Biochemical and Biophysical Research Communications 375 (2008) 241–246

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Association between the availability of environmental resources and the atomic composition of organismal proteomes: Evidence from *Prochlorococcus* strains living at different depths

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ARTICLE INFO

Article history:
Received 26 July 2008
Available online 14 August 2008

Keywords:
Amino acids
Proteome
Carbon content
Nitrogen content
Sulfur content
Resource availability
Prochlorococcus
Oceanic niches
Depth
Phosphorus

ABSTRACT

The cyanobacteria *Prochlorococcus* is a cyanbacterial genus, with some strains adapted to sea surface environments, which are poor in nutrients and have high-light intensity, and some strains adapted to deep sea conditions, which have relatively higher concentrations of nitrogen and phosphorus and lower light intensity. Here, we report pairwise comparisons between strains isolated from different depths of the same sea, which reveal a close association between atomic composition of the proteome and the availability nitrogen and phosphorus in the environment. The atomic composition of proteomes differs significantly among *Prochlorococcus* strains with different supplies of nitrogen *in vivo*; these different supplies result from different capacities for nitrogen assimilation. We repeated our whole-proteome analysis with the core proteomes of *Prochlorococcus* and obtained similar results. Our findings indicate that the elemental composition of proteomes is shaped by the availability of resources in the environment.

In natural environments, the elemental composition of available resources often does not correlate with the elemental demands of organisms. Numerous studies in ecological stoichiometry have revealed that these elemental imbalances can affect the elemental composition, metabolism, physiology, and life-history of organisms [1–3].

In unicellular organisms, proteins account for more than 50% of biomass [4,5]; in fact, a highly abundant protein may have 10^5–10^6 copies per cell [6,7]. The genetic changes that cause amino acid substitutions and alter the atomic content of proteins may thus influence the atomic budget of cells. In contrast to physiological changes, heritable genetic changes are relatively long-term and stable. A number of studies suggest that the elemental imbalances in environments may affect the content of carbon, nitrogen, and sulfur in proteins. For example, under conditions of sulfur starvation, microorganisms were found to preferentially express proteins with low sulfur content [8–11].

The atomic composition of the proteome varies considerably among species [12,13], and at least the variation in sulfur content has been found to depend on environmental conditions [14]. When an element such as carbon, nitrogen, or sulfur is in short supply, organisms are expected to increase their expression of proteins responsible for assimilating it. In *Escherichia coli*, *Saccharomyces cerevisiae,* and *Salmonella typhimurium*, the assimilating enzymes contain a lower proportion of the element that they assimilate than the proteome as a whole [15,16]. In addition, highly abundant proteins in plants and microorganisms were found to have lower atomic contents ([17] Li et al. under review). To further investigate the effect of environmental constraints in shaping the carbon, nitrogen, and sulfur) content of proteins, we studied the relationship between proteome atomic composition and environmental elemental supply among *Prochlorococcus* strains. These strains are closely related phylogenetically, but have adapted to different ecological niches.

In oceans, there are gradients of light and nutrients through the water column (Fig. 1) [18]. The surface waters are rich in light, but poor in nitrogen and other inorganic nutrients; the deep ocean, in contrast, has higher concentrations of nutrients but lower light intensity. The cyanobacteria *Prochlorococcus* is a group of marine autotrophs that generate biomass from sunlight, CO_2, and inorganic nutrients. It has both high-light strains that adapt to surface water and low-light strains that adapt to deep sea environments (>100 m) [18–23]. In total, 12 genomes of *Prochlorococcus* strains have been sequenced. By comparing *Prochlorococcus* strains isolated from different depths, we found an association between atomic composition of the proteome and the availability of environmental resources.

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doi:10.1016/j.bbrc.2008.08.011

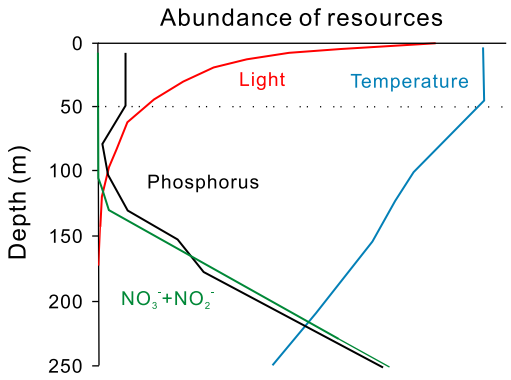

Fig. 1. Changes of resource abundance with water depth (*m*). Modified from Fig. 1b of [18].

Materials and methods

The proteome sequences of 12 *Prochlorococcus* strains (AS9601, MIT9211, MIT9215, MIT9301, MIT9303, MIT9312, MIT9313, MIT9515, MED4, NATL1A, NATL2A, and SS120) were obtained from Kettler et al. [24]. All 12 genomes share a total of 1273 genes, which can be considered the core genome [24]. For accurate pairwise comparisons, we analyzed 1221 genes that have one-to-one homologs in all 12 strains. The carbon and nitrogen content of a given protein were defined as the average number of carbon and nitrogen atoms per residue side chain. Sulfur content was calculated similarly, except that the initiating methionine residues were removed from all of the protein sequences [15].

Results

Association between the atomic composition of proteomes and sea depth

To be able to detect an association between proteome atomic content and the availability of resources in natural environments, we sought to control for other variables that might affect the atomic stoichiometry. In addition to depth, sea waters may differ in their elemental composition and light intensity because of different latitudes, different distances from the sea coast, and other environmental factors. Thus we selected four groups from the 12 sequenced *Prochlorococcus* strains, based on where they were isolated [25] (Fig. 2). It is important to take into account the fact that a strain with a greater ability to assimilate a particular element contains a higher amount of that element. These differences in availability of resources *in vivo* may mask the effects of environmental availability of resources on the atomic composition of the proteome. Different strains of *Prochlorococcus* differ most significantly in their ability to assimilate nitrogen. According to the results of Moore et al. [26] and Kettler et al. [24], MIT9211, MIT9215, MIT9301, MIT9312, MIT9515, and SS120 can utilize nitrogen only in the form of NH_4^+, while MIT9313, MIT9303, NATL1A, and NATL2A can utilize nitrogen as both NH_4^+ and NO_2^-. To compare strains with similar abilities to use nitrogen and adapted to the same environmental conditions, we defined three groups: Equatorial Pacific (including MIT9211, MIT9215, and MIT9515), Sargasso Sea (including SS120 and MIT9301), and North Atlantic (including NATL1A and NATL2A).

In the Equatorial Pacific group, MIT9211 was isolated from deep ocean waters while MIT9515 and MIT9215 were isolated near the sea surface (Fig. 2). Consistent with their depths in the water column, MIT9211 has a lower carbon content and higher nitrogen and sulfur content than MIT9215 and MIT9515; MIT9215, and MIT9515 do not differ significantly in carbon, nitrogen, or sulfur content (Fig. 3 and Supplementary Table S1).

In the Sargasso Sea group, SS120 was isolated at a depth of 30 m deeper than MIT9301 (Fig. 2). Correspondingly, SS120 has significantly lower carbon content and higher nitrogen and sulfur content than MIT9301 (Fig. 3 and Supplementary Table S1).

In the North Atlantic group, two strains (NATL1A and NATL2A) were isolated near the sea surface, at a difference of 20 m in depth (Fig. 2). Their proteomes do not differ significantly in carbon, nitrogen, or sulfur content (Fig. 3 and Supplementary Table S1).

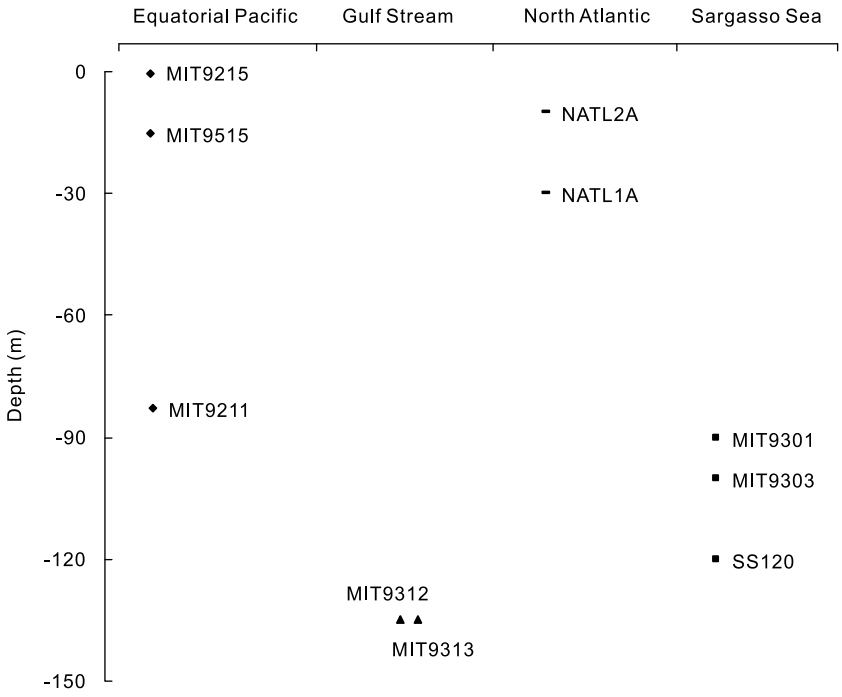

Fig. 2. Locations and depths of isolation of the *Prochlorococcus* strains analyzed in this study. Data were obtained from [25].

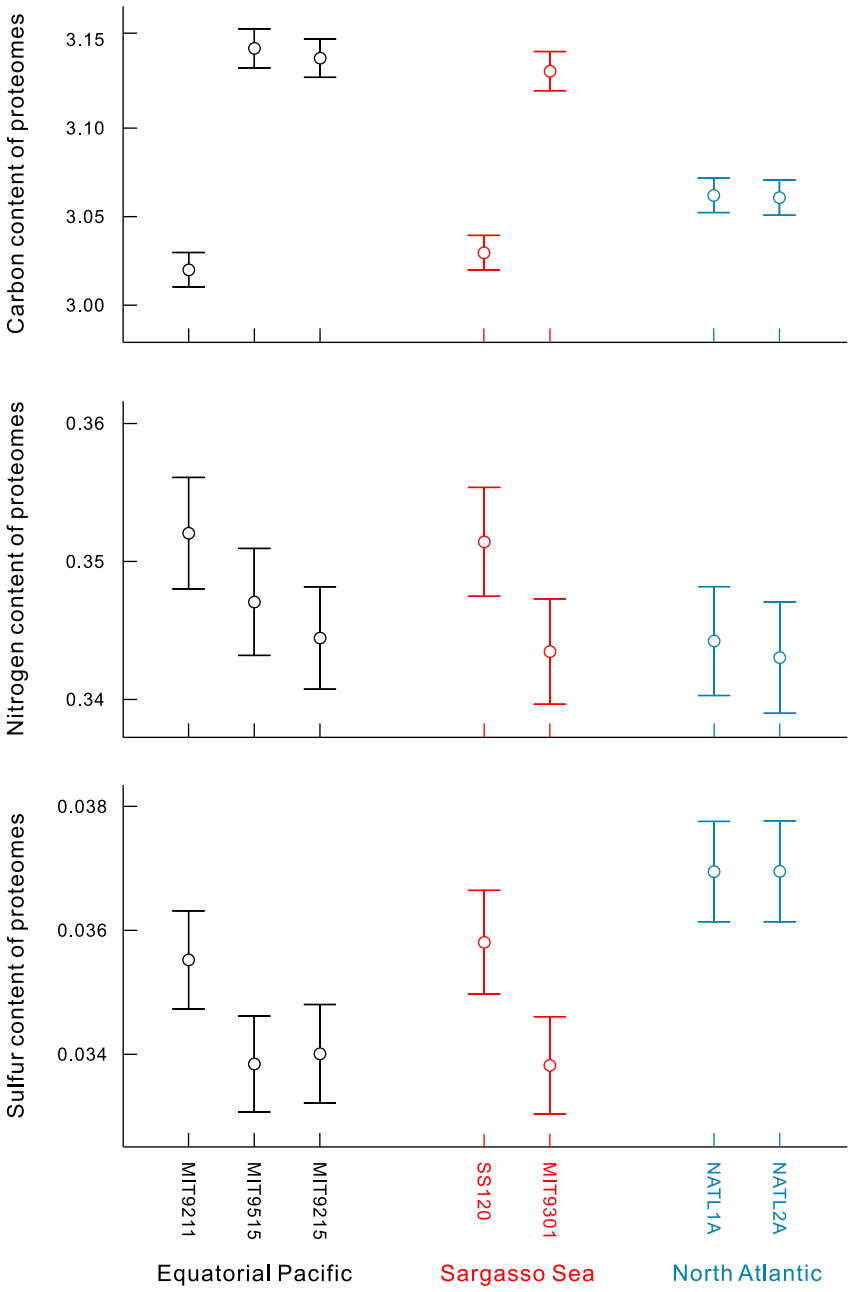

Fig. 3. Comparisons of atomic compositions of proteomes from different *Prochlorococcus* strains. Only those strains isolated from the same location and with similar abilities to assimilate nitrogen were compared. We performed Mann–Whitney *U* tests to assess the significance of the differences among strains, and results are presented in Supplementary Table S1.

Association between the atomic composition of proteomes and ability to use nitrogen

In the previous section, we proposed that the ability to assimilate nitrogen may affect the atomic composition of the proteome. MIT9312 and MIT9313 are ideal microorganisms to use to test this idea, since they both grow at a depth of 135 m in the Gulf Stream (Fig. 2) [25,27], and MIT9312 can utilize only NH_4^+, while MIT9313 can utilize both NH_4^+ and NO_2^- [24,26]. We found that MIT9313 has significantly higher nitrogen and sulfur content and lower carbon content than MIT9312 (Fig. 4 and Supplementary Table S1). These differences in sulfur and carbon content suggest that the *in vivo* nitrogen availability affects sulfur and carbon budgets; alternatively, the differences may reflect an undiscovered difference in the ability to assimilate sulfur and carbon.

Similar results from analysis of additional core proteomes

Kettler et al. [24] identified 1273 genes shared among all sequenced *Prochlorococcus* strains, which can be considered the core genome. These genes are thought to encode the essential functions of living cells. Differences in the atomic composition of these proteins are likely to reflect the environmental resource constraints experienced by specific *Prochlorococcus* strains. Restricting our analysis to the core genome is important, since the atomic composition of proteins encoded by recent horizontal gene transfer reflects, to some extent, the environmental

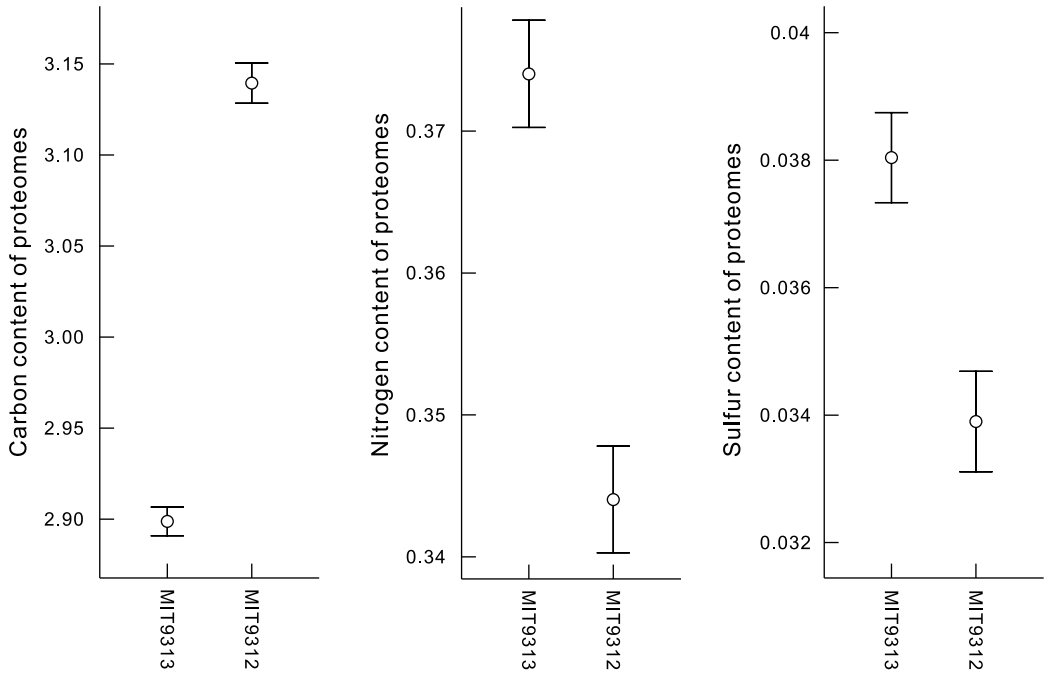

Fig. 4. Comparisons of atomic compositions of proteins from *Prochlorococcus* strains MIT9312 and MIT9313. These two strains were isolated from the same depth in the same location (Gulf Stream), but they have different abilities to assimilate nitrogen [24,26]. MIT9312 can utilize only NH_4^+, while MIT9313 can utilize both NH_4^+ and NO_2^-.

Table 1
Pairwise comparisons of atomic compositions of proteins from different *Prochlorococcus* strains*

Location	Comparison (strain a vs. strain b)	Atomic content	Percentage of proteins (%)		
			a > *b*	*a* < *b*	*P*
Equatorial Pacific	MIT9515 vs. MIT9215	Carbon content	50	47	0.403
		Nitrogen content	49	43	0.494
		Sulfur content	38	39	0.097
	MIT9515 vs. MIT9211	Carbon content	86	13	10^{-155}
		Nitrogen content	37	62	2×10^{-60}
		Sulfur content	42	53	2×10^{-19}
	MIT9215 vs. MIT9211	Carbon content	87	12	10^{-157}
		Nitrogen content	36	63	5×10^{-57}
		Sulfur content	42	54	10^{-23}
Sargasso Sea	MIT9301 vs. SS120	Carbon content	84	15	7×10^{-147}
		Nitrogen content	39	62	10^{-41}
		Sulfur content	41	54	3×10^{-24}
North Atlantic	NTAL2A vs. NTAL1A	Carbon content	39	39	0.850
		Nitrogen content	31	31	0.781
		Sulfur content	15	13	0.945
Gulf Stream	MIT9312 vs. MIT9313	Carbon content	95	5	3×10^{-193}
		Nitrogen content	21	78	2×10^{-223}
		Sulfur content	30	67	2×10^{-103}

* Only proteins with one-to-one homologs shared across all 12 *Prochlorococcus* species, i.e. proteins of the core genome of *Prochlorococcus*, were compared (1221 pairs of proteins). The significance of differences was determined using the Wilcoxon signed-rank test.

resource constraints experienced by the donor strain or species [24]. Thus, to avoid variation in proteome atomic composition arising from gene gain and loss, we compared the atomic contents of proteins encoded by the core genomes of *Prochlorococcus* strains.

As shown in Table 1, strains isolated from deeper marine waters have lower carbon content, higher nitrogen content, and higher sulfur content than strains isolated near the surface of the same location. Furthermore, differences in the ability to assimilate nitrogen are associated with the differences in atomic composition of the proteomes (Table 1). These results are consistent with those obtained by comparing whole proteomes.

Discussion

In this study we compared the proteomic atomic composition of *Prochlorococcus* strains isolated from the same location with similar abilities to assimilate nitrogen. We found a clear association between the depth of water and the proteomic atomic composition. Within the range of sea depths inhabited by *Prochlorococcus* species, the depth negatively correlates with light intensity and temperature, while it positively correlates with the concentration of nitrogen and phosphorus [18]. A logical inference is that the availability of resources affects the atomic composition of the proteome of *Prochlorococcus*.

SS120 and MIT9301 were isolated at depths differing by 30 m, while NATL1A and NATL2A were isolated at depths differing by 20 m. It is interesting that a depth difference of 30 m, but not of 20 m, leads to differences in proteomic atomic composition. As shown in Fig. 1, in waters above a depth of approximately 50 m, the concentration of inorganic nutrients and temperature do not vary noticeably (Fig. 1). NATL1A and NATL2A were all isolated from waters above a depth of approximately 50 m, which may explain their similar contents of nitrogen and sulfur.

In contrast to inorganic nutrients and temperature, light intensity attenuates rapidly at depths less than 50 m but gradually at depths greater than 50 m (Fig. 1). NATL1A and NATL2A experience quite different light densities. However, we did not find any significant difference in the atomic composition of their proteomes. This observation implies that light intensity, affecting the availability of energy, is unlikely to have a strong effect on proteome carbon content. This is consistent with our previous results in which the selection of protein building blocks, rather than energy availability, was found to explain the preferential use of low-carbon-content amino acids in highly expressed proteins (Li et al., under review). It seems that energy constraints may not affect the evolution of organisms as much as expected.

The differences in carbon content observed between the strains, such as between SS120 and MIT9301, are more likely to be a consequence of the interaction between the carbon budget and the nitrogen or phosphorus budget, as shown by Bertilsson et al. [28]. These investigators found that limiting phosphorus increases the carbon content and the carbon:phosphorus ratio of *Prochlorococcus* strain MED4.

Like the light intensity, temperature falls with increasing water depth [18]. However, Fu et al. [29] recently found that increasing temperature is not associated with significant changes in elemental ratios of *Prochlorococcus*. They also found that the elemental ratios of *Prochlorococcus* are unaffected by changes in the level of CO_2. Therefore, even if dissolved CO_2 concentration decreases with increasing water depth—an observation that still lacks strong empirical evidence—the CO_2 concentration is unlikely to affect the proteome atomic composition of *Prochlorococcus*.

In summary, we suggest that the atomic composition of *Prochlorococcus* proteomes is shaped by the environmental availability of nitrogen and phosphorus. Species that are phylogenetically closely related and that grow at different depths of the ocean have also been found in the eukaryotic genus *Ostreococcus* [30]. *O. luminaries* is adapted to the sea surface, while *Ostreococcus sp.* RCC141 is adapted to the low-light conditions of the deep sea [31]. Thus, our analysis should be repeated with these two eukaryotic algae once the genome sequence of *Ostreococcus sp.* RCC141 is available. In a sea or a lake, deep winter mixing triggers ultraphytoplankton succession every year. Species in the chain of succession are adapted to the changing availability of nutrients, primarily nitrogen and phosphorus, which varies from abundance to scarcity in the progression from spring to summer [32–34]. With the advance of genome sequencing, we may have the opportunity in the future to compare the atomic compositions of proteomes from these species.

Acknowledgments

We thank Dr. Gregory C. Kettler (Massachusetts Institute of Technology) for sending us the protein sequences of the core and flexible genomes of the annotated *Prochlorococcus*. This study was supported by NCET-07-0094 (Ministry of Education of China) and by Beijing Normal University.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.08.011.

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