

Strong interaction of CpcL with photosystem I cores induced in heterocysts of *Anabaena* sp. PCC 7120

Takehiro Suzuki¹, Haruya Ogawa², Naoshi Dohmae¹, Jian-Ren Shen², Shigeki Ehira³, Ryo Nagao^{4§}

¹Biomolecular Characterization Unit, RIKEN Center for Sustainable Resource Science, Saitama 351-0198, Japan

²Research Institute for Interdisciplinary Science and Graduate School of Natural Science and Technology, Okayama University, Okayama 700-8530, Japan

³Department of Biological Sciences, Graduate School of Science, Tokyo Metropolitan University, Tokyo 192-0397, Japan

⁴Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan

§To whom correspondence should be addressed: nagry@shizuoka.ac.jp

Abstract

Phycobilisomes (PBSs) are photosynthetic light-harvesting antennae and appear to be loosely bound to photosystem I (PSI). We previously found unique protein bands in each PSI fraction in heterocysts of *Anabaena* sp. PCC 7120 by two-dimensional blue native/SDS-PAGE; however, the protein bands have not been identified. Here we analyzed the protein bands by mass spectrometry, which were identified as CpcL, one of the components in PBSs. As different composition and organization of *Anabaena* PSI-PBS supercomplexes were observed, the expression and binding properties of PBSs including CpcL to PSIs in this cyanobacterium may be diversified in response to its living environments.

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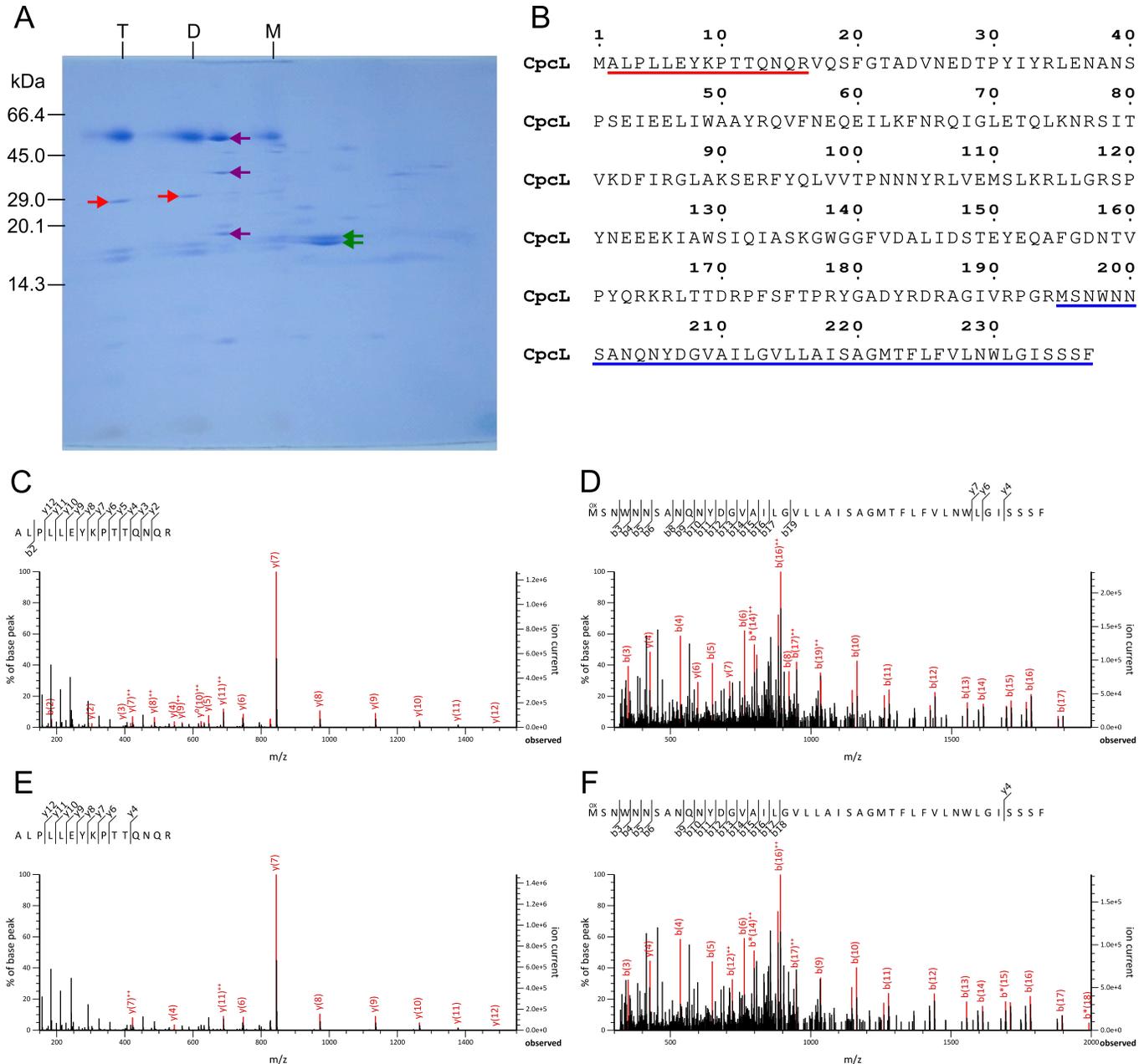


Figure 1. Identification of CpcL in the PSI tetramer and dimer fractions from heterocysts of *Anabaena* sp. PCC 7120:

(A) Two-dimensional BN/SDS-PAGE of the heterocyst thylakoids. T, PSI tetramer; D, PSI dimer; M, PSI monomer. Protein bands labeled as red arrows were analyzed by mass spectrometry. Bands labeled as purple and green arrows can be assigned to ATP synthase and PBS proteins, respectively, according to Chang et al. (2015) and Srivastava et al. (2021). This figure was modified from Nagao et al. (2022), Copyright Elsevier. (B) Amino acid sequence of CpcL. Red and blue underlines indicate N and C-terminal polypeptides, respectively, of CpcL detected by MS/MS analyses (C–F). (C, D) MS/MS spectra of ALPLLEYKPTTQNR ($m/z = 591.3284$; $z = 3+$; MASCOT score = 56; Expect value = $6.2e-6$) (C) and MSNWNNSANQNYDGVAILGVLLAISAGMTFLFVLNWLGISSSF ($m/z = 1163.5813$; $z = 4+$; MASCOT score = 70; Expect value = $2.6e-7$) (D) within the band labeled as a red arrow in the PSI tetramer fraction in panel A. (E, F) MS/MS spectra of ALPLLEYKPTTQNR ($m/z = 591.3283$; $z = 3+$; MASCOT score = 64; Expect value = $1.0e-6$) (E) and MSNWNNSANQNYDGVAILGVLLAISAGMTFLFVLNWLGISSSF ($m/z = 1163.5829$; $z = 4+$; MASCOT score = 76; Expect value = $7.8e-8$) (F) within the band labeled as a red arrow in the PSI dimer fraction in panel A.

Description

Light-harvesting antennae have been diversified in photosynthetic organisms (Blankenship 2021) and can be grouped into membrane proteins and water-soluble proteins. Phycobilisomes (PBSs) are water-soluble pigment-protein complexes and consist of linker proteins and pigment-binding phycobiliproteins (MacColl 1998; Adir 2005; Watanabe and Ikeuchi 2013; Rast et al. 2019; Adir et al. 2020). PBSs are primarily bound to photosystem II of cyanobacteria and red algae as revealed by cryo-electron microscopy (Rast et al. 2019; You et al. 2023). However, it is unclear how PBSs interact with photosystem I (PSI) to catalyze light-induced electron transfer from plastocyanin or cytochrome c_6 to ferredoxin (Golbeck 1992; Brettel and Leibl 2001; Fromme et al. 2001; Nelson and Junge 2015).

Watanabe et al. purified PSI-tetramer-PBS supercomplexes from the cyanobacterium *Anabaena* sp. PCC 7120 (hereafter referred to as *Anabaena*) grown under normal conditions (Watanabe et al. 2014), whereas we have purified PSI-dimer-PBS and PSI-monomer-PBS supercomplexes from *Anabaena* grown under iron-deficient conditions (Shimizu et al. 2023). The two studies detected CpcL in the supercomplexes, which is one of the linker proteins in PBSs and was denoted by Watanabe et al. (2014). CpcL, previously known as CpcG3 in *Anabaena*, is one of the rod-core linker proteins (Bryant et al. 1991). It contains an N-terminal linker domain and a distinctive hydrophobic C-terminal domain (Kondo et al. 2007; Watanabe et al. 2014; Hirose et al. 2017; Guo et al. 2024). In contrast, CpcL was also called CpcG2 in the cyanobacterium *Synechocystis* sp. PCC 6803 and its characteristic protein structure has been determined by cryo-electron microscopy (Zheng et al. 2023).

It is known that CpcL of *Anabaena* was highly expressed in heterocysts (Watanabe et al. 2014), which are formed under nitrogen-starvation conditions (Wolk et al. 1994; Magnuson and Cardona 2016). We recently showed that by two-dimensional blue-native (BN)/SDS-PAGE, an apparent protein band in a molecular-weight range of 20.1–29.0 kDa was observed in the PSI fractions obtained from the heterocyst thylakoids of *Anabaena*, but this band was not observed in any PSI fractions from the vegetative thylakoids of *Anabaena* (Nagao et al. 2022). The protein band is presumed to be CpcL based on its apparent molecular weight; however, it has not yet been definitively identified.

The bands presumed to be CpcL in the two-dimensional BN/SDS-PAGE of the PSI tetramer and dimer fractions from the heterocyst thylakoids (labeled as red arrows in Figure 1A) were analyzed by mass spectrometry. The results showed that the N- and C-terminal polypeptides of CpcL can be detected clearly in both PSI tetramer and dimer fractions (Figure 1B–F). Because CpcL was hardly observed in any PSI fractions by two-dimensional BN/SDS-PAGE of vegetative thylakoids from *Anabaena* (Nagao et al. 2021; Nagao et al. 2022), strong interactions of CpcL with PSI cores appear to be induced in heterocysts.

This study demonstrated that CpcL is tightly bound to at least the PSI tetramer and dimer in the *Anabaena* heterocysts, and it was hardly observed in the PSI monomer fraction (Figure 1A). Under iron-deficient growth conditions of *Anabaena*, CpcL was detected from the PSI dimer and monomer fractions but not the tetramer (Shimizu et al. 2023). Thus, the association of CpcL with PSI oligomers may be changed under different conditions; e.g., iron-deficiency and nitrogen-starvation have induced a different association pattern of CpcL with PSI oligomers. It is interesting to note that any PBS components including CpcL were hardly detected in the PSI fractions from the vegetative thylakoids of *Anabaena* by two-dimensional BN/SDS-PAGE (Watanabe et al. 2011; Nagao et al. 2021; Nagao et al. 2022), although the PSI tetramer cores together with CpcL were fractionated from the vegetative thylakoids by a milder method of sucrose density gradient centrifugation (Watanabe et al. 2014). These observations suggest that the tight association of CpcL with PSI cores occurs in *Anabaena* grown under stress conditions, such as iron deficiency and nitrogen starvation. Thus, the expression and binding properties of PBSs including CpcL to PSIs in *Anabaena* may be diversified in response to its living environments.

Methods

Heterocysts and their thylakoids were prepared as described previously (Nagao et al. 2022). Two-dimensional BN/SDS-PAGE was carried out as described previously (Nagao et al. 2021; Nagao et al. 2022). Mass spectrometry was performed according to Nagao et al. (2019) with some modifications. Briefly, the gel slices were digested with trypsin (TPCK-treated; Worthington Biochemical), and the resultant peptides were analyzed using LC-MS/MS consisting of Easy nLC 1000 and Q Exactive (Thermo Fisher Scientific). The acquired data was searched against the database of *Anabaena* sp. PCC 7120 with Mascot Version 2.8 software (Matrix Science).

References

- Adir N. 2005. Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant. *Photosynth Res.* 85: 15-32. DOI: <https://doi.org/10.1007/s11120-004-2143-y>
- Adir N, Bar-Zvi S, Harris D. 2020. The amazing phycobilisome. *Biochim Biophys Acta, Bioenerg.* 1861: 148047. DOI: <https://doi.org/10.1016/j.bbabi.2019.07.002>

- Blankenship RE (2021) Molecular Mechanisms of Photosynthesis. 3rd edn. Wiley-Blackwell, Oxford, U.K.
- Brettel K, Leibl W. 2001. Electron transfer in photosystem I. *Biochim Biophys Acta, Bioenerg.* 1507: 100-14. DOI: [https://doi.org/10.1016/S0005-2728\(01\)00202-X](https://doi.org/10.1016/S0005-2728(01)00202-X)
- Bryant DA, Stirewalt VL, Glauser M, Frank G, Sidler W, Zuber H. 1991. A small multigene family encodes the rod-core linker polypeptides of *Anabaena* sp. PCC7120 phycobilisomes. *Gene.* 107: 91-9. DOI: [https://doi.org/10.1016/0378-1119\(91\)90301-Q](https://doi.org/10.1016/0378-1119(91)90301-Q)
- Chang L, Liu X, Li Y, Liu C-C, Yang F, Zhao J, Sui S-F. 2015. Structural organization of an intact phycobilisome and its association with photosystem II. *Cell Res.* 25: 726-37. DOI: <https://doi.org/10.1038/cr.2015.59>
- Fromme P, Jordan P, Krauß N. 2001. Structure of photosystem I. *Biochim Biophys Acta, Bioenerg.* 1507: 5-31. DOI: [https://doi.org/10.1016/S0005-2728\(01\)00195-5](https://doi.org/10.1016/S0005-2728(01)00195-5)
- Golbeck JH. 1992. Structure and function of photosystem I. *Ann Rev Plant Physiol Plant Mol Biol.* 43: 293-324. DOI: <https://doi.org/10.1146/annurev.pp.43.060192.001453>
- Guo R, Xu Y-L, Zhu J-X, Scheer H, Zhao K-H. 2024. Assembly of CpcL-phycobilisomes. *Plant J.* 118: 1207-17. DOI: <https://doi.org/10.1111/tpj.16666>
- Hirose Y, Misawa N, Yonekawa C, Nagao N, Watanabe M, Ikeuchi M, Eki T. 2017. Characterization of the genuine type 2 chromatic acclimation in the two *Geminocystis* cyanobacteria. *DNA Res.* 24: 387-96. DOI: <https://doi.org/10.1093/dnares/dsx011>
- Kondo K, Ochiai Y, Katayama M, Ikeuchi M. 2007. The membrane-associated CpcG2-phycobilisome in *Synechocystis*: a new photosystem I antenna. *Plant Physiol.* 144: 1200-10. DOI: <https://doi.org/10.1104/pp.107.099267>
- MacColl R. 1998. Cyanobacterial phycobilisomes. *J Struct Biol.* 124: 311-34. DOI: <https://doi.org/10.1006/jsbi.1998.4062>
- Magnuson A, Cardona T. 2016. Thylakoid membrane function in heterocysts. *Biochim Biophys Acta, Bioenerg.* 1857: 309-19. DOI: <https://doi.org/10.1016/j.bbabi.2015.10.016>
- Nagao R, Kato K, Suzuki T, Ifuku K, Uchiyama I, Kashino Y, et al., Akita F. 2019. Structural basis for energy harvesting and dissipation in a diatom PSII-FCPII supercomplex. *Nat Plants.* 5: 890-901. DOI: <https://doi.org/10.1038/s41477-019-0477-x>
- Nagao R, Yokono M, Ueno Y, Nakajima Y, Suzuki T, Kato K-H, et al., Akimoto S. 2022. Excitation-energy transfer in heterocysts isolated from the cyanobacterium *Anabaena* sp. PCC 7120 as studied by time-resolved fluorescence spectroscopy. *Biochim Biophys Acta, Bioenerg.* 1863: 148509. DOI: <https://doi.org/10.1016/j.bbabi.2021.148509>
- Nagao R, Yokono M, Ueno Y, Suzuki T, Kato K, Kato K-H, et al., Akimoto S. 2021. Molecular organizations and function of iron-stress-induced-A protein family in *Anabaena* sp. PCC 7120. *Biochim Biophys Acta, Bioenerg.* 1862: 148327. DOI: <https://doi.org/10.1016/j.bbabi.2020.148327>
- Nelson N, Junge W. 2015. Structure and energy transfer in photosystems of oxygenic photosynthesis. *Annu Rev Biochem.* 84: 659-83. DOI: <https://doi.org/10.1146/annurev-biochem-092914-041942>
- Rast A, Schaffer M, Albert S, Wan W, Pfeffer S, Beck F, et al., Engel BD. 2019. Biogenic regions of cyanobacterial thylakoids form contact sites with the plasma membrane. *Nat Plants.* 5: 436-46. DOI: <https://doi.org/10.1038/s41477-019-0399-7>
- Shimizu S, Ogawa H, Tsuboshita N, Suzuki T, Kato K, Nakajima Y, et al., Nagao R. 2023. Tight association of CpcL with photosystem I in *Anabaena* sp. PCC 7120 grown under iron-deficient conditions. *Biochim Biophys Acta, Bioenerg.* 1864: 148993. DOI: <https://doi.org/10.1016/j.bbabi.2023.148993>
- Srivastava A, Biswas S, Yadav S, Kumar S, Rajaram H, Srivastava V, Mishra Y. 2021. Physiological and thylakoid proteome analyses of *Anabaena* sp. PCC 7120 for monitoring the photosynthetic responses under cadmium stress. *Algal Res.* 54: 102225. DOI: <https://doi.org/10.1016/j.algal.2021.102225>
- Watanabe M, Ikeuchi M. 2013. Phycobilisome: architecture of a light-harvesting supercomplex. *Photosynth Res.* 116: 265-76. DOI: <https://doi.org/10.1007/s1120-013-9905-3>
- Watanabe M, Kubota H, Wada H, Narikawa R, Ikeuchi M. 2011. Novel supercomplex organization of photosystem I in *Anabaena* and *Cyanophora paradoxa*. *Plant Cell Physiol.* 52: 162-8. DOI: <https://doi.org/10.1093/pcp/pcq183>
- Watanabe M, Semchonok DA, Webber-Birungi MT, Ehira S, Kondo K, Narikawa R, et al., Ikeuchi M. 2014. Attachment of phycobilisomes in an antenna-photosystem I supercomplex of cyanobacteria. *Proc Natl Acad Sci U S A.* 111: 2512-7. DOI: <https://doi.org/10.1073/pnas.1320599111>

Wolk CP, Ernst A, Elhai J (1994) Heterocyst metabolism and development. In: Bryant DA (ed) *Advances in Photosynthesis, The Molecular Biology of Cyanobacteria*. Springer, Dodrecht, pp 769-823 DOI: https://doi.org/10.1007/978-94-011-0227-8_27

You X, Zhang X, Cheng J, Xiao Y, Ma J, Sun S, et al., Sui S-F. 2023. In situ structure of the red algal phycobilisome-PSII-PSI-LHC megacomplex. *Nature*. 616: 199-206. DOI: <https://doi.org/10.1038/s41586-023-05831-0>

Zheng L, Zhang Z, Wang H, Zheng Z, Wang J, Liu H, et al., Zhao J. 2023. Cryo-EM and femtosecond spectroscopic studies provide mechanistic insight into the energy transfer in CpcL-phycobilisomes. *Nat Commun*. 14: 3961. DOI: <https://doi.org/10.1038/s41467-023-39689-7>

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