Available online at www.sciencedirect.com
ScienceDirect
Journal of Structural Biology 158 (2007) 116–121
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Crystallization note

Crystallization of sparingly soluble stress-related proteins from cyanobacteria by controlled urea solublization

Monica Dines [a], Eleonora Sendersky [b], Rakefet Schwarz [b], Noam Adir [a,*]

[a] *Schulich Faculty of Chemistry and Institute of Catalysis, Science and Technology, Technion, Israel Institute of Technology, Technion City, Haifa 32000, Israel*

[b] *The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel*

Received 14 August 2006; received in revised form 5 October 2006; accepted 7 October 2006
Available online 28 October 2006

Abstract

The phycobilisome photosynthetic antenna complex, found in cyanobacteria and red-algae, interacts with proteins expressed specifically to deal with different forms of physiological stress. Under conditions of nutrient starvation, the NblA protein is required for the process that leads to phycobilisome degradation and bleaching of the cells. HspA, a 16.5 kDa heat shock protein expressed in cyanobacterial cells, has been shown to provide functional stability to the phycobilisome during heat stress. We have cloned the genes encoding for these proteins into bacterial expression vectors in order to determine their three-dimensional structures. The resulting recombinant proteins were found to be sparingly soluble, limiting their usefulness in the performance of crystallization experiments. We have developed a novel protocol that utilizes relatively high concentrations of urea to afford sufficient solubility to the protein. This has lead to the successful growth of diffraction quality crystals of these proteins. Complete data sets collected to 2–2.5 Å from crystals of both proteins shows that the crystals are stable, and useful for structure determination. A preliminary structure of the NblA shows that denaturation has not occurred and specific protein-protein interactions have been preserved. We believe that this protocol may be a generally advantageous method to obtain well diffracting crystals of sparingly soluble proteins.

Keywords: Crystallization; Photosynthesis; Phycobilisome; Heat shock; Stress

1. Introduction

The photosynthetic apparatus undergoes substantial modulations in response to changing environmental parameters, such as light quality, light quantity, temperature and water/nutrient availability. These changes require the activation of specific systems resulting in the maintenance or down-regulation of the photosynthetic apparatus. Such systems typically entail changes in the levels of protein expression and can affect the pigment composition associated with photosynthetic complexes (Adir et al., 2003; Grossman et al., 1995; Kanervo et al., 2005; Monson et al., 1992; van Waasbergen et al., 2002).

Cyanobacteria possess several mechanisms for modifying and/or protecting the composition of the photosynthetic machinery with respect to environmental stress. One of most notable examples of such a system is the response of cyanobacteria to the lack of specific nutrients, especially nitrogen and sulfur. Under these conditions, the cyanobacterial cells change color from blue–green to yellow–green in a process known as chlorosis or bleaching (Allen and Smith, 1969; Yamanaka and Glazer, 1980). This is the result of the degradation of the light-harvesting pigment protein complex, the phycobilisome (PBS; Grossman et al., 1993). PBS degradation appears to occur due to expression of a number of genes encoding for stress-response elements (Grossman et al., 1993; Schwarz and Grossman, 1998; Sendersky et al., 2005; van Waasbergen et al., 2002). A principal protein component that appears

* Corresponding author. Fax: +972 4 82925703.
E-mail address: nadir@tx.technion.ac.il (N. Adir).

1047-8477/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.jsb.2006.10.021

to physically trigger PBS degradation during nutrient deprivation is the gene product of *nblA* (identified by screening non-bleaching mutants; Collier and Grossman, 1994). During nitrogen starvation the amount of NblA protein can increase up to 50-fold (Baier et al., 2001, 2004). The size of the proteins belonging to the NblA family throughout the PBS containing organisms is quite small, ranging from 54 to 65 residues, corresponding to molecular masses of about 6.5–7 kDa. The structure of an NblA protein from *Anabaena* sp. PCC 7120 was recently determined (Bienert et al., 2006). The sequence homology between NblA proteins from different species is quite low (~30%), which is remarkable since the homology of its putative substrates, the phycobiliprotein components of the PBS is quite high (>75%; Apt et al., 1995). Thus its mode of function remains unclear.

Another example of the response of cyanobacteria to a change in its environment is the protection of photosystem II (PSII) and phycocyanin (PC, a major constituent of all PBS forms) by HspA (Nakamoto and Honma, 2006; Roy and Nakamoto, 1998; Roy et al., 1999). This protein belongs to the large family of low molecular weight proteins that accumulate upon heat-shock treatment in plants and cyanobacteria. Most of the small heat shock proteins (sHSPs) investigated to date share a conserved region of about 90 residues called the "α crystalline-domain". The α crystalline-domain may be flanked by an N-terminal hydrophobic region, highly dissimilar in sequence and length, and a short C-terminal extension (Haslbeck, 2002). One of the common characteristics of all the sHSPs is their organization into large oligomeric structures, comprising 9–50 subunits. The structures of four members of the sHSPs family have been determined by X-ray crystallography (Dgany et al., 2004; Kim et al., 1998; Stamler et al., 2005; van Montfort et al., 2001), revealing in most cases hollow, globule-like structures. Additional structural information has been obtained by electron cryo-microscopy (Haley et al., 1998). Since structures of PBS components have been determined (Adir, 2005; Adir and Lerner, 2003; Adir et al., 2002; Brejc et al., 1995; Chang et al., 1996; Ritter et al., 1999), high-resolution structures of the NblA and HspA proteins may provide information on their mode of action on the PBS.

One of the major bottlenecks in obtaining high-resolution structural data by X-ray crystallography is the lack of solubility of many recombinant proteins (Pusey et al., 2005; Yokoyama, 2003). Proteins that require extensive protein–protein interactions *in vivo* (including stress-related proteins) can prove to be sparingly soluble when expressed in large quantities. In cases of sparingly soluble proteins (in many cases found expressed as inclusion bodies) steps including solubilization in a denaturing reagent followed by refolding of the protein, are typically required prior to performing crystallization trials. The problem of recombinant protein solubility has become more apparent with the advent of high-throughput structural proteomics efforts, in which targets with solubility problems are many times discarded.

We report here the successful crystallization of *Synechococcus elongatus* sp. PCC 7942 NblA (*Se*-NblA), *Thermosynechococcus vulcanus* NblA (*Tv*-NblA) and HspA from *T. vulcanus* (*Tv*-HspA). In all cases, the proteins were sparingly soluble and thus initial crystallization attempts were hampered. We have developed a simple protocol whereby the presence of a significant concentration of urea enabled us to concentrate the target proteins without denaturation. This was followed by the growth of diffraction quality crystals.

2. Experimental procedures

2.1. NblA and sHSP overexpression

The *T. vulcanus nblA* and *hspA* genes encoding for the *Tv*-NblA and *Tv*-HspA proteins were cloned into the pQE60 expression vector (Qiagen), while the *S. elongatus nblA* gene was cloned into pQE70 (Qiagen). Expression was performed in Bl21/m15 cells by growth of the cells to an OD of 0.6 at 37 °C in LB medium with 100 μg/ml ampicillin, followed by 3.5 h of induction with 1 mM isopropyl β-D-thiogalactopyranoside (IPTG)[1]. The bacterial cells were collected by centrifugation and frozen. Cells were thawed and resuspended in 50 mM Tris pH 8.0 and disrupted in a French Pressure cell. The insoluble and soluble fractions were separated by centrifugation.

2.2. Purification and solubilization of NblA and HspA proteins for crystallization

Tv-NblA and *Tv*-HspA protein were found in the soluble fraction following cell disruption. All proteins were then purified by metal-chelation chromatography on HisTrap columns (Amersham Biosciences), in an elution buffer containing 500 mM imidazole in 50 mM Tris buffer pH 8.0. Following elution, both the *Tv*-NblA and the *Tv*-HspA were insoluble and precipitate immediately or during dialysis against buffer Tris, pH 8.0. The pellet of the insoluble proteins was collected by centrifugation, and was then solubilized in buffer Tris, pH 8, with 2 M urea.

The *Se*-NblA protein was found only in the insoluble fraction and could be solubilized by treating the pellet with 2 M urea in Tris 50 mM pH 8.0. It was thus probably not in an inclusion body, rather it associated with the cell debris and membranes. It is also possible that the *Se*-NblA protein have minimal incorrectly folded regions. Following treatment with urea, the insoluble fraction was removed by centrifugation, leaving partially purified protein in the supernatant. The protein could be purified further by

[1] *Abbreviations used:* IPTG, isopropyl β-D-thiogalactopyranoside; PBS, phycobilisome; PC, phycocyanin; PSII, photosystem II; sHSPs, small heat shock proteins; *Se-NblA Synechococcus elongatus* sp. PCC 7942 NblA; *Tv*-HspA, *Thermosynechococcus vulcanus* HspA; *Tv*-NblA, *Thermosynechococcus vulcanus* NblA.

metal-chelation chromatography as above. In most cases the protein remained soluble in the absence of urea.

3. Results and discussion

In the course of our studies on the cyanobacterial PBS, we were interested in performing structural analysis of the modes of interaction between the PBS and proteins expressed in cells experiencing different forms of stress. Under conditions of nutrient starvation (both nitrogen and sulfur), cyanobacteria express different proteins that serve to disassemble the PBS (Collier and Grossman, 1992, 1994; Grossman et al., 1993). This serves to minimize excitation of the photosynthetic apparatus as well as provides a source of the nutrients lacking from the media. At elevated temperatures (heat shock), a member of the small heat shock protein family is expressed, and has been shown to protect both the integrity of the PBS as well as other functions of PSII (Nakamoto et al., 2000; Roy et al., 1999). In order to analyze these proteins further, we cloned their genes into appropriate expression vectors, and initiated protein isolation and crystallization trials. We found that both the NblA and the HspA recombinant proteins have solubility problems when isolated from *Escherichia coli* host cells. Lack of solubility is typically a measure of the formation of non-specific interactions between protein molecules as opposed to those between protein and solvent. Various measures have been proposed to alleviate this problem, including the addition of non-ionic detergents and low concentrations of chaotropic agents. It is clear however that one must avoid application of treatment that would result in protein denaturation. Thus the concentration of solubilizing additives used is typically low. On the other hand, crystallization requires the specific association of protein molecules by either ionic, polar, hydrogen-bond or hydrophobic interactions. Addition of solubilizing agents should prevent non-specific aggregation without interfering with the formation of the specific contacts needed for lattice formation.

In order to obtain the soluble protein needed for crystallization we added many different additives such as detergents, EDTA, DTT, various salts and buffers. Modifications to the conditions of expression such as temperature, induction period and the addition of osmolytes were also tried; however none of these treatments were able to positively influence the solubility of the proteins. The chaotropic reagent urea has been shown to have a favourable interaction with the polypeptide backbone (Bolen, 2004), and at high concentrations solubilizes proteins in a fashion which leads to denaturation. We decided to attempt to find the maximum concentration of urea that could provide the surrounding required for protein solubility, yet not prevent the association between molecules required for lattice formation, or denature the protein. It was observed that urea tends to increase the amplitudes and lifetimes of protein structural fluctuations (Bolen, 2004). For crystallization this is a potentially important parameter that can negatively influence the formation of nucleation centres. However, we have been able to obtain diffraction quality protein crystals at urea concentrations of 2 M.

Crystallization trials using the maximal concentration of soluble *Tv*-NblA protein (and in some cases *Se*-NblA) that could be obtained without the presence of urea did not result in the growth of crystals. We thus initiated the screening of possible crystallization conditions in the presence of urea, at protein concentrations of about 1 mg/ml. For the NblA proteins crystallization trials were initially performed using the Crystal Screen I and II kits (Hampton research, USA). NblA protein crystallization was performed using the vapor-diffusion method. Two microliters of protein were mixed with an equal volume of crystallization reservoir solutions. Crystals of the urea solubilized *Se*-NblA protein were obtained in the presence of urea (data not shown) and also in its absence (Fig. 1A) with 25% ethylene glycol as the precipitating reagent. These crystals diffracted to a maximum resolution of 2.2 Å on ID14 at the

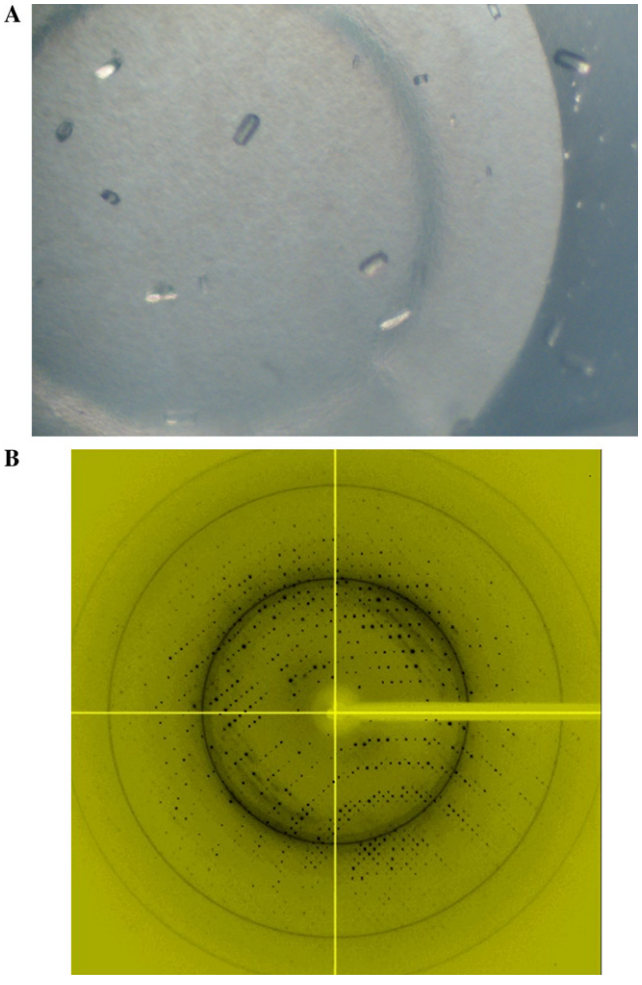

Fig. 1. (A) Crystals of NblA from *S. elongatus* sp. PCC 7942 grown in the presence of ethylene glycol. The crystals dimensions are about $0.1 \times 0.05 \times 0.04$ mm. (B) 1° oscillation diffraction pattern to 2.2 Å collected on beamline ID-14-1 at the ESRF, Grenoble.

European Synchrotron Radiation Laboratory (Fig. 1B). Analysis of the diffraction pattern showed that the crystals belong to the P4 space group with unit cell parameters $a = b = 78$ Å, $c = 70$ Å, with 6 monomers in the asymmetric unit. A complete data set has been collected and phasing by molecular replacement is under way. The crystals of *Tv*-NblA protein (Fig. 2A) were also obtained in the presence of ethylene glycol as the precipitating agent, and maximally diffracted to 2.5 Å (data not shown) and analysis of the diffraction pattern (followed by molecular replacement) showed that crystals belong to the $P3_221$ space group with the unit cell parameters $a = b = 43$ Å, $c = 148$ Å, with two monomers in the asymmetric unit. The structure of the *Tv*-NblA has been determined by molecular replacement and is now under refinement (Fig. 2B). The structure shows

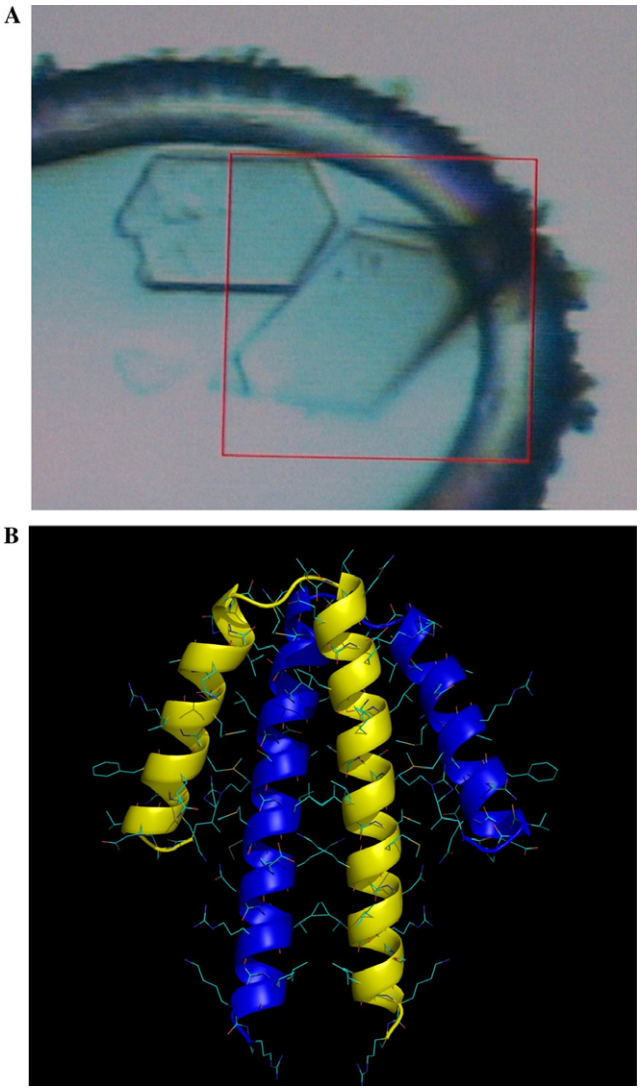

Fig. 2. (A) Crystals of NblA from *T. vulcanus* grown in presence of 2 M urea and ethylene glycol, mounted in nylon loop. The crystal dimensions are about $0.06 \times 0.04 \times 0.01$ mm. (B) Preliminary structure of *Tv*-NblA following molecular replacement, showing the preservation of secondary, tertiary and quaternary levels of structure. The two monomers are shown in yellow and blue cartoon. The figure was prepared using Pymol (DeLano, 2002).

that the presence of urea has had no deleterious effects on the secondary or tertiary structures of the protein. Indeed, formation of a dimeric structure similar to that shown for the *Anabaena* NblA (Bienert et al., 2006) shows that this concentration of urea did not affect stronger, more specific protein-protein interactions. The complete structural determination of the NblA and its potential to interact with the PBS will be described elsewhere.

When concentrated to beyond 1 mg/ml, the *Tv*-HspA precipitates. A large number of crystal trials of the *Tv*-HspA were performed at concentrations below 1 mg/ml without success. Addition of 2 M urea allowed the concentration of the protein to 10 mg/ml. The crystallization trials were initially performed in microbatch under oil and hanging drops using the Crystal Screen I and II kits, Index (Hampton research, USA), Wizard I and II (Emerald BioStructures, USA), crystals were obtained in more than 20 different conditions, most of them including various PEGs. The best crystals grown in hanging drops in 30% PEG MME 550 (Fig. 3A) and 30% PEG 2000 diffracted to between 2–2.5 Å (Fig. 3B), and analysis of the diffraction pattern and systematic absences showed that they belong to the P3 space group with cell dimensions of $a = b = 45$ Å, $c = 53$ Å, and one molecule in the asymmetric unit. A complete data set has been collected and phasing by molecular replacement is now underway.

The mode of interaction between urea and soluble proteins has been extensively studied (Bolen, 2004; Bolen and Baskakov, 2001; Street et al., 2006). The denaturing effect of urea has been proposed to primarily be a result of the association of urea molecules with the backbone of proteins, thereby forcing typically buried residues to interact with the solvent. At relatively low concentrations, urea can apparently serve as a solubilizing agent, which can even lead to crystal formation (Bolen, 2004). In this report, we show that even higher urea concentrations do not hinder the crystallization process. It is possible, that at relatively high protein concentrations, the urea merely prevents unspecific aggregation, reducing these proteins tendency to precipitate, but not nucleate.

Interestingly, the ability of 2 M urea to afford solublization without denaturation was not secondary structure dependent, since the NblA is a wholly α-helical protein, while members of the sHSP family been shown to contain extensive β-sheets (Dgany et al., 2004; Kim et al., 1998; Stamler et al., 2005; van Montfort et al., 2001). At these concentrations of urea, performance of UV spectroscopy (including the use of circular dichroism for the quantification of secondary structure in the 190–240 nm region) is difficult (Kelly et al., 2005) and thus it is possible that small conformational changes occur. However the fact that crystals were readily obtained, and the fact the *Tv*-NblA structure already determined shows no denaturative effects (Fig. 2B), suggests that this method can be used routinely in the process of crystallization trials. This is indeed important in light of the structural proteomics efforts going on world-wide. Proteins with limited solubility are typically

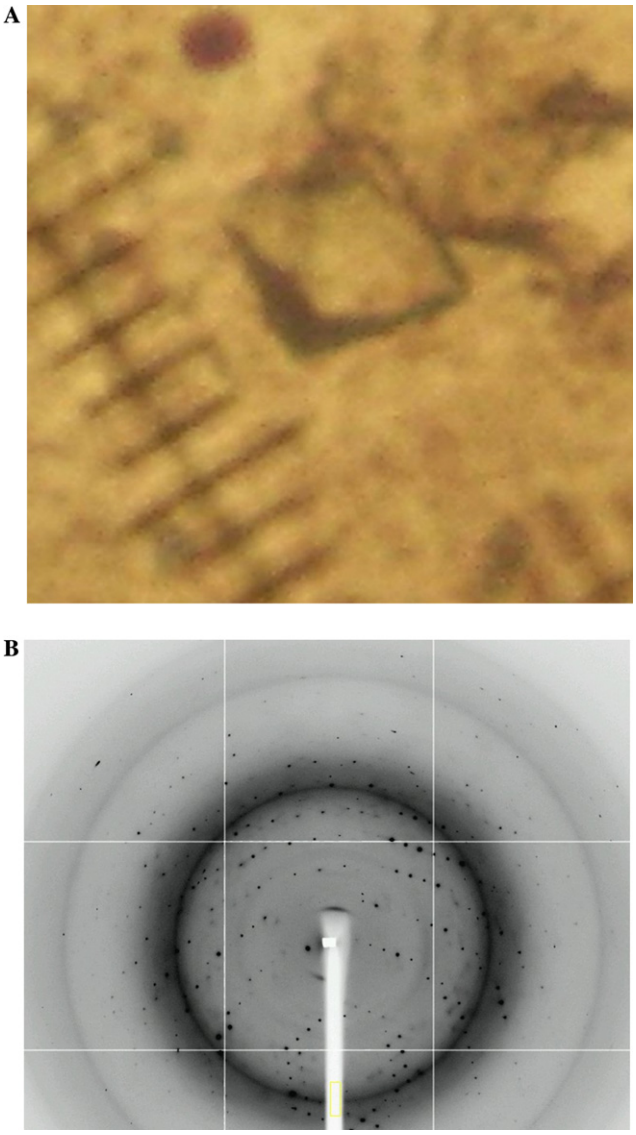

Fig. 3. (A) *Tv*-HspA crystal grown in the presence of 2 M urea and PEG MME 550. The crystals dimensions are about $0.04 \times 0.04 \times 0.04$ mm. (B) 1° oscillation diffraction pattern to 2.5 Å collected on beamline ID-29 at the ESRF, Grenoble.

discarded in efforts such as these, reducing the number of potential targets (Pusey et al., 2005). It is certainly possible that for other proteins, either higher or lower concentrations of urea may be required; however it seems likely that such a concentration can be found empirically.

Acknowledgments

We gratefully thank the staff of the European Synchrotron Radiation Facility for provision of synchrotron radiation facilities and assistance (beamlines ID29 and ID14). We would like to thank Dr. Orly Dym and the Israel Structural Proteomics Center for assistance in HspA crystallization trials. We thank Fares Salame for technical help. This work was supported by the Israel Science Foundation founded by the Israel Academy of Sciences and Humanities (438/02 and 683/03-17.1 to N.A. and R.S. respectively) and the German Israel Foundation (I-729-27.9/2002 to R.S.).

References

Adir, N., 2005. Elucidation of the molecular structures of components of the phycobilisome: reconstructing a giant. Photosynth. Res. 85, 15–32.

Adir, N., Lerner, N., 2003. The crystal structure of a novel unmethylated form of C-phycocyanin, a possible connector between cores and rods in pycobilisomes. J. Biol. Chem. 278, 25926–25932.

Adir, N., Vainer, R., Lerner, N., 2002. Refined structure of C-phycocyanin from the cyanobacterium *Synechococcus vulcanus* at 1.6Å: insights into the role of solvent molecules in thermal stability and co-factor structure. Biochim. Biophys. Acta 1556, 168–174.

Adir, N., Zer, H., Shochat, S., Ohad, I., 2003. Photoinhibition—a historical perspective. Photosynth. Res. 76, 343–370.

Allen, M.M., Smith, A.J., 1969. Nitrogen chlorosis in blue-green algae. Arch. Mikrobiol. 69, 114–120.

Apt, K.E., Collier, J.L., Grossman, A.R., 1995. Evolution of the phycobiliproteins. J. Mol. Biol. 248, 79–96.

Baier, K., Nicklisch, S., Grundner, C., Reinecke, J., Lockau, W., 2001. Expression of two nblA-homologous genes is required for phycobilisome degradation in nitrogen-starved *Synechocystis* sp. PCC6803. FEMS Microbiol. Lett. 195, 35–39.

Baier, K., Lehmann, H., Stephan, D.P., Lockau, W., 2004. NblA is essential for phycobilisome degradation in *Anabaena* sp. strain PCC 7120 but not for development of functional heterocysts. Microbiology 150, 2739–2749.

Bienert, R., Baier, K., Volkmer, R., Lockau, W., Heinemann, U., 2006. Crystal structure of NblA from *Anabaena* sp. PCC 7120, a small protein playing a key role in phycobilisome degradation. J. Biol. Chem. 281, 5216–5223.

Bolen, D.W., 2004. Effects of naturally occurring osmolytes on protein stability and solubility: issues important in protein crystallization. Methods 34, 312–322.

Bolen, D.W., Baskakov, I.V., 2001. The osmophobic effect: natural selection of a thermodynamic force in protein folding. J. Mol. Biol. 310, 955–963.

Brejc, K., Ficner, R., Huber, R., Steinbacher, S., 1995. Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the cyanobacterium *Spirulina platensis* at 2.3 Å resolution. J. Mol. Biol. 249, 424–440.

Chang, W.R., Jiang, T., Wan, Z.L., Zhang, J.P., Yang, Z.X., Liang, D.C., 1996. Crystal structure of R-phycoerythrin from *Polysiphonia urceolata* at 2.8Å resolution. J. Mol. Biol. 262, 721–731.

Collier, J.L., Grossman, A.R., 1992. Chlorosis induced by nutrient deprivation in *Synechococcus* sp. strain PCC 7942: not all bleaching is the same. J. Bacteriol. 174, 4718–4726.

Collier, J.L., Grossman, A.R., 1994. A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. EMBO. J. 13, 1039–1047.

DeLano, W.L., 2002. The PyMOL Molecular Graphics System. http://www.pymol.org.

Dgany, O., Gonzalez, A., Sofer, O., Wang, W., Zolotnitsky, G., Wolf, A., Shoham, Y., Altman, A., Wolf, S.G., Shoseyov, O., Almog, O., 2004. The structural basis of the thermostability of SP1, a novel plant (*Populus tremula*) boiling stable protein. J. Biol. Chem. 279, 51516–51523.

Grossman, A.R., Schaefer, M.R., Chiang, G.G., Collier, J.L., 1993. The phycobilisome, a light-harvesting complex responsive to environmental conditions. Microbiol. Rev. 57, 725–749.

Grossman, A.R., Bhaya, D., Apt, K.E., Kehoe, D.M., 1995. Light-harvesting complexes in oxygenic photosynthesis: diversity, control, and evolution. Annu. Rev. Genet. 29, 231–288.

Haley, D.A., Horwitz, J., Stewart, P.L., 1998. The small heat-shock protein, alphaB-crystallin, has a variable quaternary structure. J. Mol. Biol. 277, 27–35.

Haslbeck, M., 2002. sHsps and their role in the chaperone network. Cell Mol Life Sci. 59, 1649–1657.

Kanervo, E., Suorsa, M., Aro, E.M., 2005. Functional flexibility and acclimation of the thylakoid membrane. Photochem. Photobiol. Sci. 4, 1072–1080.

Kelly, S.M., Jess, T.J., Price, N.C., 2005. How to study proteins by circular dichroism. Biochim. Biophys. Acta 1751, 119–139.

Kim, K.K., Kim, R., Kim, S.H., 1998. Crystal structure of a small heat-shock protein. Nature 394, 595–599.

Monson, R.K., Jaeger, C.H., Adams, W.W., Driggers, E.M., Silver, G.M., Fall, R., 1992. Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. Plant Physiol. 98, 1175–1180.

Nakamoto, H., Honma, D., 2006. Interaction of a small heat shock protein with light-harvesting cyanobacterial phycocyanins under stress conditions. FEBS Lett. 580, 3029–3034.

Nakamoto, H., Suzuki, N., Roy, S.K., 2000. Constitutive expression of a small heat-shock protein confers cellular thermotolerance and thermal protection to the photosynthetic apparatus in cyanobacteria. FEBS Lett. 483, 169–174.

Pusey, M.L., Liu, Z.J., Tempel, W., Praissman, J., Lin, D., Wang, B.C., Gavira, J.A., Ng, J.D., 2005. Life in the fast lane for protein crystallization and X-ray crystallography. Prog. Biophys. Mol. Biol. 88, 359–386.

Ritter, S., Hiller, R.G., Wrench, P.M., Welte, W., Diederichs, K., 1999. Crystal structure of a phycourobilin-containing phycoerythrin at 1.90-Å resolution. J. Struct. Biol. 126, 86–97.

Roy, S.K., Nakamoto, H., 1998. Cloning, characterization, and transcriptional analysis of a gene encoding an alpha-crystallin-related, small heat shock protein from the thermophilic cyanobacterium *Synechococcus vulcanus*. J. Bacteriol. 180, 3997–4001.

Roy, S.K., Hiyama, T., Nakamoto, H., 1999. Purification and characterization of the 16-kDa heat-shock-responsive protein from the thermophilic cyanobacterium *Synechococcus vulcanus*, which is an alpha-crystallin-related, small heat shock protein. Eur. J. Biochem. 262, 406–416.

Schwarz, R., Grossman, A.R., 1998. A response regulator of cyanobacteria integrates diverse environmental signals and is critical for survival under extreme conditions. Proc. Natl. Acad. Sci. USA 95, 11008–11013.

Sendersky, E., Lahmi, R., Shaltiel, J., Perelman, A., Schwarz, R., 2005. NblC, a novel component required for pigment degradation during starvation in *Synechococcus* PCC 7942. Mol. Microbiol. 58, 659–668.

Stamler, R., Kappe, G., Boelens, W., Slingsby, C., 2005. Wrapping the alpha-crystallin domain fold in a chaperone assembly. J. Mol. Biol. 353, 68–79.

Street, T.O., Bolen, D.W., Rose, G.D., 2006. A molecular mechanism for osmolyte-induced protein stability. Proc. Natl. Acad. Sci. USA 103, 13997–14002.

vanMontfort, R.L., Basha, E., Friedrich, K.L., Slingsby, C., Vierling, E., 2001. Crystal structure and assembly of a eukaryotic small heat shock protein. Nat. Struct. Biol. 8, 1025–1030.

vanWaasbergen, L.G., Dolganov, N., Grossman, A.R., 2002. *nblS*, a gene involved in controlling photosynthesis-related gene expression during high light and nutrient stress in *Synechococcus elongatus* PCC 7942. J. Bacteriol. 184, 2481–2490.

Yamanaka, G., Glazer, A.N., 1980. Dynamic aspects of phycobilisome structure. Phycobilisome turnover during nitrogen starvation in *Synechococcus* sp. Arch. Microbiol. 124, 39–47.

Yokoyama, S., 2003. Protein expression systems for structural genomics and proteomics. Curr. Opin. Chem. Biol. 7, 39–43.