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A marine phytoplankton *Prymnesium parvum* upregulates the component proteins of photosystem II under iron stress

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Abstract

Iron is an essential limiting factor for primary production in many marine systems. The present study investigated differential regulation of protein expression in marine phytoplankton *Prymnesium parvum* under low Fe concentration. The phytoplankton was grown in f/2 culture medium in artificial seawater with low (0.0025 μM) and high (0.05 μM) Fe concentrations. Two-dimensional differential gel electrophoresis and matrix-assisted laser desorption–ionization–time of flight-mass spectrometer analysis were performed for protein identification and characterization. The growth of the alga declined substantially under the low Fe compared to the high Fe concentration. Under low Fe conditions, *P. parvum* upregulated 10 proteins including chloroplastic ATP synthase subunit b, D2 protein of PSII, D1 protein of PSII reaction centre, and light harvesting complex II protein, most of which are associated with photosynthetic activities in PSII. The results suggest that the marine alga *P. parvum* altered the biosynthesis of several photosynthetic proteins in order to cope with low Fe conditions.

Additional key words: Fe limitation; growth rate; photosynthesis; protein expression; *Prymnesium parvum*.

Introduction

Iron (Fe) is an essential nutrient element for autotrophic organisms. It is the most abundant transition metal in the Earth crust. Because of its key role in the redox reactions of electron transport chains in respiratory systems and photosynthesis, Fe is required for all photosynthetic organisms including phytoplankton (Chen *et al.* 2005). Iron is embodied in many redox enzymes of the intermediary metabolism and membrane-bound electron transport chains (Naumann *et al.* 2007). It is also involved in many cellular biochemical processes, such as respiration, nitrate and nitrite reduction, sulfate reduction, nitrogen fixation, and a number of other biosynthetic and derivative reactions (Geider and LaRoche 1994). Thus, Fe greatly influences the ecology, physiology, and productivity of phytoplankton.

Iron deficiency is one of the common nutritional problems in many marine systems. This is due to the fact that, despite the high abundance of Fe, its bioavailability is restricted by many environmental conditions (Naumann *et al.* 2007). The global impact of Fe deficiency on

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Abbreviations: 2–D DIGE – 2–dimensional differential gel electrophoresis; Chl – chlorophyll; HF – high Fe concentration; IEF – isoelectric focusing; LF – low Fe concentration; MALDI–TOF–MS – matrix-assisted laser desorption–ionization–time of flight-mass spectrometer; NCBI – National Center for Biotechnology Information; OD – optical density; OEE – oxygen-evolving enhancer; PBS – phosphate buffer saline; PQ – plastoquinon; ROS – reactive oxygen species.
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photosynthetic productivity has been reported in vast areas of marine systems, where Fe bioavailability is extremely low (Beardall and Raven 2004, Ryan-Keogh *et al.* 2013). Autotrophic organisms have been reported to change their morphophysiology (*e.g.*, the chloroplast structure, photosynthetic capacity, and the composition of thylakoid membranes) in response to Fe limitation (Raven *et al.* 1999, Naumann *et al.* 2007).

The tolerance of phytoplankton to Fe limitation varies widely between species (Strzepek and Harrison 2004). Generally, subnanomolar concentration of dissolved Fe in open oceans confines the growth of phytoplankton (Marchetti *et al.* 2006). However, few phytoplanktons are highly tolerant to Fe limitation and can grow in steady-state at Fe concentrations in the range of 10–30 pmol, where Fe is considered as the sum of all unchelated Fe species (Strzepek and Harrison 2004, Allen *et al.* 2008). The *Prymnesium parvum* is an ichthyotoxic phytoplankton (Haptophyta) growing in geographically widespread areas of the low nutrient, open oceans including temperate waters of both the Northern and Southern Hemispheres (Fistarol *et al.* 2003), vast areas of temperate and tropical open seas (Edvardsen and Imai 2006), the Baltic Sea (Edler *et al.* 1984), and many coastal areas (Edvardsen and Imai 2006, Carvalho and Granéli 2010). Because of its widespread distribution in low nutrient, open oceans, *P. parvum* has been used in many oceanographic studies during the last decade (Fistarol *et al.* 2003, La Claire 2006, Maki *et al.* 2008, Carvalho and Granéli 2010, James *et al.* 2011). Except of some coastal areas, *P. parvum* is an important component of the microbial community in marine systems which are low in Fe (Wells *et al.* 1995, Mills *et al.* 2004, Vuorio *et al.* 2005, Allen *et al.* 2008).

Photosynthesis, a process used by photoautotrophic organisms to convert light energy into chemical energy, is a complex process in which many proteins are involved. Phytoplankton species use Fe mainly for photosynthetic electron transport, and it is an essential component of the cytochrome (Cyt) and Fe-sulfur protein cofactors of the major photosynthetic complexes in PSII, the Cyt b_6f complex, and PSI (Raven *et al.* 1999, Strzepek and Harrison 2004). To date, understanding how low Fe availability influences phytoplankton physiology has focused on traditional metrics, such as a growth rate, elemental composition, and biophysical measurements of photosynthetic competence. A recent proteomic study by Nunn *et al.* (2013) revealed unique acclimation strategies of coastal diatom *Thalassiosira pseudonana* to Fe limitation. However, little is known about how low Fe availability affects photosynthetic activities through the alteration of peptide biosynthesis. The present study aimed to investigate the effects of the low Fe concentration on photosynthesis, particularly PSII, of marine phytoplankton, *P. parvum*, using a proteomic approach.

Materials and methods

Phytoplankton precultivation and maintenance: Marine phytoplankton *P. parvum* was collected from Fukuyama Bay, Hiroshima, Japan. The phytoplankton strain was axenic. Axenicity was assessed and monitored by the 4′,6-diamidino-2-phenylindole (DAPI) test. The axenic phytoplankton strain was precultivated for two weeks in 60-mL polycarbonate bottles with f/2 nutrient solution in artificial seawater (Table 1) without Fe (Lyman and Fleming 1940, Guillard and Ryther 1962). Modified f/2 nutrient solution in artificial seawater was sterilized by autoclaving (*MLS-3780*, *Sanyo Labo*, Japan). $FeCl_3$ (10 μM) solution (in 1.0 M HCl) was sterilized separately to avoid Fe contamination. Bottles, tips, and micropipettes used in the test were also sterilized by autoclaving. Sterilization was done at 121°C for 30 min.

The $FeCl_3$ solution was then mixed with the modified f/2 nutrient solution. Before adding Fe (as $FeCl_3$) to the f/2 sterilized nutrient solution, Fe concentration of the solution was measured and found below 0.001 μM. Thus, there was no Fe contamination during the sterilization process. Other chemicals and their sources were carefully selected, and the methods of solution preparation of the chemicals were also carefully done to avoid any possible Fe contamination. After sterilization, the materials were placed on a clean bench (*MCV – 710ATS*, *SANYO*, Japan), and they were kept under an ultraviolet ray for 25 min. Since Fe (10 μM as $FeCl_3$ in 1.0 M HCl) was added to the nutrient solution after sterilization, and the phytoplankton were grown in the solution only for 2 weeks, the possibility

Table 1. Composition of the f/2 growing medium in artificial seawater used for growing phytoplankton. *Iron was not added to this medium when it was used for the microalgal preculture. However, Fe concentrations in low and high Fe f/2 growing medium in artificial seawater were 0.0025 and 0.05 μM, respectively.

Nutrient	Concentration
$NaNO_3$	8.82×10^{-4} M
$NaH_2PO_4 \cdot 2\ H_2O$	3.85×10^{-5} M
$Na_2SiO_3 \cdot 9\ H_2O$	3.52×10^{-5} M
SeO_2	1.00×10^{-8} M
$CoSO_4 \cdot 7\ H_2O$	4.27×10^{-8} M
$ZnSO_4 \cdot 7\ H_2O$	7.30×10^{-8} M
$MnCl_2 \cdot 4\ H_2O$	9.09×10^{-7} M
$CuSO_4 \cdot 5\ H_2O$	2.80×10^{-8} M
$Na_2MO_4 \cdot 2\ H_2O$	2.89×10^{-8} M
$FeCl_3$	*
Vitamine B12	3.69×10^{-10}M
Biotin	2.05×10^{-9} M
Thiamin HCl	2.97×10^{-7} M
HEPES	5.04×10^{-6} M

of precipitation of added $FeCl_3$ was minimal, and ethylenediaminetetraacetic acid (EDTA) was not necessarily required.

Growing phytoplankton in test solutions: The two-week phytoplankton precultivation in f/2 nutrient solution in artificial seawater without Fe was repeated until the concentration of Fe in the medium reached values below 0.005 µM. Thus, the amount of Fe in the preculture can be ignored. Approximately 3×10^3 phytoplankton cells mL^{-1} of logarithmic growth phase were inoculated in 1 L of f/2 nutrient solution in artificial seawater with low (0.0025 µM, LF) and high (0.05 µM, HF) Fe concentrations (Table 1) under laminar air flow conditions. Fe concentration in LF medium was selected to be 0.0025 µM since this concentration was nearly similar to the concentration found in natural oceanic waters (Geider 1999). In order to extract sufficient amount of proteins from the cells, the phytoplankton was cultured in 14 (for LF) and 8 (for HF) 1-L polycarbonate bottles in a growth chamber. The light and dark schedule in the growth chamber was 14/10 h with the light intensity of 188 µE m^{-2} s^{-1}. Temperature in the growth chamber was 20 ± 2°C.

Growth measurement of phytoplankton: The growth of phytoplankton was measured as the optical density (OD) using a spectrophotometer (*U–2000*, *Hitachi High Technologies*, Japan) at a wavelength of 540 nm (OD_{540}) (Maki *et al.* 2008). The phytoplankton was grown for a total of 16 d from incubation (DAI), and growth data were taken every day until harvesting for protein extraction. The experiment was replicated three times.

Extraction of membrane proteins: After reaching logarithmic growth phase (14 d), the phytoplankton growth solution was transferred to 50-mL centrifuge tubes and centrifuged at 380 × *g* for 10 min at 4°C. The supernatant was removed and the phytoplankton pellet was washed twice using 2 mL of 10 mM Tris-HCl (pH 7.2). Then 1.5 mL of 10 mM Tris-HCl was added to the sample to make a phytoplankton cell suspension of which 1 mL was transferred to a 1.5-mL microtube and was sonicated for 20 s using an ultrasonic homogenizer (*UH-50*, *Surface Mount Technology*, Japan) at output 5 under ice–cold conditions. The membrane protein was separated by the method described by Enami *et al.* (1995). Briefly, the phytoplankton cell suspension was centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatant was then centrifuged at 105,000 × *g* for 30 min at 4°C and the protein pellets were washed twice with phosphate buffer saline (PBS). The pellets were then resuspended with PBS containing 1% Triton X-100. Then the membrane protein was collected from the supernatant after centrifugation at 105,000 × *g* for 30 min at 4°C. Protein quantification was performed by the Bradford (1976) assay (*Bio-Rad*, Hercules, CA, USA). The protein samples were then brought to equal concentration and were subjected to further analysis.

Two-dimensional differential gel electrophoresis (2–D DIGE): Fifty µL of trichloroacetic acid was mixed with 950 µL of protein sample of each treatment, and the mixture was centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatant was removed and the pellet was dried after washing three times with acetone. Then the sample was mixed with 400 µL of the lysis buffer [8 M urea, 2% 3-[(3-cholamidopropyl) dimethylammonio]propanesulfonic acid (CHAPS), and 30 mM Tris-HCl, pH 8.5]. The insoluble material was removed by centrifugation (15,000 × *g* at 4°C for 15 min), and the pH of the supernatant (ranging from 8 to 9) was confirmed with litmus paper. Then the sample was mixed with 400 µL of the swelling buffer [7 M urea, thiourea 2 M urea, 4% CHAPS, 0.5% immobilized pH gradient (IPG) buffer, 0.2% dithiothreitol (DTT), 0.002% bromophenol blue (BPB)], centrifuged at 10,000 × *g* for 15 min at 4°C, and the protein concentrations in the sample was measured using an *RC DC* Protein Assay Kit (*Bio-Rad*, USA). Approximately 50 µg of protein of each treatment was labeled by using *CyDye* (Cy3 and Cy5) *DIGE Fluors* (*GE Healthcare*, Tokyo, Japan), and was applied on an IPG strip (pH 3–10) in-gel rehydration protocol (Asano *et al.* 2012). Protein extracts were applied to the IPG dry strips, pH 3–10 (18 cm, *GE Healthcare*, Tokyo, Japan) during the rehydration step followed by focusing for increasing of voltage linearly from 300 to 3,500 V during 1.5 h, followed by an additional 5 h at 3,500 V using an IPGphor (*GE Healthcare*, Tokyo, Japan). After isoelectric focusing (IEF), the IPG strips were equilibrated at room temperature (22 ± 2°C) for 15 min in an equilibration buffer (50 mM Tris–HCl, pH 8.8, 6.0 M urea, 30% v/v glycerol, 2% w/v SDS and 0.002% BPB) containing 1% DTT. Next, the IPG strips were equilibrated at room temperature (22 ± 2°C) for 15 min in an equilibration buffer containing 25 mg mL^{-1} iodoacetamide. SDS – PAGE in the second dimension was carried out using 12% polyacrylamide gel. After electrophoresis, fluorescent signals were detected by a variable *Typhoon™ 9400* imager (*GE healthcare*, Tokyo, Japan). The 2-D gel images were analyzed with the *PDQuest Advanced* software (*Bio–Rad*, CA, USA) (Asano and Nishiuchi 2011). A 0.5 mg protein sample was used for identification purpose. Automated spot detection and matching was applied, followed by manual spot editing to achieve a sufficient correlation between the gels. An analysis set was created to find the spots with a minimum of around 2-fold increase or decrease between the two replicates of the LF and HF treatments. Only spots that differed significantly ($p<0.05$) in abundance according to the *Student*'s *t*-test were further investigated for the identification of the proteins.

Purification and identification of the proteins: The excised spots were subjected to in-gel digestion with trypsin, and the peptides extracted as described elsewhere (Asano and Nishiuchi 2011). Briefly, excised spots from 2-D gels were washed sequentially with acetonitrile

(100%), DTT (10 mM), and iodoacetamide (33 mM). Then the gels were washed 3 times sequentially with 100 mM NH_4HCO_3 and CH_3CN and then vacuum-dried. Twenty µL of trypsin (12.5 ng mL^{-1}) was added to the gels and they were heated overnight at 37°C. The samples were then mixed with 200 µL of a solution of 50% acetonitrile and 5% formic acid. The samples were then evaporated using a Savant SpeedVac (*VC–960*, *TAITEC*, Japan), and concentrated to a volume of 10–20 µL.

The peptide mixture was analyzed using *4800 plus MALDI–TOF/TOF™ Analyzer* (*Applied Bioscience*, Carlsbad, CA, USA). Samples were prepared for matrix-assisted laser desorption–ionization–time of flight–mass spectrometer (MALDI–TOF–MS) analysis by mixing 0.5 µL of the peptide sample with 0.5 µL α-cyano-4-hydroxycinnamic acid in 50% acetonitrile/0.1% triflouroacetic acid (TFA), and spotting the mixture on a MALDI target (Asano and Nishiuchi 2011). For protein identification, MS/MS data were evaluated by considering amino acid substitution and modification against the NCBI and *UniProt* database using the Paragon algorithm of *ProteinPilot™ v 2.0* software (*AB Sciex*, California, USA).

Statistics: The intensities of the differentially expressed protein spots were statistically analyzed to determine significant differences between their expression levels by *Student*'s *t*-test using *SPSS* (*v. 17* for *Windows*). The p-value ≤ 0.05 was considered statistically significant.

Results and discussion

Effect of Fe-limitation on phytoplankton growth: The growth of *P. parvum* did not differ up to 8 d of incubation for LF and HF conditions. Under LF, the algal growth slowed down from 9 DAI and stopped completely after 11 DAI (Fig. 1). The alga showed steady and strong growth up to the 12 DAI and then slowed down gradually until 16 DAI under HF (Fig. 1). The LF reduced the growth of phytoplankton substantially. Previous studies have also reported a decrease in growth of phytoplankton under LF condition (Imai *et al.* 1999, Liu *et al.* 2008).

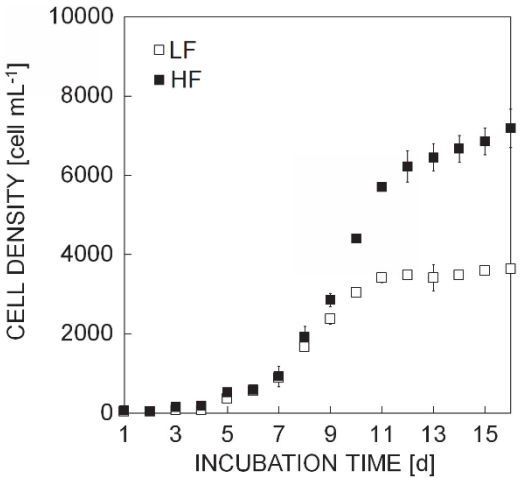

Fig. 1. Growth response of marine phytoplankton *Prymnesium parvum* to low (LF) and high (HF) Fe conditions. Fe concentrations in LF and HF culture medium were 0.0025 and 0.05 µM, respectively. The growth of phytoplankton was calculated based on the optical density (OD) at 540 nm wavelength (OD_{540}).

Differentially expressed proteins under Fe-limiting condition: The proteins extracted from phytoplankton cells of LF and HF medium were subjected to 2-D DIGE and then analyzed with the *PDQuest Advanced* software to characterize the differentially expressed proteins. After IEF, up to 10 spots of significant difference in their expression level were identified on the gels. The spots were numbered as shown in Table 2 and in Fig. 2. The differentially expressed proteins were then identified using MALDI–TOF–MS analysis. However, some spots could not be identified on the gels because they were not well resolved from neighboring spots.

Proteins involved in photosynthesis: In the present study, ten proteins were found to be upregulated between 1.2–13.2 fold under LF condition (Table 2), some of them (*e.g.*, spots 1, 3, 6, 7, and 9; Fig. 2) are component proteins of the PSII complex.

The LHCII protein (spot 6, Fig. 2) was upregulated by 2.4 ± 0.5 fold under LF conditions (Table 2). The LHCII of PSII, which is the primary protein involved in light harvesting in PSII, contains more than 80% of the light-harvesting pigments, chlorophyll (Chl) *a* and *b* (Morrissey *et al.* 1989). In a previous study, Naumann *et al.* (2007) reported that Lhcb polypeptides, which form the LHCII, remain stable and even increase in freshwater phytoplankton *Chlamydomonas reinhardtii* under Fe deficiency. Similar result has also been reported by Moseley *et al.* (2002) in the same phytoplankton. In contrast to Lhcb polypeptides, light–harvesting proteins associated with PSI (LHCI), were affected at varying degrees by Fe deficiency (Naumann *et al.* 2005).

D1 protein of PSII reaction centre (PSII D1) (spot 1, Fig. 2), which provides the binding site for the secondary plastoquinone, Q_B, was upregulated by 2.2 ± 0.6 fold under LF (Table 2). The PSII D2 protein (spots 7 and 9, Fig. 2) was upregulated by more than 2.5 fold under LF conditions (Table 2). In a previous study, Maki *et al.* (2008) identified significant changes in gene transcriptional levels (in the nucleotides of 13 specific bands) in Fe-stressed cells. Reverse Northern blot analysis showed that the transcription induction rates of nine proteins were more than 1-fold (ranging from 1.02–16.5 fold) under Fe-stressful

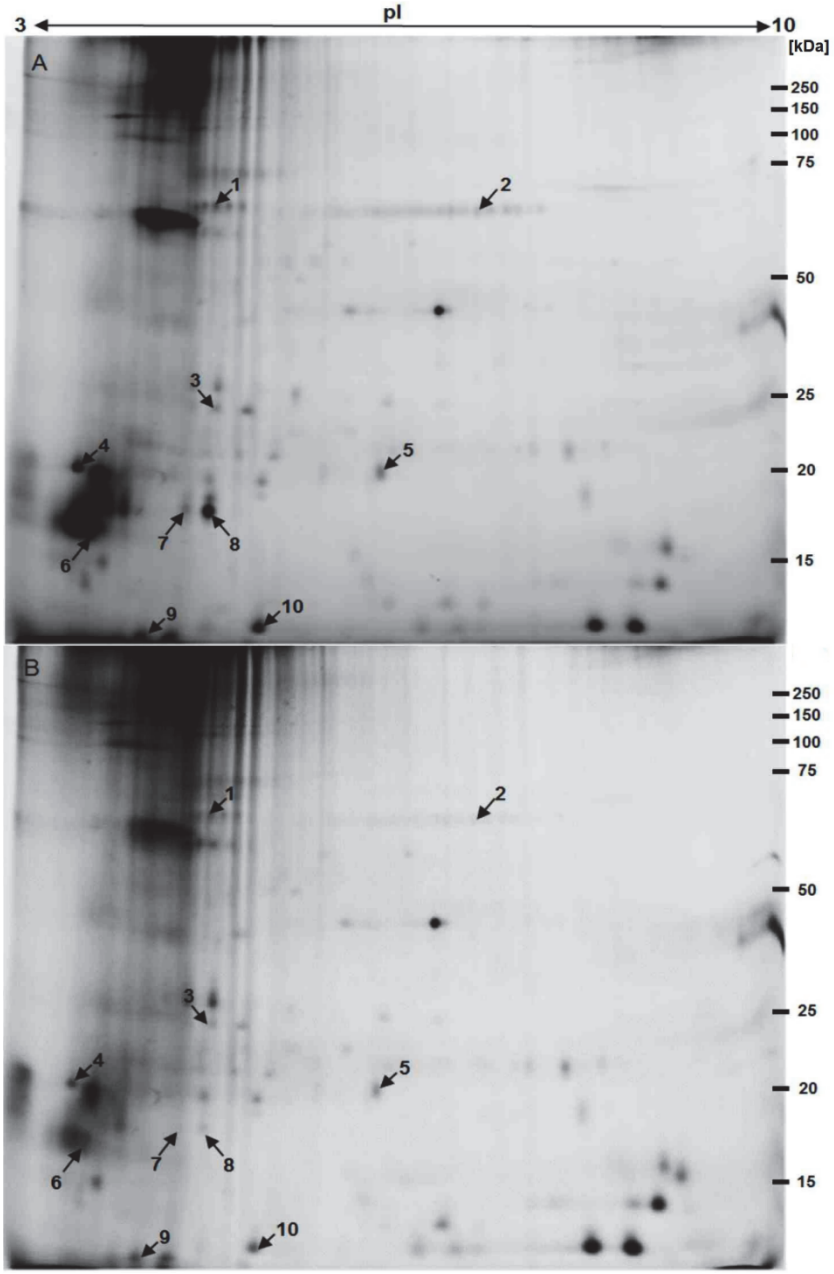

Fig. 2. Two-dimensional differential gel electrophoresis (2D-DIGE) analysis of the membrane proteins of marine phytoplankton *Prymnesium parvum* under (*A*) low (LF) and (*B*) high (HF) Fe conditions. Selected spots (1–10) represent the proteins that were differentially expressed ($p \leq 0.05$, according to the *Student*'s *t*-test) under LF and HF.

conditions. Naumann *et al.* (2007) reported 4 fold upregulation of PSII D2 protein in *C. reinhardtii* under Fe-deficient condition. Komenda *et al.* (2004) also reported upregulation of PSII D2 protein in a cyanobacterial strain *Synechocystis* PCC 6803. The expression of PSII D2 protein (upregulation) in marine phytoplankton *P. parvum* in our study was consistent with that found in freshwater phytoplankton, *C. reinhardtii* (Naumann *et al.* 2007) and in cyanobacteria *Synechocystis* (Komenda *et al.* 2004). However, slight downregulation of PSII D1 protein was observed in *C. reinhardtii* under Fe-deficient condition (Naumann *et al.* 2007), which is inconsistent with the result of present study. This may be due to the fact that the tolerance of phytoplankton to Fe limitation varies between phytoplankton species (Strzepek and Harrison 2004).

Oxygen evolving enhancer (OEE) protein (spot 3; Fig. 2) was slightly upregulated under LF (Table 2). This protein is located on the lumenal side of PSII (Fig. 3) and is thought to optimize the manganese cluster during water oxidation (Heide *et al.* 2004). Moreover, its extended structure may protect the reaction centre D1 protein in PSII from oxygen radicals (Yamamoto 2001).

Fe requirement differs for PSI and PSII in phytoplankton (Raven *et al.* 1999). The relative abundance of PSI and PSII complexes is the key determinant of Fe requirement for phytoplankton since Cyt b_6f complex (6 atoms of Fe per complex) and PSI (12 atoms of Fe per complex) are disproportionately rich in Fe compared to

Table 2. Differentially expressed proteins in *Prymnesium parvum* under low (LF) and high (HF) Fe concentration identified by MALDI–TOF–MS. The proteins were identified by comparing MS/MS data against the NCBI and *Uniprot* database using the Paragon algorithm of *ProteinPilot*. Spot IDs correspond to the 2-D electrophoresis gel spots in Fig. 1. Protein abundance was calculated based on the protein concentration/abundance in 2-D gel electrophoresis. Accession number means a number in NCBI and *Uniprot* database. Total ProtSore means, a measure of evidence of an identified protein, calculated from the confidence level of all peptides detected. ProtSore 2 indicates confidence level > 99%. The percent coverage of all amino acids from a valid peptide matches to the total number of amino acids in the protein.

Spot ID	Protein abundance (LF/HF)	Protein mass [kDa]	pI value	Name of the protein	Organism	Accession number	Total ProtSore	% Coverage
1	2.2 ± 0.6	72.4	4.8	Photosystem II reaction centre protein D1	*Cyanidium caldarium*	tr|Q6JAL3|Q6JAL3_CYACA	1.5	12.9
2	2.3 ± 0.5	70.9	7.2	ATP synthase subunit alpha, chloroplastic	*Ectocarpus siliculosus*	tr|D1J797|D1J797_ECTSI	2.2	10.6
3	1.2 ± 0.4	26.7	4.8	Oxygen evolving enhancer protein	*Rhodosorus marinus*	tr|E5RPB6|E5RPB6_9RHOD	1.7	21.3
4	13.2 ± 2.7	20.3	3.5	hypothetical protein Msip34_0367	*Methylovorus* sp. SIP3-4	gi|253998079	1.3	4.9
5	2.8 ± 0.6	19.7	6.4	Putative uncharacterized protein	*Ectocarpus siliculosus*	tr|D8LB97|D8LB97_ECTSI	0.7	5.2
6	2.4 ± 0.5	14.7	3.6	Light harvesting complex protein	*Ectocarpus siliculosus*	tr|D8LBT8|D8LBT8_ECTSI	2.4	10.5
7	2.6 ± 0.6	16.4	4.5	Photosystem II D2 protein	*prymnesiophyte* C19847	tr|D9MYL1|D9MYL1_9EUKA	2.9	6.6
8	6.6 ± 1.3	16.4	4.8	ATP synthase subunit b, chloroplastic	*Ochrosphaera neapolitana*	gi|2493071	2.0	6.2
9	2.8 ± 0.7	8.9	4.1	Photosystem II D2 protein	*prymnesiophyte* C19847	tr|D9MYL1|D9MYL1_9EUKA	6.0	12.5
10	2.4 ± 0.4	9.0	5.2	ATP synthase subunit b, chloroplastic	*Ochrosphaera neapolitana*	gi|2493071	6.0	14.0

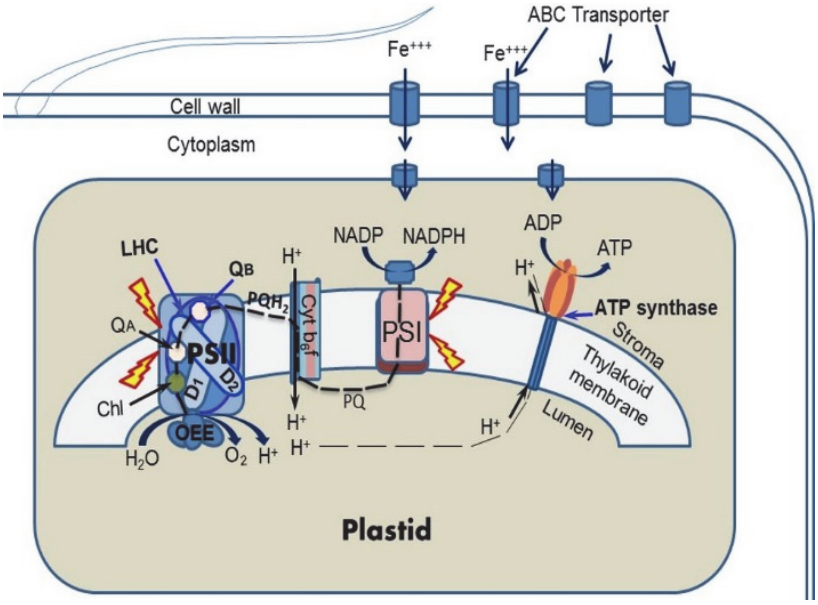

Fig. 3. Biochemical pathways and processes of photosynthesis in *Prymnesium parvum* (hypothetical) under low (LF) Fe conditions. The figure shows how upregulated proteins (*in bold*) involved in photosynthetic activities of the microalga under LF. *Thick dotted line* is the electron transport pathway in photosynthesis. Compared to photosystem (PS) I and cytochrome b_6f, the biosynthesis of PSII increased under LF.

PSII (2 atoms of Fe per complex) (Strzepek and Harrison 2004). Since PSI and Cyt b_6f require a higher amount of Fe than PSII, phytoplankton may upregulate several component proteins of PSII, as it was found in the present study, to maintain photosynthesis under LF. Previous studies have reported that the ratio of PSII to PSI is 1:1 for terrestrial plants (Raven *et al.* 1999), while it is 2:1 for phytoplankton in Fe-rich coastal areas and 10:1 for phytoplankton in nutrient poor, low Fe oceanic waters (Strzepek and Harrison 2004). As PSI and the Cyt b_6f complex contain considerably more Fe than PSII, these biochemical alterations indicate an adaptive strategy of marine phytoplankton to minimize their normal Fe requirement (Geider and LaRoche 1994, Strzepek and Harrison 2004). In response to Fe-deficiency, marine cyanobacteria and phytoplankton exhibit reduced efficiency of photochemistry in PSII (Geider and LaRoche 1994). However, compared to PSI and Cyt b_6f, high abundance of PSII is assumed to be able to effectively balance excitation energy and electron flow in the photosynthetic reactions. Marine phytoplankton, *P. parvum,* that can grow under very low Fe concentrations might employ such strategies (Strzepek and Harrison 2004).

A protein (spot 4, Fig. 2) of 20.3 kDa was highly upregulated (13.2 ± 2.7 fold) under LF. A putative uncharacterized protein (spot 5, Fig. 2) of 19.7 kDa was also upregulated under LF. The active sites and functions of these two proteins in phytoplankton are unknown. Further studies are needed to know more about these proteins.

Increased biosynthesis of ATP synthase: Biosynthesis of chloroplast ATP synthase (spots 2, 8, and 10; Fig. 2) was upregulated under LF (Table 2). Compared to HF, chloroplast ATP synthase subunit alpha and subunit b were upregulated by 2.3 ± 0.5 and 6.6 ± 1.3 fold, respectively, under LF (Table 2). Some unrecognized spots in line with subunit alpha are assumed to be the same protein or other subunits of the same protein. Fe limitation has been reported to enhance photorespiration (Allen *et al.* 2008), a process that increases ATP and NADPH requirements (3 ATP and 2 NADPH). It has been thought to be supplied by photosynthetic reactions that produce the excess of ATP and NADPH (Foyer *et al.* 2009). Under LF, the increase of chloroplastic ATP synthase in *P. parvum* might satisfy the increased need of ATP for photorespiration and other metabolic activities in the cell.

Conclusion: Under low Fe conditions, the *P. parvum* altered its cellular processes by upregulating several proteins involved in photosynthesis, particularly the PSII. The upregulation of PSII-associated proteins might result in the increased abundance of PSII complexes, while the upregulation of chloroplastic ATP synthase is likely to be a strategy of the phytoplankton to meet cellular energy requirement. *P. parvum* probably undergoes photorespiration under LF, and the phytoplankton supplies the demand of high metabolic energy (ATP and NADPH) for photorespiration by increasing ATP synthase in chloroplast. Also the LF condition was likely to induce oxidative stresses in the phytoplankton that may result in degradation of photosynthetic proteins such as PSII D1. Further studies are needed to confirm oxidative stresses in phytoplankton under LF conditions.

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