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Molybdenum assimilation by cyanobacteria and phytoplankton in freshwater and salt water

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Abstract

We measured the uptake rate of molybdate and related kinetic parameters for nine taxa of cyanobacteria and for the natural phytoplankton communities of six freshwater lakes containing planktonic N_2-fixing cyanobacteria. Molybdate uptake followed saturation kinetics and was competitively inhibited by both tungstate and sulfate. Tungstate inhibited molybdate uptake in a nearly mole-for-mole fashion; inhibition constants (K_i) were in the same concentration range (10–30 nM) as the half-saturation constants for molybdate uptake (K_m). Sulfate also inhibited molybdate uptake, but this inhibition was much less specific. The K_i for sulfate was in the mM range—three orders of magnitude above the K_m for molybdate uptake. Despite these high K_i values, however, sulfate can be an important inhibitor of molybdate uptake in many natural waters as sulfate concentrations are usually some 4–6 orders of magnitude greater than molybdate concentrations. At ambient sulfate and molybdate concentrations in most freshwater lakes, molybdate uptake would be inhibited by 1–5% due to sulfate. In marine and estuarine systems this inhibition would be 15–20% and in some saline lakes could be >70%.

Molybdenum is one of the essential cofactors for the vast majority of known N_2-fixation systems and many nitrate reductase systems (Fogg and Wolfe 1954; Manheim and Landergren, 1978; Robson et al. 1986). The function of Mo in both nitrogenase and nitrate reductase, as well as in several other enzyme systems such as formate dehydrogenase, is that it assists in the catalysis of the transfer of two, or a multiple of two, electrons to or from the substrate (Pope et al. 1980). The Mo requirements for nitrogenase activity exceed those for nitrate reductase (Fogg and Wolfe 1954).

In oxic natural waters Mo is thought to be present primarily as the oxyanion, MoO_4^{2-}, and its concentration ranges from ~1 to ~100 nM in most environments (Howarth et al. 1988*b*; Marino et al. 1990). For an organism to synthesize an active nitrogenase enzyme, it must first be able to transport MoO_4^{2-} from the environment at the ambient concentration and must be able to do so in the presence of potential inhibitors or competitors of this transport which are also present in natural waters. One potential competitive inhibitor to MoO_4^{2-} uptake is SO_4^{2-}. Sulfate resembles closely molybdate in terms of size, charge, and stereochemistry and is present in natural environments at concentrations some 4–6 orders of magnitude greater than molybdate.

Howarth and Cole (1985) proposed a two-part hypothesis concerning the interaction between sulfate and molybdate and its ecological significance. First, SO_4^{2-} is a competitive inhibitor of MoO_4^{2-} transport that lowers significantly the uptake of MoO_4^{2-} at ambient SO_4^{2-} and MoO_4^{2-} concentrations. Second, this interaction lowers the availability of Mo to N_2-fixing organisms and may limit the rate of N_2 fixation in environments such as coastal waters, where the $SO_4^{2-} : MoO_4^{2-}$ ratio is large. Thus, this interaction between molybdate and sulfate could be one of the factors (e.g. Paerl et al. 1987; Paulsen et al. 1992; Vitousek and Howarth 1991) contributing to the switch in phytoplankton nutrient limitation from P to N along salinity gradients (Caraco et al. 1987; Howarth et al. 1988*b*). The present paper addresses only the first part of the hypothesis. For the second part, we refer the reader else-

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Table 1. Freshwater lakes used to measure molybdate uptake.

Lake	Location	Sulfate (mM)	Molybdate (nM)	Chl (μg liter^{-1})	N_2 fixation (nmol ethylene liter^{-1} h^{-1})
Chodikee	41°45'N, 73°55'W	0.03	0.94	60	>100
Mirror	43°57'N, 71°42'W	0.04	0.05	1	0
Copake	42°10'N, 73°30'W	0.08	3.75	40	10–100
Taghkanic	42°07'N, 73°35'W	0.10	1.98	7	10–100
Stissing	41°50'N, 73°37'W	0.24	15.6	12	0
Oneida	43°00'N, 75°52'W	0.46	4.17	17	1–10

where (e.g. Paerl et al. 1987; Howarth et al. 1988*b*; Marino et al. 1990; Vitousek and Howarth 1991; Paulsen et al. 1992).

In previous work dealing with the first part of our hypothesis, we reported direct measurements of the kinetics of Mo uptake for only a single freshwater lake (Howarth and Cole 1985; Cole et al. 1986). Here we investigate the kinetics of Mo transport and its inhibition by sulfate and tungstate (a better known competitive inhibitor of molybdate transport in bacteria) for various cyanobacterial cultures and natural phytoplankton communities from a series of freshwater lakes, estuaries, and marine systems. We revise, substantially, our prior estimates of the kinetic parameters, V_{max}, K_m, and K_i by working with ^{99}Mo of higher specific activity, allowing us to make measurements in the ambient concentration range.

Methods

Field sampling procedure—For experiments with the natural plankton communities of lakes, we collected 20 liters of water in an acid-washed polycarbonate container with a peristaltic pump. Sampled this way, the water contacted no metallic materials. Samples were generally collected late in the day and held in the incubator overnight at ±2°C of ambient temperature with a 12 : 12 L/D cycle before beginning the experiments. The lakes sampled, their locations, and some of their characteristics are shown in Table 1.

Dissolved Mo analysis—We operationally define dissolved Mo as the total Mo that passed a Whatman GF/F filter. Concentrations of dissolved Mo were measured by graphite-furnace atomic absorption spectrophotometry (Marino et al. 1990). Dissolved Mo concentrations were measured both to determine the actual specific activity of purchased [^{99}Mo]Na_2MoO_4 and the ambient levels in the natural systems. For natural systems molybdate was concentrated, if necessary, by coprecipitation with hydrated Mn oxide or by evaporation in a closed system (Marino et al. 1990); stock solutions were run without preconcentration.

Algal cultures—We cultured cyanobacteria taxa originating in freshwater and salt water and kept them in media to which no combined nitrogen was added and which had low initial concentrations of sulfate (10–30 μM) and molybdate (~0.1–1 nM). Although planktonic marine cyanobacteria that fix nitrogen are rarely encountered in most environments (Paerl 1988; Howarth 1988; Howarth et al. 1988*a*), we obtained cultures of benthic and planktonic marine cyanobacteria from various sources (Table 2). Because individual taxa had different requirements for growth, we used several different media (Table 2). We modified media from Gorham-2 (Gorham et al. 1964), K&M (Kratz and Myers 1955), and F/2 (Guillard 1975). In all cases the modifications were designed to delete all combined nitrogen sources, reduce Mo in the trace metal solutions so that estimated final Mo concentrations were <1 nM, and replace SO_4^{2-} salts with Cl^- salts to maintain SO_4^{2-} at <30 μM. Most taxa were fixing nitrogen during the experiments.

Algal biomass and nitrogenase activity—Chlorophyll concentrations were measured fluorometrically in basic methanol extracts (Holm-Hansen and Riemann 1978) with a Turner Designs fluorometer calibrated with both purified chlorophyll (Sigma Chemical Co.) and samples measured spectrophotometrically (in acetone). Particulate C and N concentrations were measured on filtered samples (Gelman A/E) with a Carlo-Erba CNS analyzer.

The presence of nitrogenase activity in the cultures and water samples was determined by acetylene reduction (Stewart et al. 1967).

Table 2. Algal cultures used in the Mo uptake experiments. The cultures had various origins. We obtained *Anabaena oryzae* (A15), *Nodularia harveyana* (NODH2), and *Oscillatoria woronichinii* (CSIRO-52) from the Australian Collection for Marine Microorganisms and *Anabaena cylindrica* (UTEX B1611) from the collection at the University of Texas. E. Graneli provided *Nodularia spumigena* from the Baltic. *Nodularia* sp. came from a mixed culture from Pyramid Lake provided by D. Galat. We isolated *Calothrix* sp. and *Phormidium* sp. from Narragansett Bay. *Trichodesmium* sp. (NIBB-1067) was isolated from seawater by K. Ohki and colleagues (*see* Ohki et al. 1986).

Taxon	Media*	N_2 fixation (nmol ethylene liter^{-1} h^{-1})	Chl (μg liter^{-1})
O. woronichinii (brackish/coastal)	G-2; 27‰	1–10	85
Calothrix sp. (coastal marine)	G-2; 27‰	1–10	60–90
Phormidium sp. (coastal marine)	G-2; 27‰	10–100	164
A. oryzae (estuarine)	G-2 + 0.1% SW	>100	88–177
N. spumigena (Baltic)	K&M; 12‰	>100	44–69
N. harveyana (Harvey-Peel estuary)	K&M; 12‰	10–100	42–50
Nodularia sp. (Pyramid Lake)	G-2; 10‰	0	172
A. cylindrica (freshwaters)	G-2; 0‰	>100	21–190
Trichodesmium sp. (Izu peninsula, Japan)	Mod. F/2; 30‰	0	119

* G-2 is modified from Gorham-2 after Gorham et al. 1964; K&M is modified from Kratz and Myers (1955); Mod. F/2 is modified from Guillard (1975). Modifications are given in text. In the sole case of *A. oryzae* it was necessary to add 0.1% of Sargasso seawater to maintain growth.

Mo uptake kinetics—We examined the rate of incorporation of ^{99}[Mo]Na_2MoO_4 into particulate material for a range of natural plankton communities and for a variety of freshwater and salt-water cyanobacteria. The methods largely followed those of Cole et al. (1986) except that we used ^{99}Mo at extremely high specific activity (>1,000 mCi g^{-1} Mo) and extremely high purity (special order from New England Nuclear), which allowed us to measure uptake accurately at naturally occurring molybdate concentrations.

Stock solutions of radioactive molybdate were diluted upon receipt with distilled-deionized water that had been autoclaved, and the diluted stock was filtered twice through 0.22-μm pore-size Millipore filters to remove particulate contaminants. To measure the kinetic parameters for molybdate assimilation, we incubated samples (150–240 ml) in 240-ml polycarbonate flasks with a range of Na_2MoO_4 concentrations, usually from tracer amounts to ~200 nM (about twice the concentration in seawater) at eight concentration increments. Incubations were done in an incubator at 20°C for the cultures and within 2°C of ambient temperature for the lake samples. We used "cool-white" fluorescent lighting at 300–400 μEinst m^{-2} s^{-1}, and the samples were agitated continuously on a shaker table at 30–60 rpm. Incubation time ranged from 1 to 10 h. Incubations were stopped by adding 2 ml of 4 M nonradioactive Na_2MoO_4 to give a final concentration of 30–50 mM (~10^5 times the concentration of the highest assayed molybdate concentration) depending on the volume of the incubation.

Immediately thereafter, the samples were filtered through 25-mm Gelman A/E filters that had been pretreated by soaking in 1 μM Na_2MoO_4 to minimize sorption problems (Cole et al. 1986). The amount of radioactivity on each filter was measured by liquid scintillation counting with Scintiverse-II and a Beckman LS-1801 scintillation counter. Quenching was determined by H number. We waited 40 h after the end of the incubation so that excess ^{99}Tc would decay before scintillation counting (Elliott and Mortensen 1975); all samples were counted twice (about one half-life apart, 67 h) to ensure that the decay rate was identical to that for ^{99}Mo.

To measure kinetic inhibition by either sulfate or tungstate, we tested the eight molybdate concentrations (above) against three or six concentrations of Na_2SO_4 or Na_2WO_4 to create a matrix of 24 or 48 pairs of substrate and inhibitor concentrations for each inhibitor. For sulfate, the concentrations ranged from low amounts (~1 μM) to values typical for dilute freshwaters (~30 μM) and up to near seawater values (27 mM). For tungstate, we varied concentrations from zero to ~200 nM.

Several types of controls were run for the experiments. For each experiment, as an abiotic control, we heat-killed (80°C, 2 h) samples and incubated them as above. For most experiments we also incubated samples in the

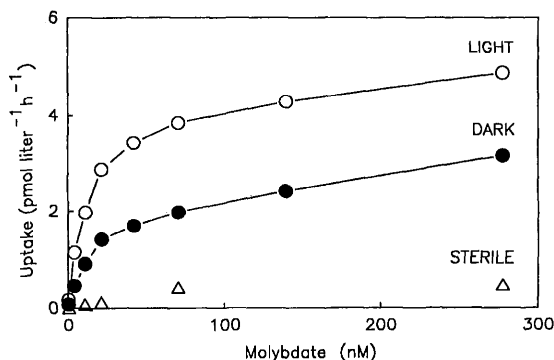

Fig. 1. Uptake of molybdate as a function of molybdate concentration for *Anabaena cylindrica*, a freshwater N_2-fixing cyanobacterium growing in G-2 media. The culture was kept at exponential growth at a Chl *a* concentration of 35 μg liter^{-1} and was fixing nitrogen (360 nmol ethylene liter^{-1} h^{-1}). Treatments: light—○; dark—●; after heat killing—▲. For the experiment in the light, K_m was 12.3 nM and V_{max} was 4.85 pmol liter^{-1} h^{-1} or 0.14 pmol (μg Chl *a*)$^{-1}$ h^{-1}.

dark to retard growth. Finally, to determine whether either the substrate or inhibitors were affecting growth rate during the incubation and to determine the effect of darkness on growth, we measured the rate of $NaH^{14}CO_3$ incorporation for each experiment at a range of substrate and inhibitor concentrations.

Calculation of kinetic parameters—The uptake of a substrate, if it follows saturation kinetics can be described by the Michaelis-Menten equation:

$$v = V_{max} \times S/(K_m + S) \quad (1)$$

where v is the uptake rate, V_{max} the uptake rate at saturating concentrations of the substrate (S), and K_m the half-saturation constant. In the absence of known inhibitors, we calculated V_{max} and K_m by linearizing the data according to the familiar Lineweaver-Burke double-reciprocal method (Rudolph and Fromm 1983) and performing linear regressions (*but see* the objections to this approach by Currie 1982).

An inhibitor for the above reaction, if it is competitive, has no effect on V_{max}, but would affect Eq. 1 as follows:

$$v = V_{max} \times S/[K_m \times (1 + I/K_i) + S] \quad (2)$$

where I is the concentration of the inhibitor and K_i the constant of inhibition. In experiments in which we used one or two concentrations of the inhibitor, the same double-reciprocal plotting procedure could be adopted and K_i could be calculated from the change in slope of the double-reciprocal plot. In experiments in which we used multiple levels of inhibitor, we obtained initial estimates of V_{max} and K_m from the no-inhibitor treatment and then by means of a modified Marquardt procedure (Statistical Graphics Corp. 1987) computed the K_i, K_m, an V_{max} that best fit the data given the initial constraints on K_m and V_{max}.

The kinetic parameters we report for the natural systems are apparent averages for the entire algal community because we do not know which organisms accomplished the uptake. Nevertheless, the parameters would adequately describe uptake in those systems.

Results

Molybdate uptake and controls—In all of the experiments we performed with cyanobacteria, the uptake of molybdate was a hyperbolic function of the molybdate concentration that could be readily described by the familiar Michaelis-Menten model of saturation kinetics (Fig. 1). In two experiments, both with marine diatoms, variance made application of any model impossible and we do not consider these experiments further here. In every experiment we performed, the uptake of molybdate was reduced to negligible levels in heat-killed cultures, indicating that uptake required living cells and suggesting active mediation. Uptake was also reduced (by 50% or more) for cells incubated in the dark (Fig. 1).

For the 27 lakes and cultures we investigated, the kinetic parameters varied considerably and independently. K_m varied from 0.7 to 199 nM and averaged 28±7.6. V_{max} varied from 0.008 to 1.4 pmol Mo (μg Chl *a*)$^{-1}$ h^{-1}; it averaged 0.26±0.06. Thus, although the total range was large for both K_m and V_{max}, each had a relatively low C.V. across organisms and environments.

Although the variation in V_{max} did not appear systematic, some of the variability in K_m could be explained by the environmental concentration of Mo from which the sample was drawn (Fig. 2). The algal communities from freshwater lakes or cultures from freshwaters (relatively low Mo environments) generally had

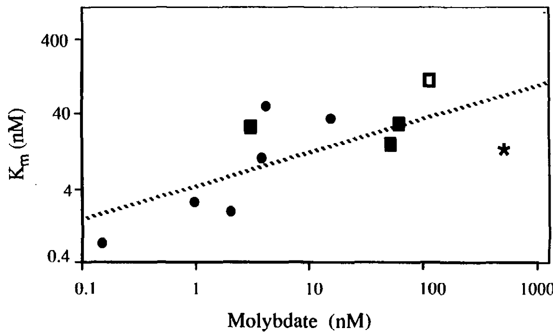

Fig. 2. Affinity constants (K_m) for systems for which we have data. Freshwater lakes—●; cultures from freshwater, estuarine, and marine systems—■. Note: the culture from Pyramid Lake (*) was plotted at the molybdate concentration reported for that lake by Howarth et al. (1988*b*) but is based on a single sample and may be in error. □—The benthic cyanobacterium, *Calothrix*. Equation for line is $\log K_m = 0.415 \times \log[MoO_4^{2-}] + 0.68$; $R^2 = 0.43$.

a lower K_m (higher affinity for Mo) than did marine or estuarine cultures. There was a significant positive relationship between $\log K_m$ and log[Mo] (Fig. 2; $r^2 = 0.43$; $P < 0.05$). To obtain this relationship for the freshwater lakes we used the measured Mo concentration; for the cultures we used the Mo concentration from the system in which the culture originated (when it was known); for the isolates of unknown freshwater, estuarine, or marine origin, we used the average Mo concentration for those environments. This relationship is curvilinear (on an arithmetic scale) so that K_m does not increase as rapidly as does the concentration of Mo.

Inhibition by tungstate—Tungstate inhibited the rate of molybdate transport in every experiment (Figs. 3, 4). The inhibition could be very adequately described by conventional competitive inhibition models. For example, Lineweaver-Burke plots of the kinetic data revealed that tungstate increased the apparent K_m but had little or no effect on V_{max} (Figs. 3, 4). Photosynthesis was not affected by the addition of tungstate in this range (0–200 nM), so the results strongly support the hypothesis that tungstate is a competitive inhibitor of molybdate transport and that the transport mechanism does not discriminate well between tungstate and molybdate. In our 12 experiments, the K_i for tungstate (constant of inhibition) ranged from 2 to 200 nM and averaged

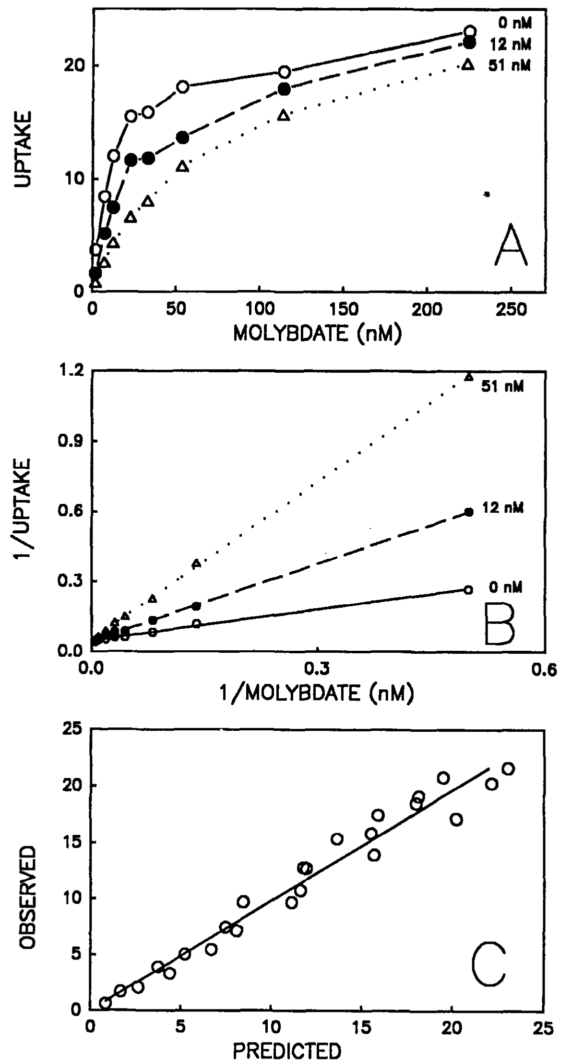

Fig. 3. Uptake of molybdate as a function of molybdate concentration for an estuarine cyanobacterium, *Anabaena oryzae*, in the presence of three levels of sodium tungstate. *A. oryzae* was maintained in G-2 media plus 0.1% seawater; salinity was adjusted to 6‰ with NaCl. The culture was kept at a chlorophyll concentration of 34 μg liter^{-1} and was fixing nitrogen (93 nmol ethylene liter^{-1} h^{-1}). A. Uptake rate (pmol Mo liter^{-1} h^{-1}) as a function of molybdate concentration. B. Lineweaver-Burke plot of the data from panel A. C. Predicted uptake of Mo (using the kinetic constants obtained in panel B) plotted against the observed rate for all three tungstate concentrations simultaneously. Best-fit kinetic parameters are $K_m = 9.5$ nM MoO_4^{2-}, $V_{max} = 22.5$ pmol liter^{-1} h^{-1}, and K_i-W = 8 nM WO_4^{2-}.

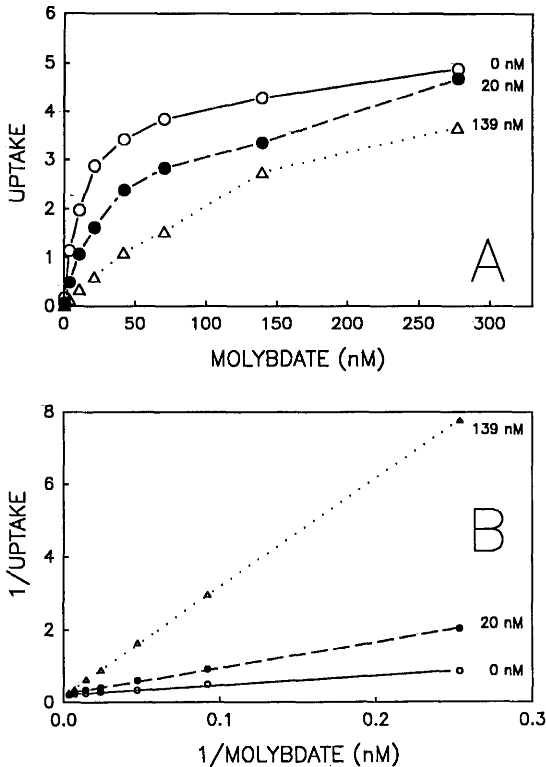

Fig. 4. Uptake of molybdate as a function of molybdate concentration for a freshwater cyanobacterium, *Anabaena cylindrica,* cultured as in Fig. 1, in the presence of three levels of sodium tungstate. A. Uptake rate (pmol Mo $liter^{-1}$ h^{-1}) as a function of molybdate concentration. B. Lineweaver-Burke plot of data in panel A.

44 ± 17. Thus, the range of K_i for tungstate was essentially the same as the range of K_m for molybdate transport. The K_i for tungstate was broadly correlated with K_m ($P < 0.01$) and a linear regression of K_i against K_m had a slope of 0.6 nm nm^{-1} ($r^2 = 0.89$, $P < 0.01$), suggesting that the uptake system has similar affinity for tungstate and molybdate.

Inhibition by sulfate—As in the case of tungstate, the inhibition of molybdate uptake by sulfate could also be adequately described by competitive inhibition models. Again, short-term photosynthesis was not greatly affected by sulfate additions over the range of interest (0.03–27 mM). Unlike tungstate, sulfate was a poor inhibitor of molybdate uptake in the nM or μM range. In all cases, we did not measure significant inhibition until sulfate reached the mM range (Figs. 5, 6). The K_i for sulfate (K_i-S) ranged from 1.2 to 65 mM for the 19 experiments we performed and averaged 10 ± 3.

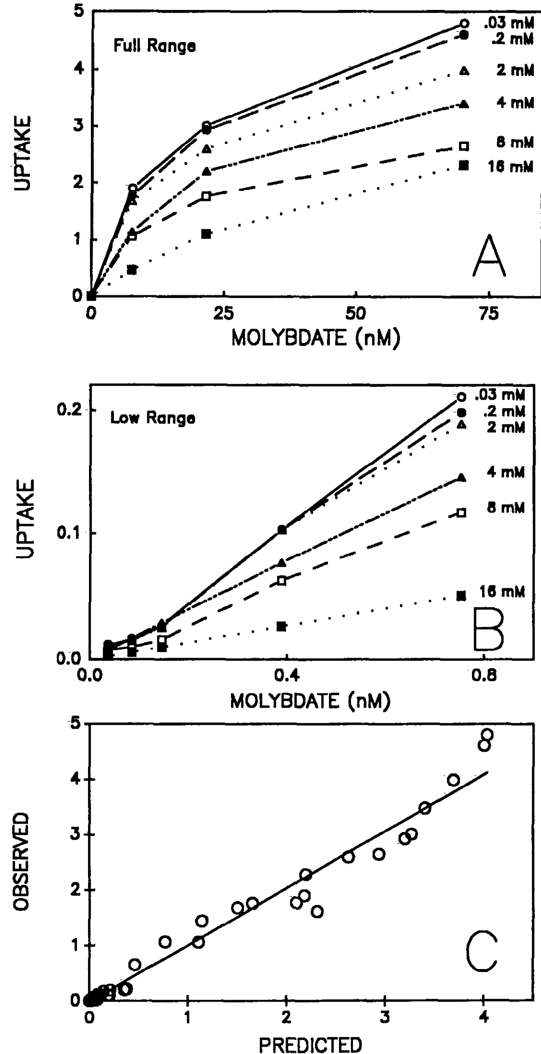

Fig. 5. Uptake of molybdate (pmol Mo $liter^{-1}$ h^{-1}) as a function of molybdate concentration for *Anabaena cylindrica* (conditions given in Fig. 1) at six levels of sodium sulfate. In this experiment chlorophyll was 21 μg $liter^{-1}$ and the N_2-fixation rate was 100 nmol ethylene $liter^{-1}$ h^{-1}. A. Full range of molybdate concentrations tested (0–70 nM). B. Low range of molybdate concentrations (0.01–0.7 nM). C. Predicted molybdate uptake rate plotted against the observed rate for all six sulfate levels. Best-fit kinetic parameters are K_m = 8 nM MoO_4^{2-}, V_{max} = 4.5 pmol $liter^{-1}$ h^{-1}, and K_i-S = 2.2 mM SO_4^{2-}.

Although the K_i-S was broadly related to K_m (linear regression; $r^2 = 0.86$; $P < 0.0001$), the slope was far from unity (5×10^5 nm nm^{-1}). These results imply that the uptake system exhibits a high degree of discrimination between sulfate and molybdate. Unlike the case for tungstate, the uptake system has several orders

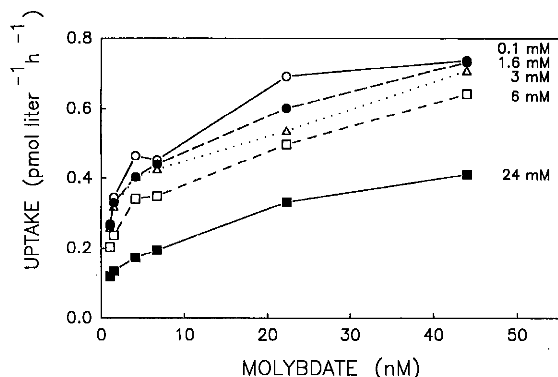

Fig. 6. Uptake of molybdate as a function of molybdate concentration for the natural phytoplankton community of eutrophic Chodikee Lake in the presence of five levels of added sodium sulfate. Best-fit kinetic parameters are $K_m = 1.75$ nM, $V_{max} = 0.5$ pmol liter^{-1} h^{-1}, and $K_i =$ 2 mM SO_4^{2-}. The chlorophyll concentration was 11.3 μg liter^{-1} and the system was fixing nitrogen (28 nmol ethylene liter^{-1} h^{-1}).

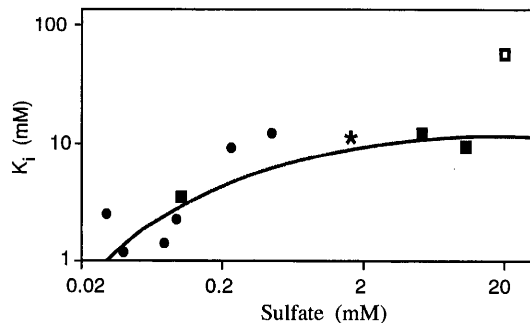

Fig. 7. Inhibition constants (K_i-S) for inhibition of the uptake of molybdate by sulfate. Freshwater lakes—●; freshwater, estuarine, and marine cultures—■. □—The benthic cyanobacterium, *Calothrix*; *—the culture from Pyramid Lake (*see cautionary note in Fig. 2*). Equation for the line is $K_i = 12 \times [SO_4^{2-}]/[0.3 + SO_4^{2-}]$. When *Calotrhix* is included, $r^2 = 0.72$; when excluded, $r^2 = 0.77$.

of magnitude greater affinity for molybdate than for sulfate.

Much of the variation in K_i-S could be explained by the sulfate concentration of the environment from which the sample was drawn (Fig. 7). A regression of log K_i-S against log sulfate for the different systems (as in the case of Fig. 2) is highly significant and explains 78% of the variation in log K_i-S ($P < 0.001$). There was good correspondence between K_i-S and the environmental sulfate concentration, but the relationship is strongly curvilinear. As sulfate increases, the ratio of sulfate to K_i increases. This ratio ($I:K_i$) is the critical term in the equation for inhibition (Eq. 2) and as this ratio increases, the degree of inhibition increases. For low sulfate systems $I:K_i$ is negligible, ~0.02; in seawater the ratio approaches 2 so that the term $(1 + I/K_i)$ from Eq. 2 roughly triples. The effect of this tripling on the uptake velocity is equivalent to tripling K_m (e.g. dividing the affinity for molybdate by 3).

Biological turnover time of molybdate—Using the measured values of V_{max}, K_m, K_i, $[MoO_4^{2-}]$, and $[SO_4^{2-}]$ we can calculate both ambient velocities of molybdate uptake (v) and a biological turnover time for molybdate. Rates of ambient specific v ranged from ~0.01 to 0.2 pmol Mo (μg Chl a)$^{-1}$ h^{-1} in the various environments, and low rates can be caused by either low concentrations of MoO_4^{2-} or high concentrations of SO_4^{2-}. In general v was well below V_{max} in most of the environments. The one exception is the *Nodularia* culture from Pyramid Lake. Our limited data suggest that Pyramid Lake has exceedingly high molybdate concentrations (Table 2) and only moderate sulfate concentrations (Howarth et al. 1988*b*), allowing V_{max} to be approached.

The biological turnover time (molybdate concentration divided by the uptake rate) is the time it would take for the average dissolved molybdate molecule to be assimilated once, in well-illuminated surface waters at an assumed steady state, in the absence of new inputs. This turnover time is generally quite long, averaging 192 d for the freshwater lakes and >3,000 d for marine systems. Within the group of lakes, however, turnover time can be quite variable and occasionally quite brief. For example, in September a cyanobacterial bloom in eutrophic Chodikee Lake, chlorophyll levels reached 70 μg liter^{-1} and N_2 fixation was 145 nmol ethylene liter^{-1} h^{-1}. At this time the calculated turnover time was only 13 d, suggesting the molybdate pool could potentially be depleted were the bloom to last for several weeks.

Discussion

One can question the validity of inferring real-world Mo uptake kinetics from culture studies. Organisms in culture can experience selective pressures often quite different from those in natural ecosystems and may well evolve enzyme systems that are different. Nonetheless, our data suggest that for a wide range of natural phytoplankton communities and phytoplankton cultures, the uptake of Mo

has some common features, all indicative of an enzyme-mediated active transport phenomenon.

Uptake followed Michaelis-Menten saturation kinetics, indicating that the number of transport sites is finite. The rate of uptake is light-dependent, suggesting that energy is necessary for assimilation. An energy requirement is in agreement with the work of Steeg et al. (1986) who found that *Anabaena oscillaroides* concentrated Mo from the environment with accumulation factors of 500 to 3,000. Finally, we find that both tungstate and sulfate appear to be competitive inhibitors of molybdate transport. Although results similar to ours have been reported for heterotrophic bacteria, (e.g. Elliott and Mortensen 1975, 1976), mammalian intestinal loops (Cardin and Mason 1976), and tomato plants (Stout and Meagher 1948), our studies are the first to examine the kinetic properties of molybdate uptake and sulfate inhibition at ambient concentrations for a range of cyanobacteria or natural phytoplankton communities containing cyanobacteria.

There may be striking differences between the transport systems of cyanobacteria reported here and the heterotrophic bacteria previously studied. First of all, researchers working with heterotrophic N_2-fixing bacteria have generally assayed molybdate uptake in the μM concentration range—a range much higher than usually found in natural waters. Such work has identified a molybdate uptake system for *Clostridium* which has K_m near 5,000 nM and K_i values for either tungstate or sulfate in the same range or higher (Elliott and Mortenson 1975). We previously reported, from measurements in a single lake, K_m of ~160 nM—far lower than that for *Clostridium* (Cole et al. 1986). Because we did not have carrier-free molybdate in those experiments, we were adding considerably more molybdate (to 500 nM) than in the present set and overestimating K_m and (V_{max} as well) by as much as 100-fold. Our new measurements for the same lake, Mirror Lake, suggest that the true K_m is probably ~1 or 2 nM at most (Fig. 2). The average K_m for all of the systems we investigated is only ~28 nM (although the range is quite large) and ~15 nM for the freshwater systems. Thus, organisms living in these relatively low molybdate environments have a molybdate transport system that is effective in the environmental concentration range.

An organism equipped with a transport system such as the *Clostridium* studied by Elliott and Mortensen (1975) would be able to transport molybdate at extremely slow rates in most natural waters or sediments, based on the kinetic data they report. In fact, the ecological significance of such low-affinity transport systems is difficult to comprehend because molybdate concentrations rarely exceed 200 nM in most natural waters (Howarth et al. 1988*b*; Marino et al. 1990). Some heterotrophic bacteria may have both a low- and a high-affinity transport system for molybdate. Maier et al. (1987) and Graham and Maier (1987) studied Mo metabolism for *Bradyrhizobium japonicum* and found a high-affinity transport system with a K_m of 11 nM (e.g. comparable to the data we report here) and a low-affinity system with a K_m of 900 nM. Mutants that lacked the high-affinity molybdate transport systems were unable to fix nitrogen at molybdate concentrations as high as 1,000 nM (Maier et al. 1987).

Other studies generally concur that tungstate is a powerful inhibitor of molybdate transport (Shah et al. 1984 and references therein). Our work here with cyanobacteria and natural phytoplankton communities agrees. We found that the K_i for tungstate was in exactly the same range as the K_m for molybdate, indicating nearly mole-for-mole inhibition. In the coastal ocean, reported tungstate concentrations are ~100-fold lower than molybdate concentrations (Pope et al. 1980; Chen and Yang 1984) and in the open ocean 2,000-fold lower (Sohrin et al. 1987), so tungstate would not be a significant inhibitor in seawater. We know of relatively few data on tungstate concentrations in freshwater; it is generally held that levels rarely are >5 nM. Molybdate concentrations, however, are often this low (*see* Howarth et al. 1988*a*), suggesting some potential for inhibition by tungstate, but we know of no direct evidence.

The inhibitory effect of SO_4^{2-} on MoO_4^{2-} uptake is probably more complex than the effect of tungstate. Cells have a substantial requirement for S which can be met by the assimilation of sulfate (Cuhel et al. 1982). Thus the inhibitor also contains an essential element for growth. At low $[SO_4^{2-}]:[MoO_4^{2-}]$ ratios (relative to the need for S and Mo) one might expect no inhibition since cells would need both elements. This dual role of sulfate may explain the increase in K_i as sulfate increases

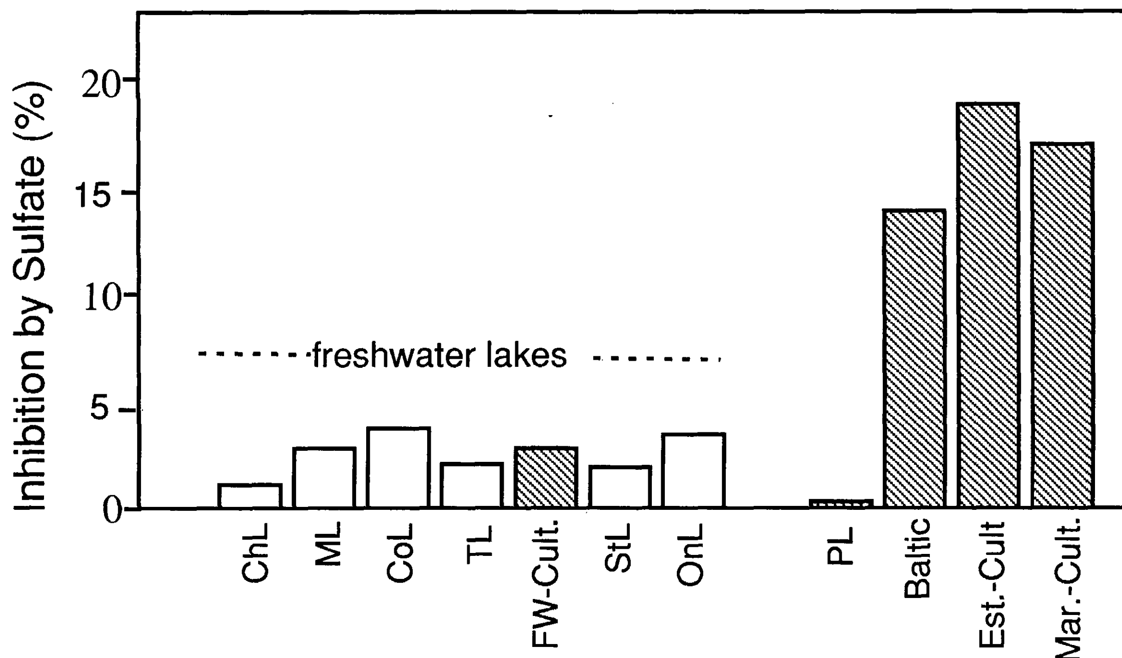

Fig. 8. Inhibition of molybdate uptake velocities at ambient sulfate and molybdate concentrations. We calculated the rate at which molybdate could be assimilated in each system if the system had no sulfate and used this theoretical "no sulfate" rate to compute how much the actual ambient rate was diminished due to inhibition by sulfate. *See cautionary note concerning Pyramid Lake in Fig. 2.* Symbols: ChL—Chodikee Lake; ML—Mirror Lake; CoL—Copake Lake; TL—Taghkanic Lake; FW-Cult.—average of freshwater cultures; StL—Stissing Lake; OnL—Oneida Lake; PL—*Nodularia* from Pyramid Lake; Baltic—*Nodularia* from the Baltic Sea; Est.-Cult.—average of estuarine cultures; Mar.-Cult—average of marine cultures.

from low to intermediate levels and the leveling-off of K_i with further increases in sulfate (e.g. Fig. 7). This duality may lead to complicating physiological adaptations in some cases.

Our results suggest that sulfate will inhibit molybdate transport in cyanobacteria and appears to do so competitively. However, sulfate is not a mole-for-mole inhibitor; cells are able to discriminate between sulfate and molybdate to a large degree. For example, if we use our average values for K_m, K_i, and V_{max} and assume a typical molybdate concentration of 4 nM for freshwaters, we find a sulfate concentration of nearly 8 mM would be required to inhibit molybdate uptake by 50%, which is consistent with our earlier observations and reasoning that sulfate is not a major impediment to Mo uptake in freshwaters (Howarth and Cole 1985; Howarth et al. 1988*a*). Thus at reasonably low molybdate concentrations the ratio (by moles) of $[SO_4^{2-}]:[MoO_4^{2-}]$ causing 50% inhibition would be $\sim 2 \times 10^6:1$. The $[SO_4^{2-}]:[Mo_4^{2-}]$ ratio ranges from 10^4 to 10^6 in natural systems (Marino et al. 1990) and although organisms can discriminate to some extent between molybdate and sulfate, molybdate uptake is, nevertheless, affected.

The kinetic parameters K_m and K_i appear to vary with the molybdate and sulfate concentrations of the systems, and molybdate and sulfate also covary. These factors tend to offset the inhibition by sulfate to some extent, especially at intermediate sulfate concentrations, but do not eliminate it at high sulfate concentrations. To illustrate this point we have plotted the percent inhibition, caused by the ambient sulfate concentration, of the velocity of molybdate uptake at the ambient molybdate concentration (Fig. 8). In this calculation we have taken into account the relationships between K_m and molybdate and K_i and sulfate (Figs. 2 and 7). In freshwater lakes ambient sulfate concentrations inhibit the uptake velocities by only 1–4%. For estuaries and in the ocean, however, molybdate uptake would be inhibited by $\sim$20% compared to what the uptake would be in the absence of sulfate. If our kinetic parameters are extrapolated to saline

lakes with high $[SO_4^{2-}]:[MoO_4^{2-}]$ ratios such as reported by Marino et al. (1990), this inhibition, using the same calculation, could be >70%.

These results support, at least in part, the first part of our original hypothesis (Howarth and Cole 1985): Mo availability is not simply a function of Mo concentration because sulfate can inhibit its assimilation. However, we find the kinetic parameters indicate that this inhibition will be marked only in ecosystems, such as some saline lakes, that have very high $[SO_4^{2-}]:[MoO_4^{2-}]$ ratios; the inhibition would be only moderate in seawater. The second part of our original hypothesis is that low availability of Mo caused by high ratios of $[SO_4^{2-}]:[MoO_4^{-2}]$ in oxic waters can limit the activity of planktonic N_2-fixing organisms. Whether this is correct cannot be inferred from the data presented here alone, since factors other than Mo availability can affect N_2-fixing organisms (Howarth et al. 1988*b*; Vitousek and Howarth 1991; Paulsen et al. 1992). Also, cells may pay an energetic cost for making molybdate-assimilating enzymes that discriminate well between sulfate and molybdate. If this cost is significant it could partially limit rates of N_2 fixation even though the cell-specific rate of molybdate uptake were only slightly affected.

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