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SILICON UPTAKE BY ALGAE WITH NO KNOWN Si REQUIREMENT. I. TRUE CELLULAR UPTAKE AND pH-INDUCED PRECIPITATION BY *PHAEODACTYLUM TRICORNUTUM* (BACILLARIOPHYCEAE) AND *PLATYMONAS* SP. (PRASINOPHYCEAE)[1]

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ABSTRACT

Phaeodactylum tricornutum *Bohlin, the one diatom known to lack a silicon requirement for growth, and the prasinophyte* Platymonas *sp. are two representatives of a taxonomically diverse group of planktonic algae that have been reported to take up Si without a demonstrable requirement for the element. For both species, removal of Si from solution during growth in batch culture has at least two components; true biological uptake throughout the growth of the culture, and spontaneous inorganic precipitation of a solid silicate phase—probably $Mg_2Si_3O_8$ (sepiolite)—under the elevated pH conditions that prevail late in batch growth. It is not clear to what extent previous observations of Si uptake by algae without siliceous frustules may be influenced by inorganic, non-cellular precipitation. The kinetics of true cellular uptake of Si are similar in* Phaeodatylum *and* Platymonas, *and different from those reported for the Si-requiring diatoms. Uptake follows hyperbolic saturation kinetics in both species, with half-saturation concentrations of 97.4 μM in* Phaeodactylum *and 80.9 μM in* Platymonas, *as compared to ca. 1–6 μM in diatoms that form siliceous frustules. Uptake by* Phaeodactylum *and* Platymonas *is not substrate-saturated until the dissolved Si concentration of the medium exceeds 200 μM. Concentrations this high do not occur in the surface layer of the ocean, and the kinetics suggest that both species deposit much less silica in nature than they can be induced to deposit in culture.*

Key index words: diatom; Phaeodactylum; Platymonas; *sepiolite; silicic acid, uptake*

During the past five years it has been reported that a considerable number of planktonic marine

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algae with no known silicon (Si) requirement incorporate substantial amounts of Si when grown in Si-enriched culture medium (Bankston et al. 1979, Fuhrman et al. 1978, D'Elia et al. 1979). These algae cover a broad taxonomic range and include certain dinoflagellates, prasinophytes and haptophytes as well as the single diatom known to lack a Si requirement. One cyanobacterium has also been reported to incorporate Si (Bankston et al. 1979). These findings have led to speculation concerning the removal of Si from the environment by algae that do not require it, and the role of this process in phytoplankton species competition and succession as well as in the oceanic silicon cycle (e.g. Fuhrman et al. 1978, D'Elia et al. 1979).

To evaluate the importance of this apparently gratuitous Si uptake in natural ecological or geochemical processes it is necessary to know the extent to which it can take place at nutrient concentrations typical of the surface ocean. The kinetics of Si uptake are well known for several diatoms (e.g. Paasche 1973b, Nelson et al. 1976, Kilham et al. 1977) and are in good general agreement with direct measurements of the concentration dependence of Si uptake by natural phytoplankton assemblages (e.g., Goering et al. 1973, Azam and Chisholm 1976, Nelson and Goering 1978). In these studies, the dependence of the uptake rate upon the external dissolved Si concentration has been described by hyperbolic saturation kinetics, with the half-saturation concentration (K_s) in the range of 0.7–6.2 μM. Comparison of these kinetic results with dissolved Si concentrations in the surface ocean (e.g. Bainbridge 1980) suggests that the rate of Si uptake by diatoms in the field may often be limited by its external concentration, but that uptake can still proceed at a substantial fraction of most diatoms' maximum rate in most marine surface waters. The concentration dependence of Si uptake by algae other than diatoms has not previously been reported, and thus it has not been clear whether such uptake can proceed at significant rates in the surface ocean.

There is also an inorganic process that may complicate the interpretation of experiments in which algae are grown to high density in Si-enriched batch culture. Silicic acid ($Si(OH)_4$), the predominant form of dissolved Si in seawater, is known to react with Mg^{2+} and OH^- ions in seawater to form the mineral sepiolite, $Mg_2Si_3O_8$ (Wollast et al. 1968). Both the direction and rate of this reaction are strongly dependent upon silicic acid concentration and pH; at silicic acid concentrations in the 100 μM range used in media such as f/2 (Guillard and Ryther 1962), the precipitation is thermodynamically possible at any pH above 8.6 (Wollast 1974). AT pH 9.0 sepiolite precipitation can occur at any dissolved Si concentration above 42 μM ($[Si(OH)_4] > 31$ μM).

Reasonably dense cultures of algae in enriched natural seawater media such as f/2 frequently achieve combinations of pH and silicic acid concentration at which sepiolite may precipitate. In dense batch cultures of both *Platymonas* sp. and *Phaeodactylum* the pH has been observed to reach 10.2. To the extent that spontaneous precipitation reactions actually take place under these conditions, direct biological interpretation of any observed removal of Si from solution would be compromised.

We report here experiments on Si uptake by *Phaeodactylum tricornutum*, the one diatom known to lack a Si requirement for growth, and by the prasinophyte *Platymonas* sp. These are the two algae for which Si uptake without a known requirement has been examined in the greatest detail to date (Fuhrman et al. 1978, D'Elia et al. 1979). Our goals in undertaking these experiments were to distinguish clearly between spontaneous inorganic precipitation and true biological uptake of Si in batch cultures of these algae, and to determine the kinetics of true cellular uptake of Si in both species.

METHODS

Experiments of four types were undertaken in this study. First, the Si concentration was monitored in batch cultures to measure the removal of Si from solution during growth. Second, the return of Si to solution upon resuspension of the algae and associated particles in isothermal, isohaline medium with low dissolved Si and pH was examined in 2 h time-course experiments. Third, the possible abiotic removal of Si in batch culture and release upon change in medium was studied in precipitation-dissolution experiments in cell-free medium. Finally, the kinetics of true cellular uptake were examined using a stable isotope tracer.

Axenic clonal cultures of *Phaeodactylum tricornutum* Bohlin (clone Pet Pd) and *Platymonas* sp. (clone Platy 1) were obtained from R. R. L. Guillard at the Woods Hole Oceanographic Institution. These are the same clones used by D'Elia et al. (1979) and Fuhrman et al. (1978) in their studies of Si metabolism in *Phaeodactylum* and *Platymonas*, respectively. No siliceous hard parts have been reported in the genus *Platymonas*, but *Phaeodactylum* is known to produce siliceous structures of at least two types. The ovate morphotype has a single siliceous valve similar to one of the paired valves found in other diatoms (Lewin et al. 1958). In the fusiform and triradiate morphotypes there is no siliceous valve, but there are numerous (up to 10) siliceous bands embedded in the cell wall (Borowitzka and Volcani 1978).

Experimental media were in all cases f/2 nutrient additions to filtered, sterile seawater (Guillard and Ryther 1962) with modification of the silicate addition, which are noted along with the description of each experiment. Algae were cultured in 250 mL polycarbonate bottles and incubated in 500 mL polycarbonate bottles. Light intensity for all culturing and incubating was 120 $\mu E \cdot m^{-2} \cdot s^{-1}$ using Sylvania "cool white" fluorescent lights. Temperature was regulated at 20 ± 1° C.

The methods used in our batch culture Si removal experiments were similar to those of Fuhrman et al. (1978) and were performed in medium with 25 μM added sodium metasilicate. For each alga replicate 250 mL batch cultures were inoculated with a small volume of a dense, but very recently grown, stock culture. Cell densities immediately after inoculation were 4.8×10^3 cells mL^{-1} for clone Pet Pd and 1.6×10^3 cells mL^{-1} for clone Platy 1. Each culture was then grown for 7 d and sampled daily for cell counts, dissolved Si analyses and pH. Cell counts were performed microscopically using a Speirs-Levy hemocytometer and counting the full 32 1 mm squares of at least 100 cells, whichever occurred first, resulting in a random counting error of <20% (Guillard 1973). For dissolved Si analysis, 25 mL samples of each culture were syringe filtered through 0.4 μm Nuclepore filters

into clean polyethylene sample bottles. All syringes were polypropylene and all filter holders polycarbonate. Samples were sealed and stored in a refrigerator at ca. 4° C until the end of the experiment, at which time they were analyzed colorimetrically for reactive silicate by the acid-molybdate method of Strickland and Parsons (1972).

On the 6th and 7th days dissolution experiments were performed to determine what fraction of the Si removed from solution during batch growth remained insoluble under conditions favoring the dissolution of sepiolite. A 10 mL aliquot from each culture was collected and centrifuged at 1000 × *g* for 15 min. The supernatant solution was taken for analysis of dissolved Si. The cells, along with any other particles that may have formed, were resuspended in 10 mL of 0.1 N HCl and shaken for 1 h. This suspension was then centrifuged again as above, and ca. 9.5 mL taken for dissolved Si analysis. The remaining particulate matter was analyzed for biogenic silica by the NaOH digestion procedure of Paasche (1973a).

A separate experiment was performed to determine what fraction of the Si removed from solution during batch growth remained insoluble when returned to medium with dissolved Si concentration and pH at reasonable levels for the surface oceans. A 2 L batch culture of each clone was grown from a small inoculum in medium with a dissolved Si concentration of 60 μM. When each culture was in the late stages of exponential growth (2.5×10^6 cells·mL^{-1} for Pet Pd; 5.3×10^5 cells·mL^{-1} for Platy 1) the growth medium was analyzed for dissolved Si concentration and the cells concentrated by centrifugation at 1000 × *g* for 15 min. For each clone ca. 100 mL of concentrated cell suspension was obtained, and 50 mL resuspended in 500 mL of sterile, isothermal, isotonic medium containing no added Si. These 550 mL suspensions were sampled immediately for cell counts and at 1, 2, 3, 5, 10, 20, 30, 45, 60, 90 and 120 min after resuspension for dissolved Si concentration.

In another experiment, cell-free medium with 100 μM added dissolved Si was used to determine whether the removal of Si from solution and its subsequent release observed in batch cultures of *Phaeodactylum tricornutum* and *Platymonas* sp. could take place in the absence of cells. Twelve 250 mL batches of this medium were prepared in 250 mL polycarbonate culture bottles. The pH of these samples was adjusted to 8.2, 8.9, 9.6, 10.1 and 10.3 in pairs, and 10 mL samples were taken daily, filtered through 0.4 μm Nuclepore filters and analyzed for dissolved Si. If necessary, the solutions were readjusted daily with concentrated NaOH to maintain the desired pH. In all media at pH ≧ 9.6 a white precipitate formed concurrently with the removal of Si from solution (see Results). A separate batch of this precipitate was produced in 250 mL of identical cell-free, 100 μM Si medium at pH 10.0 over 6 days, concentrated to ca. 10 mL by centrifugation at 1000 × *g* for 20 min and resuspended in 225 mL of isothermal, sterile low-Si (<1 μM) seawater. The pH of the seawater added was 8.17 and that of the resultant seawater/solid mixture initially 8.25, rising to 9.4 as the solid phase dissolved over the next 3 h. Samples of this suspension were filtered after 1, 3, 5, 11, 15, 30, 60, 122 and 180 min for dissolved Si analysis.

Two series of ^{30}Si tracer experiments were performed to determine the rate of true biological uptake of dissolved Si by both species as a function of the external substrate concentration. A preliminary experiment with *P. tricornutum* indicated that these experiments would have to consider a much broader concentration range than have previous kinetic studies of dissolved Si uptake by diatoms (e.g. Paasche 1973b, Nelson et al. 1976, Kilham et al. 1977). In view of this, dissolved Si additions up to 500 μM were used.

For each species a 2.5 L batch culture was grown from a small inoculum in medium containing 60 μM dissolved Si. Growth was monitored by daily cell counts. When the culture was in the latter stages of exponential growth (ca. 5×10^5 cells·mL^{-1} for *Platymonas*; ca. 1×10^6 cells·mL^{-1} for *Phaeodactylum*) the cells were concentrated by centrifugation at 1000 × *g* for 15 min and resuspended in 3.7 L medium containing no added Si. This cell suspension was sampled for cell density and dissolved Si concentration analysis. It was then left undisturbed for 1 h so that any rapid release of dissolved Si could go to completion. After 1 h the culture was re-sampled for dissolved Si concentration analysis. In each experiment the dissolved Si concentration of the 3.7 L culture immediately after addition of the concentrated cell suspension was ca. 4 μM. Dissolved Si concentrations 1 h later were 9.0 μM in the *P. tricornutum* experiment and 13.4 μM in the *Platymonas* sp. experiment.

The prepared cultures were divided into 200 mL aliquots and inoculated with 5, 10, 15, 20, 30, 40, 50, 75, 100, 150, 200, 250 and 500 μM dissolved Si using a ^{30}Si-labeled solution (95.20 atom% ^{30}Si). Additional suspensions of heat-killed and Formalin-killed cells were inoculated with 100 μM to measure the rate of any abiotic Si uptake, such as sepiolite precipitation or adsorption to particle surfaces. All samples were incubated for 2 h. The samples were then filtered through 0.4 μM Nuclepore filters. The filters were sealed in plastic petri dishes, dried at 65° C for 24 h and then analyzed for ^{30}Si enrichment by conversion of all Si to barium fluosilicate, followed by solid-sample mass spectrometry (Nelson and Goering 1977) using a Nuclide Corp. model 3-60 (3-inch radius, 60 degree deflection) magnetic sector mass spectrometer fitted with a direct insertion probe for solid samples.

The specific uptake rate of Si (V) was calculated from the ^{30}Si enrichment of the particulate Si, as described by Nelson and Goering (1977). V has dimensions of (time^{-1}) and represents the number of moles of Si taken up per mole of particulate Si initially present per unit time. This specific uptake rate was then used along with the particulate Si concentration of the cell suspension (Si_p) (also obtained from the mass spectrum; Nelson and Goering 1977) and the cell count (N) to compute the uptake rate per cell per unit time (ρ):

$$\rho(\text{mol Si·cell}^{-1}\text{·h}^{-1}) = \frac{\text{Si}_p(\text{mol Si·mL}^{-1}) \times \text{V(h}^{-1})}{\text{N(cells·mL}^{-1})} \qquad (1)$$

RESULTS

In batch cultures of both *Phaeodactylum tricornutum* and *Platymonas* sp. there was significant removal of Si from solution concurrent with growth over a 7 day period (Fig. 1). During this period the pH of all cultures increased from 8.1 to ca. 10.0 (Fig. 1). Depletion of dissolved Si was detectable only after day 3, when pH had risen to ca. 8.9 in both cultures of *P. tricornutum* and ca. 9.3 in both cultures of *Platymonas* sp. The total amount of Si removed from solution per cell produced over the 7-day time course was $6.6 \pm 0.2 \times 10^{-15}$ mol·cell^{-1} for *P. tricornutum* and $3.6 \pm 0.2 \times 10^{-14}$ mol·cell^{-1} for *Platymonas* sp. If all cells produced contained these amounts of Si, one would expect dissolved Si to be depleted by 2.5–3 μM in the *Phaeodactylum* cultures, and by 5–6 μM in the *Platymonas* cultures, during the first 3 days of growth. No depletion of dissolved Si by either alga was detectable over this time interval.

Analysis of the particulate silica produced during these growth experiments (Table 1) indicates that 80–90% of the particulate silica produced in the *P. tricornutum* cultures, and 90–95% of that produced in the *Platymonas* sp. cultures redissolved within 1 h in 0.1 N HCl. These conditions would not dissolve biogenic silica (e.g. Lewin 1961, Hurd 1972) but would favor the dissolution of sepiolite (Wollast et

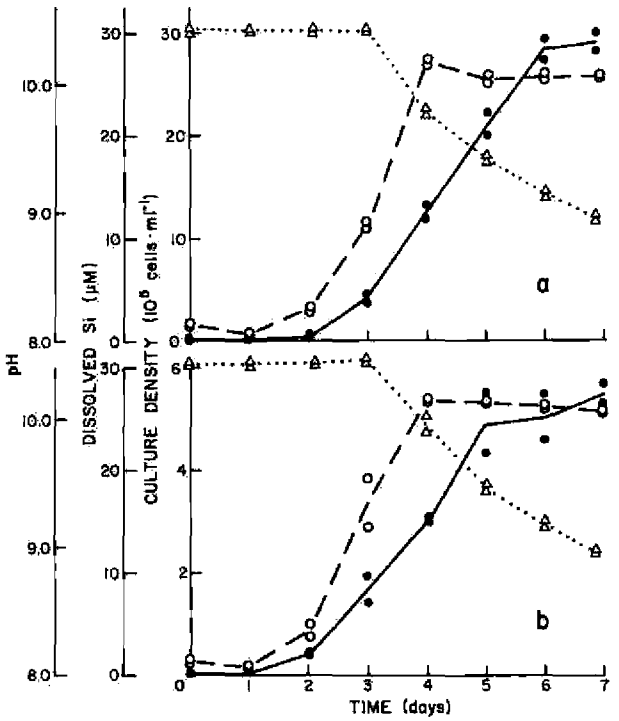

FIG. 1a, b. Time course of cell density (●) dissolved Si concentration (△) and pH (○) during growth in batch cultures of *Phaeodactylum tricornutum* (a) and *Platymonas* sp. (b).

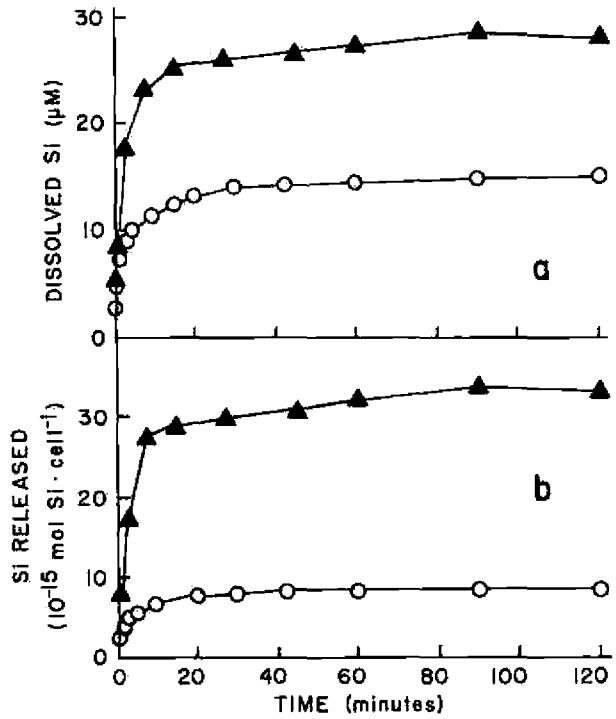

FIG. 2a, b. Release of Si from batch cultures of *Phaeodactylum tricornutum* (○) and *Platymonas* sp. (▲) after resuspension in isothermal, isotonic medium with low dissolved Si concentration at pH <8.5. a, dissolved Si concentration measured in cell suspensions; b, calculated release of Si per cell.

al. 1968) and other acid-soluble silicate phases, and would also lyse cells. Resuspension of cells in isothermal, isotonic, low Si medium at a pH of ca. 8.2 also resulted in rapid return of Si to solution (Fig. 2). Under these less drastic conditions, which would not be expected to lyse cells, 50–60% of the Si removed from solution during 6 days batch growth was returned to solution in 20 min. Dissolution of diatom silica in seawater is much slower than this (e.g. Lewin 1961, Nelson et al. 1976). Thus, most Si depletion during batch growth of *P. tricornutum* and *Platymonas* sp. must have resulted from precipitation of an acid-soluble silicate phase, cellular uptake of Si that was not accompanied by deposition of siliceous hard parts, or both.

TABLE 1. *Si mass balance for 7-day growth experiments on* Phaeodactylum tricornutum *and* Platymonas *sp. in batch culture. All values are in μmol Si·L⁻¹. Entries report the mean and range for duplicate cultures.*

	Depleted from medium	Extractable in 0.1 N HCl	Not extractable in 0.1 N HCl, soluble in 0.2 N NaOH
Phaeodactylum tricornutum			
By day 6	16.8 ± 0.2	13.7 ± 1.4	1.2 ± 0.1
By day 7	18.9 ± 0.1	17.5 ± 0.4	0.8 ± 0.2
Platymonas sp.			
By day 6	16.9 ± 0.3	15.9 ± 0.2	undetectable*
By day 7	19.4 ± 0.1	18.0 ± 0.5	undetectable*

* Less than 0.2 μmol·L⁻¹.

Inorganic precipitation of Si from cell-free medium (Fig. 3) was undectable over a 7 day period at pH 8.2 and 8.9. At pH values ≧ 9.6 there was a continuous removal of Si from solution, the rate increasing with increasing pH. The precipitate that formed at pH 10.0 released Si readily into isother-

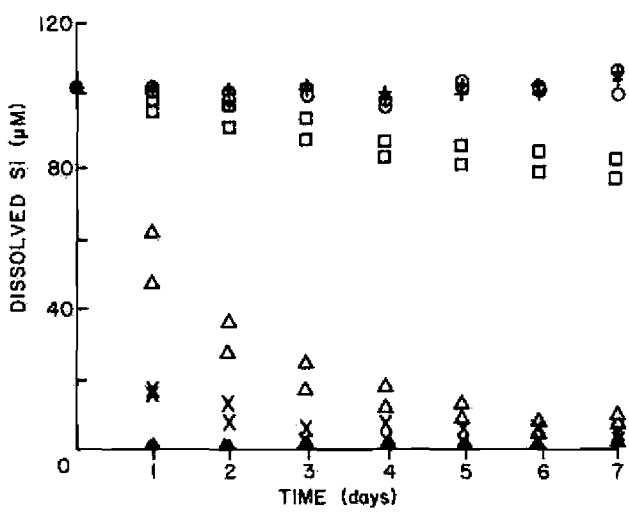

FIG. 3. Time course of dissolved Si concentration in cell-free f/2 medium adjusted to pH values of 8.2 (+), 8.9 (○), 9.6 (□), 9.9 (△), 10.1 (×) and 10.3 (▲).

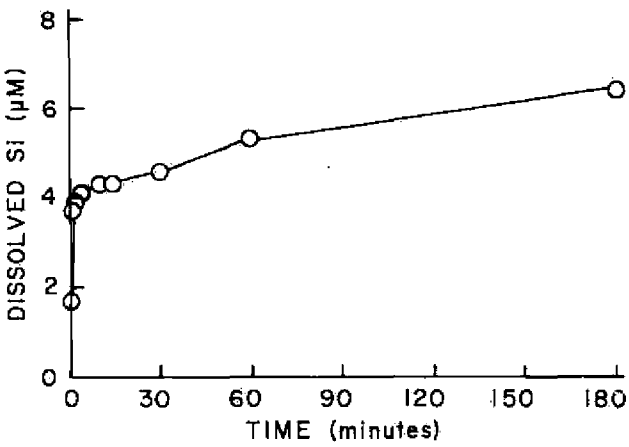

FIG. 4. Time course of Si release from particulate matter precipitated in cell-free f/2 medium with 100 μM added Si at pH 10.0 upon resuspension in cell-free isohaline, isothermal medium with pH 8.16 and dissolved Si 1.7 μM.

mal, isohaline, low pH, low Si medium (Fig. 4). The time course of Si release from this precipitate was similar to that observed when concentrated cell suspension of *P. tricornutum* and *Platymonas* sp. batch cultures were resuspended in similar medium (Fig. 2).

The ^{30}Si tracer experiments were conducted in the 8.2–8.5 pH range, and should thus have avoided inorganic precipitation. Under these conditions uptake of Si by both *P. tricornutum* and *Platymonas* sp. was observed, and the rate of uptake increased with increasing dissolved Si concentration. Fig. 5 presents the uptake rate per cell (ρ) as a function of the extracellular dissolved Si concentration for both algae. No significant ^{30}Si enrichment was found in the heat- or Formalin-killed cultures; thus ρ at all dissolved Si additions ≤ 100 μM represents true cellular uptake and not adsorption or precipitation. The lack of large increase in ρ for either species over the 100–500 μM enrichment range indicates that inorganic precipitation and adsorption of Si were insignificant at all dissolved Si concentrations in these 2 h incubation experiments.

The dependence of ρ upon dissolved Si concentration appears generally to follow hyperbolic saturation kinetics. This is a very common relationship between the uptake rate of a nutrient and its external concentration, and has often been described by the Michaelis-Menten equation for enzyme kinetics (Dugdale 1967). The equation is of the form:

$$\rho = \frac{\rho_{max} \cdot [\text{dissolved Si}]}{K_s + [\text{dissolved Si}]} \qquad (2)$$

where ρ_{max} represents the substrate-saturated rate of uptake and K_s the dissolved Si concentration at which $\rho = \rho_{max}/2$. To test the fit of eq. (2) to our data and obtain preliminary estimates of ρ_{max} and K_s [dissolved Si]/ρ was plotted as a function of [dissolved Si]. This plot is based upon a linear transformation of eq. (2):

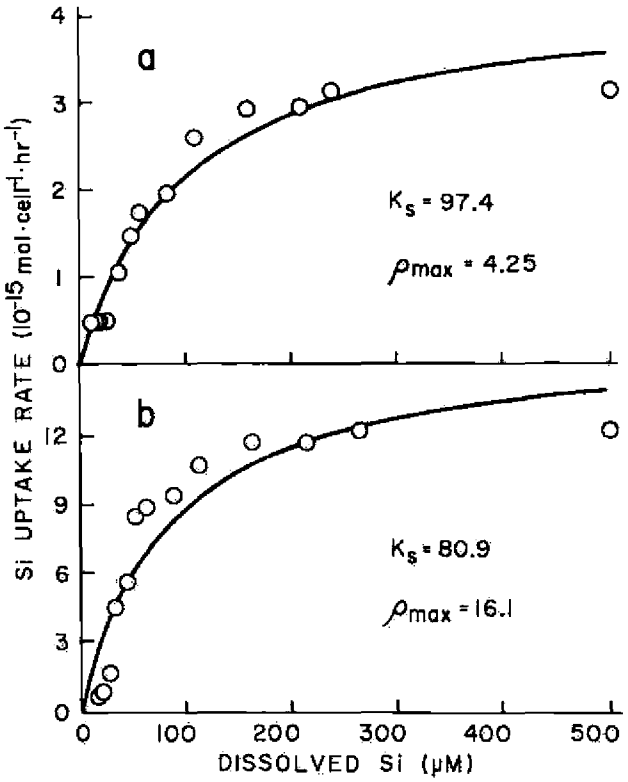

FIG. 5a, b. Si uptake rate of *Phaeodactylum tricornutum* (a) and *Platymonas* sp. (b) as a function of extracellular dissolved Si concentration. Curves were fitted using the Michaelis-Menten equation (eq. 2), and ρ_{max} and K_s values obtained are indicated.

$$\frac{[\text{dissolved Si}]}{\rho} = \frac{K_s + [\text{dissolved Si}]}{\rho_{max}} \qquad (3)$$

Values of ρ_{max} and K_s obtained by fitting eq. (3) to our data were then used as preliminary estimates so that the hyperbolic curve (eq. 2) could be fitted directly to our data by an iterative technique (Wilkinson 1961). The curves fitted by the iterative method are plotted along with the data in Fig. 5. The K_s values of 97.4 μM for *P. tricornutum* and 80.9 μM for *Platymonas* sp. are ca. 10–100 times higher than previously reported values of the K_s of Si uptake by diatoms (e.g. Paasche 1973b, Nelson et al. 1976, Kilham et al. 1977).

DISCUSSION

Although both *Phaeodatylum tricornutum* and *Platymonas* sp. have been shown to lack any significant Si requirement for growth, significant removal of Si from solution has been reported to take place concurrent with growth of each alga in nutrient-enriched batch cultures (Fuhrman et al. 1978, D'Elia et al. 1979). Our results confirm these findings and thus would seem to agree with those showing uptake of Si by numerous planktonic algae with no apparent Si requirement (Bankston et al. 1979). However, the thermodynamic possibility that inorganic silicate phases may precipitate spontaneously under condi-

tions typical of batch culture experiments (Wollast et al. 1968) means that neither the observed disappearance of Si from solution nor the observed appearance of silicon in the particulate phase can be interpreted unambiguously as true biological uptake in all cases. We have evidence of both inorganic precipitation and true cellular uptake of Si in our experiments with *Phaeodactylum tricornutm* and *Platymonas* sp.

Conditions for inorganic precipitation. We observed no detectable removal of Si from solution in batch cultures of *P. tricornutum* or *Platymonas* sp. until after the pH exceeded 8.9 and 9.3, respectively (Fig. 1). If Si were being incorporated into cellular material at a constant amount per cell throughout these experiments, depletion from the medium would have been detectable much sooner than it was. Data presented in Table 1 show that between 80 and 95% of the Si removed from solution during batch growth of either species could be rapidly redissolved in 0.1 N HCl; conditions that would have little or no effect on diatom silica (e.g. Lewin 1961, Hurd 1972). Thus >80% of the observed depletion of dissolved Si resulted from processes other than biogenic silica deposition. One of these processes may have been true cellular uptake into acid-extractable intracellular pools (at least we have no evidence that it was not). However, our results are most consistent with a conclusion that there was significant precipitation of sepiolite or some other acid-soluble inorganic silicate phase during the time course of growth in batch culture, once cell growth had elevated the pH above ca. 9.5.

The experiments in cell-free f/2 medium show that significant removal of Si from solution can occur over the course of 7 days at pH levels ≧9.6 via spontaneous inorganic precipitation. Figure 6 shows the calculated equilibrium concentration of total dissolved Si in seawater in equilibrium with sepiolite as a function of pH. Calculations were made using MICROQL (Westall, 1979), an iterative chemical equilibrium program written in BASIC, and implemented on an Apple II+ computer. Constants for the calculations were taken from Stumm and Morgan (1970) and Wollast et al. (1968), and where needed corrected to the ionic strength of seawater with single ion activity coefficients from Garrels and Thompson (1962). Note that at very high pH values solutions as low as 5–10 μM can be supersaturated. Above pH 9.0 another pair of solid phases may form, brucite ($Mg(OH)_2$) and another active form of $Mg(OH)_2$ (Stumm and Morgan 1970). These minerals would compete with sepiolite for Mg, and tend to raise the equilibrium solubility of Si. The calculations in Figure 6 do not consider the $Mg(OH)_2$ minerals, and thus should be considered the lowest dissolved Si concentration at a given pH at which sepiolite could precipitate. Silica may also be coprecipitated with or adsorbed to $Mg(OH)_2$ precipitates.

The pH and dissolved Si concentrations of our six cell-free experiments are also shown in Figure 6; the results conform to the thermodynamic prediction in five of the six cases, but at pH 8.9 our solutions apparently remained supersaturated with respect to sepiolite for 7 days without forming any precipitate that contained Si. The cell-free experiments did not match any of the batch culture experiments exactly with respect to the time course of Si removal or redissolution. However, the experiments over the 9.6–10.3 pH range indicate that inorganic Si precipitation can take place over the same approximate time period as that usually used in batch growth experiments (several days). In addition, the resuspension experiments summarized in Figures 2 and 4 show that this precipitate dissolves to a significant degree over the same time period (minutes or hours) during which we observed return of Si to solution when batch cultures of *P. tricornutum* and *Platymonas* sp. were resuspended in isothermal, isotonic but low-Si medium at a pH <8.5. Thus the cell-free and batch culture results combine to indicate that in all probability inorganic precipitation was at least as important as true biological uptake in removing Si from solution during batch growth of both *P. tricornutum* and *Platymonas* sp. It thus appears necessary to consider the possibility of abiotic removal of Si from solution in any Si-enriched growth medium during experiments where culture growth may result in elevated pH.

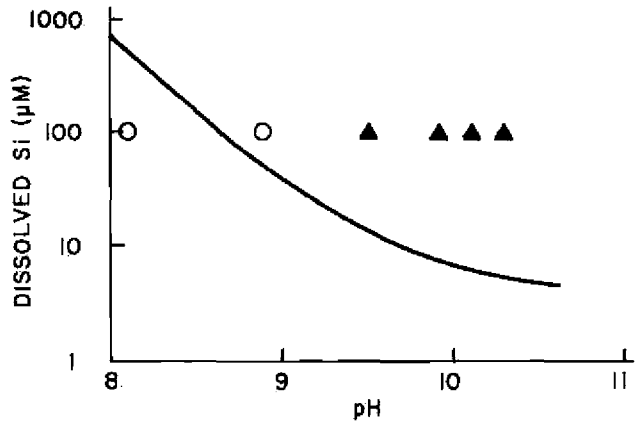

FIG. 6. Calculated total dissolved Si concentration of seawater in equilibrium with sepiolite ($Mg_2Si_3O_8$) over the pH range 8.0 to 10.6. Also shown are the dissolved Si/pH combinations used in our six precipitation experiments in cell-free f/2 medium. Circles represent experiments in which no removal of Si from solution was observed over a 7 day period; triangles represent the conditions under which a Si-containing precipitate formed.

Kinetics of true cellular uptake. From a biological perspective, the above discussion of conditions under which Si can precipitate inorganically is directed mostly at eliminating this phenomenon so that studies of biological uptake of Si are not impaired. The batch growth, resuspension and cell-free medium experiments (Figs. 1–4) were necessary so that tracer

experiments reported in Figure 5 could be designed. We found that the rate of true cellular uptake of Si can be measured over a wide range of dissolved Si concentrations in short-term (2 h) ^{30}Si tracer experiments, using cell suspensions dilute enough to keep the pH from exceeding 8.5. Under these conditions the heat-killed and Formalin-killed controls indicate the absence of significant surface sorption or inorganic precipitation of Si.

The true cellular uptake of Si by both *P. tricornutum* and *Platymonas* sp. can be described approximately by hyperbolic saturation kinetics (Fig. 5), but the K_s values for uptake are ca. 10–100 times higher than those that have been reported for diatoms having a Si requirement for growth (e.g. Paasche 1973b, Nelson et al. 1976, Kilham et al. 1977). The Si uptake systems of both *P. tricornutum* and *Platymonas* sp. thus appear to require such high external substrate concentrations that the ecological importance of Si uptake by either alga is open to serious doubt. Neither species is saturated with respect to Si uptake until the external dissolved Si concentration exceeds 200 μM—a concentration that is never achieved in near-surface ocean water. Our kinetic results indicate that over the 0–5 μM range that characterizes most marine surface waters (Bainbridge 1980) $\rho < 0.08\ \rho_{max}$ for *Platymonas* sp. and $\rho < 0.05\ \rho_{max}$ for *P. tricornutum*.

If the Si uptake kinetics of *P. tricornutum* and *Platymonas* sp. are typical of those planktonic algae that take up Si without an apparent requirement, then those algae are kinetically incapable of competing successfully for Si with those diatoms that require it for growth. The role of Si deposition by algae kinetically similar to *P. tricornutum* and *Platymonas* sp. in the global oceanic Si cycle is also likely to be insignificant compared with that of the Si-requiring diatoms.

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