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The Possibility of Regulating the Species Composition of Marine Phytoplankton Using Organically Complexed Iron

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Anthropogenic carbon dioxide emissions, derived from the burning of fossil fuels, land-use change and agriculture, are increasing the atmospheric concentration of carbon dioxide.[1] It is predicted that the growth in the carbon dioxide concentration will lead to a dangerous interference with the climate system in the coming centuries. Therefore, reducing carbon dioxide in the atmosphere has become an international priority, and various projects to mitigate the atmospheric emissions and the concomitant enhancement of the atmospheric greenhouse effect are being undertaken throughout the world.[2,3]

One possibility for carbon sequestration is to export it to the ocean's depths and the seabed by phytoplankton through photosynthesis.[4] More photosynthetic production being exported from the ocean surface would result in more carbon being retained in the oceans and less returned to the atmosphere. This process has the potential to reduce the atmospheric concentration of carbon dioxide and have mitigative effects on the global climate. In the last 1980s, Martin and colleagues hypothesized that phytoplankton growth in the major nutrient-rich waters is limited by an inadequate iron supply in several large regions of the world's oceans, where the major plant nutrients remain replete but standing stocks of phytoplankton remain lower than expected.[5-7] They further suggested that fertilizing the ocean with iron may lead to an increased flux of carbon from the surface and a subsequent decrease in atmospheric carbon dioxide. The validity of this hypothesis was demonstrated by a series of iron fertilization experiments in which enhanced iron input increased phytoplankton growth rates and biomass.[8,9] However, some problems remain unresolved. It was reported that the microbial decomposition[10] and the grazing pressure of zooplankton[11] would prevent the efficient export of carbon to the ocean's depths. It is necessary to increase the species of phytoplankton from which large and rapidly sinking particles are made in the oceans.

We thus propose to regulate both the amount of photosynthesis production and the composition of the phytoplankton community using organically complexed iron. For iron incorporation under iron-limited conditions, many microorganisms have a specific strategy in which they release high affinity and low molecular weight ligands called siderophores.[12,13] We have studied several species of marine phytoplankton for the production of iron-complexing ligands and discussed the possibility of regulating the dominant species of phytoplankton in the oceans using iron-siderophore complexes.

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Experimental

Axenic cultures of *Chattonella ovata* (Raphidophyceae), *Chattonella antiqua* (Raphidophyceae), *Gephyrocapsa oceanica* (Haptophyceae), *Oltmannsiellopsis viridis* (Chlorophyceae) and *Rhodomonas ovalis* (Cryptophyceae) and a non-axenic culture of *Pleurochrysis carterae* (Haptophyceae) were used. Before the experiments, the algal cultures were maintained in the same medium for 1 – 2 weeks until cells were at an exponential phase of growth. Experimental cultures were grown at 20°C under a 12:12 h L/D photoperiod at a light intensity of 50 μmol m^{-2} s^{-1} provided by cool white fluorescent lights. Thirty-milliliter capacity acid-washed polycarbonate bottles containing 20 ml of sterilized f/2 medium, modified by reducing the concentration of iron without EDTA, were incubated with acclimated exponential phase cells. Cultures were grown for a period of 1 – 3 weeks.

Samples were taken from each bottle, and filtered through 0.4-μm filters. Immediately after sampling, a 0.5-ml volume of the sample solution was mixed with 0.5 ml of the Chrome Azurol S (CAS) assay solution (CAS, 2×10^{-4} M; $FeCl_3$, 2×10^{-5} M; hexadecyltrimethylammonium bromide (HDTMA), 1.6×10^{-3} M; 1,4-piperazinediethanesulfonic acid (PIPES), 1.0×10^{-1} M; pH 5.8). After 30 min, the absorbance was measured at 655 nm. The concentrations of iron-complexing ligands were calculated from the decrements of the absorbance at 655 nm (ΔA_{655}; Fe(III) $CAS^{3-\lambda}$, $\varepsilon = 105000$ (M^{-1} cm^{-1})).

Phytoplankton growth was measured by fluorometrical measurements on a fluorometer (TD-700, TURNER DESIGNS). The axenic nature was verified frequently by 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) direct straining and examination under an epifluorescent microscope.

Results and Discussion

Table 1 shows the concentrations of iron-complexing ligands released from several species of phytoplankton. We determined the iron-complexing ligands as CAS-positive compounds using an improved Chrome Azurol S (CAS) assay,[14] which was

Table 1 Concentrations of CAS-positive compounds from marine phytoplankton

Species	$[Fe]_0$[a]	Day	[Chl-*a*]	[L]	[L]/[Chl-*a*]
	(μmol/l)	(day)	(μg/l)	(nM)	(mmol/g)
Gephyrocapsa oceanica	0.5	20	22	340	15
	2.0	20	71	630	8.8
	4.0	20	91	630	6.9
Rhodomonas ovalis	2.0	10	95	880	9.3
Oltmannsiellopsis viridis	2.0	10	58	320	5.5
Chattonella antiqua	2.0	10	15	40	2.7
Chattonella ovata	2.0	10	12	20	1.7
Pleurochrysis carterae	2.0	10	35	180	5.1

a. Initial concentrations of iron in the culture media.

modified for application to seawater samples.[15] This method permits the determination of strong ligands that remove Fe(III) from Fe(III)CAS complex:

$$Fe(III)CAS^{3-\lambda} + L^{\kappa-} \longrightarrow Fe(III)L^{3-\kappa} + CAS^{\lambda-},$$

where L represents CAS-positive compounds. The detection limits are approximately 20 nM of CAS-positive compounds, when it is assumed that Fe(III) and L form 1:1 complexes.

In cultures of *Gephyrocapsa oceanica*, the algal growth was limited by the iron concentrations, and the production of CAS-positive compounds was affected by the iron concentration (Table 1). Under low-iron conditions ($[Fe]_0$ = 0.5 μM), the cells of *G. oceanica* released 15 mmol/g of CAS-positive compounds into the medium. Under high-iron conditions ($[Fe]_0$ = 4.0 μM), CAS-positive compounds produced in the medium were 6.9 mmol/g, although the cells grew more than under low-iron conditions. The ability of the other phytoplankton to synthesize CAS-positive compounds was also stimulated under iron-deficient conditions. Many research groups have reported that insufficient iron accelerates the biosynthesis of siderophores by bacteria and fungi.[12,13,16] The CAS-positive compounds observed in our experiments were, therefore, considered to be siderophores from phytoplankton.

The concentration range of CAS-positive compounds in the culture media (Table 1) was up to 880 nM in the culture media. Trick *et al*. have reported the production of 10 – 70 μg NH_2OH l^{-1} hydroxamate siderophores by *Prorocentrum* sp., *Thalassiosira pseudomona* and *Dunaliella tertiolecta*, that were determined by a Csaky test.[17] Boye and van den Berg recently showed that *Emiliania huxleyi* released 6 – 11 nM of iron-complexing ligands other than siderophores, determined by cathodic stripping voltammetry.[18] The iron-complexing ligands observed in our laboratory are consistent with Trick's results with respect to the behavior of the release type and the concentration range.

Most siderophore-mediated iron uptake studies of microorganisms have exhibited a remarkable specificity that membrane located transport systems recognize the molecular structure and conformation of siderophores.[12,13,19] This is associated with the ability of microorganisms to uptake siderophore-mediated iron. Siderophores facilitate iron uptake by the siderophore producer, but limit access to iron by other organisms. The specificity of siderophores for iron uptake systems is expected to lead to a method for selectively controlling the dominant species of phytoplankton in the major nutrient-rich iron-limited regions of the oceans. Moreover, siderophore-iron complexes are superior to inorganic iron as iron donors supplying iron to phytoplankton. On the other hand, some phytoplankton has another strategy for iron acquisitions. A recent study showed that eukaryotic phytoplankton prefers iron bound to porphyrin complexes over other iron complexes.[20] This may suggest that iron-complexing ligands other than siderophores are suitable for the regulation of photosynthesis.

The regulation of species composition in phytoplankton blooms is the key to establish the technical feasibility of carbon sequestration into the oceans through photosynthesis. Such regulation enables carbon dioxide to transform the large, rapidly sinking particles that are formed out of large size plankton, such as diatoms. It is possible to control the ocean pH by regulating the fraction of algal production and to increase the flux of carbon dioxide absorbed into the oceans. These methods reduce the cost of carbon sequestration and the risk of serious negative effects for marine environments. Despite the importance of iron availability to phytoplankton, there are many unanswered questions surrounding the chemistry of organically complexed iron in seawater. Additional observations are needed to thoroughly elucidate the iron transport systems of phytoplankton.

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