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RELATIONSHIP BETWEEN NUCLEOSIDE DIPHOSPHATE KINASE ACTIVITY AND LIGHT-LIMITED GROWTH RATE IN THE MARINE DIATOM *THALASSIOSIRA PSEUDONANA* (BACILLARIOPHYCEAE)[1]

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ABSTRACT

Light-limited cultures of the marine diatom Thalassiosira pseudonana *(Hustedt) Hasle and Heimdal (3H clone) were grown over a range of growth rates between 0.06 and 1.64 d^{-1}. Variations in cell volume, cell quotas of carbon, nitrogen, and protein, and maximal activity of the enzyme nucleoside diphosphate kinase (NDPK) were measured and examined as a function of growth rate. NDPK from* T. pseudonana *showed K_m values of 0.24 and 0.68 mM for thymidine 5′-diphosphate and adenosine 5′-triphosphate (ATP), respectively, which are similar to those found for NDPK from a variety of organisms, from bacteria to mammals. An apparent activation enthalpy of 3.52 $kCal \cdot mol^{-1}$ was determined from Arrhenius plots. No thermodynamic transition points were noted over a temperature range from 10° to 25°C. NDPK activity was significantly correlated with growth rate but not with cell volume, carbon, nitrogen, or protein; for interspecific comparisons, normalization of enzyme activity to cell number may be most meaningful. NDPK activity per cell versus growth rate followed a U-shaped relationship, being relatively constant between 0.5 and 1.0 d^{-1} and rising at higher and lower growth rates. Over this range, enzyme activity may be regulated by substrate concentration (ATP or other nucleoside triphosphates) or by adenylate energy charge. At higher growth rates where energy charge and substrate concentrations are probably high, changes in enzyme concentration appear to be required. The reasons for a rise in enzyme activity at low growth rate is unclear. Simultaneous measurement of nucleoside di- and triphosphate levels alongside NDPK measurements may help clarify the relationship, but these preliminary experiments indicate that NDPK is of limited usefulness as an index of* in situ *growth rate.*

Key index words: Bacillariophyceae; chemical composition; enzyme activity; enzyme kinetics; growth rate; nucleoside diphosphate kinase; Thalassiosira pseudonana

Accurate determination of the *in situ* growth rate of marine phytoplankton is critical to our understanding of trophodynamics and carbon cycling in the world oceans (Goldman 1989, Furnas 1990). Although the standard ^{14}C-incorporation technique has provided a great deal of valuable information, it requires that algae be contained for an incubation period. This has severe drawbacks including alteration of light climate, nutrient limitation, metal contamination, and enhanced grazing by microzooplankton, which may all lead to errors in the growth rate estimate (Gieskes and Kraay 1982, Leftley et al. 1983, Harris 1984). As a result, alternative means of assessing the *in situ* growth rate have been suggested (see Furnas 1990). For example, rates of protein synthesis (Lean et al. 1989, De Madariaga et al. 1991), analyses based on distinctive cell cycle characteristics (Chang and Carpenter 1988, 1990, Antia et al. 1990), adenylate nucleotide content (Sheldon and Sutcliffe 1978, Karl 1980), DNA dye-binding (Falkowski and Owens 1982), coulometric methods (Irwin 1991), cell absorption characteristics (Eppley and Sloan 1966), RNA:DNA ratios (Dortch et al. 1983), and correlations between growth rate and cell composition (Goldman 1986) have all been examined with varying degrees of success.

Another approach involves the use of activity measurements of enzyme proteins. In theory, an enzyme measurement is related to an *in situ* rate. A single measurement could therefore provide a rate estimate without requiring timed incubations. Nitrate reductase activity has been extensively measured as an index of nitrate assimilation rate (Eppley et al. 1969, Dortch et al. 1979), but it may not be applicable to systems where other nitrogen forms are important. Enzymes involved in nitrogen and carbon assimilation have also been considered. Glutamate dehydrogenase (Ahmed et al. 1977), glutamine synthetase (Bressler and Ahmed 1984), and carboxylases (Glover and Morris 1979, Mortain-Bertrand et al. 1988) have been measured, but each of these processes may be uncoupled from cell growth under various circumstances. Electron transport activity has also been examined to estimate respiration rates (Kenner and Ahmed 1975, Blasco et al. 1982, Packard 1985) but not in the context of growth rate prediction.

Ideally, an enzyme useful in growth rate prediction should be involved in pathways specific to biosynthesis. Nucleoside diphosphate kinase (E.C. 2.7.4.6., NDPK) appears to be such an enzyme. NDPK catalyzes the reversible reaction:

$$ATP + NDP \rightleftharpoons ADP + NTP,$$

where NDP and NTP are the high-energy di- and triphosphate forms of the nucleosides cytidine, guanosine, uridine, or thymidine. With the exception of adenosine 5′-triphosphate (ATP) and a small portion of GTP, all nucleoside triphosphates are syn-

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thesized by this enzyme (Ingraham and Ginther 1978). Because energy for biosynthesis of cell components is provided as a nonadenine nucleoside (e.g. GTP for protein synthesis or CTP for fatty acid synthesis) and nonadenine nucleosides are required for DNA and RNA synthesis (Parks and Agarwal 1973), an increase in biosynthesis may require an increase in NDPK.

Although NDPK has been measured in a wide range of organisms from bacteria to higher plants to mammalian cells (Parks and Agarwal 1973), we are aware of only one report of a measurement in a unicellular autotroph (Klein and Follmann 1988) and no cases of measurements in marine phytoplankton. There has been speculation about the importance of NDPK in cell growth processes during development (Dickinson and Davies 1971), including a correlation with growth rate in mammalian tumor cells (Koyama et al. 1984) and evidence of relationships between NDPK and growth rate in crustaceans (Berges 1989, Berges et al. 1990). In multicellular organisms, however, the relationship between NDPK and growth is complicated by changes in body size and composition during development. Such relationships may be clearer in a unicellular organism.

The objectives of our study were to examine (a) the general characteristics of NDPK in a marine diatom, (b) the relationship between maximal NDPK activity (which should be proportional to enzyme concentration) and growth rate under light (energy) limitation, and (c) the relationship between various cell components and growth rate in order to determine to which biomass parameter NDPK activity is best scaled.

MATERIALS AND METHODS

The marine diatom *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal (3H clone) was obtained from the Northeast Pacific Culture Collection, Department of Oceanography, University of British Columbia. Cultures were grown in semicontinuous batch culture in enriched artificial seawater based on the recipe by Harrison et al. (1980). Sodium glycerophosphate was replaced with an equimolar concentration of sodium phosphate, and ferrous ammonium sulphate was replaced with an equimolar concentration of ferric chloride. Selenite, nickel, and molybdate were added to achieve 1 nM final concentration. Temperature was maintained at 17.5° ± 0.5° C using a circulating water bath. Cultures were grown in 1-L glass flasks, stirred at 60 rpm with Teflon-coated stir bars, and bubbled with air filtered through a 0.22-μm membrane filter. Continuous illumination was provided by Vita-lite® fluorescent tubes and attenuated by distance or neutral density screening to give a range of irradiances from 6 to 120 μmol quanta·m^{-2}·s^{-1} measured in air inside empty culture vessels using a LiCor model 185 meter. During the course of experiments, cultures were never dense enough to reduce average irradiance by more than 10%. Growth rates were followed by *in vivo* fluorescence and measured twice daily using a Turner Designs® Model 10 fluorometer, and cell counts were made using a Coulter Counter® model TAII equipped with a population accessory. All sampling was conducted in early to mid logarithmic growth phase.

Cell composition. In all experiments, cell carbon and nitrogen quotas were determined by filtering samples onto precombusted 13-mm Gelman type AE glass fiber filters and analyzing them using a Carlo Erba CNS analyzer. Samples for protein determination were collected on precombusted Whatman GF/F filters. Homogenates were prepared as described by Dortch et al. (1984). They were ground with 3% trichloroacetic acid and solublized in 1 N NaOH. Protein was determined by the method of Bradford (1976) using the microassay procedure of the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, 500-0001) with bovine serum albumin (BSA, Sigma Chemical Co., A 7638) as a standard. Cell volumes were calculated from Coulter Counter measurements and calibrated using 5-μm latex microspheres, following Thompson et al. (1991).

Cell homogenization and enzyme assay. Samples were collected on 25-mm Whatman GF/F glass fiber filters using filtration pressures less than 100 mmHg. Filters were immediately placed in 1 mL of ice cold extraction buffer consisting of 50 mM imidazole (pH 7.4), 2 mM dithiothreitol, ethylenediaminetetraacetate (EDTA), 1% BSA, and 0.1% Triton X-100. Cells and filters were ground in a 5-mL glass-Teflon tissue homogenizer for 2 min. Homogenates were centrifuged in a Sorval RCB-2B centrifuge at 4° C for 5 min at 750 g and used immediately in assays. Preliminary experiments showed that no NDPK activity remained in the pellet.

Assay conditions were adapted from Berges et al. (1990). Assays were conducted in 1-mL volumes in disposable plastic cuvettes. All assay components were obtained from Sigma Chemical Co. and were the purest grade available. ADP produced in the NDPK reaction was coupled to nicotinamide adenine dinucleotide, reduced (NADH), oxidation through pyruvate kinase and lactate dehydrogenase (Agarwal et al. 1978). Substrate concentrations were optimized by increasing the concentration of each reaction component until no further increase in NDPK activity was observed. This was routinely verified over the course of the experiments in cultures growing at low and high irradiances. Further increases in substrate concentrations were avoided, since they also increased rates of side reaction and thus decreased precision. Final concentrations in the assay were 50 mM imidazole buffer (pH 7.4), 0.2 mM NADH, 20 mM $MgCl_2$, 70 mM KCl, 1.1 mM phosphoenol pyruvate, 2.0 mM ATP, 0.7 mM TDP, 10 units (U) lactate dehydrogenase (Sigma L 2500), and 1 U pyruvate kinase (Sigma P-1506). Reactions were started by adding TDP. Controls were run without homogenate and without TDP, and rates were corrected accordingly (Agarwal et al. 1978). Reactions were followed by monitoring the decrease in absorbance at 340 nm due to NADH oxidation using an LKB Ultrospec II UV spectrophotometer with a six-position water-cooled turret. Typically, it was necessary to monitor reactions 5–10 min to establish the initial, linear rate of reaction. Temperature was maintained at 17.5° ± 0.1° C using a Lauda RM6 water circulating bath. NDPK activity was expressed in units, where 1 U represents the quantity of enzyme catalyzing the conversion of 1 μmol of substrate to product per minute, using a millimolar extinction coefficient of 6.22.

Enzyme characterization. Assays were conducted over a range of ATP and TDP concentrations to determine K_m values for the algal enzyme. NDPK versus substrate concentration curves were fit to a Michaelis–Menten model using a nonlinear fitting routine (NONLIN; Wilkinson 1990). Assays were also conducted over a range of temperatures from 10° to 25° C. An Arrhenius transformation was used to calculate an apparent activation enthalpy ($\Delta H‡$) of the enzyme (Hochachka and Somero 1984).

Steady-state experiments. On six separate occasions, four to six algal semicontinuous batch cultures were grown at different irradiances ranging from 6 to 120 μmol quanta·m^{-2}·s^{-1}. Cultures were acclimated for a minimum of 10 generations except in cultures growing at $\mu < 0.4$, where 6–8 generations were allowed. Cultures were sampled for cell volume, nitrogen and carbon quo-

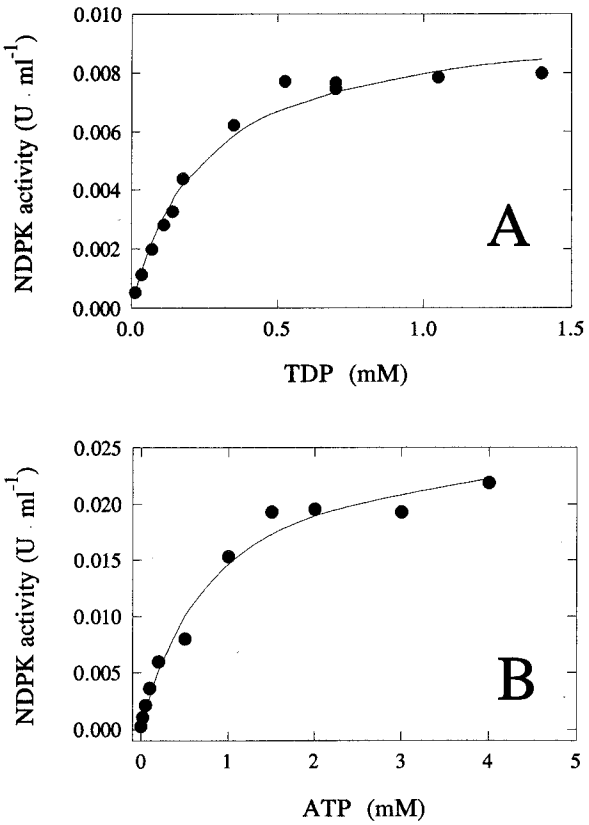

FIG. 1. NDPK activity versus substrate concentration for A) TDP and B) ATP in homogenates of *Thalassiosira pseudonana*. Curves are fit to rectangular hyperbolae. K_m values are 0.24 mM for TDP and 0.86 mM for ATP.

ta, protein, and NDPK activity. These parameters were plotted against growth rate and examined using linear correlation analyses (Wilkinson 1990).

Transition experiments. On two occasions, six *T. pseudonana* cultures were acclimated in the same manner as in steady-state experiments: three to 15 μmol quanta·m^{-2}·s^{-1} and three to 135 μmol quanta·m^{-2}·s^{-1}. Samples identical to those in the steady-state experiment were taken; then, the cultures were transposed. In the first experiment, cultures were sampled at 24-h intervals for 72 h. In the second experiment, sampling continued for 210 h after the transition. Changes over time in carbon and nitrogen quota, cell volume, growth rate, protein, and NDPK activity were examined.

RESULTS

K_m values for the substrates, calculated from six separate homogenates, were 0.24 ± 0.01 mM for TDP and 0.86 ± 0.06 mM for ATP (Fig. 1).

The slope of the regression lines of Arrhenius plots of log NDPK activity versus the inverse of temperature gave an apparent activation enthalpy of 3.52 ± 0.11 kCal·mol^{-1} (Fig. 2).

Steady-state growth rates versus irradiance data were collected over a period of 18 months and demonstrate the similar behavior of cultures over the experimental period (Fig. 3). Fitting a Michaelis–Menten-type curve to the data gave a μ_{max} of 1.64

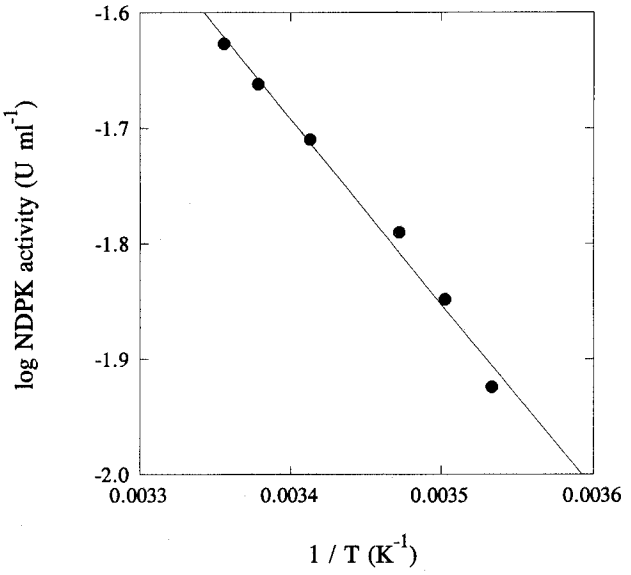

FIG. 2. Arrhenius plot of NDPK from *Thalassiosira pseudonana*. The solid line represents a least-squares regression fit to the data. Apparent activation enthalpy is 3.52 kCal·mol^{-1}.

d^{-1} and a half-saturation constant (K_I) of 23 μmol quanta·m^{-2}·s^{-1}. From Figure 3, I_k was estimated to be approximately 40 μmol quanta·m^{-2}·s^{-1}.

Carbon, nitrogen, and protein cell quotas (pg·cell^{-1}) were not significantly correlated with growth rate ($P > 0.3$, $P > 0.06$, and $P > 0.5$, respectively) (Fig. 4); however, there was a significant positive linear relationship between cell volume and growth

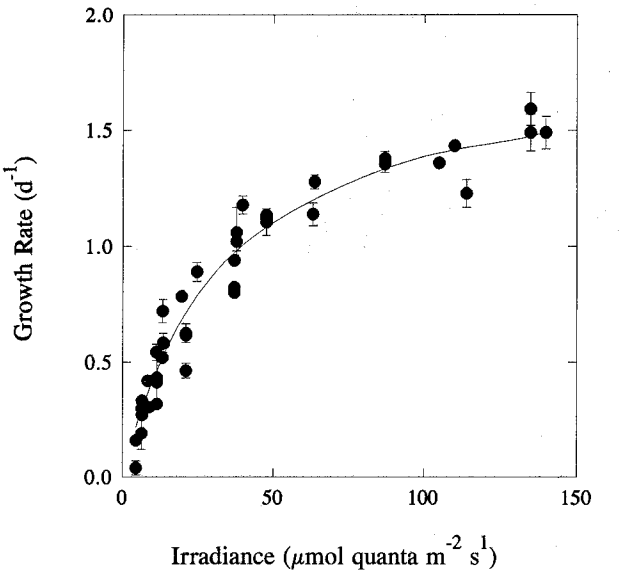

FIG. 3. Growth rate versus irradiance curve for *Thalassiosira pseudonana*. Curve is fit to a rectangular hyperbola. μ_{max} = 1.64 d^{-1} and K_I = 23 μmol quanta·m^{-2}·s^{-1}. Each point represents a single culture. Error bars represent the standard error of the mean of three to six growth rate measurements.

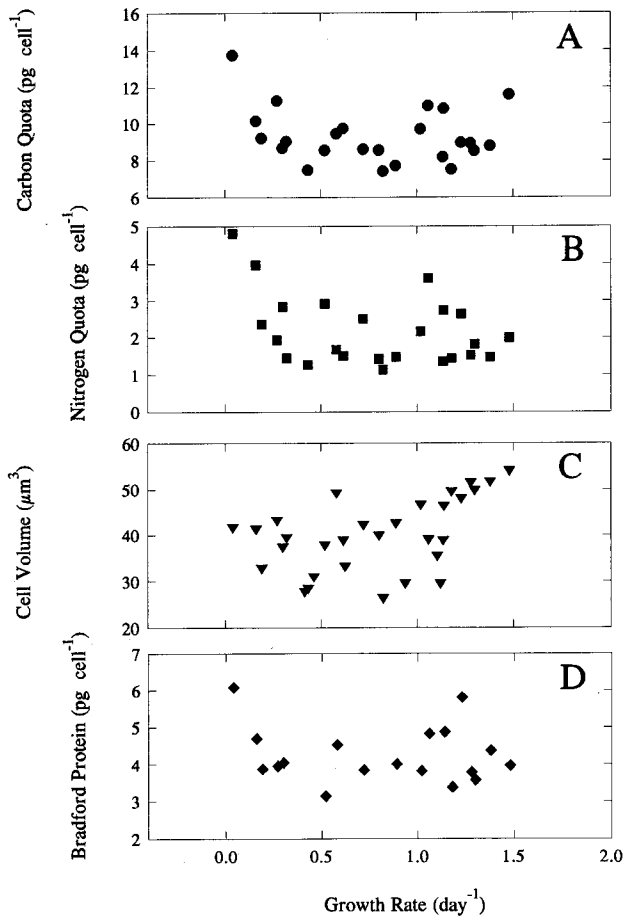

FIG. 4. Cell composition versus light-limited specific growth rate for *Thalassiosira pseudonana*. A) Carbon cell quota, B) nitrogen cell quota, C) cell volume, and D) protein cell quota. Each data point represents the mean of duplicate determinations from a single culture.

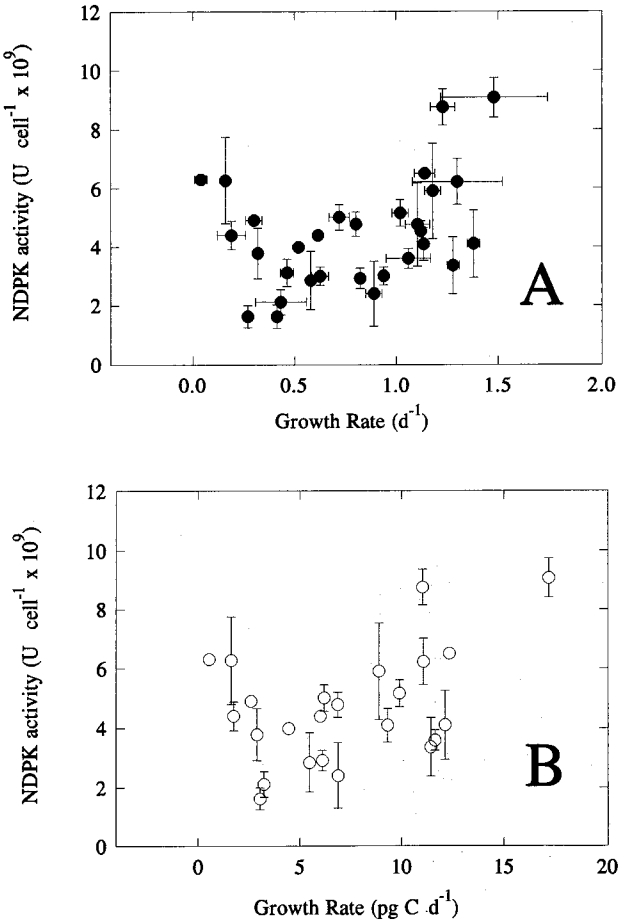

FIG. 5. NDPK activity versus A) light-limited specific growth rate and B) growth rate in terms of carbon, for *Thalassiosira pseudonana*. Each data point represents a single culture. Error bars show the standard error of the mean of two enzyme assays or a minimum of three growth rate determinations.

rate ($P < 0.01$). If carbon, nitrogen, and protein were expressed per unit cell volume (i.e. pg·μm^{-3}), there were significant negative relationships with growth rates for carbon ($P < 0.05$) and nitrogen ($P < 0.05$), but not protein ($P > 0.09$). In addition, there were no significant correlations between NDPK activity (on a per cell basis) and carbon cell quota, nitrogen cell quota, protein cell quota, or cell volume ($P > 0.2$ in all cases; data not shown).

The relationships between NDPK activity on a per cell basis and either specific growth rate or growth rate in terms of carbon (the product of specific growth rate and carbon cell quota, which is analogous to a ^{14}C measurement) were highly variable (Fig. 5). However, NDPK activity was significantly and positively correlated with growth rate. When a linear model was used, NDPK activity per cell was significantly correlated with specific growth rate ($P < 0.05$) and carbon growth rate ($P < 0.04$). NDPK activity at low growth rates ($\mu < 0.4$) appeared to increase. Using a quadratic model, the correlation improved ($P < 0.01$ for both cases). Expressing NDPK activity per unit cell volume, carbon, nitrogen, or protein did not change the pattern of the relationship, although the variability increased significantly.

Transition experiments provided another way to assess whether or not growth rate and NDPK activity were related. By measuring NDPK activity in individual cultures before and after a transfer from low to high light or vice versa, changes in enzyme activity could be followed and an approximate time for changes to occur determined. Transition experiments were repeated twice. In the first case, the time course was followed for only 72 h, in the second case for 210 h. Results were nearly identical in both experiments; for clarity, only the results of the 210-h time course are presented. Under steady-state conditions, the growth rate of high light cultures was 1.45 d^{-1}, while low light cultures grew at 0.56 d^{-1} (Fig. 6). Cultures were switched at t = 32 h, and growth rates had changed by 48 h. Composition between treatments was compared within each sampling time using paired *t*-tests. Results were similar

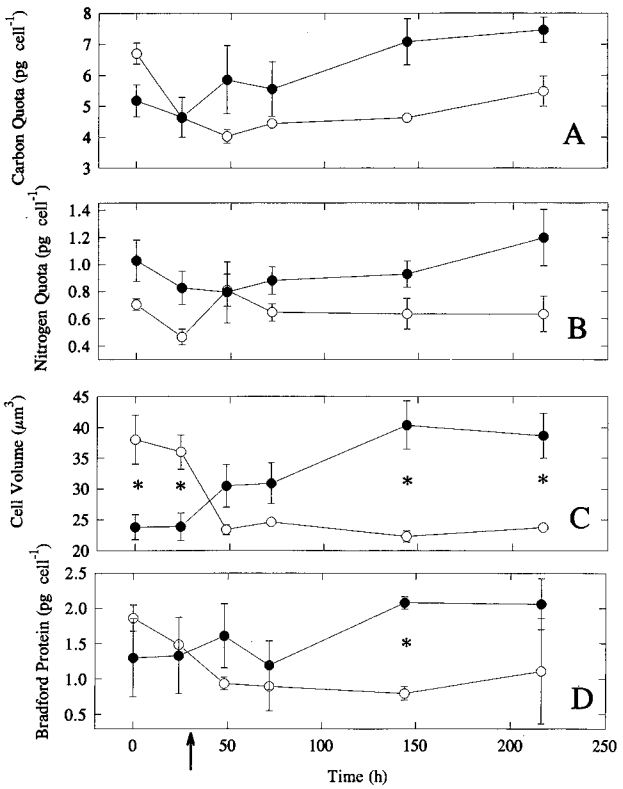

FIG. 6. Cell composition versus time for transition experiments for A) carbon cell quota, B) nitrogen cell quota, C) cell volume, and D) protein cell quota determined by the Bradford method using BSA as a standard, in *Thalassiosira pseudonana.* ○ = Cultures grown under high light (135 μmol quanta·m^{-2}·s^{-1}) and moved to low light (15 μmol quanta·m^{-2}·s^{-1}) at t = 32 h (marked by the arrow); ● = cultures grown under low light and moved to high light at t = 32 h. Each data point represents the mean of three replicate cultures. Error bars represent standard errors of the mean. Statistically significant differences ($P < 0.05$) are indicated by asterisks (*).

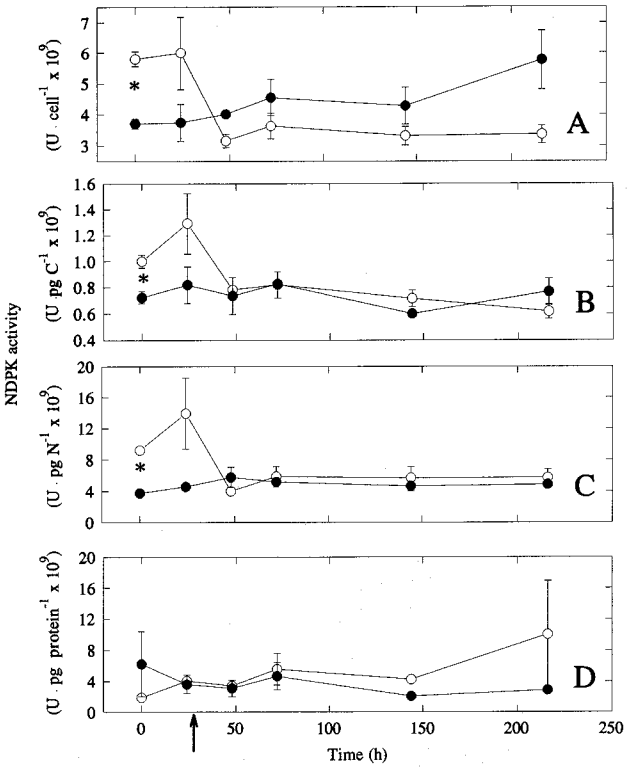

FIG. 7. NDPK activity scaled to A) cell number, B) carbon cell quota, C) nitrogen cell quota, and D) protein cell quota, versus time for transition experiments with *Thalassiosira pseudonana.* Symbols are the same as those in Figure 6.

to the steady-state experiments in that there were no differences between high and low light cells for carbon quota or nitrogen quota. Protein quota differed only in one case. Cell volume was significantly higher in high light-grown cells before the transition, and by 150 h cell volumes in transition cultures were effectively the same as those found under the corresponding low or high light steady state. Steady-state NDPK activities were significantly higher in high light cultures scaled to cells, carbon, or nitrogen but not significantly different when scaled to protein (Fig. 7). Following the transition, although the NDPK activity dropped significantly for high light to low light transition, the treatments were not significantly different at the end of the experiment.

DISCUSSION

Although NDPK is found in a broad range of living organisms, its characteristics are very similar (Parks and Agarwal 1973). Differences between characteristics of the enzyme from *Thalassiosira pseudonana* and those published in the literature might have been anticipated, since the majority of work has been done on the purified enzyme. The algal NDPK is almost certainly a combination of isoforms (although not classical isozymes), as is the case in many organisms (Parks and Agarwal 1973). Despite these differences, the kinetic and thermodynamic constants appear almost identical to those published. We obtained a K_m for TDP of 0.24 mM, which is within the range of 0.11–0.55 mM found for NDPK in human erythrocytes and near the value of 0.25 mM found for the most abundant isoform (Agarwal et al. 1978). For ATP, we obtained a K_m of 0.86 mM. Nomura et al. (1991) found that the two isoforms of NDPK in spinach leaves had K_m values of 2.0 and 0.89 mM, while the human erythrocyte isoforms range from 0.08 to 3.0 mM, with the abundant forms near 1.0 (Agarwal et al. 1978). Arrhenius plots of the enzymes often display diphasic behavior, although this depends on the particular isoform (Agarwal et al. 1978). It is possible that this was obscured in our study by a mixture of isoforms or because of the relatively low number of temperatures assayed. The pI 7.3 isoform of NDPK from human erythrocytes shows two phases with a break at 31° C; the activation enthalpies above and below the break are 4.8 and 10.3 kCal·mol^{-1}, respectively (Agarwal and Parks 1971). For NDPK from *Bacillus*

subtilis, a transition occurs at 25° C with activation enthalpies of 4.2 and 8.5 kCal·mol^{-1} above and below the break, respectively (Sedmak and Ramaley 1971). In all cases, the optimal substrate concentrations and assay conditions are remarkably similar; conditions determined for crustacean tissue (Berges et al. 1990) proved optimal for NDPK from *Thalassiosira pseudonana*.

We found no relationship between light-limited growth rate and carbon, nitrogen, or protein quota. Cell volume, however, was positively correlated with growth rate. Although there were significant relationships between carbon and nitrogen per cell volume, these are probably caused by the significant change in volume alone. This illustrates a potential pitfall in using such ratios (see Packard and Boardman 1988). Raven (1981) points out that variation in cell volume may be an important metabolic adaptation; to maintain the proper cellular concentration of metabolites and catalysts, it may be necessary to change cell volume. Thompson et al. (1991) provide a detailed review of carbon and volume relationships with growth rate and show that there is a general, positive relationship for a variety of species. For *Thalassiosira pseudonana*, in particular, our volume-growth rate relationships agree well with theirs, but, in contrast, Thompson et al. (1991) found a strong positive relationship between carbon quota and growth rate. The reason for these differences is unclear, although the experiments in the present study were conducted over a much shorter period of time than those of Thompson et al. (1991), during which large differences in cell volume and carbon quotas were seen. Such variability may result, in part, from size changes related to the sexual cycle of diatom species, although we observed no evidence of sexual reproduction in any of our cultures. Various authors have also demonstrated size and carbon quotas that decrease with growth rate (Thompson et al. 1991).

There is evidence in our data of increases in cell volume and cell quotas of carbon, nitrogen, and protein at very low growth rates ($\mu < 0.25$). Very little data are available for such low growth; Thompson et al. (1991), for example, has only one culture in this range. Sakshaug and Andresen (1986) report increases in cell carbon and nitrogen quotas at low irradiance in cultures of *Skeletonema costatum*, but this effect is only prominent when cells are grown on light–dark cycles with short day lengths. Increased NDPK activity at low growth rate was also found in our study. This is not due solely to increases in cell size, since the pattern persists even if data are scaled to cell volume or carbon, nitrogen, or protein cell quotas.

Composition data for the transition experiments agree well with the steady-state data; only cell volume changed consistently through the transitions. This volume change is in agreement with data presented by Thompson et al. (1991), although these authors also found significant changes in carbon quota. In a study similar to ours, Post et al. (1985) noted that in turbidostat-grown cultures of *Thalassiosira weisflogii*, changes in carbohydrate occurred during light transitions, but no significant changes in protein were found. Similarly, Claustre and Gostan (1987) found changes in volume but not in protein during transitions with *Isochrysis* and *Hymenomonas* species.

A discussion of the causes and meaning of these cell composition differences is beyond the scope of this paper; however, such changes have important implications for selecting a biomass variable on which to scale enzyme activity. Since there was no indication that NDPK activity was correlated with any index of cell composition measured, the usefulness of normalizing to facilitate comparisons within this species is questionable. Because NDPK activity per cell varied over a factor of 6 or 7 while carbon or cell volume only varied by a factor of 2, scaling enzyme activity to either carbon or volume does not substantially change the relationship between NDPK and growth rate and may, in fact, add variation to the measurement. In addition, if the relationship between enzyme activity and carbon, cell volume, or nitrogen is complex, the scaled enzyme activity becomes much more difficult to interpret (Packard and Boardman 1988). Protein is commonly selected as a scaling variable in enzyme studies, but diatom species have many potentially interfering compounds such as amino acids, which complicate such a measurement (Dortch et al. 1984, Berges et al. 1992). Cell volume may also be unsuitable as a scaling factor because of methodological biases. If, as Thompson et al. (1991) speculate, short-term diatom volume increases (such as those found in this study) are achieved by addition of intercalary bands, then this implies a change in geometry that will result in an error if the volume is measured by a particle counter such as a Coulter Counter (see Kubitscheck 1987). For the present, when a single species is considered, expressing activity per cell and providing data on cell composition seems to be the most reasonable course. In cases where interspecific comparisons must be made the issue is clearly more complex.

Although statistically significant, the relationship between NDPK activity and growth rate is relatively poor and therefore of limited use in a predictive sense. The implications of these findings for control of cell metabolism are, however, quite interesting.

Growing evidence from a variety of sources indicates that organisms growing at higher rates tend to have higher enzyme activities. Pedersen et al. (1978) reported that in the bacterium *Escherichia coli*, 102 of 140 proteins (representing ⅔ of the protein mass of the cell) cataloged on chromatography plates showed nearly linear increases with increasing growth rate. In yeast cells, Sebastian et al. (1973) demonstrated a correlation between RNA polymer-

ase I activity and growth rate, while Yao et al. (1985) found the ornithine decarboxylase activity in the ciliate *Tetrahymena thermophila* was also correlated with growth.

Although there is a strong rationale for expecting a correlation between growth rate and the synthesis of NTP compounds, it is possible that NDPK does not control this process. In fact, it has been suggested that NDPK is not a regulatory step in the conversion of ATP to other nucleoside triphosphates (Parks and Agarwal 1973). Even if this is true, it does not mean that NDPK activity will not follow growth rate. Brown (1991) argues that there are adaptive pressures on cells to minimize their protein content (since protein is usually near the solubility limit within the cell). Thus, if a given enzyme was not rate-limiting and it was in greater concentration than necessary, there would be an advantage to reducing its concentration. As a result, even non-rate-limiting enzymes should respond as the fluxes through metabolic pathways change.

Alternatively, it is possible that NDPK is rate-limiting but that the maximal activity, measured in the present study and assumed to be proportional to enzyme concentration, is not equivalent to the *in vivo* enzyme activity. In addition to responding to changes in enzyme concentration, enzyme activity can be regulated by factors including substrate concentration, allosteric affectors, and a variety of activation/inactivation mechanisms.

If NDPK was substrate-limited (i.e. if reaction rates were a function of substrate and not enzyme concentration), ATP and NTP concentrations within the cell would be expected to fluctuate as a function of growth rate. For ATP this does not appear to be true; a review by Karl (1980) showed that ATP levels per cell are relatively constant over a wide range of growth conditions for prokaryotes, autotrophs, and heterotrophs. While some studies have demonstrated a correlation between growth rate and ATP pools, this depends on whether ATP is scaled to cell number or carbon quota, and there is still controversy (Chapman and Atkinson 1977, Karl 1980, Sakshaug and Andresen 1986). The relationship may also depend on what is limiting growth. Karl (1980) cited data showing that in the diatom *Thalassiosira fluviatilis* ATP correlates with growth rate under nitrate or phosphate limitation but not when cells are limited by light or ammonium. Laws et al. (1983) showed that for the diatom *Thalassiosira weisflogii* the ratio of ATP : carbon was constant over a wide range of light- and nutrient-limited growth rates. They speculated that ATP turnover, as opposed to concentration, might be a critical factor. In bacterial systems, the concentration of ATP and other nucleotides are, at best, a weak function of growth rate (Marr 1991). Karl (1980) suggested that adenine nucleotides are at or near saturating levels for most respiratory and metabolic enzymes. For other nucleotides there is also disagreement. Chapman and Atkinson (1977) found that other nucleotides followed patterns of ATP and did not vary with growth rate. Interestingly, they attributed this to rapid equilibration of other nucleotide pools and ATP through NDPK. Data presented by Marr (1991) support this view. Alternatively, Karl (1980) demonstrated that certain NTP pools, particularly GTP, vary with biosynthesis and growth rate. He suggested that the ratio of GTP:ATP might be useful as an index of growth rate. Pall (1985) also assigned GTP a key regulatory role in anabolic processes within the cell.

At the level of enzyme regulation, phosphorylation control of NDPK has been suggested (Pall 1985) but has not been demonstrated. There is evidence, however, that the adenylate energy charge plays a role in controlling enzyme activity. Thompson and Atkinson (1971) showed that for bovine liver NDPK, activity of the enzyme is maximal when the energy charge is near 1.0 and rapidly drops off as the ratio falls. Laws et al. (1983) demonstrated a significant positive correlation between energy charge and growth rate in *Thalassiosira weisflogii* cultures under a variety of limitations. This may explain the pattern in activity with growth rate observed in our study. At moderate growth rates (between about 0.5 and 1.0 d^{-1}), there is little change in the *in vitro* activity of the enzyme. Over this range, either substrate concentration or energy charge may be regulating activity. At higher growth rates, energy charge may be high and substrates saturating, so that further increases in growth rate may necessitate enzyme concentration increases. In contrast, Dolezal and Kapralek (1976) showed that in a bacterium grown in a chemostat between 7 and 60% of maximal growth rate, there was little change in adenylate levels and no change in energy charge. This has also been shown in the diatom *Skeletonema costatum,* where cell content of ATP increased only when growth rates were 50% of μ_{max} or greater (Sakshaug 1977). This discrepancy might be explained by the fact that these studies used chemostats, and therefore the cells were nutrient-limited, whereas growth rates were light-limited in the present study. The reason for an apparent increase in NDPK activity at very low growth rates is unclear. The regulation of NDPK may be even further complicated by the presence of isoforms, each of which may respond to different regulation. NDPK isoforms do exist in a variety of species (Parks and Agarwal 1973), but their function is unclear.

Another possible source of variability is the stage of cell division. Berges (1989) found strong relationships between NDPK activity and growth rate in the brine shrimp *Artemia franciscana,* but such relationships were specific to different developmental stages. However, Klein and Follmann (1988) showed that for the green alga *Scenedesmus obliquus* NDPK activity was constant throughout the cell division cycle.

It is apparent that neither NDPK activity nor nucleotide concentrations are entirely satisfactory as indices of *in situ* growth rate. If, however, the measurements were combined, it is possible that their predictive value would improve, particularly if adenylate energy charge, ATP turnover rates, or substrate concentration regulate NDPK activity over a range of growth rates. Furthermore, measurement of nucleotides and NDPK activity could provide insight into the specific growth rate limitation that cells experience *in situ*. Our study has examined only light-limited growth rates. Since data presented by Karl (1980) suggest that light, phosphorus, nitrate, or ammonium limitation result in different ATP–growth rate relationships, examining NDPK activity with respect to these cases would also be interesting.

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