# Molecular responses of phytoplankton to iron limitation


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*Doctoral Dissertation*

# Molecular responses of phytoplankton to iron limitation

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# ABSTRACT

The present study focused on the proteomic study of phytoplankton to Fe limited conditions. It was found that marine phytoplankton *Prymnesium parvum* employ several strategies to compensate Fe stress. It was found that marine phytoplankton biosynthesize different proteins under low total Fe and ligand-induced Fe-limited conditions. To understand responses of phytoplankton to Fe deficiency, responses to nitrogen and phosphate deficiency were also studied and was found that several proteins were differentially expressed in marine phytoplankton *P. parvum* in response to different exposure levels of nitrate, phosphate and iron. The expression levels of an 83 kDa protein in *P. parvum* can be used as biomarker of N-status, while a 121 kDa protein can be used as a biomarker of P-deplete condition in aquatic systems. In addition, two protein can be used as biomarker of Fe-status (deplete or replete conditions) in aquatic systems. Under Fe-limited condition, *P. parvum* may increase Fe uptake efficiency by increasing ABC transporters. Under Fe-limited condition, the phytoplankton may also increase photorespiration which needs high metabolic energy. The phytoplankton may satisfy the demand of high metabolic energy (for photorespiration and ABC transporter) by increasing ATP synthase in chloroplast. Fe stress may cause oxidative stress in phytoplankton which is thought to be defended by up-regulating oxidative stress response proteins MnSOD and STK. Carbohydrate degradation and glycolytic activity was thought to be increased under Fe-limited conditions. Marine phytoplankton *P. parvum* also alters its cellular biochemical processes by up-regulating several proteins involved in photosynthesis. The phytoplankton also increased biosynthesis of some PSII component proteins under Fe-limited conditions.

**Experiment 1:**

In the first experiment, growth and proteomic responses of three marine phytoplankton strains (*Pleurochrysis roscoffensis, Prymnesium parvum* and *Skeletonema marinoi-dohrnii* complex) under Fe limitation by low total Fe and ligand induced Fe limitation condition were studied. Compared to Fe-rich conditions (Fe = 1 μM), the growth of phytoplankton decreased substantially under low total

Fe-limited condition (Fe = 0.07 μM for *P. roscoffensis* and *S. marinoi-dohrnii complex*, and Fe = 0.03 μM for *P. parvum*). The marine phytoplankton express different proteins under Fe-limited and Fe-rich conditions and the protein expression differ among the phytoplankton. In low total Fe condition, *P. parvum* expressed three proteins (19, 32 and 42 kDa), which were very similar to those expressed by *P. roscoffensis* in the same condition. In addition, *S. marinoi-dohrnii complex* expressed a 55 kDa protein, which was not expressed by the other phytoplankton in low total Fe condition. The phytoplankton expressed different proteins under ligand induced Fe-limited conditions. Both *P. parvum* and *S. marinoi-dohrnii complex* expressed a common protein (19 kDa), while *S. marinoi-dohrnii complex* produced an additional protein of 33 kDa under desferrioxamine B (DFO-B) induced Fe-limited conditions. A 19 kDa protein was expressed by *P. roscoffensis* under low total Fe and ligand-induced Fe-limited conditions; however, the *P. roscoffensis* expressed a new protein of 27 kDa under diethylenetriamine-N,N,N',N'',N''-pentaacetate (DTPA) induced Fe-limited conditions instead of the 33 kDa protein that was expressed under low total Fe and DFO-B-induced Fe-limited conditions. The results indicate that marine phytoplankton alters their Fe acquisition strategy under low total Fe and ligand-mediated Fe-limitations by expressing different proteins.

**Experiment 2:**

Nitrogen (N), phosphorus (P) and Iron (Fe) are important nutrients for phytoplankton, and are key limiting nutrients in marine systems. In the second study, growth and protein expression of marine phytoplankton *Prymnesium parvum* under different nitrate, phosphate and iron conditions were investigated in order to evaluate whether proteins and their expression level can be used as biomarker of N, P, and Fe conditions in aquatic systems. The growth of *P. parvum* increased with the increase of nitrate, phosphate and iron concentrations in the culture medium. Protein expression levels also differed significantly ($p < 0.001$) for different nitrate, phosphate and iron conditions in the culture medium. The expression level of an 83 kDa protein at 0 and 5 μM nitrate treatments differed significantly ($p < 0.001$) from

those at 20, 30, 50 and 100 μM nitrate treatments, indicating the expression levels of this protein as a biomarker of N status in the culture medium. A 121 kDa protein was expressed at phosphate stress conditions ([P] ≤ 1.0 μM), while this protein was not expressed at phosphate replete conditions ([P] ≥ 5 μM). Therefore, the expression of 121 kDa protein in *P. parvum* is indicative of phosphate deplete condition in aquatic systems. The expression level of a 42 kDa protein was significantly higher ($p < 0.01$) at Fe-stress condition ([Fe] = 0.01 μM) than Fe-replete conditions ([Fe] ≥ 0.1 μM). In addition, a new protein of 103 kDa was only expressed under Fe-deplete condition ([Fe] = 0.01 μM). Therefore, the 42 and 103 kDa proteins can be used as a biomarker of Fe-limitation condition of aquatic systems. However, further studies (two dimensional gel electrophoresis and mass spectrometry) are needed to identify and characterize these proteins in *P. parvum*.

**Experiment 3:**

Iron is a vital limiting factor for phytoplankton in vast regions of oceans, notably the high nutrient low chlorophyll (HNLC) regions. Therefore, it is needed to be acquainted with the adaptation mechanisms of marine phytoplankton under Fe-limited condition. In third experiment, *Prymnesium parvum* was grown under Fe-deplete (0.0025 μM) and Fe-rich (0.05 μM) conditions, and proteomic responses were compared. Compared to 0.05 μM Fe concentration (Fe-rich condition) *P. parvum* showed substantially reduced growth under 0.0025 μM Fe concentration (Fe-limit condition). In sodium dodecyl sulfate gel electrophoresis, 7 proteins (16, 18, 32, 34, 75, 82, and 116 kDa) were highly expressed under Fe-deplete condition, while one protein (23 kDa) was highly expressed under Fe-rich condition. The proteins were subjected to 2-dimensional gel electrophoresis to differentiate individual proteins, and were identified by MALDI-TOF-MS analysis. The results showed that under Fe-deplete condition *P. parvum* increases the biosynthesis of ABC transporters and a flagellar associated protein which may change their Fe acquisition strategy in order to facilitate Fe acquisition under Fe stress condition. Under Fe-deplete condition, *P. parvum* increases the synthesis of RuBisCO and/or pyruvate dehydrogenase, phosphoribosylaminoimidazole-succinocarboxamide

synthase, malate dehydrogenase, glycosyl hydrolase, glyceraldehyde-3-phosphate dehydrogenase, and two Fe-independent oxidative stress response proteins, MnSOD and Serine threonine kinase. These proteins are assumed to be involved in a number of cellular biochemical processes, such as photorespiration, glycolysis followed by degradation of stored polysaccharides, and managing of iron limitation induced oxidative stresses that facilitate marine phytoplankton to cope with Fe-limitation.

**Experiment 4:**

Iron (Fe) is essential for photosynthesis, a process used by autotrophic organisms to convert light energy into chemical energy, of autotrophic organisms. Fe limitation may influence the growth and productivity of microalgae by reducing photosynthetic efficiency. In the fourth experiment, the effect of Fe-limitation on growth and photosynthetic activities of marine microalga (*Prymnesium parvum*) were investigated. Marine microalga *P. parvum* was grown in f/2 medium in artificial seawater under Fe-limit (0.0025 μM) and Fe-rich (0.05 μM) conditions. Compared to Fe-rich condition (the highest of 156 cell $mL^{-1}$ $d^{-1}$ at $10^{th}$ day), *P. parvum* showed substantially reduced growth rate under Fe-limit condition (the highest of 97 cell $mL^{-1}$ $d^{-1}$ at $8^{th}$ day). Proteomic responses of *P. parvum* to Fe-limitation were also studied for understanding the strategies of marine microalgae that the organism employ in order to maintain photosynthetic activity and productivity. Under Fe-limit condition, *P. parvum* was found to up-regulate eleven proteins, which were identified by matrix-assisted laser desorption-ionization-time of flight-mass spectrometer (MALDI-TOF-MS) analysis. Results showed that *P. parvum* increases the biosynthesis of several proteins associated with photosystem II (PSII), which is assumed to be a strategy of the microalga in order to cope with the Fe-limitation. The up-regulation of chloroplast ATP synthase biosynthesis would be a strategy of the microalga to meet the cellular energy under Fe-limit condition. Thus, microalgae alter the biosynthesis of several photosynthetic proteins in order to sustain Fe-limit condition.

# 学位論文審査結果の要旨

提出学位論文について、各審査委員が個別に審査した後、平成25年7月19日に審査員による予備審査会を実施するとともに、平成25年8月6日に口頭発表会と論文審査委員会を開催し、以下のように判定した。

海洋植物プランクトンは、必須元素である鉄が不足すると、栄養欠乏状態に対応するために細胞内の分子組成を変化させる。このメカニズムを利用して、本研究では、沿岸海域における藻類バイオマス育成において、鉄に対する栄養状態の程度を判断するための新しい指標物質の探索とその分析法の確立に取り組んだ。海洋植物プランクトンの室内培養試験において、鉄や窒素、リンが欠乏する条件で多く発現する複数の細胞内タンパク質を見出した。蛍光標識二次元ディファレンスゲル電気泳動解析法、及びマトリックス支援レーザー脱離イオン化/飛行時間型質量分析法を用いてタンパク質を同定し、鉄欠乏状態では細胞膜のトランスポータータンパク質が増加すること、また、鉄欠乏によって生じた酸化ストレスを軽減する代謝機構が細胞内で促進されること等を示した。更に、鉄濃度に応答する細胞内タンパク質を迅速に測定する手法として、蛍光誘導体化高速液体クロマトグラフィー法を確立した。

以上、本研究は、鉄制限状態における海洋植物プランクトンの細胞内分子応答を化学的に解析したものであり、微量鉄化学種を活用した海洋沿岸域における環境保全と藻類バイオマス有効利用技術に対して有用な知見を提供する研究として博士（学術）の学位に値するものと判断した。