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Iron and light limitation of carbohydrate production by phytoplankton in the Southern Ocean van Oijen, Tim

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Summary

The Southern Ocean is a net sink of carbon dioxide (CO₂), nowadays considered as the major greenhouse gas. This apparent sink function is remarkable because the Southern Ocean is a major upwelling area where deep waters with high CO₂ content are brought to the surface. However, these deep waters are also high in essential major nutrients (such as nitrogen and phosphorus) supporting fixation of CO₂ by phytoplankton. Part of the photosynthetically fixed carbon in surface waters is exported to the deep sea by the sinking of phytoplankton cells or aggregates, a mechanism often referred to as the 'biological pump'. This leads to undersaturation of CO₂ in the surface waters and, consequently, uptake of CO₂ from the atmosphere.

However, phytoplankton in the Southern Ocean do not always fully utilise the major nutrients that are available to them. In large parts of this ocean province, high concentrations of these nutrients remain in the surface waters in association with low standing stocks of phytoplankton. Shipboard and *in situ* iron addition experiments have demonstrated that in these regions insufficient iron availability limits phytoplankton growth. Iron is involved in many processes inside phytoplankton cells. It is of crucial importance for photosynthesis, because iron-sulphur clusters facilitate the transport of electrons in the photosynthetic membranes of the chloroplasts. Also, the iron-containing enzyme ferredoxin is needed for the generation of reducing power. Finally, iron is a component of enzymes involved in the nitrogen metabolism.

Next to iron, other factors including temperature and light are known to control phytoplankton productivity in the Southern Ocean. This thesis was written to get a better understanding of the relative impact of iron and light limitation on Antarctic phytoplankton growth. The research focused on the production of storage carbohydrates. These carbohydrates accumulate in phytoplankton cells at high light levels. They are subsequently respired when light availability is low to supply energy and carbon skeletons for continued protein synthesis. Storage carbohydrates are considered to be essential for phytoplankton so they can grow fast in a dynamic light environment. A variable light climate is typical of the Southern Ocean where deep wind-induced mixing is the rule rather than the exception. The short-term dynamics in storage carbohydrates, a major part of which is water-extractable, have hardly been studied for natural pelagic phytoplankton populations so far.

The main questions addressed in this thesis were:

- *Do water-extractable carbohydrates serve as short-term storage products in Antarctic phytoplankton?*
- *What is the effect of iron limitation on the carbohydrate metabolism of Antarctic phytoplankton?*
- *How is this effect influenced by light conditions?*

Various approaches have been used to answer these questions: laboratory culture experiments, shipboard incubation experiments with natural phytoplankton populations, *in situ* measurements, and an *in situ* iron enrichment experiment. The field experiments extended the knowledge of the seasonal and regional variations in the relative role of iron and other factors, especially light, in limiting phytoplankton growth in the Southern Ocean. At the start of the project a method was developed for the measurement of storage polysaccharides. Based on the chemical properties of glucan, the most abundant storage polysaccharide in phytoplankton, a hot-water-extraction of particulate matter was used to separate storage polysaccharides from structural and mucus polysaccharides. The polysaccharide concentration in the extract was analysed using the TPTZ (2,4,6-tripyridyl-s-triazine) method.

Do water-extractable carbohydrates serve as short-term storage products in Antarctic phytoplankton?

The amount of water-extractable polysaccharides exhibited strong day-night variations in cultures of the Antarctic diatom *Chaetoceros brevis* and in natural phytoplankton populations of the Atlantic sector of the Southern Ocean. These findings were consistent with the role of these carbohydrates supplying energy and carbon skeletons for continuous protein synthesis. Additional evidence for this function was provided by ^{14}C incubation experiments with subsequent biochemical fractionation of the incorporated ^{14}C into several pools, including 'glucan' and 'proteins'. Using a model describing the dynamics in the glucan pool, it was calculated that the nocturnal respiration rate of glucan was high, almost two times higher than the diurnal respiration rate. The nocturnal consumption of glucan was found to be accompanied by continued protein synthesis.

The diurnal polysaccharide production decreased with decreasing irradiance, both for *C. brevis* cultures and for natural phytoplankton populations. In accordance, *in situ* there was a decrease in the Chl *a*-normalised polysaccharide concentration with depth. This decrease may also be explained by the respiration of polysaccharides that were accumulated during time spent near the surface. At the Antarctic Polar Front during austral autumn 1999, mixing was not deep relative to the depth of the euphotic zone (z_{cu}). The reserve of carbohydrates was probably only important for a patch of phytoplankton that got trapped in deeper and dimmer waters. At the end of the EisenEx/CARUSO experiment in spring 2000 mixing was much deeper than z_{cu} . In this situation all phytoplankton cells probably needed a carbohydrate reserve to continue growth during the time intervals they spent at depth while being mixed up and down the water column.

How does iron limitation affect the carbohydrate metabolism of Antarctic phytoplankton?

The effect of iron limitation on carbohydrate production was studied in a laboratory experiment performed with *C. brevis* and during the research expeditions. In the laboratory experiments and during the *in situ* fertilisation experiment, the diurnal production of water-extractable carbohydrates, the first products of photosynthesis, was reduced by iron limitation. In case the cells were nitrogen-limited they would accumulate storage polysaccharides. Therefore, the observed changes in the storage carbohydrate metabolism provide evidence that photosynthesis is the primary target of iron starvation. This implies that the diel change in the cellular polysaccharide content, not the average content is an indicator of iron stress.

The photochemical characteristics of iron-stressed phytoplankton cells supported the notion that photosynthesis was the primary target of iron limitation. During the laboratory experiments, low iron concentrations led to a decrease in the amount of light harvesting complexes in cells of *C. brevis*. This was accompanied by a reduced capacity to absorb light and a lower maximal quantum yield of photochemistry in PSII ($F_v:F_m$). In agreement, during the *in situ* iron fertilisation experiment there was an increase in $F_v:F_m$ inside the Fe-fertilised patch. The apparent cross section of photosystem II (σ_{PSII}) decreased in response to the Fe-release, as did the time constant for re-oxidation of the first quinone acceptor of PSII, Q_a .

The nitrogen quota of *C. brevis* cells decreased in response to iron limitation. This observation is in line with those of another research group, who found indications that the nitrogen assimilation of iron-stressed diatoms is limited by the supply of photosynthetically derived reductant for enzymatic reactions. Alternatively, nitrogen assimilation may be restricted by the supply of carbon skeletons from storage carbohydrates because of the low diurnal production of water-extractable polysaccharides in iron-limited cells. The ratio of C:N differed little between iron-deplete and iron-replete *C. brevis* cells. This, combined with the observed decrease in carbohydrate consumption during the dark period in iron-stressed cells, suggests that C- and N-metabolism were still tightly coupled in these cells.

How is the effect of iron limitation on the carbohydrate metabolism of Antarctic phytoplankton influenced by irradiance?

In order to study the combined effects of iron and light limitation, *C. brevis* was cultured at two different photon irradiances, with and without the addition of iron. The production of storage carbohydrates was lowest for cells that were exposed to low irradiance. Iron stress reduced the carbohydrate production even more, which demonstrated that the production was co-limited by iron and light. A stronger impact of iron on carbohydrate production at low irradiance was hypothesised, because at this irradiance more iron is required to extend the photosynthetic apparatus. However, no such interactive effect was evident. Especially at low irradiance, there was an increase in the Chl *a*-specific light absorption in iron-limited cells which counteracted the impact of iron stress on photosynthetic efficiency. The increase in absorption was related to a decrease in cellular pigment content that reduced the package effect (the self-shading of pigments).

As suggested above, a buffer of storage carbohydrates may allow phytoplankton to maintain high growth rates under conditions of deep vertical mixing. Since iron limitation was found to reduce the accumulation rate of storage carbohydrates it may affect their function as a source of energy during times spent in deep, dim, waters. It was also observed that iron-stressed *C. brevis* cells that were cultured at low irradiance, were sensitive to photoinhibition. This may lead to photodamage in phytoplankton during time spent near the ocean surface. The combination of these effects may explain the absence of phytoplankton blooms (especially of diatoms) in areas with low iron concentrations.

Seasonal and regional variations in the role of iron and light in controlling Southern Ocean phytoplankton growth

The fieldwork presented in this thesis was carried out in the Atlantic sector of the Southern Ocean during austral autumn (1999) and spring (2000), which allows a comparison of the role of iron and light limitation during these seasons. While phytoplankton responded to iron fertilisation in spring, there was no response to iron addition during deck incubations of the resident phytoplankton community in autumn. Indeed, ambient iron concentrations were somewhat elevated compared to those measured in spring. In autumn, iron-enriched deep water is transported into the surface layer by entrainment during the deepening of the surface mixed-layer. The low biomass that was encountered in autumn was likely caused by light limitation due to the deep wind mixed layer (>40 m). This was supported by the observation of a strong reduction in carbohydrate production by phytoplankton during incubations at an irradiance corresponding to the one at 20-45 m *in situ* depth.

During the *in situ* iron fertilisation experiment in spring, three weeks after the first iron release, the areal amount (integral between 0-100 m depth) of water-extractable carbohydrates had doubled inside the fertilised patch, while remaining roughly constant in the surrounding waters. Deck incubation experiments revealed that the diurnal chlorophyll *a*-specific production rates of water-extractable polysaccharides were significantly higher for “in-patch” than for “out-patch” samples. Still, deep mixing induced by several severe storms deteriorated the light climate to such extent that light and iron availability were co-limiting the carbohydrate production. The polysaccharide production was light-limited when cells were incubated at irradiance levels corresponding to those at 20-30 m depth *in situ*, whereas at certain times the upper mixed layer was several-fold deeper due to storms. Most of the increase in biomass inside the iron-fertilised patch was associated with the fraction of large (>10 μm) phytoplankton cells, consistent with the shift in the community structure towards larger diatoms. A stronger response for this fraction was expected, since large cells have a higher half-saturation constant for iron uptake because of their low surface/volume. Still, measurements of diel dynamics in cell size, fluorescence and carbohydrate amount during the deck-incubation experiments showed that even small phytoplankton cells responded to iron addition. Yet, there was only a minor increase in cell numbers of small species in response to *in situ* iron fertilisation. It appears likely that their biomass was controlled by microzooplankton grazing. The grazing pressure of mesozooplankton on diatoms does not increase because copepods and other large zooplankton groups have long generation times.

During autumn there was a high spatial variation in phytoplankton abundance and community composition. At the Antarctic Polar Front phytoplankton biomass was relatively high and the community was dominated by diatoms. In contrast, in the surrounding waters of the Antarctic Circumpolar Current biomass was extremely low and small species dominated the community. Iron supply may have been higher at the Antarctic Polar Front than in the surrounding waters because of upwelling of iron-rich water, but the differences in iron concentrations were only minor. Likely, the differences in phytoplankton biomass between the front and the waters south of it are related to a difference in mixing depth. The front is a feature with high vertical stability, while in the Antarctic Circumpolar Current beyond the influence of fronts mixing sometimes was >100 m. Possibly, the dominance of diatoms at the front resulted from differential grazing pressure on small phytoplankton cells and diatoms.

Final remarks

One of the overarching goals of iron fertilisation experiments is to test the Iron Hypothesis, in which glacial-interglacial changes in the atmospheric CO₂ concentration are related to changes in phytoplankton productivity and carbon export to the deep sea, induced by variations in the supply of iron-rich dust. All eight *in situ* iron fertilisations that have been performed in various ocean regions to date resulted in an increase in phytoplankton biomass. In spite of all the effort it is not clear whether this increase results in enhanced carbon export. In fact, no significant export of carbon has been measured after iron fertilisation in any of the experiments. Apart from one, the experiments did not last long enough to capture the final collapse of the bloom so the carbon export associated with this event could not be recorded.

Carbohydrate production by phytoplankton may partly determine the export production, since the cellular carbohydrate amount affects the buoyancy of the cells. Also, the extracellular release of carbohydrates plays a role in the formation of large aggregates that sink fast. Furthermore, extracellularly released carbohydrates contribute to the dissolved organic carbon pool and may be exported by downwelling of surface water. Obviously, more research is necessary if we want to sketch a full picture of what happens to a phytoplankton population after iron enrichment.