J. Phycol. **21**, 168–171 (1985)

NOTE

SILICON UPTAKE BY ALGAE WITH NO KNOWN Si REQUIREMENT. II. STRONG pH DEPENDENCE OF UPTAKE KINETIC PARAMETERS IN *PHAEODACTYLUM TRICORNUTUM* (BACILLARIOPHYCEAE)[1]

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ABSTRACT

Silicon uptake kinetics of the diatom Phaeodactylum tricornutum *(Bohlin) were examined at pH 8.8 ± 0.1 and pH 9.7 ± 0.1. Uptake follows hyperbolic saturation kinetics at both pH's, but at the higher pH the half-saturation constant for uptake is 11.8 μM, as opposed to 54.8 μM at the lower pH. When the uptake rate is examined as a function of the calculated concentration of the monovalent conjugate base, $SiO(OH)_3^-$, the half-saturation constant for uptake is 6.6 μM at either pH.*

Key index words: diatoms; pH; Phaeodactylum tricornutum; *silicic acid; uptake kinetics*

In a previous paper (Nelson et al. 1984) we reported on aspects of Si uptake by two algae with no known Si requirement, the diatom *Phaeodactylum tricornutum* (Bohlin) and the prasinophyte *Platymonas* sp. Both species demonstrated hyperbolic saturation kinetics for Si uptake with half-saturation constants considerably higher than those previously found for Si-requiring diatoms (e.g. Paasche 1973, Nelson et al. 1976, Kilham et al. 1977). In this paper we report on the Si uptake kinetics of *Phaeodactylum tricornutum* as a function of pH over a pH range that alters the chemical speciation of dissolved Si to a large degree.

Phaeodactylum tricornutum (clone Pet Pd, obtained from R. R. L. Guillard, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts) was grown to 6 × 10^5 cells·mL^{-1} in f/2 medium (Guillard and Ryther 1962) with 60 μM added Si at a temperature of 20° C and under continuous illumination of 170 μE·m^{-2}·sec^{-1}. The cells were centrifuged at 1000 × *g* for 15 min and resuspended in f/2 without added Si (background [Si] = 1.5 μM). The cells were then centrifuged again and resuspended in two 2 L flasks of f/2 medium without added Si, one with the pH adjusted to 8.5 and one to pH 9.6. Before the uptake experiments were begun these cultures were left undisturbed at least 1 h, thus allowing any rapidly dissolving inorganic silicate phases to dissolve (Nelson et al. 1984). For uptake experiments 250 mL aliquots were taken from the cell suspension and inoculated with ^{30}Si-labeled silicic acid solution (95.2 atom % ^{30}Si) to the desired levels. The cultures were then incubated at 170 μE·m^{-2}·sec^{-1} at 20° C for 2 h.

At the end of the incubations the samples were filtered through 0.8 μm polycarbonate membrane filters (Nucleopore Inc.). Sample preparation and ^{30}Si analysis were as described by Nelson and Goering (1977). The pH of the cultures was measured at the beginning and end of the incubations with an Orion model 801 pH meter, equipped with a Corning 476050 glass/Ag/AgCl electrode, standardized at pH 7.0 and 10.0. A slight increase in the pH was observed over the duration of the incubations, about 0.1 unit in the low series, and 0.05 unit in the high series, due to photosynthesis. A trend of increasing pH with the amount of ^{30}Si added, about 0.3 unit in the low pH series and 0.1 pH unit in the high series, was due to the alkalinity of the isotope solution. The dissolved Si concentration of the medium prior to the ^{30}Si addition was determined by the method of Strickland and Parsons (1972) on replicate samples.

The concentrations of the various species of silicic acid in the experimental flasks were calculated from the mean pH over the incubation period, the measured concentration of dissolved Si and the dissociation constants (Sjöberg et al. 1981):

$$\frac{[Si(OH)_3^-]a_{H^+}}{[Si(OH)_4]} = 10^{-9.47} \quad (1)$$

$$\frac{[SiO_2(OH)_2^{2-}]a_{H^+}}{[SiO(OH)_3^-]} = 10^{-12.6} \quad (2)$$

In the pH ranges and dissolved Si concentrations of our experiments undissociated silicic acid ($Si(OH)_4$) and the first conjugate base ($SiO(OH)_3^-$) together are predicted to make up more than 99% of the total Si. Silica polymers are not thermodynamically stable at these low dissolved Si concentrations (Stumm and Morgan 1970). In a few high-pH, high-Si cultures sepiolite ($Mg_2Si_3O_8$) saturation is predicted (Wollast et al. 1968), but our previous experiments (Nelson et al. 1984) have shown evidence of actual precipitation only at higher concentrations.

The cell-specific rate of Si uptake (ρ) is presented in Fig. 1A for both pH series as a function of total (initial + added) dissolved Si concentration. The dependence of ρ upon the total dissolved Si concentration can be approximated by hyperbolic saturation kinetics as described by the Michaelis-Menten equation (Dugdale 1967):

$$\rho = \frac{\rho_{max} \cdot S}{K_s + S} \quad (3)$$

where S is the concentration of the substrate under consideration and K_s is the concentration of that substrate at which $\rho = \rho_{max}/2$. The results were fitted by iterative nonlinear regression (Wilkinson 1961) to determine K_s and ρ_{max} and their associated confidence limits. The kinetic parameters obtained by the direct fit were used to define the solid lines in Fig. 1A–C as well.

In both series *P. tricornutum* exhibited hyperbolic saturation kinetics for Si uptake. In the high pH

[1] *Accepted: 16 August 1984.*

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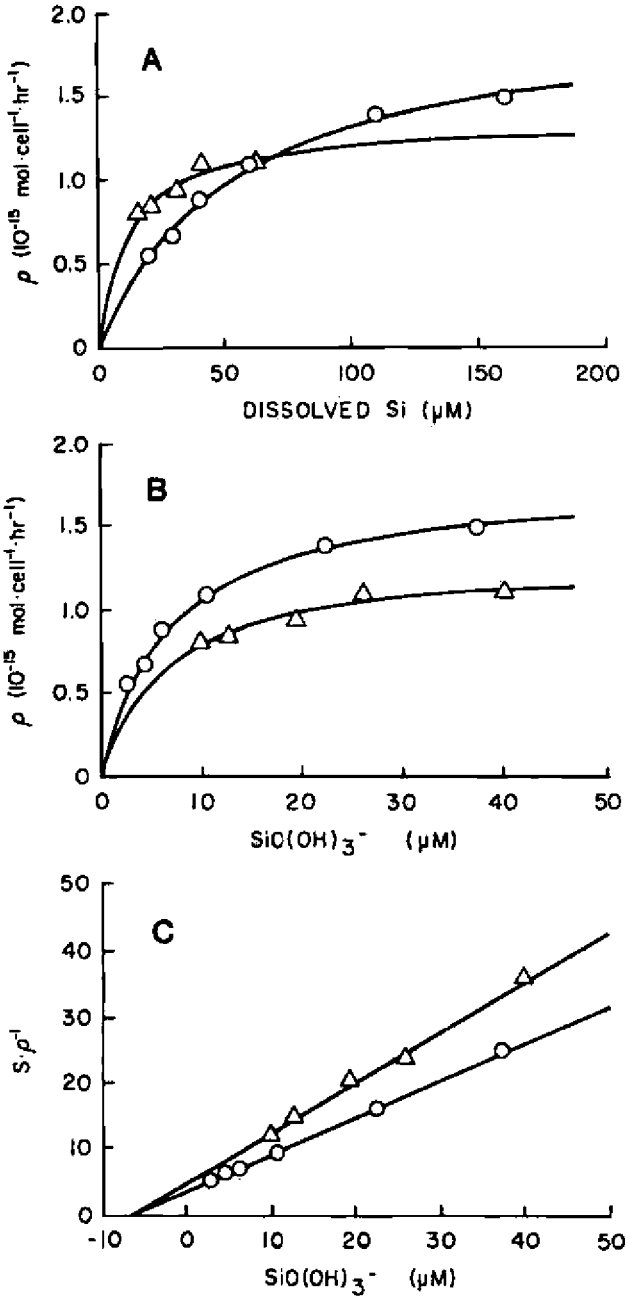

FIG. 1A–C. The rate of uptake of Si by *Phaeodactylum tricornutum* as a function of substrate concentration at two different pH ranges, 8.6 (○) and 9.5 (△). A) Uptake rate versus total reactive silica. B) uptake rate versus calculated concentration of $SiO(OH)_3^-$ ion. C) Hanes-Woolf plot with $SiO(OH)_3^-$ ion as the substrate.

series both the K_s and the ρ_{max} are lower than in the low pH series when total dissolved Si is considered as the substrate. Thus, at the high pH Si uptake proceeds more rapidly at low concentrations, but less rapidly at high concentrations than at the lower pH.

The differences between the K_s in the two series are considerable. Using total Si as the substrate, the

TABLE 1. *Half-saturation constants (K_s) for silicon uptake using total Si, and calculated concentrations of undissociated silicic acid, and the first and second conjugate bases as substrates (±95% confidence interval).*

pH series	Si (μM)	$Si(OH)_4$ (μM)	$SiO(OH)_3^-$ (μM)	$SiO_2(OH)_2^{2-}$ (nM)
Low	54.8 ± 12.7	50.1 ± 12.6	6.65 ± 1.04	0.81 ± 0.13
High	11.8 ± 7.0	5.36 ± 3.56	6.62 ± 3.69	6.8 ± 3.43

K_s values are 54.8 μM in the low pH series, and 11.8 in the high pH series. Considering the calculated $SiO(OH)_3^-$ concentration as the substrate the estimated K_s values are 6.65 and 6.62 μM for the high and low pH series, respectively. The uptake rates versus the calculated concentration of $SiO(OH)_3^-$ are shown in Fig. 1B. These results are also shown as Hanes-Woolf plots (Fig. 1C), which linearize the Michaelis-Menten expression and set the x intercept equal to $-K_s$, the y intercept equal to K_s/ρ_{max}, and the slope equal to ρ_{max}. As further evidence of the relationship between the K_s and the concentration of $SiO(OH)_3^-$ we have estimated the K_s values for Si uptake using the calculated concentrations of four possible substrates, total dissolved Si, undissociated $Si(OH)_4$, $SiO(OH)_3^-$ and $SiO_2(OH)_2^{2-}$ (Table 1). Only for $SiO(OH)_3^-$ is there agreement of the K_s values for the two pH ranges. Dependence of enzyme kinetics on the acid/base chemistry of the substrate is well known (Segel 1976).

The near equality of K_s for two different pH values only when $SiO(OH)_3^-$ is considered the substrate could be interpreted to suggest that $SiO(OH)_3^-$ rather than $Si(OH)_4$, is the substrate that binds to the Si-transport protein. However, on closer examination a number of mechanisms not requiring initial binding of $SiO(OH)_3^-$ can be constructed which predict the same experimental result. For example, when analyzed analogously to the original Michaelis-Menten derivation, the reactions:

$$\underset{\text{(external)}}{E + Si(OH)_4} \overset{k_1}{\underset{k_2}{\rightleftharpoons}} E\text{-}SiO(OH)_3^- + H^+ \overset{k_3}{\rightarrow} \underset{\text{(internal)}}{E + Si(OH)_4} \quad (4)$$

(where E represents a Si transport protein unaffected by pH over the range of consideration, E-$SiO(OH)_3^-$ the transport protein-substrate complex, and k_1, k_2 and k_3 the rate constants for the individual reactions), can be shown to follow the rate law:

$$\rho = \frac{\rho_{max}[Si(OH)_4]}{K_m[H^+] + [Si(OH)_4]} \quad (5)$$

in which:

$$K_m = \frac{k_2 + k_3}{k_1} \quad (6)$$

K_m is a dimensionless quantity analogous to the Michaelis-Menten constant in the more simple Michaelis-Menten derivation, and is by definition independent of pH. The product ($K_m[H^+]$) has units of concentration and comparison of equations 3 and 5 show that this product is equal to the experimentally obtained half-saturation constant (K_s) when undissociated silicic acid is considered as the substrate. Thus, the observed K_s must decrease with increasing pH. However, using the equilibrium relation between $Si(OH)_4$, $SiO(OH)_3^-$ and H^+ (eq. 1), this rate law can be rearranged to:

$$\rho = \frac{\rho_{max}[SiO(OH)_3^-]}{10^{-9.47} K_m + [SiO(OH)_3^-]} \quad (7)$$

which has a half-saturation concentration for $SiO(OH)_3^-$ that does not vary with pH.

Si uptake mechanisms that predict constant K_s with varying pH when $SiO(OH)_3^-$ is considered the substrate can be constructed using any species of dissolved Si as the reacting substrate. However, they all have in common the formation of an E-$SiO(OH)_3^-$ complex. Thus, $SiO(OH)_3^-$ is not necessarily the species in solution that reacts with the Si transport protein, but our results indicate it may be the species transported across the cell membrane. Recent models for the observed dependence of metal uptake by phytoplankton on free ion activity also invoke mechanisms in which, through ligand exchange, various organic and inorganic species of metals may actually react with the metal binding site, but only the metal ion is actually bound and/or transported (Anderson and Morel 1982). The advantage of such a mechanism to an organism is that it allows an uptake system to operate on substrates at far higher concentrations than would be the case if the mechanism used the apparent substrate.

The hypothesis that $SiO(OH)_3^-$ is the true substrate for Si uptake depends on the assumption that the K_s of the Si-transport protein remains constant over the pH range 8.8–9.7. While changing pH can change the K_s of an enzyme by altering the active site or the tertiary structure, it is by no means certain over any particular pH range. For example, the digestive enzyme chymotrypsin exhibits a constant K_s for the substrate acetyl-L tryptophane amide over the pH range 6.6 to 8.0, while the V_{max} increases with pH due to noncompetitive inhibition by H^+ (Bernhard 1968). In our case, the observed fit between the change in the K_s with pH and the dissociation of silicic acid could arise from coincidental changes in the K_s with pH. However, we believe the probability of such a fortuitous match to be far smaller than the probability of the K_s for the true substrate remaining constant over a 1 pH unit interval.

The ρ_{max} for Si uptake is somewhat higher for the low pH series. The observed decrease in ρ_{max} at the higher pH could be interpreted in terms of a noncompetitive inhibition by hydroxyl ion, once it is assumed that the K_s for the true substrate ($SiO(OH)_3^-$) is constant over the pH range (Segel 1976). However, *Phaeodactylum tricornutum* responds to elevated pH with reduced growth (Hayward 1968), so the observed reduction in the maximum Si uptake rate may simply be the result of a lower rate imposed on all assimilatory pathways at high pH.

Phaeodactylum tricornutum is a diatom, albeit a very unusual one in that it does not require Si for growth, and its Si uptake system may reasonably be suspected to be evolutionarily related to that of Si-requiring diatoms. This would imply that Si-requiring diatoms may also show dependence of Si uptake parameters on pH. Azam et al. (1974) and Bhattacharyya and Volcani (1980) have shown that the rate of silicon uptake by *Nitzschia alba* at fixed total Si concentrations increases with increasing pH over the pH range 4–9. Although kinetic parameters in their experiments cannot be determined at different pH values due to the experimental design, these results are consistent with a decrease of K_s for $Si(OH)_4$ or total dissolved Si with pH as in our experiments.

In the surface waters of the open ocean the extreme pH range is between 7.5–8.5 (Parsons and Takahashi 1973), while the total Si content of surface seawater is often close to the measured K_s for Si uptake for marine diatoms (Bainbridge 1980, Nelson et al. 1981). If the Si uptake systems of Si-requiring diatoms have K_s values that vary with pH as does that of *Phaeodactylum tricornutum*, the variations of pH alone could have significant impact on the uptake rate of Si and the growth rate of diatoms in the oceans. In freshwater, where pH ranges are greater, the pH dependence of Si uptake kinetics could have even more profound effects. If the K_s of Si uptake is proven to vary with pH for diatoms in general, this variable must be included in future studies of the Si uptake parameters of diatom populations in both the laboratory and the field.

The authors thank Dr. J. R. Lara-Lara and R. Millan-Nunez for their help in earlier phases of this study, M. Brzezinski for the nonlinear kinetic curve fitting, and Drs. R. R. L. Guillard, F. M. M. Morel and P. A. Wheeler for valuable discussions during manuscript preparation. HBF contribution no. 434.

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J. Phycol. **21**, 171–175 (1985)

NOTE

CHROOCOCCOID CYANOBACTERIA IN LAKE ONTARIO: VERTICAL AND SEASONAL DISTRIBUTIONS DURING 1982[1]

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ABSTRACT

Chroococcoid cyanobacteria (0.7–1.3 μm in diameter) were discovered to be a significant component of the Lake Ontario plankton. Using epifluorescence microscopy, the densities of these microorganisms were found to vary by four orders of magnitude with a single large peak in abundance (6.5×10^5 cells mL^{-1}) corresponding to the time of maximum water temperature. The morphology and abundance of these cyanobacteria were similar to those previously found in oceanic systems. They constituted 10% of the bacterial numbers in the epilimnion during this period, approximately 40% of the biomass of prokaryotes less than 2.0 μm, and 30% of the biomass of all microorganisms less than 20 μm in size. Size fractionation studies indicated that they were responsible for approximately 38% of the total primary production during times of peak abundance, and were important in phosphorus uptake. Cyanobacteria observed in the food vacuoles of heterotrophic microflagellates and in the guts of rotifers suggest that the latter organisms may be important consumers of this prokaryote population.

Key index words: Lake Ontario plankton; cyanobacteria; microbial abundance

Chroococcoid cyanobacteria (0.5–2.0 μm) are photosynthetic, prokaryotic microorganisms that have only recently been shown to be an important component of oceanic plankton communities (Johnson and Sieburth 1979, Waterbury et al. 1979, Krempin and Sullivan 1981). They were overlooked in earlier studies because the conventional Utermöhl settling technique for counting phytoplankton

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