of magnitude below the respiration rate in vegetative cells. The estimate of respiration rate among quiescent cysts is even lower, at approximately 1.5% of the vegetative rate (Table 2).

The loss of membranous cytoplasmic components and disruption of chloroplasts observed by Bibby and Dodge (1972) during *W. tylota* encystment most probably reflects reductions in respiratory and photosynthetic capacity analogous to those observed here in *S. trochoidea*. The apparent elevation of metabolic activity in young (dormant) cysts relative to older (quiescent) cysts in *S. trochoidea* is consistent with the relatively rapid disappearance of storage products observed in *Gonyaulax tamarensis* cysts during the first few weeks of storage (Anderson 1980) and supports the suggestion that the mandatory dormancy period observed in many dinoflagellate cysts may not represent a period of "rest" as much as it does a period of development (Dale 1983).

Germination. Not surprisingly, increased metabolic activity accompanied germination in *S. trochoidea* cysts (Figs. 3, 4, Table 2). The respiration rate calculated from carbohydrate loss in activated cysts is comparable to that measured directly, although differences between the germination kinetics in each experiment make direct day to day comparisons impossible. The stimulation of respiratory activity began within 12 h of activation and thus preceded protein and chl *a* synthesis and the increase in photosynthetic activity. The relationship between these events cannot be rigorously established with the data at hand, but it seems reasonable that nitrogen uptake and protein synthesis would be dependent upon energy derived from respiratory metabolism and that chl *a* synthesis and the overall reactivation of photosynthesis would in turn require newly synthesized enzymes and photosynthetic proteins. The drop in chl *a* content observed 96 and 120 h after activation (at which point more than 50% of the cysts had germinated) most likely reflects the composition of those cysts which had not yet germinated (and might

never do so), rather than a net decrease of chl *a* within these cysts over time.

The overall dependence on endogenous energy reserves in *S. trochoidea* cysts during germination is indicated by the relatively low net photosynthetic rates observed in activated cysts (Fig. 4). These remained less than or equal to 0 until just prior to germination. The general correspondence between carbohydrate disappearance and measured oxygen consumption suggests that carbohydrate acts as the major respiratory substrate during this period. This conclusion is further supported by the lack of significant changes in the lipid content of cysts over the same interval.

Although respiratory and photosynthetic activity increase dramatically prior to excystment, the fact that both are still below the rates measured in vegetative cells (Table 2) argues that the full restoration of vegetative metabolism in these cells is not complete until after excystment. The extent to which the observed changes in activity are prerequisites for germination remains uncertain. The ability of *S. trochoidea* cysts to germinate in the dark after only brief exposure to light (Binder and Anderson 1986) demonstrates that photosynthetic activity is not required for germination. Likewise, germination in nitrogen-depleted medium (Binder and Anderson 1987) suggests that net protein synthesis is not a requirement, although turnover of proteins or N-rich compounds and resynthesis of required proteins is by no means excluded. These observations notwithstanding, germination rate and/or frequency is often improved by the provision of light or a nitrogen source (Binder 1986); thus, while neither photosynthesis nor bulk protein synthesis appears to be an absolute requirement for germination, both can influence its rate and extent.

The extent to which respiratory activity is a prerequisite for germination is not known. Reports of germination by *Ceratium hirundinella* cysts under anoxic conditions suggest that such activity may not be required for germination in this species (Huber and Nipkow 1923, Krupa 1981). In contrast, the apparent lack of anoxic germination in cysts of other dinoflagellates suggests that aerobic respiration could be a prerequisite for germination in many species (Endo and Nagata 1984, Anderson et al. 1987).

Comparisons with other algal resting stages. Resting stages are known among members of most classes of phytoplankton (Fryxell 1983). Those for which the most physiological data are available include cyanobacterial akinetes (Nichols and Carr 1977, Nichols and Adams 1982), diatom resting cells and spores (Hargraves and French 1983), and chlorophyte akinetes (Coleman 1983).

The accumulation of storage products, as observed in *S. trochoidea* cysts, occurs in all of these resting forms, and in fact in dormant stages of a wide variety of organisms (Sussman and Douthit 1973, Bewley and Black 1983). In cyanobacterial akinetes, glycogen and cyanophycin (composed of polypeptides) serve as storage products (Wildman et al. 1975, Sutherland et al. 1979), while lipid and/or starch are accumulated in the resting cells of diatoms, chlorophytes, and cryptophytes (Anderson 1975, Berkaloff and Kadar 1975, Lichtlé 1979, Doucette and Fryxell 1983, O'Neal and Lembi 1983).

Although reduced metabolic activity is another characteristic common among dormant stages (Sussman and Halvorson 1966, Sussman and Douthit 1973, Bewley and Black 1982), the metabolic profile of algal resting stages is quite variable. Thus, while decreases in *Nostoc* akinete respiration were comparable to those reported here in *S. trochoidea* cysts (Chauvat et al. 1982), respiration rate in akinetes of another cyanobacterium, *Anabaena cylindrica,* were actually greater than those in vegetative cells (Fay 1969a, Yamamoto 1976). Likewise, respiration rates in *Pithophora oedogonia* (Chlorophyceae) akinetes were greater than or equal to vegetative rates (O'Neal and Lembi 1983). Respiration in diatom resting stages is generally less than that in vegetative cells (Anderson 1976, French and Hargraves 1980).

Generalizations concerning resting stage photosynthetic capacity are equally difficult to make. Although reductions in photosynthetic capacity similar to that observed in *S. trochoidea* cysts are evident in chlorophyte and cyanobacterial akinetes and in some diatom resting cells (Fay 1969a, Yamamoto 1976, Chauvat et al. 1982, O'Neal and Lembi 1983), photosynthesis in other diatom resting spores is apparently comparable to that in vegetative cells (French and Hargraves 1980, Hollibaugh et al. 1981). Despite their reduced photosynthetic capacity, cyanobacterial akinetes contain chl *a* at levels comparable to vegetative cells (Fay 1969b, Sutherland et al. 1979, Chauvat et al. 1982). Diatom resting spores and vegetative cells also have similar chl *a* content (Anderson 1975, French and Hargraves 1980, Doucette and Fryxell 1983).

Differences in resting stage metabolic capacities notwithstanding, the general pattern of metabolic activity during germination in many algal resting stages is similar to that observed here in *S. trochoidea* cysts. In particular, an early increase in respiratory activity, a dependence on respiration for energy during initial stages, and a delayed but marked increase in photosynthetic activity characterize germination in many algae (Hommersand and Thimann 1965, Chauvat et al. 1982, O'Neal and Lembi 1983). As suggested above for *S. trochoidea,* this series of observed events may reflect a developmental sequence involving a stimulation of respiration, the use of the resulting energy in RNA and protein synthesis, and finally the application of newly synthesized enzymes and protein components to the reconstitution of the photosynthetic system. Chauvat et al. (1982) presented more direct evidence for just such a cascade of events in germinating *Nostoc* akinetes.

The germination of *Chaetoceros* resting spores pro-

vides a conspicuous exception to this paradigm: spores of this species begin to photosynthesize at relatively high rates immediately upon exposure to light, even after 167 days of cold storage (Hollibaugh et al. 1981). Interestingly, these diatom resting spores appear to require photosynthesis for germination, whereas chlorophyte akinetes and dinoflagellate cysts do not (Huber and Nipkow 1923, Neal and Herndon 1968, Anderson and Wall 1978, Binder and Anderson 1986).

In overview, cysts of *Scrippsiella trochoidea* appear to represent resting stages as "restful" as any observed among the algae. The extent of reduction in respiratory and photosynthetic capacity they display, combined with their significant accumulation of storage compounds, is consistent with their often presumed (though heretofore untested) role as "dormant" perenniating cells.

This research was supported in part by National Science Foundation grant OCE-8400292, by the Education Program and Coastal Research Center at the Woods Hole Oceanographic Institution, and by the Donaldson Charitable Trust. Contribution No. 7209 from the Woods Hole Oceanographic Institution.

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