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REGULAR PAPER

Photosynthetic carbon acquisition in *Sargassum henslowianum* (Fucales, Phaeophyta), with special reference to the comparison between the vegetative and reproductive tissues

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Abstract The photosynthetic oxygen evolution characteristics were examined in both vegetative (blade) and sexual reproductive (receptacle) tissues of *Sargassum henslowianum* (Fucales, Phaeophyta) from the Shenao bay of Nanao Island, China, to establish the mechanism of photosynthetic acquisition of inorganic carbon (Ci) in this species. In natural seawater (pH 8.1, ca. 2.2 mM Ci), irradiance-saturated net photosynthetic rate (NPR) was greater by 25.3% in blade than receptacle, whereas dark respiratory rate (DR) was 2-fold higher in receptacle than blade. NPR at pH 8.1 was nearly saturated with the 2.2 mM Ci for both blade and receptacle. However, the values of the half-saturation constant for Ci were sharply increased at pH 9.0. NPR was significantly affected, but DR was remained unchanged, with the variation of the pH values in seawater. The data from the final pH value derived from the pH-drift experiments and the comparison between the measured and theoretically estimated photosynthetic rates suggested that both blade and receptacle were capable of acquiring HCO_3^- in seawater. The inhibitors experiments showed that a HCO_3^- dehydration mechanism mediated by external carbonic anhydrase activity occurred in both the blade and receptacle tissues of *S. henslowianum*. The proton buffer TRIS had no inhibitory effect on NPR at normal pH value in natural seawater (pH 8.1), but it significantly depressed NPR at pH 9.0. This suggested that proton transport occurred at the outside of the plasma membrane facilitated the operation of the carbon acquisition at pH 9.0. It was proposed that the strategy of photosynthetic carbon acquisition at higher pH would prevent the alga from the damage of over-excitation and photoinhibition in case of sunshine and calm water. We concluded that the blade and receptacle tissues of *S. henslowianum* have similar mechanism of acquisition of exogenous Ci from seawater to drive photosynthesis; yet they are differentiated more or less with the photosynthetic properties.

Keywords *Sargassum henslowianum* · Brown algae · Inorganic carbon acquisition · Photosynthesis · Receptacle · Marine macroalgae

Abbreviations

Ci	Inorganic carbon
NPR	Net photosynthetic rate
DR	Dark respiration
CA	Carbonic anhydrase
AZ	Acetazolamide
DIDS	4,4′-Diisothiocyano-stilbene-2,2′-disulfonate
TRIS	Tris(hydroxymethyl)aminomethane
APC	Apparent photosynthetic conductance

Introduction

Marine macroalgae acquire exogenous inorganic carbon (Ci) to drive photosynthesis for growth and development

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from the bulk of dissolved Ci existing in the ocean. In air-equilibrium natural seawater, with an average pH of approximate 8.1, the bulk of total dissolved Ci is HCO_3^- (ca 2.0 mM), and CO_2 (only 10 μM at 20°C) is less than 1% of the total dissolved Ci. Given this, it is not surprising that a large number of species of marine macroalgae have developed mechanisms that permit the acquisition of the most abundant source of Ci in seawater, HCO_3^-, as an exogenous carbon source for photosynthesis (Johnston 1991; Beer 1994; Axelsson et al. 1995, 1999; Raven 1997; Larsson et al. 1997; Mercado and Niell 1999; Zou et al. 2003, 2004). Two basic processes have been generally proposed for HCO_3^- acquisition by macroalgae: (1) catalysis of HCO_3^- dehydration by extracellular carbonic anhydrase (CA; EC 4.2.1.1), supplying CO_2 for transporting into the cells; (2) direct uptake of ionic HCO_3^- across the plasma membrane facilitated by anion-exchange proteins. However, there are many modified ways and strategies in marine macroalgae facilitating the acquisition of exogenous Ci from the seawater. For example, an inducible system for Ci utilization at high pH is shown to be in marine green macroalgae (Carlberg et al. 1990; Axelsson et al. 1995). In brown macroalge, acquisition of Ci for photosynthesis appears to be depended on the presence of blue light (Schmid and Dring 1996). A P-type H^+-ATPase (proton pump) located in plasma membrane has also been shown to facilitate the external CA-catalyzed conversion of HCO_3^- to CO_2 in the brown algae *Laminaria saccharina* (Axelsson et al. 2000; Mercado et al. 2006) and *L. digitata* (Klenell et al. 2002), the red alga *Coccotylus truncates* (Snoeijs et al. 2002), and the green alga *Cladophora glomerata* (Choo et al. 2002). Additionally, the extent to which marine macroalgae are able to acquire HCO_3^- for photosynthesis varies among taxa and/or species, and the special strategies by which the alga acquire Ci is closely related to habitat, conferring as adaptational advantage to the alga (Surif and Raven 1989; Maberly 1990; Larsson et al. 1997; Mercado et al. 1998; Snoeijs et al. 2002; Murru and Sandgren 2004).

Sargassum is a very large genus in the Phaeophyta, being distributed throughout the tropical and temperate coastal waters around the world (Nizamuddin 1962; Phillips 1995). Many species of *Sargassum* thrive and even give rise to "*Sargassum*-bed", providing the sheltering nursery grounds for the numerous marine animals such as fish and invertebrtates, and also acting as a food source for herbivores (Ang 1987; Ornellas and Coutinho 1998; Tanake and Leite 2003). Some species of *Sargassum* are economically important, being not only widely used as animal fodder and manure in agriculture, but also being the most potential important seaweeds resource for the industrial use of alginates production (Tseng 1984; Ang 1987; Tseng and Lu 2000). *Sargassum* species are also of interest for pharmaceutical and cosmetic application owing to their natural substance contents (Zubia et al. 2003; Stiger et al. 2004). Given the ecological and economic importance and the world-wide distribution of *Sargassum* species, considerable investigations have been carried out on the taxonomy, distribution, reproduction and phenology, population dynamics and ecology for *Sargassum* (e.g. Rico and Fernandez 1997; Andrew and Viejo 1998; Steen 2004; Yoshida et al. 2004; Engelen et al. 2005, 2008; Ang 2006; Rivera and Scrosati 2006; Sjøtun et al. 2007; Mattio et al. 2008a, b; Noiraksar and Ajisaka 2008; Monteiro et al. 2009). Some author also concern the photosynthetic physiology of *Sargassum.* For example, Schofield et al. (1998) examined the acclimation of photosynthetic machinery in *S. natans* to high light environment. They demonstrated that *S. natans* minimizes irradiance stress by energy dissipation through xanthophyll cycle pigments and a rapid down-regulation of PSII activity. Schaffelke and Klumpp (1998) showed that short-term nutrient pulses could enhance the growth and net photosynthetic rates of the coral reef macroalga *S. baccularia* in the Great Barrier Reef, Australia. Häder et al. (2001) showed that in a Atlantic *S. vulgare* and two other common Atlantic brown macroalgae the photosynthetic quantum yield was effected by exposure to solar radiation, and UV radiation had a significant share in the effect. However, there are much fewer studies up to now focus on the mechanism of photosynthetic acquisition of Ci for *Sargassum* species, regardless of the their widely distribution in the world.

A plant of *Sargassum* possesses a holdfast, a "stem" from which main "branches" (or main axes) are formed, which give rise to blades (leaves), lateral branches, and vesicles. Blades are the main photosynthetic tissues. In addition to blades, other part in *Sargassum* plants may possess photosynthetic capacity. Various parts of macroalgae are differentiated with different photosynthetic properties (Küppers and Kremer 1978; Gao and Umezaki 1988, 1989; Gao 1989, 1991). For example, it was shown that photosynthetic activity was highest in leaves, intermediate in vesicles and lowest in the holdfast and primary laterals in *Sargassum horneri* (Gao 1991) and *S. thunbergii* (Gao and Umezaki 1989). Vesicles of *Sargassum horneri* and *S. thunbergii* possess high photosynthetic capacity in addition to supplying floatation. Gao (1989) stated that photosynthetic production of a *Sargassum* plant seems to be carried out almost by leaves when it is young, and it contributed more and more by vesicles as it grows larger until maturation period. During the fertile season, the sexual reproductive tissues, receptacles, are born largely on the lateral branches of the main axis. Antheridia and oogonia develop within the receptacles. There is some evidence that receptacle tissue of Fucoid macroalgae had the capacity to photosynthesize (Brenchley et al. 1997; Zou and Gao 2005), and its photosynthetic activity was even higher than that of the vegetative tissue in

Himanthalia elongate (Brenchley et al. 1997) and *Hizikia fusiformis* (Zou and Gao 2005). However, it has hardly been paid attention on the photosynthetic comparison between different parts in macroalgae with regard to the mechanism of photosynthetic carbon acquisition.

This genus of *Sargassum* constitutes an important part of the seaweed flora along the coastline of China (Tseng 1984; Tseng and Lu 1992, 2000). *Sargassum henslowianum* C. Agardh is one of the most common dioecious *Sargassum* species grown in the shoreline of Nanao Island, Shantou, China. Here it grows luxuriantly from January to June per year, forming a densely populated zone in the shallow subtidal. The plants are non-fertile from January to March, and begin to show signs of receptacles in the middle of April. The peak sexual reproductive phase is in May to June, with receptacles being 5–10 cm in length. The reproductive phase ends in mid-July, followed by the occurrence of senescence and rupture of the thalli (Personal observation). It is of interest to determine the photosynthetic characteristics of reproductive and vegetative tissues of fertile plants of *S. henslowianum*. In the present study, we examine the mechanism of exogenous Ci acquisition in blade and receptacle tissues of *S. henslowianum* to characterize the photosynthetic physiology in this species.

Materials and methods

Plant materials

Sargassum henslowianum C. Agardh samples were collected from subtidal zone at Shengao bay, Nanao Island, Shantou, China (23°20′ N, 116°55′ E) in May 2007 when the thalli were fertile. The collected plants, cleaned of epiphytes and rinsed of sediments, were placed in the plastic barrel with seawater and transported to the laboratory within 3 h. They were maintained in filtered seawater vigorously aerated with ambient air at 25°C and 180 μmol $m^{-2}s^{-1}$ (fluorescent illumination, LD cycle 12 h:12 h). The leaf-like blades and the female ripe receptacles (no eggs had been released yet) from the middle part of the plants were cut into small segments with a sterilized shape razor and used for experiments as described below. To minimize the possible effects of cutting damage (wound respiration), the segments of blade and receptacle were maintained about at least 2 h before the photosynthetic measurements under the same conditions for laboratory maintenance of the whole individuals.

Irradiance-dependent photosynthetic oxygen evolution

Rate of net photosynthetic O_2 evolution (NPR) was measured by using a Clark-type oxygen electrode (YSI Model 5300, USA) in a photosynthetic chamber, which was encircled by a water jacket connected to a cooling circulator (Cole Parmer, USA) for temperature control. The illumination was provided by a halogen lamp and photon irradiance was measured with a quantum sensor (SKP 200, ELE International, UK).

The filtered seawater (pH 8.1, total dissolved Ci ca. 2.2 mM) was used as the medium for photosynthetic measurement. Sample of 0.1–0.3 g fresh weight (FW) was introduced into the chamber containing 8 ml of seawater medium that was magnetically stirred. Dark respiration (DR) measurements were carried out at 100% air-equilibrium oxygen concentrations in seawater, while photosynthetic measurements were in a limited oxygen concentrations range (below 50% of air-equilibration oxygen concentrations) by sparging with nitrogen to avoid the possible oxygen tension. The samples were allowed to equilibrate in the darkness until the rate of oxygen consumption was constant, usually for approximately 4–6 min, and the rate of DR was monitored. The samples were then exposed to progressively increasing photon irradiance by altering the distance between the light source and the photosynthetic chamber. The NPR versus irradiance (*P–I*) curves were then obtained.

Inorganic carbon-dependent photosynthetic oxygen evolution

Before the measurement, Ci-free seawater with pH 8.1 or 9.0 was prepared. Ci was removed from the natural seawater by reducing pH to less than 4.0 with the addition of 0.5 M HCl, and then sparging for at least 2 h with high purity N_2 gas. The pH was adjusted as 8.1 or 9.0 with freshly prepared 0.5 M NaOH and 0.5 M HCl. All manipulations were under N_2.

Samples were transferred to the photosynthetic chamber containing Ci-free seawater with pH 8.1 or 9.0. The samples were left to photosynthesize to deplete the Ci present in the medium and in the algal cells till no further O_2 evolved, which took about 20 min. Different aliquots of $NaHCO_3$ stock solution were then injected into the chamber in order to create increasing Ci concentrations in the reaction medium. O_2 evolution was recorded within 5 min after each addition of $NaHCO_3$. The NPR versus Ci concentrations (*P–C*) curves were then obtained.

The effects of pH values on the oxygen exchange

Oxygen exchanges under the darkness and saturating irradiance were measured in natural seawater with various pH values at constant dissolved Ci concentration (ca. 2.2 mM) in order to evaluate the pH effects on DR and NPR. Several values of pH were used (6.0, 7.0, 8.0, 9.0, and 10.0), which

were adjusted by adding appropriate quantities of freshly prepared HCl and NaOH. When the seawater with desired pH values were prepared, they were quickly stoppered to avoid CO_2 outgassing when the pH was less than 8.0 or CO_2 dissolving when the pH was above 8.0. Before each respiratory or photosynthetic measurement, algal samples were pre-incubated in seawater with the actual pH for 15 min for a short-term adaptation. After this, the medium was replaced and the samples allowed to acclimatize another 4–6 min, before the respiratory or photosynthetic measurements took place. This latter incubation duration did not cause significant change in the pH of the reaction medium (less than 0.05 units).

The effects of buffer and inhibitors on photosynthetic oxygen evolution

Buffers are often used to maintain constant pH values for the reaction media for photosynthetic measurements. However, it has been shown that the buffers per se could inhibit the photosynthetic carbon acquisition in some macroalgal species such as the marine brown alga, *Laminaria saccharina* (Axelsson et al. 2000) and the seagrass species such as *Zostera marina* (Hellblom et al. 2001). This buffer-sensitive mechanism was though to be that proton buffers could eliminate proton extrusion forming acid zones in the external HCO_3^--dehydration on the thalli surface (Axelsson et al. 2000; Hellblom et al. 2001; Beer et al. 2002). Therefore, we investigated the possible inhibitory effect of buffer per se on the photosynthesis of *E. binghamiae*. The buffer used here was tris(hydroxymethyl)aminomethane (TRIS, biological buffers, Sigma). NPR was determined at pH 8.1 and 9.0 with and without the addition of TRIS buffer, respectively. The final TRIS concentrations in the media varied from 0 to 120 mM. The buffer response curves were then obtained.

NPR in natural seawater (ca. pH 8.1 and Ci 2.1 mM) was determined in the presence of various inhibitors. Acetazolamide (AZ, Sigma) and 4,4′-diisothiocyanostilbene-2,2′-disulfonate (DIDS; Sigma) were used. It is generally thought that AZ cannot penetrate into the algal cells and inhibits only the extracellular CA activity (Moroney et al. 1985); while DIDS inhibits the direct HCO_3^- uptake of the photosynthetic cells by means of acting the plasmalemma-located anion-exchange protein (Drechsler et al. 1993; Axelsson et al. 1995, 1999). AZ stock solution (50 mM) was prepared with 0.5 M NaOH and the final concentration used was 200 μM, while DIDS were directly dissolved in the seawater to give the final concentrations of 400 μM used in the experiments. Seawater media containing AZ (final concentration 200 μM) and TRIS (final concentration 50 mM) were also used. For all the inhibitors experiments, the pH changes during measurement were not more than 0.05. Such a small pH change made ignorable effect on the photosynthetic measurement, so as to avoid the unnecessary interpretation on the pH effect.

Assays of pH compensation point

To obtain the pH compensation point, pH-drift experiments were conducted in sealed glass vials containing 1.0 g fresh samples and 20 ml natural seawater at 25°C and 450 μmol photons m^{-2} s^{-1}. The final pH values (i.e. pH compensation points) were obtained when there were no further increases (Maberly 1990), which took 8–10 h.

Calculations and statistics

For *P–I* curves, the apparent photosynthetic efficiency (α) was estimated as the irradiance-limited slope. The irradiance compensation point (I_c) and irradiance saturation point (I_k) were calculated as DR/α and (NPR_{max} + DR)/α, respectively (Henley 1993). Here NPR_{max} was the irradiance-saturated maximum NPR, which was calculated as the mean in the asymptote region of the *P–I* curve; DR was the dark respiration rate. For *P–C* curves, values of $K_{0.5}$ (apparent half-saturation constants, i.e., the Ci concentration required to give half of Ci-saturated maximum rate of photosynthetic O_2 evolution) were estimated from the fit of the *P–C* curve to the Michaelis–Menten equation. The apparent photosynthetic conductance (APC), i.e. the initial slope of *P–C* curve, was calculated by linear regression over the range of 0–0.55 mM Ci. This value was used to indicate how effectively the algae use low concentration of Ci, as discussed by Johnston et al. (1992).

The theoretical maximum photosynthetic rate of the samples incubated in the chamber were estimated from the rate of CO_2 production derived from spontaneous (uncatalyzed) dehydration of HCO_3^- in seawater according Miller and Colman (1980) and Matsuda et al. (2001) by the following equations: $d[CO_2]/dt = K_1 \times [DIC]/A + K_3 \times [DIC] \times [H^+]/K_{H_2CO_3}/A$, and $A = 1 + [H^+]/K_1 + K_2/[H^+]$, where [DIC] is the concentration of dissolved Ci in seawater. K_1 and K_3 are the rate constants of reactions $HCO_3^- \rightarrow CO_2 + OH^-$ and $H_2CO_3 \rightarrow CO_2 + H_2O$, respectively. $K_{H_2CO_3}$ and K_2 represent the dissociation constants of the reactions $H^+ + HCO_3^- \leftarrow\rightarrow H_2CO_3$ and $H^+ + CO_3^{2-} \leftarrow\rightarrow HCO_3^-$, respectively. The assumption was made that the alga consumed CO_2 at a rate causing the CO_2 concentration to approach zero. This gave a theoretical maximal rate of conversion of CO_2 from HCO_3^- in seawater.

All data plotted on graphs were mean values with standard deviations (SD). Statistical significance of the data was tested with analysis of variance (ANOVA) or *t* test. The level of significance was set at 0.05.

Results

Figure 1 showed the rates of net photosynthetic oxygen evolution (NPR) versus irradiance (*P–I*) curves of vegetative (blade) and reproductive (receptacle) tissues in fertile *Sargassum henslowianum* plants, and the Table 1 presented the photosynthetic parameters of *P–I* curves. In natural seawater (pH 8.1, ca. 2.2 mM Ci), irradiance-saturated net photosynthetic rate (NPR_{max}) was greater ($P < 0.01$) by 25.3% in blade than receptacle. However, dark respiratory rate (DR) was 2-fold higher in receptacle compared with blade. As a result, the ratio of DR to gross photosynthesis (NPR_{max} plus DR) was significantly higher in receptacle than blade (Table 1). Compared with receptacle, blade tissue exhibited a higher apparent photosynthetic efficiency (α), but a lower light compensation point (I_c). The light saturation points (I_k) for photosynthesis were similar between the two tissues.

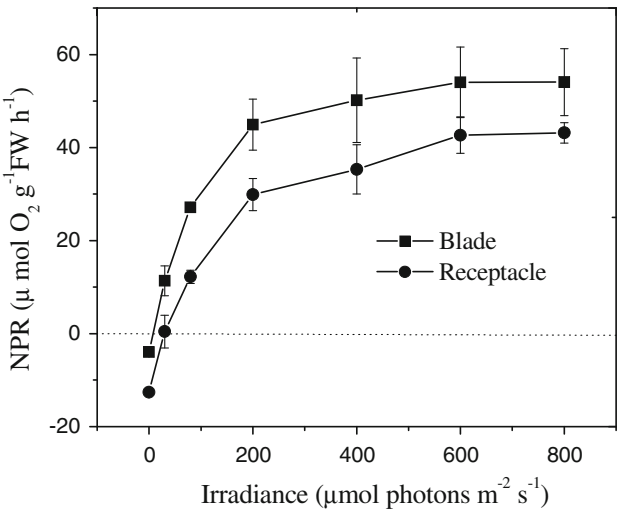

Fig. 1 Rate of net photosynthetic oxygen evolution (NPR) versus irradiance (*P–I*) curves in blade and receptacle of *Sargassum henslowianum*. Measured in natural seawater (pH 8.1, Ci ca. 2.2 mM) and at 25°C. *Vertical bars* represent ± standard deviation (SD) of the means ($n = 4$)

Table 1 Photosynthetic parameters of the *P–I* curves in blade and receptacle tissues of *Sargassum henslowianum*

Parameters	Blade	Receptacle
NPR_{max} (μmol O_2 g^{-1} FW h^{-1})	54.1 ± 7.2	43.2 ± 2.2
DR (μmol O_2 g^{-1} FW h^{-1})	−4.0 ± 0.6	−12.6 ± 0.6
α [(μmol O_2 g^{-1} FW h^{-1})/(μmol photons m^{-2} s^{-1})]	0.38 ± 0.01	0.30 ± 0.01
I_c (μmol Photons m^{-2} s^{-1})	10.4 ± 1.7	41.7 ± 2.5
I_k (μmol Photons m^{-2} s^{-1})	152.4 ± 22.6	184.5 ± 12.5
DR/gross photosynthesis*100%	6.9 ± 1.1	22.6 ± 2.0

Values were derived from Fig. 1. Values are means ± SD ($n = 4$)

The responses of NPR to Ci concentration of seawater were tested at two pH values: the normal seawater pH (8.1) and a higher value, 9.0 (Fig. 2). Oxygen evolution rates at pH 8.1 were nearly saturated with the 2.2 mM Ci (i.e., the normal concentration in seawater) for both the blade and receptacle. This was in accordance with the half-saturation constant for Ci ($K_{0.5}$, ca. 1.3 mM Ci) of them (Table 2). In contrast, NPR at pH 9.0 increased in an almost linear way with the increasing Ci concentrations over the range of Ci concentration used in the experiment. The values of $K_{0.5}$(Ci) at pH 9.0 were increased by one order of magnitude relative to those at pH 8.1 (Table 2). The apparent photosynthetic conductance (APC) was significantly higher ($P < 0.01$) in blade than receptacle for both pH 8.1 and 9.0. In addition, APC was considerably reduced with the rise of pH from 8.1 to 9.0.

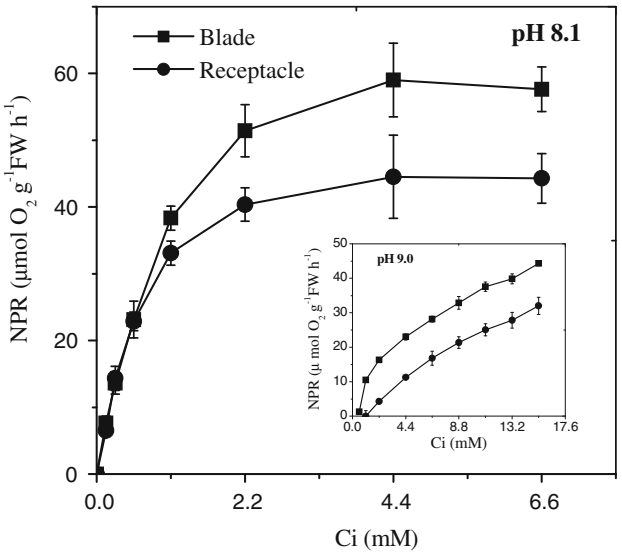

Fig. 2 Rate of net photosynthetic oxygen evolution (NPR) versus Ci (*P–C*) curves in blade and receptacle of *Sargassum henslowianum*. Measured in seawater with ambient (8.1) and elevated (9.0) pH values, respectively, and at 25°C. *Vertical bars* represent ± standard deviation (SD) of the means ($n = 4$)

Table 2 Parameters of the *P–C* curves in blade and receptacle tissues of *Sargassum henslowianum*

Parameters	Blade	Receptacle
pH 8.1		
$K_{0.5}$ (mM)	1.31 ± 0.40	1.26 ± 0.19
APC	32.9 ± 2.1	26.2 ± 1.0
pH 9.0		
$K_{0.5}$ (mM)	10.9 ± 0.9	20.4 ± 3.3
APC [(μmol O_2 g^{-1}FW h^{-1})/(mM)]	8.6 ± 0.4	3.4 ± 0.4

Values were derived from Fig. 2. Values are means ± SD ($n = 4$). $K_{0.5}$, the concentration of inorganic carbon supporting half of the carbon-saturating maximum photosynthetic rate; *APC* apparent photosynthetic conductance

Figure 3 showed the photosynthetic and respiratory rates in blade and receptacle tissues of *S. henslowianum* plants measured in seawater adjusted at various pH values. The pH values caused a significant ($P < 0.01$) effect on NPR for both the blade and receptacle tissues. NPR increased gradually from pH 6.0 to pH 8.0, followed by a steep decrease at pH 9.0. Nearly zero or even negative NPR were found when the pH rose to 10.0. Contrary to photosynthesis, DR for both blade and receptacle tissues were remained unchanged ($P > 0.1$) with the variation of the pH values in seawater.

The theoretical photosynthetic rates calculated by the uncatalyzed supply of CO_2 in the bulk medium were 1250, 191, 17.7, 1.24, and 0.46 μmol O_2 g^{-1}FW h^{-1} at pH 6.0, 7.0, 8.0, and 9.0, respectively. They were compared with the measured values of NPR presented in Fig. 3. While the theoretical rates could high enough to account for the measured NPR at pH 6.0 and 7.0 for either blade or receptacle tissues, the theoretical rates were much lower than the measured rates of photosynthesis at pH 8.0 and 9.0. This indicated that both the blade and receptacles tissues were capable of acquiring HCO_3^- in the bulk medium as an external source of Ci to drive photosynthetic oxygen evolution. Additionally, the pH-drift experiment showed that blade and receptacle could reach the similar final value of pH (9.38 ± 0.15 and 9.36 ± 0.09 for blade and receptacle, respectively).

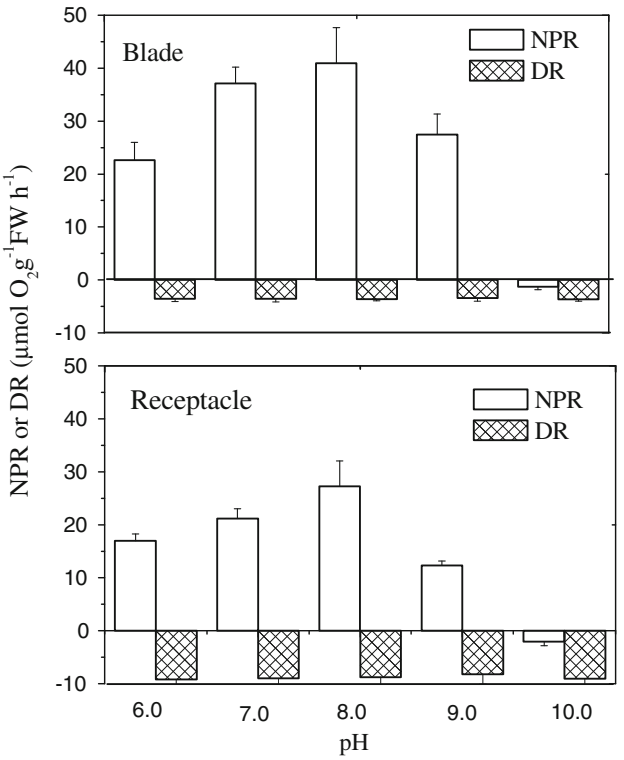

Fig. 3 Rates of net photosynthetic oxygen evolution (NPR) and dark respiration (DR) in blade and receptacle of *Sargassum henslowianum* as a function of pH values in seawater with constant Ci concentration. The experimental medium was natural seawater containing ca 2.2 mM Ci, and the pH value was adjusted by HCl and NaOH. Vertical bars represent ± standard deviation (SD) of the means ($n = 4$)

It was evident that NPR in seawater with the normal pH value (8.1) exhibited no inhibition by TRIS (Fig. 4). However, in seawater at pH 9.0, a gradual increase in TRIS buffer concentration produced a decrease in net photosynthesis for both blade and receptacles tissues of *S. henslowianum* (Fig. 4). At around 40 mM, the degree of inhibition leveled off (about 80% for both blade and receptacles tissues), and additional buffer had no significant further inhibition.

The degree of NPR persisting after addition of DIDS, AZ, or TRIS was measured in natural seawater (ca. 2.2 mM Ci) at two pH value: 8.1 and 9.0 (Fig. 5). The

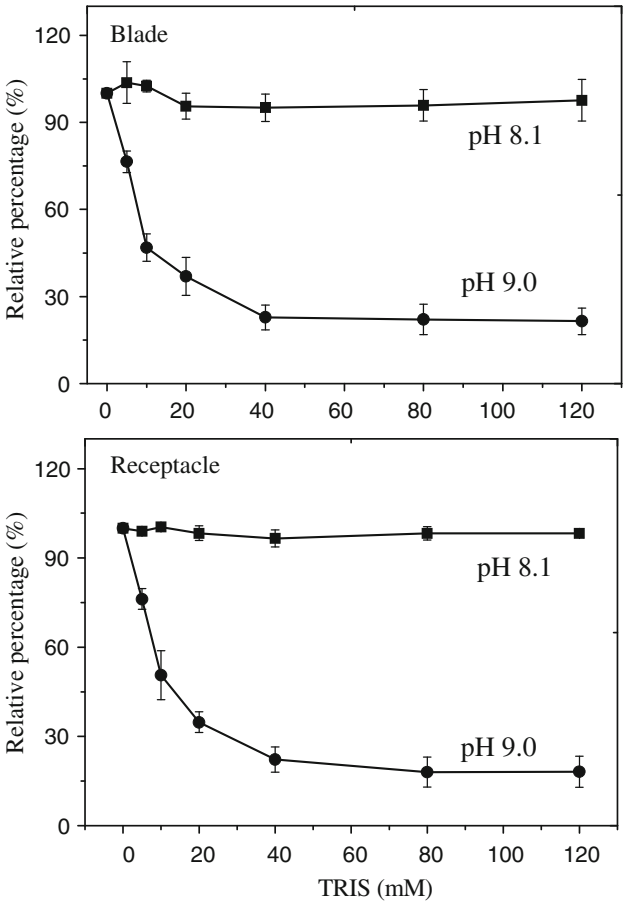

Fig. 4 Relative percentage of net photosynthetic oxygen evolution rate (NPR) in blade and receptacle of *Sargassum henslowianum* as a function of TRIS buffer concentration. Measured in natural seawater (ca. 2.2 mM Ci) adjusted to two pH values: 8.1 and 9.0. *Vertical bars* represent ± standard deviation (SD) of the means ($n = 4$). When the bars are absent, the SD smaller than the symbol size. NPR in blade and receptacle at pH 8.1 are 42.2 ± 4.2 and 34.2 ± 3.6 μmol O_2 g^{-1} FW h^{-1}, respectively; and at pH 9.0 are 18.0 ± 4.5 and 6.4 ± 0.8 μmol O_2 g^{-1} FW h^{-1}, respectively

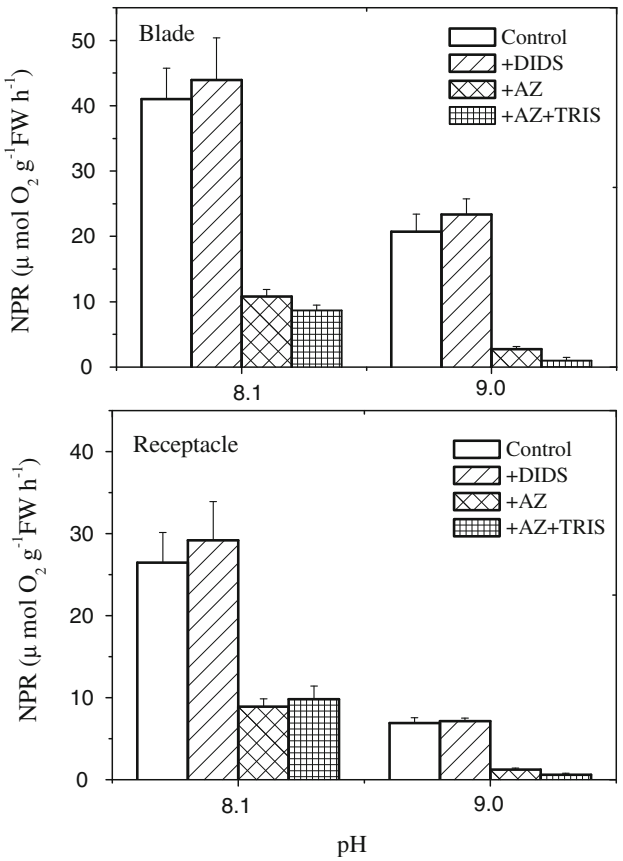

Fig. 5 Net photosynthetic oxygen evolution rate (NPR) in blades and receptacles of *Sargassum henslowianum* after addition of anion-exchanger inhibitor (DIDS, 400 μM of final concentration), external CA inhibitor (AZ, 200 μM of final concentration), or TRIS buffer (50 mM of final concentration). The control was the NPR obtained in seawater without the addition of the inhibitors or TRIS. Measured in natural seawater (ca. 2.2 mM Ci) adjusted to two pH values: 8.1 and 9.0. *Vertical bars* represent ± standard deviation (SD) of the means ($n = 4$)

anion-exchanger inhibitor, DIDS, produced no inhibitory effect ($P > 0.1$) on NPR for both blade and receptacle tissues of *S. henslowianum* at the two pH values. In contrast, addition of AZ, the inhibitor of external CA activity, depressed significantly ($P < 0.01$) the NPR for both the pH values. The inhibition by AZ increased from 73.7 to 86.8% in blade tissue, or from 66.2 to 81.9% in receptacle tissue, when the pH was changed from 8.1 to 9.0. The inhibitory effect of AZ was more marked with the simultaneous addition of TRIS for both blade and receptacle tissues at pH 9.0. However, TRIS did not result in an additional inhibition when the pH was 8.1 (Fig. 5).

Discussion

The present study showed that in addition to blade (i.e., the general recognized main photosynthetic tissue), receptacle (the sexual reproductive tissue) of *Sargassum henslowianum* was capable of photosynthesizing, although it photosynthesized at a lower rate than the blade tissue did. Our results also demonstrated that *S. henslowianum* had a much higher rate of dark respiration in the receptacle compared with the blade, reflecting a strong metabolic sink and a fairly high requirement of assimilation products such as energy and carbon skeletons associated with the reproductive tissue. When the receptacle is ripe and the ambient environmental conditions are favorable (i.e. an advantageous temporal and spatial window for fertilization success occur), the gametes will be discharged from the numerous spherical conceptacles which distributed subepidermally in the receptacles. The developing receptacles usually act as resource (carbon skeleton and energy) sinks within the fertile plants, which draw upon the vegetative button for any excess photoassimilates (Brenchley et al. 1997; Zou and Gao 2005). Photosynthesis of receptacle per se may, therefore, contribute, at least partly, to the photoassimilate requirements for its development. Furthermore, the gametes release from receptacle in fucoid algae requires the process of photosynthetic competence of receptacle (Serrão et al. 1996; Pearson et al. 1998). An important consequence of photosynthesis in seawater (during potentiation) appeared to be intracellular accumulation of K^+ and Cl^- by nearly all cells in the receptacle (Speransky and Brawley 2001). Thus, the photosynthesis of receptacle in *S. henslowianum* may play an essential role in the development and functioning of receptacle.

The ability of a seaweed to raise the final pH of the medium to above 9.0 (equivalent to 0.6 μM CO_2) has been generally viewed as an indicator of HCO_3^- use in the species (Maberly 1990; Johnston et al. 1992). The pH-drift experiments in this study showed that both the blade and receptacle tissues of *S. henslowianum* produced a final pH value of 9.4. This suggested that both blade and receptacle were capable of acquiring HCO_3^-, and the two different tissues exhibited a similar capacity to extract Ci in seawater. Additionally, in seawater of pH 8.0 and 9.0 with constant Ci concentration (2.2 mM) of our photosynthetic measuring system in this study, the free CO_2 is rather low, being ca. 12 μM at pH 8.0, and less than 1 μM at pH 9.0. The theoretically calculated maximum photosynthetic rates which could be maintained exclusively by the CO_2 supply coming from the spontaneous uncatalyzed dehydration of HCO_3^- in the bulk medium were considerably lower than the measured rates of photosynthetic O_2 evolution for both blade and receptacle. This gave further evidence that *S. henslowianum* had to acquire HCO_3^- in the bulk medium in order to drive photosynthetic O_2 evolution at the normal seawater pH value (8.0) and above.

A common mechanism by which HCO_3^- is acquired in marine macroalgae involves the extracellular dehydration

of HCO_3^- via activity of surface-bound carbonic anhydrase (CA), followed by uptake into the photosynthesizing cells of the formed CO_2 (e.g. Björk et al. 1992; Johnston et al. 1992; Haglund et al. 1992a, b; Axelsson et al. 1995; Mercado and Niell 1999; Zou et al. 2003, 2004). From our results, it is evident that such a HCO_3^- dehydration mechanism also occurred in both the blade and receptacle tissues of *S. henslowianum*, because the membrane impermeable CA inhibitor, AZ, drastically depressed the photosynthetic O_2 evolution at pH 8.1 (i.e. the normal seawater pH value) and pH 9.0.

Use of HCO_3^- by means of direct uptake has also been reported in some macroalgae, and such a HCO_3^- uptake mechanism can be almost totally inhibited by the plasmalemma-located anion-exchange (AE) inhibitor, DIDS (e.g. Drechsler et al. 1993; Beer 1994; Axelsson et al. 1995, 1999; Larsson et al. 1997). However, our results demonstrated that a DIDS-sensitive AE-type HCO_3^- transporter was unlikely to be present in Ci acquisition of *S. henslowianum*, because the photosynthesis in either blade or receptacle tissue of this alga was not affected by DIDS. The significant decrease of photosynthetic rate in paralleled with the pH increase (from 8.1 to 9.0) in *S. henslowianum* was an additional indication for a lack of direct uptake for HCO_3^-, as pointed by Mercado et al. (1998), and Mercado and Niell (1999). However, the effects of DIDS on indirect measures of Ci acquisition should be interpreted cautiously since DIDS may produce non-specific effects on the whole cell function, and thereby affect transport processes not directly related to HCO_3^- uptake (Young et al. 2001). That there was no inhibition of photosynthesis by DIDS suggested DIDS had no effect on the photosynthesizing cells in either blade or receptacle of *S. henslowianum*.

It is interesting to note that AZ and the proton buffer TRIS, alone were both capable of depressing major part of photosynthesis of *S. henslowianum* at pH 9.0. This suggested that either AZ or TRIS manipulated the same HCO_3^- acquisition system at high pH (9.0) in *S. henslowianum*, which was sensitive not only to AZ, but also to proton buffers, as in another brown seaweed species, *Laminaria saccharina* reported by Axelsson et al. (2000). It is known that AZ hardly penetrate into cells, inhibiting extracellular CA, and thereby inhibits external CA-mediated HCO_3^- dehydration related to Ci acquisition (Moroney et al. 1985; Axelsson et al. 1995), while the TRIS buffer sensitivity per se suggested that proton transport occurred at the outside of the cell membrane which facilitated the operation of the external CA-dependent HCO_3^- utilization (Axelsson et al. 2000). The local protons' zone (close to the external CA) would not only support the dehydration of HCO_3^- catalyzed by external CA, but also facilitate the entry into the cells of the formed CO_2 (Axelsson et al. 2000). It was likely that the TRIS buffer dissipated such a locally zone of protons excretion (low pH) and thereby inhibited HCO_3^- acquisition. A plasmalemma-associated H^+-ATPase might pump protons to the outside of the plasma membrane (Fernández et al. 1999; Hellblom et al. 2001; Young et al. 2001). Additionally, it was reported that an electron transport chain location in the cell membrane of the diatom *Skeletonema costatum* was capable of providing protons and reducing power outside the cell membrane (Nimer et al. 1998) and Axelsson et al. (2000) also suggested that such an electron transport chain might also occur in *L. saccharina*. Whether the protons excretion in photosynthesizing cells in *S. henslowianum* was created by H^+-ATPase or by electron transport chain is a matter of further investigation.

S. henslowianum is a fast-growing species, commonly forming a densely populated zone in the sublittoral as well as the lower parts of the eulittoral in Shenao Bay of Nanao Island, Shantou. During the growth reason (from January to June), this species has a rather high photosynthetic productive, and is capable of enduring and utilizing even the fairly high solar irradiances it may be exposed to when growing close to the water surface. The pH value of the seawater surrounding the algae might rise due to the process of uptake of Ci and nutrients such as NO_3^- by algae, especially under conditions of high standing stock and/or slow mainstream flows. *S. henslowianum* would benefit greatly from a HCO_3^- acquisition mechanism that functions at higher pH. Furthermore, because maintenance of the extracellular CA activity and proton accumulation outside the cell membrane by ATPase or electron transport chain are energy-consuming processes, HCO_3^- acquisition in *S. henslowianum* would prevent the alga from the damage of over-excitation and photoinhibition in case of sunshine and calm water.

It should be noted that although the blade and receptacle tissues of *S. henslowianum* have similar mechanism of acquisition of exogenous Ci from seawater to drive photosynthesis, they are differentiated more or less with the properties of photosynthesis versus irradiance and/or Ci relationships. The present results showed that light-saturated net photosynthesis in ambient seawater was higher in blade than receptacle of the fertile *S. henslowianum* plants. Similar results were also shown in in *Fucus spiralis* and *F. vesiculosus* (Küppers and Kremer 1978) and *F. serratus* (Brenchley et al. 1997). In contrast, the photosynthetic activity of receptacle was reported to be higher than that of vegetative tissue in *Himanthalia elongata* (Brenchley et al. 1997) and *Hizikia fusiformis* (Zou and Gao 2005). The present results also showed that receptacle of *S. henslowianum* had a lower apparent photosynthetic efficiency and a lower apparent photosynthetic conductance than the blade tissue, suggesting a lower efficiency of Rubisco carboxylation and a lower efficiency of harvesting and

transferring light energy in receptacle. Additionally, receptacle required a higher level of irradiance to make the net photosynthesis positive than blade did. Therefore, *S. henslowianum* is differentiated with differed photochemical reactions and carboxylation between the blade and receptacle tissues. Such difference of photosynthetic properties between blade and receptacle could be ascribed to the morphological differentiation (i.e. cystidioid receptacle vs. leaf-like blade) and the difference of the structure of the photosynthetic apparatus between these two photosynthetic tissues. The exact cytological and biochemical mechanisms underpinning the difference of photosynthetic properties between blade and receptacle are waiting to be further investigated.

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