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Carbohydrate production by phytoplankton and degradation in the marine microbial food web

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Summary

In this thesis I describe studies relating to the cycling of the algal storage glucan chrysolaminaran. Chrysolaminaran is the most abundant type of storage carbohydrate in marine phytoplankton. I choose it as a model substrate to study factors influencing the cycling of carbohydrates, one of the most important processes in the marine carbon cycle. Carbohydrates comprise an important fraction of the biomass of phytoplankton. Upon release into the environment, they are usually readily degraded by prokaryotes in the microbial loop. Sometimes, however, degradation may be hampered and carbohydrates end up in refractory dissolved organic matter (Benner et al. 1992), in marine particles, and in sediments (Cowie and Hedges 1984). Research into factors hampering the degradation of carbohydrates is complicated by a number of factors, such as 1) the vast diversity of naturally occurring carbohydrates, 2) the low *in-situ* rates of degradation, and 3) the low concentrations. By studying the cycling of a relatively simple carbohydrate that is structurally well characterized, some of these problems can be circumvented. Moreover, chrysolaminaran is one of the most abundant carbohydrates in the marine system, yet little is known about factors controlling its production and degradation.

The aim of this study was to elucidate factors influencing production and degradation rates of carbohydrates in the marine ecosystem by following the steps of the chrysolaminaran cycle. In **chapter 1** three research questions were formulated:

- How do nutrient concentrations and irradiance levels influence the production of chrysolaminaran by phytoplankton?
- What are the characteristics of bacterial enzyme systems involved in degradation of chrysolaminaran?
- What is the influence of a massive release of carbohydrates on the abundance and activity of prokaryotes?

Some of the principles found by studying this relatively simple carbohydrate provide further insights into the carbohydrate production and degradation patterns observed in marine ecosystems.

How do nutrient concentrations and irradiance levels influence the production of chrysolaminaran by phytoplankton?

Phaeocystis is a cosmopolitan microalga genus often dominating the phytoplankton population in temperate and polar waters, and known for the production of copious amounts of carbohydrates. It produces two major pools of carbohydrates; extracellular mucopolysaccharides in the colony matrix and storage glucan, of which chrysolaminaran is the main constituent. In **chapter 2** the question how nutrient concentrations and irradiance levels influence the production of storage glucan and mucopolysaccharides is addressed. This was studied during a spring bloom of *Phaeocystis pouchetii* in a mesocosm near Bergen, Norway. Chapter 2 describes a partial precipitation method to separate both pools and study their dynamics in response to variations in nutrients and irradiance. During the growth phase of the bloom the glucan content of the colonies showed a diel cycle, the amplitude of which increased with higher light levels, whereas the mucopolysaccharide content was unaffected. Following nutrient limitation, the bloom reached a stationary phase, during which the carbohydrate to carbon ratio of the colonies increased. This was mainly caused by an increase in glucan content. It was shown that the *Phaeocystis* colony matrix is built with a relatively small but constant amount of carbohydrates, whereas the glucan contents is highly variable and increases upon nutrient limitation and high light levels. At the end of the bloom glucan contributed up to 60% to the *Phaeocystis* carbon. Since a major part of *Phaeocystis* primary production is recycled in the water column by prokaryotes, this suggests that glucan and hence chrysolaminaran is an abundant substrate for prokaryotes after a *Phaeocystis* bloom.

Diatoms are responsible for as much as 40% of the annual marine CO_2 uptake (Nelson et al. 1995). Chrysolaminaran is the principal storage glucan of diatoms and therefore represents a major part of their biomass. The effect of excessive light conditions on the production of chrysolaminaran in the marine diatoms *Thalassiosira weissflogii* and *T. antarctica* was investigated in **chapter 3.** This was part of a laboratory study of the effects of high-light acclimation on tolerance to excessive photosynthetically active (PAR) and ultraviolet (UVR) radiation light conditions. Cultures acclimated to either low or high PAR conditions were subjected to simulated surface irradiance (SSI) that mimicked irradiance around noon, including UVR. A number of physiological indicators of stress related to high-light conditions were determined after 30 min SSI and during 120 min recovery in low irradiance. In both *T. weissflogii* and *T. antarctica* the chrysolaminaran content of high-light acclimated cells was higher when compared to low-light acclimated cells, consistent with the results of chapter 2. In high-light acclimated cells the pool of xanthophyll pigments was higher and the efficiency of PSII was lower compared to low-light acclimated cells. The SSI treatment caused a decline in the PSII efficiency, coinciding with de-epoxidation of diadinoxanthin. During recovery these processes were reversed, but in low-light acclimated cells reversion was not completed within three hours. This resulted in a reduction in carbohydrate build-up in low-light acclimated cells. In high-light acclimated cells reversion was complete after one hour of recovery. In high-light acclimated *T. antarctica* the chrysolaminaran content was unaffected by both the SSI treatment and the control treatment, whereas in high-light acclimated *T. weissflogii* the chrysolaminaran content decreased after both treatments. The incapability to build up chrysolaminaran during

the recovery period was probably caused by the reduced efficiency of PSII in high-light-acclimated cells. In *T. weissflogii* the carbon and energy stored in the chrysolaminaran was likely mobilized during recovery. The UVR component of the SSI treatment had no influence on the chrysolaminaran content of any of the cultures.

What are the characteristics of bacterial enzyme systems involved in degradation of chrysolaminaran?

This question of the characteristics of bacterial enzyme systems involved in degradation of chrysolaminaran was the base of **chapter 4**. After a bloom of *Phaeocystis globosa* in the Dutch coastal North Sea several bacterial strains capable of utilizing laminarin as a sole carbon source were isolated. On average 26% of prokaryotes detected by epifluorescence counts were able to grow in Most Probable Number (MPN) dilution series on laminarin as a sole carbon source. Several bacterial strains were isolated from different dilutions and phylogenetic characterization revealed that they were belonging to different phylogenetic groups. The activity of the laminarin degrading enzyme systems was further characterized in three strains of *Vibrio* sp. that were able to grow on laminarin as a sole carbon source. At least two types of activity were detected during degradation of laminarin: the release of glucose and the release of glucans larger than glucose. At saturating substrate concentrations the rate of the glucan release exceeded the rate of the glucose release, resulting in accumulation of glucan intermediates during degradation of high concentrations of laminarin. At low concentrations, however, the ratio glucose/ glucan release increased, as a result of a higher substrate affinity of the glucose releasing enzymes than of the glucan releasing enzymes. Therefore, at low substrate concentrations no accumulation of glucan intermediates occurred. The kinetic properties of the laminarin degrading enzyme system may thus explain the observation that bacterial hydrolysis of polymers of aggregates and uptake of low molecular weight compounds are sometimes uncoupled processes, resulting in release of free polymers from particles into the surrounding water mass (Cho and Azam 1988; Smith et al. 1992; 1995; Unanue et al. 1998; Azúa et al. 2003). In aggregates the carbohydrate concentrations are high (Azúa et al. 2003), leading to high substrate concentrations for glucosidases. If the kinetic properties of the laminarin degrading enzymes were exemplary for other hydrolase systems, this would explain the release of polymers from particles.

Laminarinase enzymes showed a minimal activity on substrates with similar glucosidic bonds, but a different size, secondary, and /or tertiary structure. In an aqueous environment polymers tend to self-assemble into hydrogels (Chin et al. 1998; Verdugo et al. 2004), thereby altering their tertiary structure and accessibility of the linkages for the enzymes. If the difference in degradation potential of laminarin versus the substrates with a different size and/or structure is exemplary for the difference in degradation potential of “free” polymers and polymers embedded in a gel structure, turnover times may be slowed down from days to years.

What is the influence of a massive release of carbohydrates on the abundance and activity of prokaryotes?

The effect of a spring bloom of *Phaeocystis globosa* on the microbial community is investigated in **chapter 5** to study what is the influence of a massive release of carbohydrates on the abundance and activity of prokaryotes. MICRO-CARD-FISH analysis was applied to samples collected from the coastal North Sea over the course of a spring and summer season to determine the abundance and activity of different groups of prokaryotes. The abundance, as well as the fraction of active prokaryotes increased during the initial development of the *P. globosa* bloom. At the peak of the bloom, the prokaryote numbers decreased, but increased again during the decline of the bloom. The activity of the prokaryotes remained high throughout the spring and summer. Bacteria belonging to the Bacteroidetes were dominating the prokaryote community during the wax and wane of the *P. globosa* bloom. They have been associated with degradation of complex carbohydrates (Kirchman 2002), and therefore are probably involved in degradation of *Phaeocystis* carbohydrates. Members of the *Roseobacter* clade always showed the highest fraction of active cells, however, they comprised a minor part of the prokaryote community. Two groups of archaea, crenarchaea and euryarchaea, were detected throughout the study period. Their combined contribution was on average 2% of the total prokaryotic community, and the active fraction of archaea was always lower than the fraction of active bacteria. Therefore we conclude that archaea do not play a major role in the biogeochemical cycles of the coastal North Sea during spring and summer.

Conclusions: The carbohydrates of *Phaeocystis* and their degradation in the microbial food web – a review

The conclusions from the previous chapters are integrated in **chapter 6** to discuss the production of carbohydrates by *Phaeocystis* and their degradation in the microbial food web. Since chrysolaminaran comprises an important fraction of *Phaeocystis* biomass, especially at the end of a bloom when nutrients are limited, it is an important component of the biogeochemical cycle following a *Phaeocystis* bloom. Hence, it should be considered alongside the mucopolysaccharides that have received much more attention so far. Lysis of *Phaeocystis* cells and deterioration of colonies releases the biomass as dissolved organic matter (DOM) during the wane of a bloom. Laboratory studies have revealed that both mucopolysaccharides and chrysolaminaran are potentially readily degradable by heterotrophic bacteria (Janse 2000b). In particular bacteria belonging to the Bacteroidetes benefit from the carbohydrate rich DOM. Observations of accumulation of DOM and foam after blooms of *Phaeocystis* (Eberlein et al. 1985; Lancelot et al. 1987a), however, indicate that the DOM may be persistent. One of the reasons put forward is the high C/N and C/P ratio of *Phaeocystis* organic matter, which may lead to nutrient limitation during microbial degradation, thereby prolonging degradation times. Upon prolonged degradation times carbohydrates can self-assemble into hydrogels (Chin et al. 1998). This may have a profound effect on carbon cycling, since hydrogels provide a vehicle to move DOM up the size spectrum to sizes subject

to sedimentation. In addition, it changes the physical nature and microscale structure of the organic matter encountered by bacteria, thereby affecting the degradation potential of the *Phaeocystis* organic matter.