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Interaction of a small heat shock protein with light-harvesting cyanobacterial phycocyanins under stress conditions

Hitoshi Nakamoto*, Daisuke Honma

Department of Biochemistry and Molecular Biology, Saitama University, 255 Shimo-Okubo, Sakura-ku, Saitama 338-8570, Japan

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Abstract **Phycobiliproteins such as phycocyanins are the most abundant proteins found in cyanobacteria which are assembled to form the phycobilisome. Here, we showed that a small heat shock protein, HspA, interacts directly with phycocyanins from the cyanobacterium *Synechococcus* sp. strain PCC 7942 in vitro and suppresses inactivation of their light-harvesting functions due to heat denaturation in the presence of hydrogen peroxide. Under the denaturing conditions, phycobilisomes were de-assembled to lighter complexes and then aggregated. HspA associated with phycocyanins in the dissociated complexes, and suppressed the aggregation. The specific interaction between a small heat shock protein and phycocyanins was further supported by the fact that HspA and α-crystallin protected isolated phycocyanins from denaturation, while HtpG and lysozyme did not. The maximum protection was observed at a molar ratio of four HspA monomer per one phycocyanin (αβ) monomer.**

Keywords: Phycobilisome; Phycocyanin; Small heat shock protein; α-Crystallin; Cyanobacterium

1. Introduction

Phycobilisomes are the most abundant soluble protein complexes and the major light-harvesting antennae for photosynthesis in cyanobacteria and red algae [1–7]. Their abundance and spectroscopic properties provide unique advantages for biochemical analyses. They are highly ordered, supramolecular assemblies of $5–20 \times 10^6$ Da, directly attached to the stromal surfaces of thylakoid membranes, where they absorb photons and efficiently transfer excitation energy to the photosynthetic reaction centers. Phycobiliproteins which carry covalently linked open-chain tetrapyrrole chromophores, bilins, are the primary constituents of phycobilisomes. On the basis of their visible absorption properties, phycobiliproteins have been assigned to four classes, phycoerythrocyanin, phycoerythrins, phycocyanins, and allophycocyanins. Each phycobiliprotein is composed of two different subunits, α and β, that associate to form a heterodimer (αβ), called a monomer. Monomers form disk shaped trimers ($\alpha_3\beta_3$), and hexamers ($\alpha_6\beta_6$). The hexamers are formed by face-to-face assembly of the trimers. These oligomers are the building units for the assembly of phycobilisomes. In addition to phycobiliproteins, linker polypeptides participate in the assembly of phycobilisomes [8]. Linker polypeptides stabilize the phycobilisome structure and modulate the absorption and fluorescence characteristics of different phycobiliprotein oligomers with which they are specifically associated, thus contributing to the optimization of the excitation energy transfer to the reaction centers.

In general, phycobilisomes consist of two morphologically distinct substructures, the core and peripheral rods [1–7]. The core is composed primarily of allophycocyanins and is in direct contact with thylakoid membranes. The membrane/phycobilisome association is mediated by a large chromoprotein present within the phycobilisome core, which also has linker polypeptide features; it is referred to as the anchor protein or core–membrane linker polypeptide. Multiple sets of rods radiate from the core, which are made up of phycocyanins, phycoerythrins, and/or phycoerythrocyanin.

Phycobilisomes from the unicellular cyanobacterium *Synechococcus* sp. strain PCC 6301 have been studied in detail because of their simple phycobiliprotein composition [9]. Linker polypeptides represent ~12% of the protein of the phycobilisomes; phycocyanin, ~75%, and allophycocyanin, ~12%. In the present study, we used *Synechococcus* sp. strain PCC 7942 as an experimental organism. The cyanobacteria *Synechococcus* sp. strain PCC 7942 and *Synechococcus* sp. strain PCC 6301 are genetically, closely related [10]. However, strain PCC 7942 is more readily transformable, and has been used extensively for genetic manipulation.

It is generally thought that phycobilisomes are assembled spontaneously. In fact, phycobilisomes are reassembled in vitro from purified linker polypeptides and phycobiliproteins [11]. So far, no evidence of molecular chaperone function in phycobilisome assembly or de-assembly has been reported [12].

The small heat shock proteins (Hsps) constitute a ubiquitous family of molecular chaperones characterized by a conserved α-crystallin domain of about 80 amino acid residues [13]. We have previously cloned the *hspA* gene encoding a 16.5-kDa small Hsp from the cyanobacterium *Synechococcus vulcanus*, and introduced it into strain PCC 7942 through a shuttle vector, for constitutive expression of the protein [14]. The transformed cells, strain ECT16-1, exhibited elevated tolerance to heat stress, as compared with a reference strain, ECT. Heat shock treatment at 50 °C for 15 min led to a great decrease of a maximal light absorption due to phycocyanins in the ECT strain [14]. On the other hand, there was almost no absorption decrease in the ECT16-1 strain, indicating that constitutive overexpression of HspA can protect the light-harvesting functions of phycocyanins under heat stress. The loss of absorption was not reversible at least

*Corresponding author. Fax: +81 48 858 3384.
E-mail address: nakamoto@post.saitama-u.ac.jp (H. Nakamoto).

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within 24 h after the heat treatment. These results strongly suggest that phycocyanins were irreversibly denatured and/or degraded under the heat stress. The HspA protein which forms a large homo-oligomer of 24 subunits in its native state can suppress irreversible aggregation of thermally denatured model protein substrates [15]. Thus, we hypothesized that HspA recognizes phycocyanins as major cellular targets in order to protect them from irreversible denaturation, contributing to a great increase in survival at a lethal temperature. To test the hypothesis, we decided to set up experiments which reproduce the phycocyanin photo-bleaching at 50 °C in vitro. HspA suppressed the photo-bleaching by direct interaction with phycocyanins.

2. Materials and methods

2.1. Materials

Synechococcus sp. strain PCC 7942 cells were cultured photoautotrophically in BG-11 inorganic liquid medium [16]. The BG-11 was modified to contain 5 mM Tes-NaOH, pH 8.0, and 50 mg/l Na_2CO_3. The liquid culture in a flat glass vessel was continuously aerated. Unless otherwise indicated, cultures were grown at 30 °C under a light intensity of 20 $\mu E/m^2/s$.

Lysozyme from chicken egg white was obtained from Sigma (St. Louis, MO, USA). Bovine α-crystallin was obtained from StressGen (Victoria, British Columbia, Canada).

2.2. Preparation of phycobilisomes

Phycobilisomes from exponentially growing cells of *Synechococcus* sp. PCC 7942 were isolated by a previously described procedure [9]. The soluble supernatant fraction that was obtained by incubation of the broken cell suspension in the presence of 1% Triton X-100 for 30 min followed by centrifugation for 30 min at $31\,000 \times g$ was layered onto a sucrose step gradient of 11.5 ml consisting of 2.0, 1.0, 0.75, 0.5, and 0.25 M solutions of sucrose in 0.75 M NaH_2PO_4/K_2HPO_4 containing 1 mM NaN_3 and 1 mM 2-mercaptoethanol ($NaKPO_4$ buffer), pH 8.0. Centrifugation was performed at $102\,262 \times g$ at 18 °C for 17 or 18 h.

2.3. Bleaching of phycobilisomes and phycocyanins in vitro

Each solution containing phycobilisomes, phycocyanins, HspA, HtpG, or lysozyme was dialyzed against the 0.75 M $NaKPO_4$ buffer. Phycobilisomes or phycocyanins were incubated in either 0.75 M (in the case of phycobilisome denaturation) or 20 mM $NaKPO_4$ (in the case of phycocyanin denaturation) buffer containing 0.3% H_2O_2 at 50 °C for 15 min, and then chilled on ice. For spectroscopic analysis, the denatured phycobilisome or phycocyanin mixture was filled up to 1 ml with either 0.75 M or 20 mM $NaKPO_4$ buffer, and then centrifuged at $15\,000 \times g$ at 4 °C.

2.4. Purification of phycocyanins

Phycocyanins from *Synechococcus* sp. PCC 7942 were purified by a previously described procedure [11]. Isolated phycobilisomes were dialyzed at 4 °C to reduce the concentration of the $NaKPO_4$ buffer to 4.8 mM, and purified by a linear gradient (10–150 mM $NaKPO_4$) elution from Toyopearl 650S (Tosoh Corp., Tokyo, Japan). The phycocyanin fractions were pooled, dialyzed against 1 mM $NaKPO_4$ buffer containing 0.1 M NaCl, and then applied to a hydroxyapatite column, HP40-100 (Asahi Optical Co. Ltd., Tokyo, Japan). Phycocyanins were eluted with linear gradients of $NaKPO_4$ (from 1 mM to 80 mM) and NaCl (from 0.1 M to 1 M).

2.5. Purification of HspA

Recombinant *S. vulcanus* HspA without any fused tag was expressed in *Escherichia coli*, and purified to apparent homogeneity by a combination of ammonium sulfate fractionation, DEAE-Toyopearl 650S, Hydroxyapatite HP40-100, and a second DEAE-Toyopearl chromatography [15].

2.6. Purification of HtpG

Recombinant HtpG from *Synechococcus* sp. PCC 7942 was expressed in *E. coli*, as a His-tagged protein and was purified to apparent homogeneity by metal chelate affinity chromatography. The details for the purification procedure will be described elsewhere.

2.7. Spectroscopy

Absorption spectra were recorded at room temperature on a Hitachi 557 double wavelength double beam spectrophotometer (Hitachi Koki, Tokyo, Japan). Absorption at 620 nm was measured by a Shimadzu UV-1200 double beam spectrophotometer (Shimadzu, Kyoto, Japan).

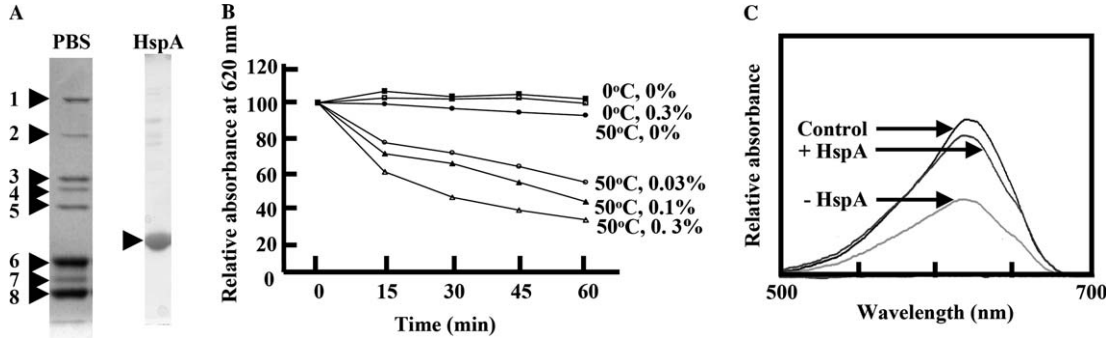

Fig. 1. Bleaching of isolated phycobilisomes in vitro and suppression of the bleaching by a small Hsp, HspA. (A) Purified phycobilisomes (PBS) from *Synechococcus* sp. strain PCC 7942 and HspA from *S. vulcanus*. 14 μg of isolated phycobilisomes and 7 μg of HspA were applied to SDS–PAGE, and then the gel was CBB-stained. All the polypeptide bands corresponding to the phycobilisome components and HspA are indicated by arrowheads. Band 1, the anchor protein (the core–membrane linker polypeptide) of 75 kDa; Band 2, ferredoxin-$NADP^+$ oxidoreductase; Band 3, 33-kDa rod linker polypeptide; Band 4, 30-kDa rod linker polypeptide; Band 5, 27-kDa rod–core linker polypeptide; Band 6, 19-kDa phycocyanin β; Band 7, 18.2-kDa allophycocyanin β; Band 8 contains both phycocyanin α and allophycocyanin α of 17.7 kDa. The size of HspA is 16. 5 kDa. (B) Time courses of phycocyanin bleaching in vitro in the presence or absence of H_2O_2 at 0 or 50 °C. 50 μg of phycobilisomes in 150 μl of 0.75 M $NaKPO_4$ buffer containing various concentrations of H_2O_2 were incubated at 0 or 50 °C for various times, and then diluted to 1 ml by 0.75 M $NaKPO_4$ buffer for absorption measurements. (C) Absorption spectra of phycobilisomes prior to (Control) or after incubation at 50 °C in the presence (+) or absence (−) of HspA. 30 μg of phycobilisomes in 50 μl of 0.75 M $NaKPO_4$ buffer containing 0.3% H_2O_2 were incubated at 50 °C for 15 min in the presence or absence of 30 μg HspA. The mixture was diluted to 1 ml by 0.75 M $NaKPO_4$ buffer, centrifuged, and the supernatant fraction was analyzed.