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Light-independent biosynthesis and assembly of the photosystem II complex in the diatom *Chaetoceros gracilis*

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ABSTRACT

Diatoms can survive for long periods in the dark. However, how biosynthesis of photosynthetic proteins contributes to survival in the dark is poorly understood. Using a radiolabeling technique, we examined whether de novo biosynthesis and assembly of photosynthetic proteins differs in light-adapted vs. dark-adapted marine diatoms (*Chaetoceros gracilis*). In light-adapted cells, D1 protein was heavily radiolabeled owing to rapid turnover of photosystem II (PSII). In dark-adapted cells (>24 h), the radiolabeling patterns of PSII components changed, but the PSII dimer still formed. Therefore, diatoms may regulate the biosynthesis of photosynthetic proteins for long-term survival in the dark.

1. Introduction

As members of phytoplankton communities in aquatic ecosystems, diatoms have an important role in the global carbon cycle [1]. They evolved by a secondary endosymbiosis event in which a photosynthetic red alga was engulfed by a heterotrophic eukaryotic host [2,3]. Consequently, diatom chloroplasts are surrounded by four membranes [4,5]. Another feature of marine diatoms is that they can survive for long periods in the dark, i.e., months to years [6–8], while retaining pigments and viable photosynthetic machinery [9,10]. However, whether the diatom photosynthetic protein complexes are newly biosynthesized and assembled during prolonged darkness is unclear.

The photosystem II (PSII) complex responds dynamically to changes in environmental conditions, e.g., non-photochemical quenching and photoinhibition under variable environmental conditions, whereas the PSI complex does not. Specifically, de novo assembly and turnover of PSII is central to the dynamic regulation of the photosynthetic apparatus. The PSII complex is composed of the reaction center (RC) complex that includes the proteins D1, D2, and certain small subunits, and the peripheral proteins including the chlorophyll (Chl)-binding subunits CP47 and CP43 and other small subunits [11]. PSII assembly appears to follow a distinct sequence of steps [12–14]. Briefly, the D2-cytochrome b_{559} subcomplex forms first [15,16], and it binds to the precursor D1 (pD1)–PsbI subcomplex to form the RC complex [16,17]. CP47 then binds the RC complex to form the RC47 complex, with concomitant processing of the D1 precursor to its mature form [15]. Subsequently, CP43 associates with the RC47 complex to form a PSII monomer, and finally two PSII monomers associate to form a PSII dimer. Some of these steps proceed under illumination but not in darkness [18]. On the other hand, photodamaged D1 proteins are replaced during photoinhibition with newly biosynthesized ones at the stage of assembly intermediate, the RC47 complex [14].

Two-dimensional blue native (BN)/SDS–PAGE combined with radiolabeling is a powerful tool for the precise assessment of the de novo biosynthesis and assembly of PSII complexes [12,17]. For the study reported herein, we examined the consequences of short-term radiolabeling of photosynthetic protein complexes in light- and dark-adapted cells of the marine diatom *Chaetoceros gracilis*, which has been well studied [19–22]. We found that rapid turnover of PSII clearly occurred under illumination, and dimerization of PSII was detected under prolonged darkness.

Abbreviations: BN–PAGE, blue-native PAGE; Chl, chlorophyll; FCP, fucoxanthin chlorophyll *a*/*c*-binding protein; PS, photosystem; RC, reaction center; RubisCO, ribulose 1,5-bisphosphate carboxylase/oxygenase

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2. Materials and methods

2.1. Cell culture

C. gracilis Schütt cells (UTEX LB 2658) were grown in artificial seawater at 25 °C under continuous low-light illumination (30 μmol photons m^{-2} s^{-1}) and with air bubbling [19,21]. For analytical experiments, cells (180–200 × 10^5 cells ml^{-1}) were inoculated into a new culture (50 ml) at a final cell density of 1.0–2.0 × 10^5 cells ml^{-1} (OD_{750} = 0.01–0.02). Cell density was determined using a Thoma hemocytometer. Chlorophyll (Chl) concentrations were determined in 90% acetone using the equation of Jeffrey and Humphrey [23].

2.2. Short-term radiolabeling

Cells (~20–30 × 10^5 cells ml^{-1} per sample) that were illuminated (30 μmol photons m^{-2} s^{-1}) or held in the dark were cultured at 25 °C for 24 h with air bubbling. Samples of both types of cells (1 ml, ~10 and 5 μg Chl ml^{-1} in light- and dark-adapted cells, respectively) were incubated with [^{35}S]L-methionine (43.5 TBq $mmol^{-1}$; American Radiolabeled Chemicals, USA) at a final concentration of 0.37 MBq ml^{-1} for 30 min in the light (30 μmol photons m^{-2} s^{-1}) or dark. Labeling was stopped by addition of 2.5 mM non-radioactive L-methionine and 500 μg ml^{-1} chloramphenicol. Cell samples were also cultured in the dark for 48 or 72 h and then radiolabeled in the dark as described above. The cells were immediately frozen in liquid nitrogen and stored at −80 °C prior to protein analysis.

2.3. BN–PAGE and two-dimensional BN/SDS–PAGE

BN–PAGE was performed as described [24]. Cells that had been stored at −80 °C were centrifuged at 40 000×*g* for 5 min at 4 °C, and then suspended in 0.4 M sucrose, 40 mM MES–NaOH (pH 6.5). The suspended cells were disrupted by freeze–thawing and sonication, the combination of which increased cell breakage. Whole-cell extracts were solubilized by addition of 2% (w/v) dodecyl-maltoside at a concentration of 0.5 mg Chl ml^{-1} for 10 min on ice in the dark. After centrifugation at 40 000×*g* for 10 min, each supernatant (corresponding to 5 μg Chl) was subjected to BN–PAGE (4–16% acrylamide gradient). A standard molecular marker set of proteins (NativeMark; Invitrogen, USA) was used for calibration of the BN–PAGEs. After electrophoresis, the proteins in the gels were stained with Coomassie Brilliant Blue R-250. Then, the gels were dried and subjected to autoradiography (BAS2500; Fujifilm, Japan).

For two-dimensional BN/SDS–PAGE, BN–PAGE lanes were cut out of the gels, and the proteins contained within were denatured with 2% (w/v) lithium lauryl sulfate and 2% (v/v) 2-mercaptoethanol in 0.4 M sucrose, 40 mM MES–NaOH (pH 6.5) at 25 °C for 30 min and then subjected to SDS–PAGE (16% acrylamide and 7.5 M urea). The proteins in each two-dimensional gel were silver-stained [25]. The gels were then dried and subjected to autoradiography (BAS2500).

3. Results

3.1. Cell growth under light and dark conditions

Fig. 1 shows the photoautotrophic increase in *C. gracilis* cell density when the cells were continuously exposed to 30 μmol photons m^{-2} s^{-1} (solid line). Doubling time was ~11–12 h during the logarithmic phase (0–72 h). When cells that had been grown for 48 h were incubated for 24 h in the dark, cell density

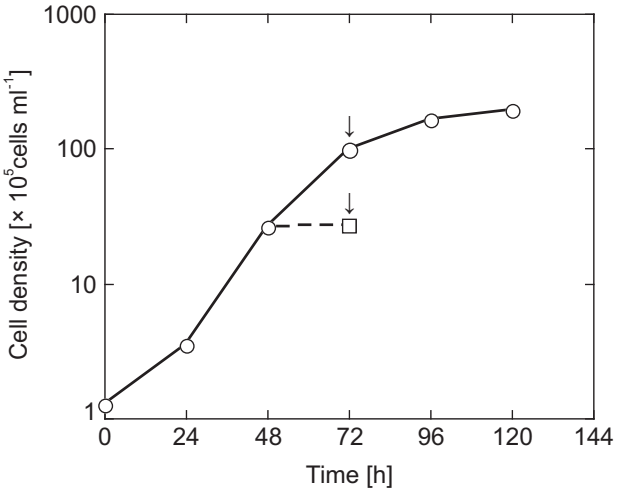

Fig. 1. Population growth curve for *C. gracilis* cells cultured in the light (solid line) and in the dark (dashed line). Arrows indicate the cells used for radiolabeling.

was unchanged (dotted line). The dark-adapted cells recovered logarithmic growth when exposed to 30 μmol photons m^{-2} s^{-1} (data not shown).

3.2. Effects of light and dark conditions on de novo biosynthesis of photosynthetic proteins

We examined light-dependent and -independent de novo biosynthesis and assembly of photosynthetic proteins by labeling 24-h light- and dark-adapted cells for 30 min with [^{35}S]L-methionine (the cell populations indicated by the arrows in Fig. 1). After cell breakage, six discrete protein bands were separated by BN–PAGE (Fig. 2, bands I–VI) and were further resolved by two-dimensional BN/SDS–PAGE (Figs. 3 and 4). Bands I–VI were identified as PSI-FCP, PSII dimer, RubisCO, PSII monomer, FCP-A oligomer, and FCP-B/C trimer, respectively. When the 24-h light-adapted cells were radiolabeled for 30 min while illuminated (Fig. 3; 24L/L), D1 from the PSII dimer and monomer was heavily radiolabeled, whereas D2 and CP43 were moderately radiolabeled. Notably, labeling of CP47 was very low. The RC47 complex was clearly labeled (asterisk in Figs. 3 and 4), but its silver-stained form could hardly be visualized, suggesting that the assembly of PSII

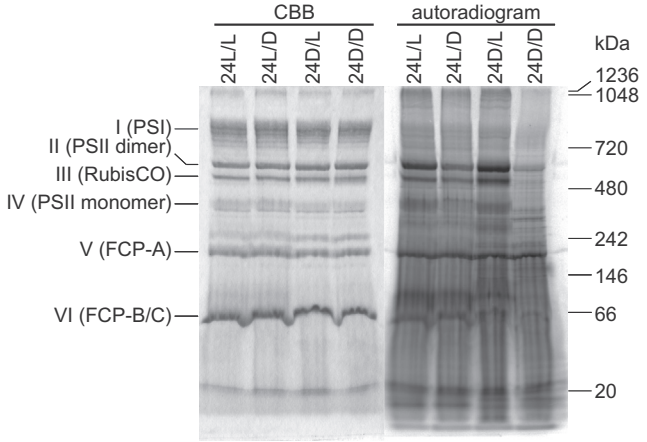

Fig. 2. De novo biosynthesis and assembly of photosynthetic proteins in light- or dark-adapted cells. 24L/L, 24-h light-adapted cells radiolabeled for 30 min in the light; 24L/D, 24-h light-adapted cells radiolabeled for 30 min in the dark; 24D/L, 24-h dark-adapted cells radiolabeled for 30 min in light; 24D/D, 24-h dark-adapted cells radiolabeled for 30 min in the dark. CBB, Coomassie Brilliant Blue.