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Phycobilisome composition in *Chondrus crispus* (Gigartinales, Rhodophyta) from a wild type strain and its vegetatively derived green mutant

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Intact phycobilisomes from a wild-type red *Chondrus crispus* and its vegetatively derived green mutant were isolated by centrifugation through a discontinuous sucrose density gradient. Pigment composition was subsequently characterized by spectrophotometry. Vegetative thalli of the two strains grown together for six months in the laboratory resulted in different pigment profiles. Two pigmented phycobilisome bands appeared in the sucrose gradient of the wild-type alga, a purple coloured one, and a pink one, whereas only a single blue band appeared in the gradient of the green mutant. Spectrophotometric and fluorescence analyses identified the phycobiliprotein composition of the purple band as the typical phycoerythrin–phycocyanin-allophycocyanin complement in the wild-type, but there was no detectable phycoerythrin present in the blue band of the green mutant. Sodium dodecyl sulphate, preparative polyacrylamide gel electrophoresis analysis confirmed the presence of allophycocyanin subunits in all extracts, but firm evidence of an R-phycoerythrin linker polypeptide in the blue band was missing. These results highlight the ability of *C. crispus* to adapt to a phycoerythrin deficiency by adjusting light harvesting pigment ratios.

Key Words: *Chondrus crispus*; green mutant; phycobilisomes; phycoerythrin; pigmentation

INTRODUCTION

Phycobilisomes (PBS), the supramolecular structures of pigment-protein complexes of red algae and cyanobacteria, serve as integral light harvesting modules (e.g., Arteni et al. 2008, Shi et al. 2011). The functional versatility of PBS is critical to the evolutionary fitness of their hosts. For over a billion years they have enabled the red algae and cyanobacteria to adapt to fluctuations in life sustaining irradiance, effectively enhancing the organisms' repertoire of adaptation mechanisms. The full complement of phycobiliproteins (PBP) contained in a typical red algal PBS includes R-phycocyanin (R-PC), allophycocyanin (APC), and three forms of phycoerythrin: B-phycoerythrin (B-PE), present in the more primitive red algae, R-phycoerythrin (R-PE), occurring primarily in the higher red algae, and C-phycoerythrin (Lee 2008). The PBP are each composed of two subunits, α and β, and their associated structural components (Grossman et al. 1993).

In nature, well-nourished *Chondrus crispus* are deep reddish brown thalli, the colour providing visual evi-

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dence that the PBS are present and nutritional levels are adequate. Some red algae have two kinds of PBS, presumably to predispose them to better acclimation strategies by providing an intermediary organization step of light harvesting pigments (Algarra et al. 1990). Lüder et al. (2001) isolated intact PBS from *Palmaria decipiens* (Reinsch) R. W. Ricker and *Palmaria palmata* (Linnaeus) Weber & Mohr. They demonstrated that the absorption spectra of the phycobiliprotein components in each of the two PBS pools separated on a sucrose density gradient were not significantly different from one another. This result suggests the possibility of a physiological role in photoacclimation attributed to the two distinct structures of PBS. When they isolated the pigment proteins using sodium dodecyl sulphate, preparative polyacrylamide gel electrophoresis (SDS-PAGE) analysis, three low molecular weight polypeptides were found in one of the PE-subunits and assumed to be dissociation products of a missing γ-subunit. The γ-subunit usually contained in PE contributes to the expansion of the absorbance range of red algae, allowing for increased light harvesting ability (Liu et al. 2008). Algarra et al. (1990) isolated two distinct PBS structures in *Porphyra umbilicalis* (L.) J. Ag. and determined that one of the structures was characterized by a lower relative content of R-PE in relation to R-PC and APC compared to the second one. These authors suggest that the presence of differentially enriched PBS within the same alga could function to enhance the response time as a light acclimation strategy.

Phycobiliprotein complexes are actively formed, dismantled, and rearranged in PBS in response to light conditions and nutrient status. As the most peripheral phycobiliprotein to the APC core, the uncoupling of energy transfer often begins with PE as it becomes dissociated from the PBS complex. Under higher ionic conditions however, the initial uncoupling can occur between PC and APC. These dissociation patterns are thought to be species specific (Gantt et al. 1979, Grossman et al. 1993). Phycoerythrins are the most abundant PBP in many red algae and they display rapid binding and dissociation abilities (Algarra et al. 1990, Glazer 1994, Talarico 1996). The presence of two different PBS structures in some red algae may enhance their ability to acclimate quickly to changing light environments by maintaining a pool of PE PBP in a state of metabolic flux (Algarra et al. 1990, Lüder et al. 2001).

In the present study, intact PBS were isolated from *C. crispus* wild type plants in a discontinuous sucrose density gradient and compared to those of a vegetative green colour mutant. As shareholders in the same genotypical parent, an analysis of the degree of pigment variability between the two clones would be instructive. It could lead to the identification of specific PBS subunit complexes responsible for the expression of the unique blue-green colour of the mutant, which is made visually apparent by a shortage of red pigment. Furthermore, if the presence of differentially organized, but well-coupled PBS in an alga functions as an acclimation strategy, then it is reasonable to expect that two pools would be present in *C. crispus*, an intertidal species found in cool temperate climates.

This study compared the PBP complement of a wild type red alga to that of a green colour mutant that arose vegetatively on a frond of the original wild type plant. Ascertaining that the green mutant was PE deficient, and quantifying the resultant ratios of PC and APC would be instructive in establishing commercial value of the alga as a crop. Certain algal pigments have specialized applications, such as in clinical and immunological fluorescence analyses, as natural colorants, and as therapeutic agents for diseases associated with chronic oxidative imbalances (Farooq et al. 2006, Sekar and Chandramohan 2008, Ivanova et al. 2010). Unique pigment ratios contained in the commercially cultivated green mutant should be identified and characterized for potential market application. The potential for improving growth rate by understanding the light harvesting capabilities of the green alga is also of commercial interest.

MATERIALS AND METHODS

Algal material and culture conditions

A green colour mutant was derived vegetatively from a red (wild-type) strain of *Chondrus crispus* that has been cultivated in the laboratory at Acadian Seaplants Ltd. (Cornwallis, NS, Canada) for over 20 years (Fig. 1). Both the original red strain and the green mutant strain are proprietary to Acadian Seaplants Ltd. For this study, equivalent amounts of biomass of each strain were grown in pasteurized seawater supplemented with f/2 nutrients (McLachlan 1973) in a 15 L culture vessel at 17 ± 2°C. Medium was changed weekly. Cultures were initially exposed to an irradiance of 155-165 μmol m^{-2} s^{-1} at 14 : 10 L : D provided by 34 W cool white fluorescent lamps. Conditions were held constant for six weeks to produce enough biomass so that the same stocking density could be maintained in larger (60 L) culture vessels. In the combined culture, the green mutant grew at about 75% of the rate of the red wild type (L. Cornish unpublished data).

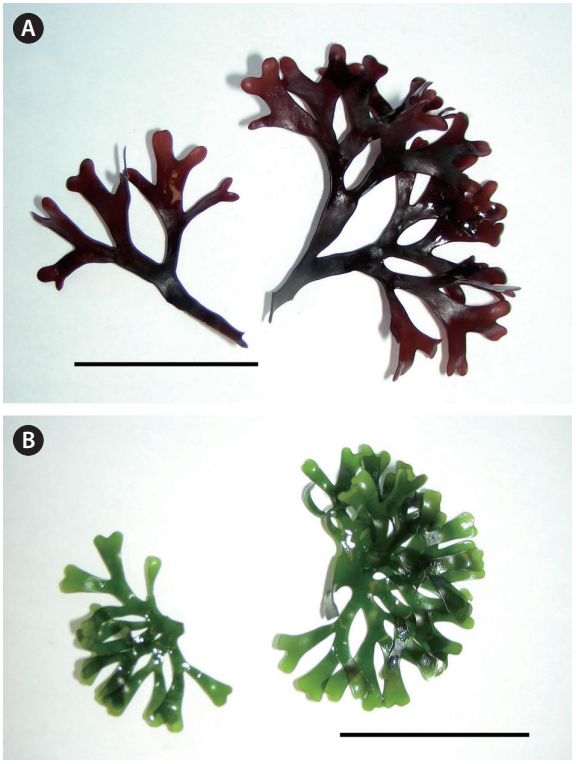

Fig. 1. Fronds *Chondrus crispus* showing red, wild-type (A) and its green mutant strain (B). Scale bars represent: A & B, 5 cm.

Irradiance was then reduced to 75-80 µmol m^{-2} s^{-1} at 16 : 8 L : D for another six weeks, to encourage pigment development.

Isolation of PBS

The PBS extraction procedure was adapted from Algarra et al. (1990), and Gray and Gantt (1975). The algal samples (20 g fresh weight) were washed in pasteurized seawater and rinsed twice in 0.65 M phosphate buffer before placing them into a -80ºC freezer for approximately 24 h. After removal from the freezer, 5.0 g of each algal sample was ground in liquid nitrogen and approximately 2 g of the ground material was added to 50.0 mL extraction solution consisting of 49.0 mL Sorensens' phosphate buffer (Bozzola and Russell 1999) at pH 7.0, 7.0 µL β-mercaptoethanol, 10 protease deactivator tablets (Ref. #11 836 170 001; Roche Diagnostics GmbH, Mannheim, Germany) and 1.0 mL Triton X-100 in a 100 mL beaker. The samples were stirred gently in the dark at 4°C for 30 min. Cell debris was removed by centrifugation at 20,000 rpm (33,600 ×g) for 15 min at 4°C (Beckman J2-M1 centrifuge with fixed angle rotor JA20; Beckman Inc., Palo Alto, CA, USA), and 4-5 mL aliquots of the pigmented supernatant were layered on top of a discontinuous sucrose step gradient (similar to Gantt and Lipschultz 1972) in 0.65-M phosphate buffer (4.0 mL 1.5 M, 7.0 mL 1.0 M, 8.0 mL 0.75 M, 7.0 mL 0.5 M, and 7.0 mL 0.25 M sucrose). The remaining supernatants were retained as whole crude extracts and analyzed separately. Discontinuous sucrose density centrifugation was performed at 27,000 rpm (96,508 ×g) for 17.5 h (Beckman Coulter Le-Bok "Optima" Ultracentrifuge with SW28 swinging bucket rotor) at 4°C. After centrifugation, each of the pigmented PBS bands isolated from the algal samples were withdrawn from the sucrose gradient using a B-D 1M11/2 23GTW needle (Becton Dickinson and Co., Rutherford, NJ, USA) and a 5.0 mL syringe, and collected into 15 mL Fisherbrand plastic vials, covered with aluminum foil to prevent light penetration and frozen at -20°C.

SDS-PAGE

PBS samples were thawed and diluted with 0.65 M phosphate buffer solution at pH 6.97 and centrifuged at 3,300 ×g through an Amicon Ultra Centrifugal Filter Devices in a 1EC Central MP4R SB centrifuge (Millipore, Billerico, MA, USA) for 20 min at 4°C. The PBS were retained on the filter while buffer and sucrose passed through. Retained PBS were re-suspended in fresh phosphate buffer and the centrifugation step was repeated three times to further concentrate the pigment proteins and to minimize the interference effects of the sucrose and some of the other compounds in the samples, such as Triton-X.

A 12% polyacrylamide resolving gel was prepared with a 4% stacking gel and 10 µL samples of whole crude PBS extracts from red and green thalli as well as concentrated PBS samples isolated from sucrose density gradient bands were diluted 1 : 1 in SDS-PAGE sample buffer and loaded into separate lanes in the stacking gel for protein subunit analysis. Samples were loaded by volume and were not normalized for equivalent protein concentrations. Precision Plus (Bio-Rad Laboratories Inc., Richmond, CA, USA) protein standards were resolved in adjacent lanes for molecular mass determination. SDS-PAGE was conducted as per standard operating protocol (Laemmli buffer system). When the majority of the samples reached the bottom of the resolving gel, the gel was removed and washed three times in double distilled (dd) H_2O then placed in Biosafe Coomassie stain (Bio-Rad Laboratories Inc.) overnight, and subsequently destained with dd H_2O.

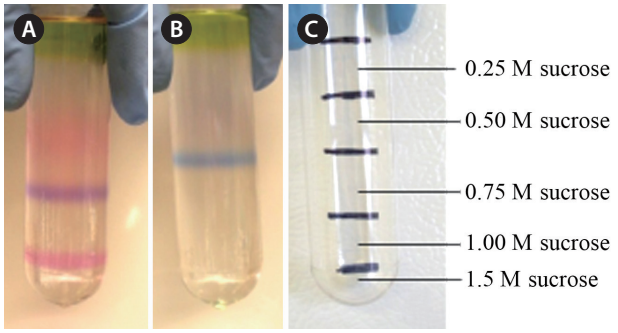

Fig. 2. Phycobilisome bands isolated by sucrose density gradient from members of a cultivated clonal population of *Chondrus crispus*. Natural red wild-type (A), a green colour mutant (B), and an empty tube showing sucrose gradient position (C).

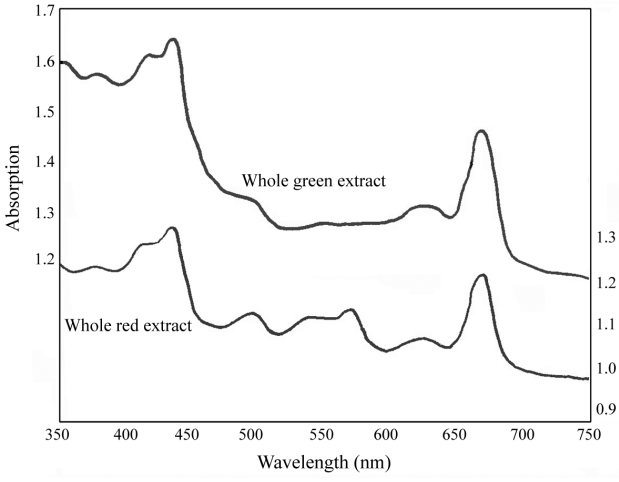

Fig. 3. Absorption spectrum of the whole red extract (lower spectrum) the whole green extract (upper spectrum) before separation in the sucrose density gradient.

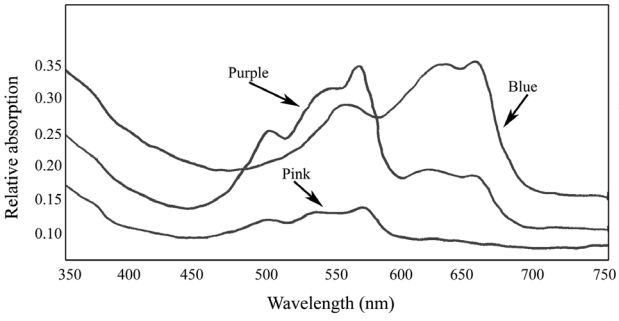

Fig. 4. Absorbance spectra of isolated pigment bands from red and green *Chondrus crispus* extracts.

Absorption and fluorescence

The absorption spectra for the pigment samples were recorded at room temperature with a SpectraMax Plus Spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). The fluorescence emission spectra were carried out with a SpectraMax GeminiXS Spectrafluorometer (Molecular Devices) at room temperature with an excitation of 450 nm (similar to Algarra et al. 1990). Absorbance values at 498.5, 614, and 651 nm of both the crude PBS extracts and of the pigmented PBS bands isolated from the sucrose gradient were used to calculate phycobiliprotein content by the following equations (Kursar et al. 1983, Beer and Eshel 1985):

$$PE\ (mg\ mL^{-1}) = [(A_{564} - A_{592}) - (A_{455} - A_{592}) \times 0.20]\ 0.12$$
$$PC\ (mg\ mL^{-1}) = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51]\ 0.15$$
$$APC\ (\mu g\ mL^{-1}) = 181.3 \times A_{651} - 22.3 \times A_{614}$$

RESULTS

PBS isolation profiles resolved by discontinuous sucrose density gradient centrifugation of crude extracts from each algal strain were clearly different from one another. The wild type *C. crispus* sample exhibited two differentially pigmented zones, a purple PBS band in the 0.75 M sucrose layer and a pink PBS band located just above the 1.5 M layer (Fig. 2). A diffuse pink tint was also visible throughout the 0.5 M sucrose layer. In contrast, the green mutant yielded only a single, bright blue PBS band located above the 0.75 M sucrose layer (Fig. 2). There was no obvious evidence of carotenoids or phycoerythrin proteins in the green extracted samples, and there was a relatively wide green pool on the top of the gradient, presumably chlorophyll. A bluish tint was barely discernable throughout the 0.25 and 0.5 M sucrose layers.

The absorption spectra of the extracts before separation in the sucrose density gradient indicated the presence of phycoerythrin (565 nm) in the wild type red plant (Fig. 3). There was no apparent absorbance maximum for PE in the green mutant (Fig. 3), compared to the red strain. The whole red extract also displayed absorption peaks at 495 nm and 540 nm, suggesting the presence of R-phycoerythrin type I (Gantt and Lipschultz 1972). In both the red and green whole extracts, there was an obvious absorbance maximum at ~650 nm, indicative of APC.

Absorbance spectra of the pigment proteins isolated by the sucrose density gradient showed that the purple band from the wild type *C. crispus* consisted primarily of

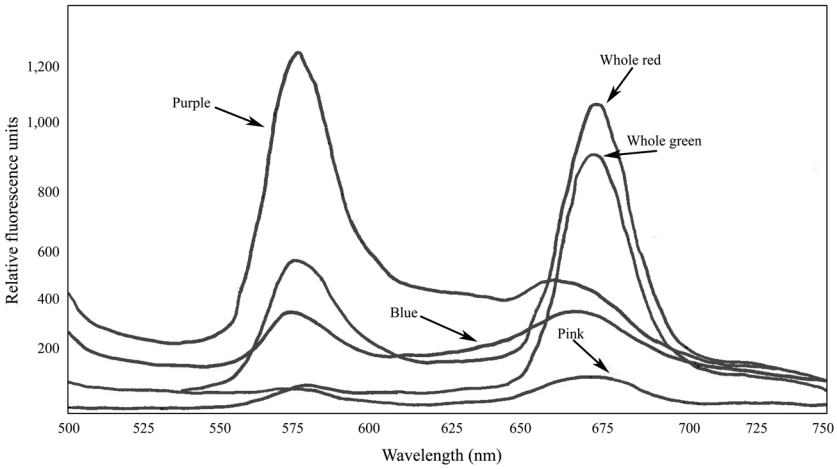

Fig. 5. Fluorescence spectra of *Chondrus crispus* pigment extracts excited with 450 nm light at room temperature.

R-PE type I (3 peaks, 498, 540, and 565) (Su et al. 2010*b*) with some absorption at approximately 620 nm and 650 nm, indicating the presence of PC and APC (Fig. 4). The pink band also consisted of PE, but there was no clear evidence of PC or APC maxima. Conversely, the bright blue band isolated from the green mutant demonstrated strong absorbance at about 620 and 650 nm, corresponding most likely to PC and APC. The APC content in the blue band was twice as much as in the purple band (Table 1).

Fluorescence spectra of the whole crude extracts indicated the presence of functional PBS in the samples of both *C. crispus* strains evidenced by well-identified APC emission peaks at 670 nm (Fig. 5). However, a smaller B-PE peak (574 nm) in the red extract suggests the presence of some dissociated PBS as well. It is important to note that sample preparation steps included freezing, which can cause PBS to dissociate. Fluorescence spectra of the pigmented bands indicate that the isolated PBS were mostly dissociated, although there was some fluorescence around 670 nm in all cases, suggesting that a relatively small number of intact PBS were still present (Fig. 5). While phycoerythrin content at 9.10 μg mL^{-1} appeared to be present in a substantial amount in the whole extract from the red *C. crispus* plants, the phycocyanin content was relatively low at 1.40 μg mL^{-1}. The whole crude green extract did not appear to contain PE, but was shown to have 4.10 μg mL^{-1} of PC, and 202.9 μg mL^{-1} of APC, compared to 158.0 μg mL^{-1} of APC in the whole red extract (Table 1).

Table 1. Pigment concentrations in whole crude *Chondrus crispus* extracts and in samples of each isolated pigment band

Sample[a]	PE (μg mL^{-1})	PC (μg mL^{-1})	APC (μg mL^{-1})
Whole red	9.1	1.4	158.0
Whole green	-	4.1	202.9
Purple band	20.4	1.4	27.1
Pink band	5.8	0.6	13.3
Blue band	-	2.5	53.9

PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin.
[a]Whole red and whole green samples represent extracts isolated in the initial crude extraction, before isolation of phycobilisomes on the sucrose gradient. The purple and the pink samples were bands isolated from the whole red extract, and the blue sample was isolated from the whole green extract. The units in μg mL^{-1} are derived from the formulae for phycobiliproteins concentrations in extracts.

Pigment content calculated for the coloured bands indicated that the purple band extracted from the red *C. crispus* had similar PE and APC contents compared to the other samples, but was relatively low in PC content, 20.4, 27.1, and 1.4, respectively. The pink band from this strain had 5.8 μg mL^{-1} of PE but very little PC, 0.6 μg mL^{-1}, and 13.3 μg mL^{-1} of APC. The blue band, also devoid of PE, demonstrated the presence of PC, 2.5 μg mL^{-1}, and APC, 53.9 μg mL^{-1} (Table 1).

The whole red extracted samples had a thin pool of carotenoids on top of the sucrose gradient, followed immediately below by a green chlorophyll zone. Below this was a narrow section of clear area, followed by a zone of pale pink, concentrated mostly in the 0.5 M sucrose segment. It is expected that the primary constituents of this pinkish zone were bits of PBS debris and dissociated PE components. The purple band isolated from the red *Chondrus* strain had similar amounts of PE and APC, giving a 0.753 ratio and almost 15 times as much PE to PC at 14.570 (Table 2). This ratio differed from that of the

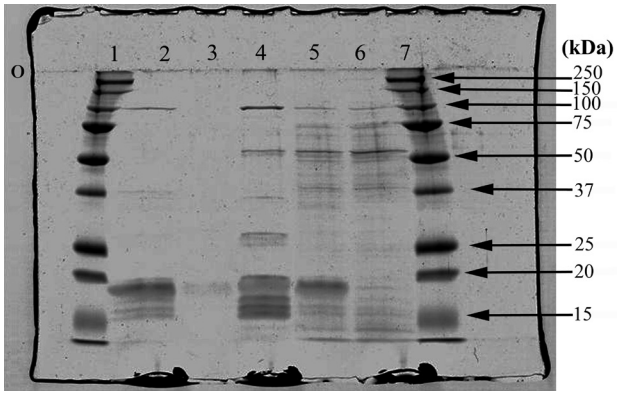

Fig. 6. Subunit complexes of isolated pigment proteins of wild type *Chondrus crispus* and its vegetative green mutant as resolved by sodium dodecyl sulphate, preparative polyacrylamide gel electrophoresis. Lanes: 1, Precision Plus Protein Standard; 2, sample (10 μL) of purple phycobilisomes (PBS) band; 3, sample (10 μL) of pink PBS band; 4, sample (10 μL) of blue PBS band; 5, whole extract (10 μL) from red clone; 6, whole extract (10 μL) from green clone; 7, Precision Plus Protein Standard.

whole red extract at 6.490 PE to PC. With the exception of the whole extracts, all the pigment samples had a PC to APC ratio between 0.045 and 0.052. Dry matter content of the frozen algal samples was not measured and the PBS extractions were not quantitative, therefore the pigment measurements cannot be related back to the original tissue concentrations.

The apparent molecular masses of the dissociated constituents of the protein complexes of all samples are listed in Table 3. The results of the SDS-PAGE analysis further demonstrate some differences among the phycobiliprotein subunit complexes in the extracts collected (Fig. 6).

DISCUSSION

The use of colour mutants has proved a valuable tool in red algal biology (see review by van der Meer 1990). Such mutations have played an important role in uncovering

Table 2. Ratios of the phycobiliproteins isolated from wild type red *Chondrus crispus* and its green mutant

Strain	PBS sample	Ratios of phycobiliproteins		
		PE : APC	PC : APC	PE : PC
Wild type red	Whole crude extract	0.058	0.009	6.49
	Purple band	0.753	0.052	14.57
	Pink band	0.436	0.045	9.67
Green clone	Whole crude extract	-	0.020	-
	Blue band	-	0.046	-

PBS, phycobilisome; PE, phycoerythrin; APC, allophycocyanin; PC, phycocyanin.

Table 3. Apparent molecular masses of phycobiliprotein subunit complexes from phycobilisome bands isolated from wild type red *Chondrus crispus* and its vegetative green mutant

Mass (kDa)	Purple	Pink	Blue	Whole red	Whole green	Comments
15	+		+	+	+	
15.9	+		+			
16				+	+	
17.2			+	+	+	R-PCα subunit
17.5			+	+	+	APCα subunit
18.6-19.5	+	+	+	+	+	APCβ subunit
20			+	+ (faint)	+	
25	+ (faint)		+	+	+	
26			+			
36			+	+	+	
38	+ (faint)			+	+	R-PE linker
49				+	+	
50			+	+	+	
73-74			+	+	+	
75				+	+	
100	+		+	+	+	

R-PC, R-phycocyanin; APC, allophycocyanin; R-PE, R-phycoerythrin.

the details of red algal life histories and development, in particular in the genera *Palmaria* (van der Meer and Todd 1980), *Porphyra* and *Pyropia* (Ma and Miura 1984, Mitman and van der Meer 1994, Yan et al. 2005, Niwa 2010 as *Porphyra*, Niwa and Abe 2012). In *Porphyra*, segregation of the green mutation was a key feature in identifying the position of meiosis (Ma and Miura 1984). Colour mutants have also provided important model systems for studying mechanisms of inheritance in red algae in a variety of genera including *Chondrus* (e.g., van der Meer 1981) and *Gracilaria* (Plastino et al. 1999, 2004, Costa and Plastino 2011). Some of these mutants may be artificially produced by various chemical (e.g., Yan et al. 2000) or radiation treatments (e.g., Niwa et al. 2009), or spontaneously occur in culture (e.g., *Chondrus*) (van der Meer 1981, Staples et al. 1995) such as the *Chondrus* used in this study. While green mutants may be common in nature, with frequencies of up to 19% recorded for a *Gracilaria* from Brazil (Guimaraes et al. 2003), green mutant strains of *Chondrus* (Staples et al. 1995) and *Hypnea* (Yokoya et al. 2007) generally do not perform as well as wild-type strains in culture. However, despite obvious pigment deficiencies, these mutants may be better adapted for certain light conditions. For example, a green mutant of *Hypnea musciformis* grew better at higher irradiances than its wild type progenitor (Yokoya et al. 2007). While these green mutant strains may be deficient in some phycobilin pigments as shown here, these mutants may even be deficient in the formation of PBS. For example, Pueschel and van der Meer (1984) were unable to detect them in their *Palmaria* mutant. The establishment of intact PBS associated with thylakoids remains to be established in the *Chondrus* strain used in this study. Given the variety of green mutations in *Chondrus* evaluated by van der Meer (1981), such strains would be useful to further characterize the pigment / protein composition of PBS.

The composition of red algal PBS typically consists of an APC core and several peripheral rods containing PC or PC/PE (Arteni et al. 2008, Su et al. 2010*b*). PE is the outermost pigment protein and its function is to increase the light harvesting capacity of the alga. This study has provided a clear explanation for the reduced growth rate of the natural green clone (Cornish unpublished data). An apparent inability to produce a full complement of functionally active PE proteins, accentuated by a deficiency of R-PE linker peptides would account for this inability to maximize light harvesting. Without PE, the functionality of the light-harvesting complex in the green mutant is compromised, and initial steps of light absorption must begin with PC.

The linker polypeptides are believed to mediate the assembly of PBP into highly ordered arrays within the PBS, and to facilitate the interactions between the PBS and the thylakoid membrane (Liu et al. 2005). With limitations on the amount and / or structure of PE, the green mutant would require an alternate means of energy capture and utilization. The purple PBS band from the wild type red *C. crispus* had the highest ratio of PE to APC, a condition contributing to the better growth rate in that strain. The blue band from the green *Chondrus* strain contained twice as much APC as the purple band, suggesting that APC functioned more in the energy absorption and transfer in the absence of significant amounts of PE. Based upon its absorption spectrum, the APC in the blue extract was determined to be in its trimeric form, and SDS-PAGE confirmed the purity of the APC by elucidating the α and β subunits (Su et al. 2010*a*). This, along with the increased concentration, would help account for the more pronounced staining of the APC components in the SDS-PAGE analysis of the blue band (Fig. 6), relative to the other extracts.

Unlike the two distinct pigment zones isolated by Lüder et al. (2001) from *P. palmata*, the two pigment bands isolated from the red *C. crispus* appeared in different sucrose gradient concentrations (Fig. 2). Both *P. palmata* pigment zones were present in the 35% (w/v) sucrose layer, whereas in the present study, the purple band appeared just above the 1.0 M layer, and the second band, the pink one, appeared just above the 1.5 M layer. The data from the present study agrees with Algarra et al. (1990) who isolated two pink coloured bands from *Porphyra umbilicalis* in the 0.75 M and the 1-1.5 M layers of the sucrose density gradient, although the blue band isolated from the green mutant, differed from all of the others in terms of its location just above the 0.75 M layer.

Not all the phycobiliprotein subunits and structural components such as linker polypeptides have been identified in the samples, but by making comparisons to data collected by others, some potential explanations can be proposed. Of particular interest is the polypeptide that seems to appear in the blue band only, with a molecular mass around 26 kDa. Liu et al. (2005) have identified a Rod-core linker peptide at 26.8-31.9 kDa. Furthermore, both the blue and the pink extracts appear to be missing clear evidence of an important R-PE linker protein, which suggests the PE in the pink band may be partially dissociated. This is consistent with Algarra et al. (1990) and Lüder et al. (2001) who proposed an enhanced acclimation strategy in some red algae, facilitated by a pool of PE proteins present in an intermediate state of assembly.

SDS-PAGE analysis was performed on samples after washing and concentrating them numerous times to minimize potential contaminants, such as Triton-X and sucrose. Attempts to wash and concentrate the pink-pigmented band may have contributed to some PBP losses, since this band had been isolated in the densest layer of the sucrose gradient, relative to the others, and it demonstrated significant PBS dissociation. Consequently, the sample protein from the pink band analyzed for subunit complexes was quite dilute, although this should not negate the possibility that the pink zone consisted of a pool of PE in a state metabolic transition.

Kursar et al. (1983) found that colour mutants of *Gracilaria tikvahiae* exhibiting a bright green phenotype contained little PE, and their chlorophyll free aqueous extracts were typically bright blue. In their analysis of a number of nuclear and non-nuclear controlled colour alterations, they determined that a wide variety of algal pigment mutations can exist based upon a number of possible biliprotein assemblies.

The specific phycobilisome composition of the *C. crispus* green mutant in this study differed from its clonal 'parent' plant at the cellular level, demonstrated by an absence of PE. By adjusting relative pigment ratios, the green mutant appears to be able to adapt to the PE deficiency by more fully utilizing PC and APC for light harvesting, and subsequent electron transport to PSII. Only in the normal red, wild type alga was a second pool of PE based proteins identified, and these may represent an auxiliary reserve of light absorption resources, enhancing the repertoire of adaptation mechanisms in *C. crispus*.

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