Photosynthetic responses of thalli and isolated protoplasts of *Bryopsis hypnoides* (Bryopsidales, Chlorophyta) during dehydration*

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**Abstract** *Bryopsis hypnoides* Lamouroux is a unique intertidal siphonal green alga whose extruded protoplasm can aggregate spontaneously in seawater to form numerous new cells that can develop into mature algal thalli. In this study, the photosynthetic responses during dehydration of both the thalli and protoplasts isolated from *B. hypnoides* were measured using a Dual-PAM (pulse amplitude modulation)-100 fluorometer. The results show that photosynthetic rates of *B. hypnoides* thalli were maintained for an initial period, beyond which continued desiccation resulted in reduced rates of PSII and PSI. However, the photosynthetic performances of the isolated protoplasts dehydrated in air (CO2 concentration 600–700 mg/L) showed a slight increase of Y(II) at 20% water loss, but the rates decreased thereafter with declining water content. When protoplasts were dehydrated in CO2 deficient conditions (CO2 concentration 40–80 mg/L), the values of Y(II) declined steadily with increased dehydration without an initial rise. These results indicated that the thalli and isolated protoplasts of this alga can utilize CO2 in ambient air effectively, and the photosynthetic performances of the isolated protoplasts were significantly different from that of the thalli during dehydration. Thus the protoplasts may be an excellent system for the study of stress tolerance.

**Keyword:** *Bryopsis hypnoides*; chlorophyll fluorescence; dehydration; protoplasts; pulse amplified modulation fluorescence system

1 INTRODUCTION

*Bryopsis*, a genus of siphonal green algae, offers an excellent biological system for studying the regeneration of protoplasts, the interaction of various cell organelles, and the *de novo* synthesis of cell membranes and walls (Kim et al., 2001; Ye et al., 2005). As intertidal inhabitants, *Bryopsis* are exposed for 2–3 h between low and high tides, during which the thalli are exposed to direct solar radiation, desiccation and dramatic changes of temperature. Moreover, *Bryopsis* spp. are unicellular algae, and damage to their thalli (which would be the entire cell) could be potentially fatal, e.g. all the cytoplasm leaking out of the cell when it is cut. *Bryopsis* have evolved an effective way to “heal” themselves when damaged. This distinctive regeneration phenomenon has been widely investigated (Gibor, 1965; Tatewaki and Nagata, 1970; Kobayashi and Kanaizuka, 1985; Menzel, 1988; Pak et al., 1991; Kim et al., 2001; Ye et al., 2005), but the effects of desiccation on this alga in particular have received less attention.

Photosynthesis is a fundamental process common to all ecosystems, and desiccation has been shown to significantly influence this process. Photosynthetic responses to desiccation have been studied extensively for intertidal marine macroalgae. In the case of *Caloglossa leprieurii* (Johnson et al., 1974), *Gastroclonium coulteri* (Hodgson, 1981), *Porphyra haitanensis* (Zou and

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Gao, 2002) and Porphyra katadai (Lin et al., 2009), desiccation negatively influenced photosynthetic rates that significantly decreased linearly with declining water content. However, some studies demonstrated an increase of photosynthetic activity in certain intertidal algae under moderate desiccation (Quadir et al., 1979; Johnston and Raven, 1986; Gao and Aruga, 1987; Madsen and Maberly, 1990; Lipkin et al., 1993; Beach and Smith, 1997; Pena et al., 1999; Sampath-Wiley et al., 2008), although further desiccation resulted in decreased photosynthetic rates. Although desiccation has generally been viewed as adverse for algal development (Yabe et al., 1995; Stapel et al., 1997), the physiological responses of intertidal algae during desiccation can not be attributed simply to the tolerances of destructive stresses. Nevertheless, most desiccation tolerance studies have focused on the photosynthetic response of the intact thalli, with few examining this at the protoplast level.

It was reported that the protoplasts isolated from Bryopsis can survive in air for several hours, and after natural seawater was added, the protoplasts could aggregate into some viable spheres and subsequently grow into mature individuals (Ye et al., 2005). Thus, those living protoplasts should be good experimental material for studying various problems in cell physiology. Gibor (1965), working on cytoplasts from Acetabularia, reported that they remained alive for about 2 weeks and maintained photosynthesis. Protoplasts isolated from winter rye, oats, Arabidopsis and Jerusalem artichoke tubers have been used successfully to study mechanisms of freezing damage (Webb et al., 1994; Uemura et al., 1995; Murai and Yoshida, 1998). Recently, protoplasts were used in studies of desiccation tolerance (Xiao and Koster, 2001; Koster et al., 2003), and it has been proposed that they could be used to develop a system for the study of desiccation tolerance.

Bryopsis hypnoides Lamouroux, an alga with seasonal growth, is widely distributed along the Qingdao coast during June to December. Therefore, it may play an important role in the marine photosynthetic and carbon cycle. In this study, we employed a Dual-PAM-100 fluorometer, which can simultaneously investigate the photosynthetic performance of PSI and PSII, to monitor the photosynthetic performance of B. hypnoides thalli and isolated protoplasts during dehydration. The aim of the study was to compare the photosynthetic responses of the thalli and isolated protoplasts during dehydration and to examine the desiccation tolerance of B. hypnoides protoplasts.

2 MATERIAL AND METHOD

2.1 Algal materials and protoplast isolation

Bryopsis hypnoides were sampled from the intertidal zone of Huiquan Bay (36°0’N, 120°2’E), Qingdao, China, rinsed with plenty of autoclaved seawater and brushed by a soft brush to remove the surface microbial and epiphytic organisms. Algal thalli were maintained in a seawater aquarium at 20°C, illuminated by a light of 60 to 80 μmol/(m²-s) (photosynthetically-active radiation) and aerated with normal air.

After blotting surface water from the fresh algae, the thalli were cut into small pieces, and the protoplasts were extruded onto eight layers of sterilized gauze.

All experiments were carried out in a constant humidity room where the temperature and velocity of the air was 20°C and 40% RH, respectively. Dehydration assays of the thalli and isolated protoplasts were conducted within 2 h.

2.2 Intact thalli dehydration and re-hydration studies

For dehydration experiments, the water content was expressed in terms of absolute water content (AWC, in %) according to the formula of Lin et al. (2009). Because the dehydration process in air is slow, we spread the thalli thoroughly and placed two layers of filter paper along the thalli to accelerate dehydration. After dehydration, the thalli were put back into seawater to re-hydrate for 1 h, 2 h, 3 h and 4 h to estimate photosynthetic parameters respectively.

2.3 Protoplast dehydration and viability studies

Two ranges of CO₂ concentration (600–700 mg/L and 40–80 mg/L) were selected to study the effect of CO₂ concentration in ambient air on the photosynthetic activity of the protoplasts during dehydration. The lower CO₂ concentration range was obtained by passing ambient air through a saturated sodium hydroxide solution into an air bag (1 m³). The higher CO₂ concentration range (600–700 mg/L) was obtained by pumping ambient air together with pure CO₂ into the air bag. The same air bag was used in all measurements.