Expression, purification and preliminary crystallization study of RpaC protein from *Synechocystis* sp. PCC6803

E. CSÉFALVAY[*,+], M. LAPKOUSKI[*], O. KOMÁREK[**]

Department of Structure and Function of Proteins, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic and Institute of Physical Biology, University of South Bohemia in České Budějovice, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic[*]
Department of Biological Dynamics, Laboratory of Ecology and Physiology of Plants, Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic and Institute of Physical Biology, University of South Bohemia in České Budějovice, Zámek 136, CZ-373 33 Nové Hrady, Czech Republic[**]

Abstract

State transitions in cyanobacteria are physiological adaptation mechanisms that change the interaction of the phycobilisomes with the photosystem I and photosystem II core complexes. This mechanism is essential for cyanobacteria at low light intensities. Previous studies of cyanobacteria have identified a gene named *rpa*C, which appears to be specifically required for state transitions. The gene product of *rpa*C is very probably a transmembrane protein that is a structural component of the phycobilisome-photosystem II supercomplex. However, the physiological role of RpaC protein is unclear.

Here we report the construction of an expression system that enables high production of fusion protein TrxHisTagSTag-RpaC, and describe suitable conditions for purification of this insoluble protein at a yield of 3 mg per 1 dm^3 of bacterial culture. Cleavage with HRV 3C protease to remove the TrxHisTagSTag portion resulted in low yields of RpaC-protein (~ 30 μg/dm^3 of bacterial culture), therefore the applicability to structural studies was tested for the fusion protein only. Several preliminary conditions for crystallization of TrxHisTagSTag-RpaC were set up under which microcrystals were obtained. This set of conditions will be a good starting point for optimization in future crystallization experiments. TrxHisTagSTag-RpaC protein may prove useful in biochemical studies where the small size of RpaC protein is limiting the investigation of interactions with significantly larger parts of the photosynthetic apparatus. Furthermore, the purification procedure described here might also be applied to the production and purification of other small membrane proteins for biochemical and structural studies.

Additional key words: affinity chromatography; photosystem; phycobilisome – PSII supercomplex.

Introduction

The state transitions are a well known and clearly described phenomenon in higher plants and green algae, as well as in cyanobacteria, particularly *Synechocystis* PCC 6803. State transitions regulate (optimize) the light harvesting of phototrophic organisms under low light conditions (Bonaventura and Myers 1969, Murata 1969, Fork *et al*. 1983). In general the principle of this phenomenon is the coupling and uncoupling of the light harvesting complex (LHC) or antenna complex (AC) with photosystem II (PSII) (State 1) or photosystem I (PSI) (State 2) (Horton *et al*. 1980, Horton *et al*. 1981, Allen *et al*. 1981).

In green plants and green algae the underlying biochemical principle of state transitions is based on the activation or deactivation of a protein kinase which phosphorylates a part of the pool of LHCII light-harvesting complexes and the redox state of the plastoquinone pool (van Thor *et al*. 1998). This leads to redistribution of LHCII between PSII and PSI (Allen *et al*. 2001). LHCII kinase has also been identified in green alga

Received 12 August 2008, *accepted* 25 May 2009.
[+]Corresponding author; fax: +420386361279, e-mail: ecsefalvay@nh.usbe.cas.cz
Acknowledgements: Authors thank to Dr. Rüdiger Ettrich for his scientific help and language corrections. Support from the Ministry of Education, Youth and Sports of the Czech Republic (MSM6007665808), the Academy of Sciences of the Czech Republic (AVOZ60870520, KJB500870701), and the Grant agency of the Czech Republic (206/07/0917). We thank EMBL for access to the X11 beamline at the DORIS storage ring of DESY in Hamburg.

Chlamydomonas reinhardtii (Depege *et al*. 2003), but has no obvious orthologue in cyanobacteria (Mullineaux *et al*. 2005).

In cyanobacteria the state transitions enable the distribution of energy between PSII and PSI; phycobilisome antenna has the major harvesting role over both of the photosystems (Mullineaux 1994, Rakhimberdieva *et al*. 2001). Lacking the LHCII complex the exhibition of state transitions in cyanobacteria remains unclear up to now. Thus the mechanism of state transition must be accomplished by a different way. It appears to involve migration of phycobilisomes from PSII to PSI and phosporylation of one or more proteins triggered by plastoquionone pool (van Thor *et al*. 1998).

A random mutagenesis study in cyanobacterium identified a gene named *rpa*C (GeneBank accession No. SLL1926), which appeared to be specifically required for state transitions. Analysis of the *rpa*C deletion mutant in comparison with a wild type protein supports the hypothesis that state transitions are important under low light conditions. A previous study demonstrated that the *rpa*C gene ortholog is essential for the cell viability in *Synechococcus* sp. PCC7942. In the absence of *rpa*C the photosynthetic system of *Synechocystis* is in state 1 while in the absence of *rpa*C in *Synechococcus* the phycobilisome antenna is permanently disconnected from PSII, indicating a permanent shift to state 2 (Joshua *et al*. 2005).

The authors of the latter study propose that the *rpa*C gene product controls the stability of the phycobilisome-PSII super-complex, and is probably a structural component of the complex that regulates the stability of phycobilisome-PSII interaction (Joshua *et al*. 2005).

In this paper we describe the cloning and expression of the gene encoding RpaC in *E. coli.* The purification procedures of the expressed recombinant RpaC protein have been optimized to yield sufficient amounts of highly pure fusion TrxHisTagSTag-RpaC protein for the usage in further biochemical and biophysical studies which should elucidate the crucial role of this protein in state transitions.

Materials and methods

All chemicals used in the following experiments for which the manufacturer is not specified are of biochemical or electrophoresis grade and produced by either *PENTA* Prague, CZ or *Sigma-Aldrich*, St. Louis, MO, USA.

Generation of an expression plasmid: The *rpa*C gene (255 bp) was PCR amplified using genomic *Synechocystis* sp. 6803 DNA [0.5 μg DNA, 1 μM N-terminal primer, 1 μM C-terminal primer, 1 × Pfu buffer, 200 μM dNTP, and 1.5 U Pfu polymerase (Promega U. S. Madison, WI, USA) in 50 mm^3 of reaction] by using specific primers (N-terminal primer 5´-**GCTCTTGAA GTCCTCTTTCAGGGACCC**GGATCCGAAAGAGA CTTACCCAAATATC-3´ and C-terminal primer AAAA AAGAATTC*TCATCA*ATCGGACTCCACCGGGAC-3´, *Sigma-Aldrich*, St. Louis, MO, USA) containing the sequence encoding for the HRV 3C protease recognition site (*bold*), BamHI or EcoRI recognition sequences (underlined), and two stop codons (*italics*). PCR cycling conditions were 95 °C/300 s, 30 × (95 °C/60 s, 50 °C/30 s, 72 °C/25 s), and 72 °C/300 s. The amplified fragment was digested by EcoRI endonuclease (*New England Biolabs*, Ipswich, MA, USA) and cloned into vector pET32a (*Novagen, an Affiliate of Merck KGaA*, Darmstadt, Germany) digested with EcoRV (*New England Biolabs*, Ipswich, MA, USA) and EcoRI (*New England Biolabs*, Ipswich, MA, USA). The *rpa*C insert of the resulting plasmid pET32a-3C*rpa*C was verified by sequencing (using T7 terminator primer, *Sigma-Aldrich*, St. Louis, MO, USA).

Expression of fusion TrxHisTagSTag-RpaC protein: *E. coli* BL21 (DE3)-RIPL (*Novagen, an Affiliate of Merck KGaA*, Darmstadt, Germany) cells were transformed with pET32a-3C*rpa*C to obtain BL21-(DE3)-RIPL - pET32a-3C*rpa*C. Overnight cultures of BL21-(DE3)-RIPL - pET32a-3C*rpa*C in Luria-Bertani medium (20 cm^3, *ForMedium*, Norfolk, UK) with 100 μg cm^{-3} ampicillin (*ForMedium*, Norfolk, UK) were used for the inoculation of expression cultures (1 dm^3) grown in the same media to an $OD_{600nm,}$ of ~ 0.6 in a 10 mm cuvette. Expression of fusion protein TrxHisTagSTag-RpaC was induced by addition of IPTG (isopropyl β-D-1-thio-galactopyranosid, *ForMedium*, Norfolk, UK) to a final concentration of 1 mM, and cultures were grown at room temperature overnight.

Purification of fusion protein TrxHisTagSTag-RpaC: Cells were harvested by centrifugation (4000 × g, 600 s, 4 °C), resuspended in 50 cm^3 of buffer NPI-10 (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8) and incubated with lysozyme (50 μg cm^{-3}, *SERVA Electrophoresis GmbH*, Heidelberg, Germany) for 30 min on ice. Cells were disrupted two times using a French press (138 MPa, *Thermo Fisher Scientific Inc*.,Waltham, MA, USA). Benzonase nuclease (5 U, *Sigma-Aldrich*, St. Louis, MO, USA) was added to the lysate to digest nucleic acids. After incubation at 37 °C for 1800 s Triton X-100 [4-(1,1,3,3-tetramethylbutyl)] phenyl-polyethylene glycol, *Sigma-Aldrich*, St. Louis, MO, USA) was added to a final concentration of 1 % and the mixture was incubated at 37 °C for an additional 1 800 s. Cell debris was pelleted by centrifugation (15 000 × g for 1 800 s, room temperature). The supernatant was loaded onto a Co^{2+} sepharose column (20 cm^3, *Sigma-Aldrich*, St.

Louis, MO, USA) which was equilibrated with 5 column volumes (CV) of NPI-10 buffer. The column was washed with 5 CV of buffer NPI-20 (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazol, pH 8) to remove unbound proteins. TrxHisTagSTag-RpaC protein (27,8 kDa, pI 5.12) was subsequently eluted with buffer NPI-250 (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazol, pH 8) at a flow rate of 1 cm^3 min^{-1}. Fractions containing protein were analyzed by 12 % sodium dodecyl sulphate-polyacryl amide gel electrophoresis (SDS-PAGE). The fractions containing TrxHisTagSTag-RpaC protein were combined and the protein was concentrated using *Amicon Ultra* (10 kDa cut off) centrifugal filter devices (*Millipore Corporation*, Billerica, MA, USA). After 3 washing steps with heparin low salt buffer (50 mM Tris, 50 mM NaCl, 5 mM EDTA, pH 8) the sample was applied to a heparin column (22 cm^3, *Sigma-Aldrich*, St. Louis, MO, USA) using a ratio of 1:10 (V_{sample}/V_{column}). The column was washed with 5 CV heparin low salt buffer, and the protein was eluted with 2.5 CV of a linear gradient of NaCl (50–500 mM) at a flow rate of 1 cm^3/ 60 s. Fractions (5 cm^3) containing protein were analyzed by SDS-PAGE. Fractions containing pure TrxHisTagSTag-RpaC protein were collected, concentrated as described above, and then washed 3 times by 1 × HRV 3C protease buffer (150 mM NaCl, 50 mM Tris, pH 7.5) and stored at –20 °C. Concentrated samples were measured spectrophotometrically (OD_{280}, ε_{280} = 240 275 $M^{-1}cm^{-1}$ in a 10 mm cuvette).

Cleavage and purification of RpaC from fusion TrxHisTagSTag-RpaC protein: The RpaC protein (9.5 kDa, pI 4.76) was separated from the TrxHisTagSTag by cleavage with HRV 3C protease (*Novagen, an Affiliate of Merck KGaA*, Darmstadt, Germany), 1 U per 30 µg of protein, 57 600 s at 4 °C. RpaC protein was purified (from uncut fusion protein, anchor protein and protease) using Co^{2+} sepharose. Co^{2+} sepharose was equilibrated with 5 volumes of 1 × protease buffer. After addition of the cleavage reaction mixture containing protease and protein to the resin, the sample was incubated 1800 s on ice. The cleavage was performed in a 1.5 cm^3 reaction tube with equal volumes of Co^{2+} sepharose and cleavage reaction. The mixture was then centrifuged for 60 s at 14000 × *g* and the supernatant was collected. RpaC protein was eluted from the Co^{2+} sepharose with 3 equal volumes of protease buffer. After the elution steps the residual fusion protein, TrxHisTagSTag anchor protein, and HRV 3C protease were washed from Co^{2+} sepharose by NPI-250 buffer. All elution and wash fractions were analyzed by Tris-Tricine PAGE (Schägger *et al*. 1987). Fractions containing RpaC protein were collected, concentrated using *Amicon Ultra* (3 kDa cut off) centrifugal device (*Millipore Corporation*, Billerica, MA, USA), and then washed 3 times with 50 mM Tris, pH 7.5, and stored at –20 °C. Concentration of the sample was measured spectrophotometrically (OD_{280nm}, ε_{280} = 9 970 $M^{-1}cm^{-1}$ in a 10 mm cuvette).

Crystallization of fusion TrxHisTagSTag-RpaC protein: The purified fusion protein TrxHisTagSTag-RpaC (15 mg cm^{-3}) was used for crystallization. Initial experiments were performed in 24-well Linbro plates (*Hampton Research*, Aliso Viejo, CA, USA) at two temperatures, 4 and 21 °C. The standard hanging-drop vapour-diffusion method was used for all experiments. A 1 mm^3 protein solution was mixed with an equal volume of reservoir solution taken from the crystallization kits *Crystal Screen 1* and *2* (*Hampton Research*, Aliso Viejo, CA, USA), *JBScreen Mixed* and *JBScreen 1* (*Jena Bioscience*, Jena, Germany), *Wizard III* random sparse matrix crystallization screen (*Emerald BioSystems*, Bainbridge Island, WA, USA), Clear *Strategy Screen 1* (*Molecular Dimemsions Ltd.*, Suffolk, UK), and *MemStart Kit* (*Molecular Dimemsions Ltd*., Suffolk, UK). Drops were equilibrated over a 500 mm^3 reservoir solution.

Results

Construction of expression system: The DNA fragment encoding *rpa*C was amplified from genomic *Synechocystis* 6803 DNA as described in Materials and methods and cloned into pET32a (*Novagen, an Affiliate of Merck KGaA*, Darmstadt, Germany) (Fig. 1). The pET32a vector is constructed for expression of proteins fused to thioredoxin, which in many cases increases solubility. Clones were verified by sequencing and subsequently tested for expression of TrxHisTagSTag-RpaC fusion protein.

Expression of TrxHisTagSTag-RpaC protein and testing of suitable purification conditions: Expression strain *E. coli* BL21 (DE3) was transformed with vector pET32a-3C*rpa*C. The expression of TrxHisTagSTag-RpaC was tested at different temperatures, 37 °C, 30 °C, and 20 °C, with different concentrations of IPTG (0.2 mM, 0.5 mM, and 1 mM). Crude extracts were prepared by resuspension of harvested cells in 100 mM sodium phosphate buffer. Cells were disrupted enzymatically by lysozyme and mechanically using a French press (138 MPa, *Thermo Fisher Scientific Inc*., Waltham, MA, USA). The insoluble fraction was separated by centrifugation. Both pellets and crude extracts were analyzed by SDS-PAGE for all preps. In all instances the fusion protein was found to be in the insoluble pellet (data not shown).

To solubilize the fusion protein we used 8 M urea and 0.1 % SDS. Surprisingly, 8 M urea solubilized TrxHisTagSTag-RpaC from cells grown at 37 °C, but not

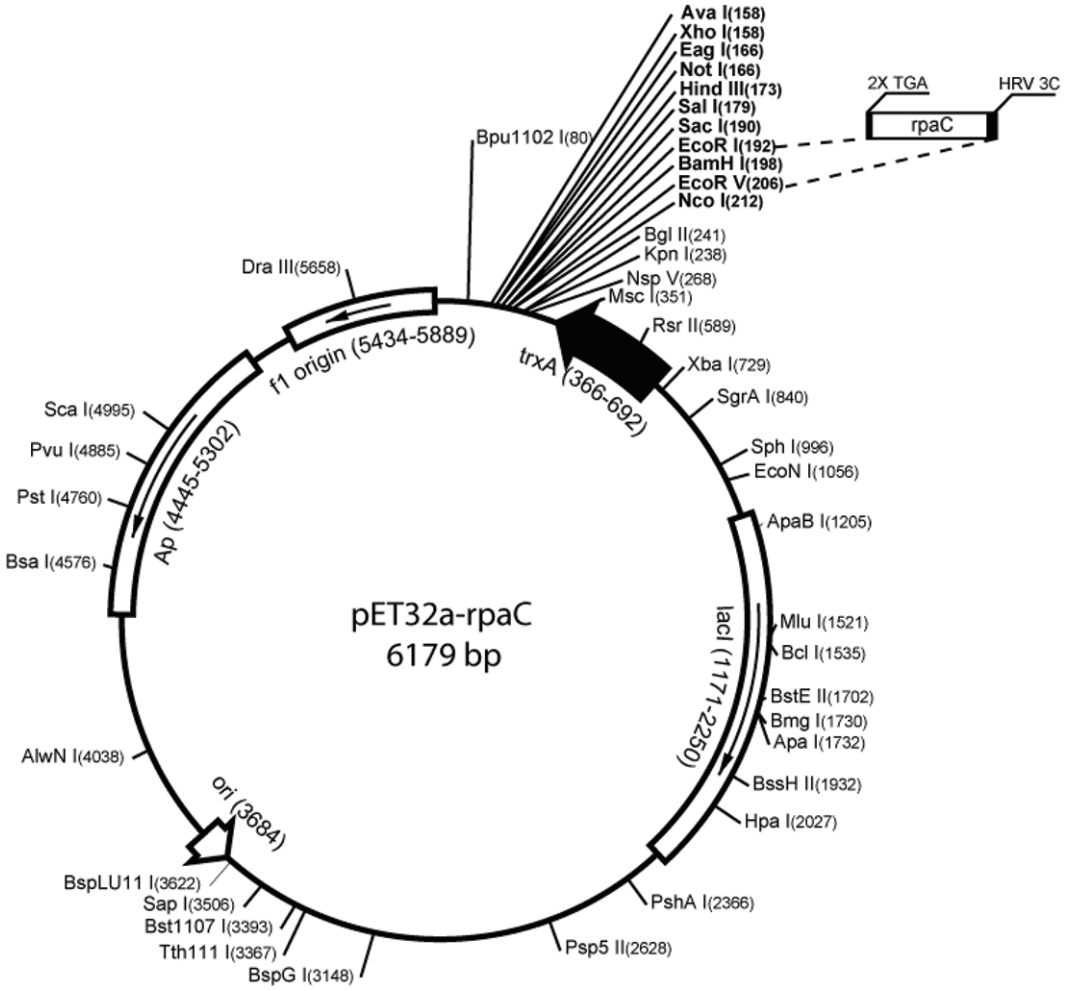

Fig. 1. Schematic representation of the expression plasmid pET32a-3C*rpa*C. The plasmid was constructed by insertion of a PCR derived *rpa*C fragment fused to the HRV 3C protease recognition sequence (285 bp) between the EcoRV and EcoRI restriction sites of plasmid pET32a (5900 bp, *Novagen, an Affiliate of Merck KGaA*, Darmstadt, Germany). Ap: ampiciline resistance, lacI: lactose coding sequence, ori: origin of replication.

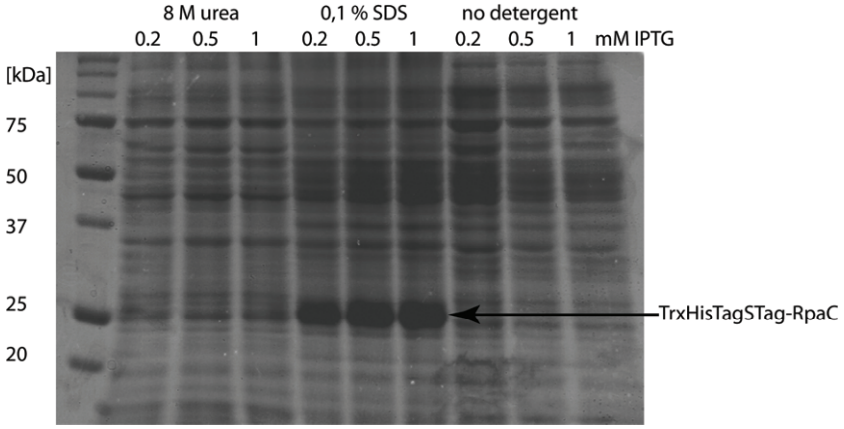

Fig. 2. Influence of denaturating reagents and IPTG concentration (both indicated on top) on solubility of TrxHisTagSTag-RpaC. *Lane 1*: molecular weight marker, fragment masses are indicated on the left; *lanes 2–4*: crude extracts containing 8 M urea; *lanes 5–7*: crude extracts containing 0.1 % SDS; lanes *8–10*: crude extracts containing no detergent from cells *E. coli* BL21(DE3) - pET32a-3C*rpa*C induced by 0.2, 0.5, and 1 mM IPTG respectively, for 20 h at 30 °C. Soluble fusion protein TrxHisTagSTag-RpaC found in crude extracts containing 0.1 % SDS is indicated by an arrow.

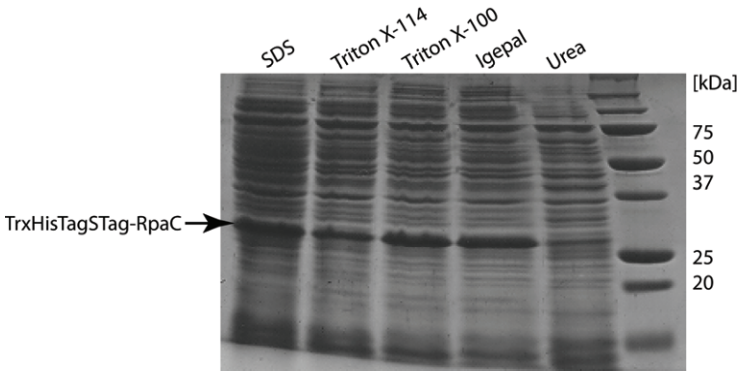

Fig. 3. Influence of detergents on the solubility of TrxHisTagSTag-RpaC (indicated on top). *Lane 1*: crude extract containing 0.1 % SDS; *lane 2*: crude extract containing 1 % Triton X-114; *lane 3*: crude extract containing 1 % Triton X-100, *lane 4*: crude extract containing 1 % Igepal; *lane 5*: crude extract containing 8 M urea from cells *E. coli* BL21(DE3) - pET32a-3C*rpa*C induced by 1 mM IPTG for 20 h at 30 °C; *lane 6*: molecular weight marker, fragment masses are indicated on the right. Presence of soluble fusion protein TrxHisTagSTag-RpaC in the crude extracts is indicated by an arrow.

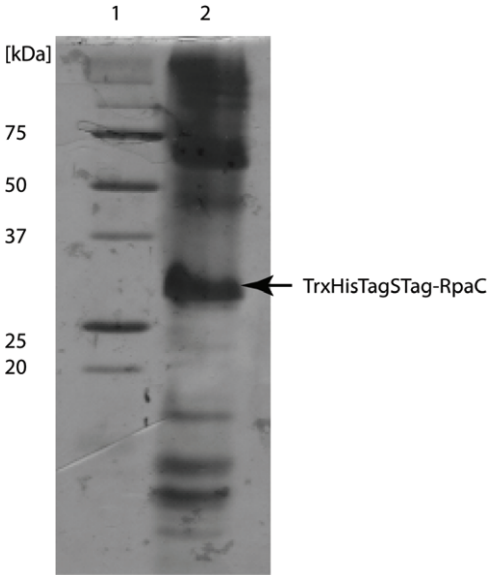

Fig. 4. TrxHisTagSTag-RpaC fusion protein sample after purification on Co^{2+} sepharose. *Lane 1*: molecular weight marker, fragment masses are indicated on the left; *lane 2*: TrxHisTagSTag-RpaC, ~ 10 µg. The band corresponding to TrxHisTagSTag-RpaC is indicated by an arrow.

the protein from cells grown at 30 °C and room temperature. In contrast, 0.1 % SDS alone solubilized protein from 30 °C and room temperature cultures but did not increase the solubility of protein from 37 °C cultures. We did not observe any influence of different IPTG concentrations on protein solubility (Fig. 2). Solubilized fusion protein from these various preps were purified using affinity chromatography (Co^{2+} sepharose). However, a problem occurred at this stage: protein solubilized in 8 M urea and purified under denaturing conditions hardly renatured and easily precipitated, while protein solubilized by 0.1 % SDS hardly bound to the column resin. Therefore, other detergents were tested. In all subsequent trials expression of TrxHisTagSTag-RpaC was induced by 1 mM IPTG at 30 °C. After overnight induction, lysis, and pelleting the protein was solubilized by either 1 % Triton X-100, Triton X-114, or Igepal [4-(1,1,3,3-tetramethylbutyl)] phenyl-polyethylene glycol, (1,1,3,3-tetramethylbutyl) phenyl-polyethylene glycol, or polyoxyethylene (2) isooctylphenyl ether, *Sigma-Aldrich*, St. Louis, MO, USA). Protein was well solubilized in all cases (Fig. 3).

For all further experiments Triton X-100 was therefore used in the purification procedure as it is most compatible with Co^{2+} sepharose. Protein yields were increased when the 'codon plus' expression strain BL21(DE3) RIPL was used as a host.

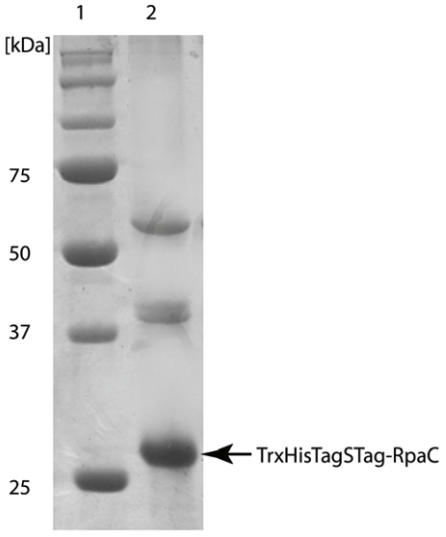

Fig. 5. TrxHisTagSTag-RpaC fusion protein sample after purification on heparine sepharose. *Lane 1*: molecular weight marker, fragment masses are indicated on the left; *lane 2*: the TrxHisTagSTag-RpaC sample (5 µg) used for crystallization experiments. The band corresponding to TrxHisTagSTag-RpaC is indicated by an arrow. The additional two bands most probably represent aggregate forms of TrxHisTagSTag-RpaC.

Purification of TrxHisTagSTag-RpaC protein: TrxHis TagSTag-RpaC was purified by using two-step affinity chromatography as described in Materials and methods and fractions were tested on SDS-PAGE. Co^{2+} sepharose was used in the first purification step. However, the eluted fractions still contained significant amount of impurities (cellular proteins) (Fig. 4). To increase purity of the fusion protein a heparin column was used in a second purification step. The sample was eluted from the heparin column by a linear NaCl gradient (50–500 mM). Purified protein (Fig. 5) was concentrated and stored in 1 × HRV 3C protease buffer. The final yield was approximately 3 mg of protein per 1 dm^3 of culture.

Cleavage and purification of RpaC from fusion TrxHisTagSTag-RpaC protein: Protein RpaC (85 amino acid residues) was separated from the fusion protein as described in Materials and methods. The amount of HRV 3C protease required for sufficient digestion of TrxHisTagSTag-RpaC was determined to be 1 U per 30 µg of fusion protein. RpaC after purification on Co^{2+} sepharose was visualized on Tris-Tricine PAGE (Fig. 6). However, as shown in Fig. 6, a large percentage of the RpaC (9.5 kDa) was found in wash and elution fractions also containing residual fusion protein (27.8 kDa), TrxHisTagSTag anchor (18.4 kDa) and protease (22 kDa). After concentration of the RpaC elution fraction, the protein concentration was approximately 7-fold lower than in the source TrxHisTagSTag-RpaC sample and the final yield was ~ 30 µg/dm^3 of source bacterial culture. Therefore, the RpaC sample was not suitable for crystallization experiments. Further purification attempts using other chromatography methods such as gel and ionex chromatography did not lead to improved purity of RpaC as we again saw diffusion of the protein amongst all fractions (data not shown). Hence we decided to use fusion protein TrxHisTagSTag-RpaC for crystallization experiments.

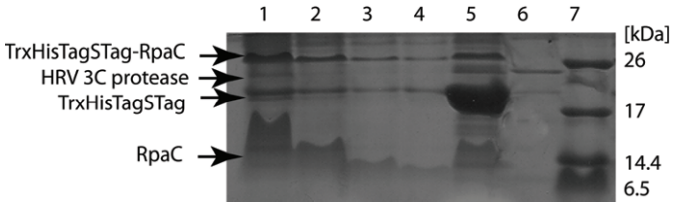

Fig. 6. Purification of RpaC protein from the protease restriction mixture by using Co^{2+} sepharose. *Lanes 1–4*: elution fractions; *lanes 5–6*: wash fractions; *lane 7*: molecular weight marker, fragment masses are indicated on the right. Bands corresponding to TrxHisTagSTag-RpaC, TrxHisTagSTag, RpaC proteins, and HRV 3C protease are indicated by arrows.

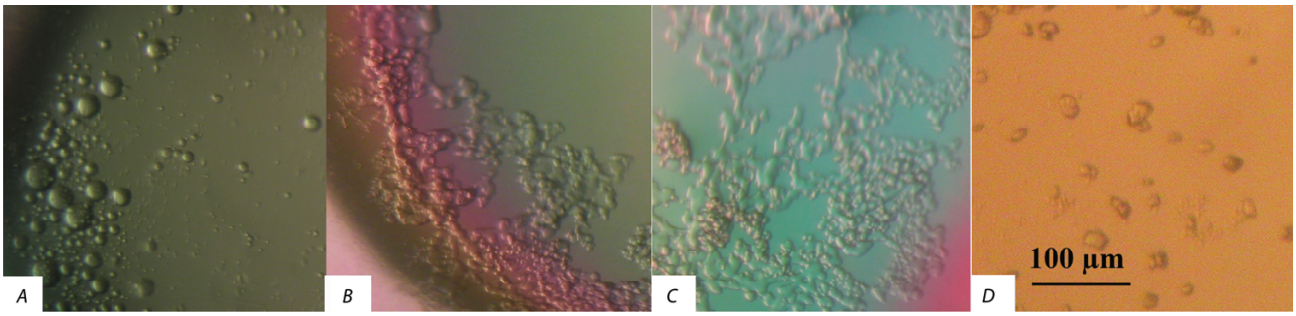

Fig. 7. Crystallyzation trials of TrxHisTagSTag-RpaC using *MemStart Kit. A*: Spherulites obtained under conditions: 0.1 M trisodium citrate/citric acid, 1 M magnesium sulphate, pH 5.6. *B,C*: Quasi crystals grown under conditions: 0.1 M ADA/NaOH, 0.1 M lithium sulphate, and 1 M magnesium sulphate, pH 6.5; and 0.1 M Tris-HCl, 0.2 M magnesium chloride, 12 % w/v polyethylene glycol 4000, pH 8.5. *D*: Microcrystals obtained under condition: 0.1 M MES/NaOH, 0.2 M calcium acetate, 10 % w/v polyethylene glycol 8000, pH 6.5.

Crystallization of TrxHisTagSTag-RpaC fusion protein: Several commercially available kits were used as starting points for screening and optimization of crystallization conditions for alpha type transmembrane proteins using the vapor diffusion method as described in Materials and methods. Crystallization conditions were also carried out at the High-throughput crystallization facility (Mueller-Dieckmann 2006) at the European Molecular Biology Laboratory (EMBL), Hamburg. Screening with the *MemStart Kit* (*Molecular Dimemsions Ltd.*, Suffolk, UK) led to suitable initial conditions for crystal production. Using precipitant solutions from the *MemStart Kit*, spherulites and quasi crystals were obtained under the following conditions: 1) 0.1 M trisodium citrate/citric acid and 1 M magnesium sulphate, pH 5.6; 2) 0.1 M ADA/NaOH, 0.1 M lithium sulphate, and 1 M magnesium sulphate, pH 6.5; and 3) 0.1 M Tris-HCl, 0.2 M magnesium chloride, 12 % w/v polyethylene glycol 4000, pH 8.5 (Fig.7*A,B,C*). Possible microcrystals were grown from a solution of 0.1 M MES/NaOH, 0.2 M calcium acetate, and 10 % w/v polyethylene glycol 8000, pH 6.5 (Fig. 7*D*). Therefore the conditions found in the *Mem Start Kit* would be a good starting point for attempting to grow crystals suitable for X-ray diffraction experiments.

Discussion

The thylakoidal RpaC protein at the time of its discovery was considered the regulatory protein for the so named state transitions photosynthetic process in cyanobacteria. It has been proposed that the *rpaC* gene product controls the stability of the phycobilisome - PSII supercomplex and is probably a structural component of the complex (Joshua *et al*. 2005).

RpaC from *Synechocystis* 6803 has been recently characterized as an 85 amino acid residue protein (Mullineaux *et al*. 1997). Although being relatively small it has a quite remarkable domain structure as predicted from computational analysis of its amino acid sequence. These computer simulations have the N and C termini located in the cytoplasm while the two transmembrane hydrophobic alpha helices, which are connected *via* a short 6 amino acids stretch, extend to the lumenal side. This topology model has not yet been experimentally verified.

So far orthologues of the RpaC protein have been identified in three cyanobacteria species: *Synechocystis* 6803 (Emlyn-Jones *et al*. 1999), *Synechococcus* 7942 (Joshua *et al*. 2005), and *Anabaena* (Kaneko *et al*. 2001). The N-terminal sequence is particularly strongly conserved in all cyanobacteria RpaC orthologues. However, a putative *Synechococcus* 7942 *rpa*C gene product has a C-terminal extension of 21 amino acids as compared to *Synechocystis* 6803 RpaC, implicating possible differences in the C-terminal regions between species. Interestingly, in contrast to *Synechococcus* 7942, the RpaC protein is not essential for viability of *Synechocystis* 6803 under normal light conditions (Joshua *et al*. 2005).

In the work described above we purified recombinant RpaC protein from *Synechocystis* 6803. We obtained large amounts of highly pure TrxHisTagSTag-RpaC fusion protein, which was used for crystallization experiments. Preliminary crystallization experiments demonstrate that it is possible to crystallize TrxHisTagSTag-RpaC fusion protein. Overall, however, low crystallization efficiency was observed which may be caused by the propensity of the fusion protein to form more-or-less unordered aggregates. This problem might be overcome by using suitable detergents in low concentration. Also, alternative biophysical methods such as Raman spectroscopy or NMR could be additionaly used for structure determination. However, since these methods can only be applied to RpaC without fusion parts, the limiting factor for these experiments is the insufficient amount and low purity of RpaC protein obtained. Difficulties in optimizing the purification process after the removal of fusion components could not be solved even by using high-resolution chromatographical methods such as HPLC. At this time we have simply been unable to find a suitable method for separation of RpaC from its fusion form. With high probability this problem could stem from the rather hydrophobic properties of RpaC and its tendency to form aggregates. Unfortunately, detergents such as 1 % Triton X-100 were not able to improve the purification performance.

Future biochemical studies may help to provide deeper insight into the state transition process and purified TrxHisTagSTag-RpaC fusion protein may be an especially helpful tool for studying the protein-protein interactions required to switch the photosynthetic apparatus between state 1 and 2. For such studies the utilization of purified native RpaC protein is not feasible due to its very low molecular weight in relation to the other parts of the photosynthetic apparatus, which supposedly directly contact RpaC protein. The large size of TrxHisTagSTag-RpaC may circumvent this problem. Another advantage of fusion TrxHisTagSTag-RpaC is its possible immobilization, which could dramatically simplify biochemical experiments. Lastly, the fusion protein may also help to examine the presence of RpaC protein in other organisms by using immunological assays.

References

Allen, J.F., Bennett, J., Steinback, K.E., Arntzen, C.J.: Chloroplast protein phosphorylation couples plastoquinone redox state to distribution of excitation energy between photosystems. – Nature **291**: 25-29, 1981.

Allen, J.F. and Forsberg, J.: Molecular recognition in thylakoid structure and function. – Trends Plant Sci. **6**: 317-326, 2001.

Bonaventura, C. and Myers, J.: Fluorescence and oxygen evolution from *Chlorella pyrenoidosa*. – Biochim. Biophys. Acta **189**: 366-383, 1969.

Depege, N., Bellafiore, S., Rochaix, J.D.: Role of chloroplast protein kinase Stt7 in LHCII phosphorylation and state transition in *Chlamydomonas*. – Science **299**: 1572-1575, 2003.

Emlyn-Jones, D., Ashby, M.K., Mullineaux, C.W.: A gene required for the regulation of photosynthetic light harvesting in the cyanobacterium *Synechocystis 6803*. – Mol. Microbiol. **33**: 1050-1058, 1999.

Fork, D.C., Satoh, K.: State I-state-II transitions in the thermophilic blue-green-alga (cyanobacterium) *Synechococcus lividus*. – Photochem. Photobiol. **37**: 421-427, 1983.

Horton, P., Allen, J.F., Black, M.T., Bennett, J.: Regulation of phosphorylation of chloroplast membrane polypeptides by the redox state of plastoquinone. – FEBS Lett. **125**: 193-196, 1981.

Horton, P., Black, M.T.: Activation of adenosine 5′ triphosphate-induced quenching of chlorophyll fluorescence by reduced plastoquinone. The basis of state-I-state-II transitions in chloroplasts. – FEBS Lett. **119**: 141-144, 1980.

Joshua, S., Mullineaux, C.W.: The *rpa*C gene product regulates phycobilisome-photosystem II interaction in cyanobacteria. –

Biochim. Biophys. Acta **1709**: 58-68, 2005.
Kaneko, T., Nakamura, Y., Wolk, C.P., Kuritz, T., Sasamoto, S., Watanabe, A., Iriguchi, M., Ishikawa, A., Kawashima, K., Kimura, T., Kishida, Y., Kohara, M., Matsumoto, M., Matsuno, A., Muraki, A., Nakazaki, N., Shimpo, S., Sugimoto, M., Takazawa, M., Yamada, M., Yasuda, M., Tabata, S.: Complete genomic sequence of the filamentous nitrogen-fixing *Cyanobacterium anabaena* sp. strain PCC 7120. – DNA Res. **8**: 205-213, 2001.
Mueller-Dieckmann, J.: The open-access high-throughput crystallization facility at EMBL Hamburg. – Acta Crystallogr. **62**: 1446-1452, 2006.
Mullineaux, C.W.: Excitation energy transfer from phycobilisomes to photosystem I in a cyanobacterial mutant lacking photosystem II. – Biochim. Biophys. Acta **1184**: 71-77, 1994.
Mullineaux, C.W., Emlyn-Jones, D.: State transitions: an example of acclimation to low-light stress. – J. Exp. Bot. **56**: 389-393, 2005.
Mullineaux, C.W., Tobin, M.J., Jones, G.R.: Mobility of photosynthetic complexes in thylacoid membranes. – Nature **390**: 421-424, 1997.
Murata, N.: Control of excitation transfer in photosynthesis. I. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum*. – Biochim. Biophys. Acta **172**: 242-251, 1969.
Rakhimberdieva M.G., Boichenko V.A., Karapetyan N.V., Stadnichuk I.N.: Interaction of phycobilisomes with photosystem II dimmers and photosystem I monomers and trimers in the cyanobacterium *Spirulina platensis*. – Biochemistry **40**: 15780-15788, 2001.
Schägger, H., von Jagow, G.: Tricine-sodium sulfate-polyacrylamide gel electroforesis for the separation of proteins in the range from 1 to 100 kDa. – Anal. Biochem. **166**: 368-379, 1987.
van Thor, J.J., Mullineaux, C.W., Matthijs, H.C.P., Hellingwert, K.J.: Light harvesting and state transitions in cyanobacteria. – Bot. Acta **111**: 430-443, 1998.