S4-028

Cloning and expression of a cDNA encoding the extrinsic 20 kDa protein of photosystem II from a red alga *Cyanidium caldarium*

H. Ohta, M. Ueno, T. Suzuki, A. Satoh, A. Okumura, M. Kamo, I. Enami

Department of Biology, Science University of Tokyo, Shinjuku-ku, Tokyo 162-8601, Japan.
ohta@rs.noda.sut.ac.jp

Keywords: oxygen evolution, photosystem II, extrinsic proteins, 20kDa protein, cloning

Introduction

Oxygen-evolving PSII complex purified from a red alga, *Cyanidium caldarium*, contains four extrinsic proteins, namely, 12 kDa, 20 kDa, 33 kDa proteins and cytochrome *c*-550 (Enami et al. 1995, 1998). Among these four proteins, the 20 kDa protein is unique in that it has been found only in the red algal PSII but not in cyanobacterial and higher plant PSII. Release-reconstitution experiments with the purified *C. caldarium* PS II and its extrinsic proteins suggested that the 20 kDa protein is required for maximum binding of cyt *c*-550 and the 12 kDa protein to PSII, but may not function directly in the red algal oxygen evolution (Enami et al. 1998). In order to better understand the function of the 20 kDa protein and its role in the evolutionary process of PSII extrinsic proteins, it is essential to clone the gene and determine its sequence. In this work, we cloned the gene for the 20 kDa protein from the red alga, *Cyanidium caldarium*, based on the N-terminal amino sequences of the mature protein. The cloned gene was expressed successfully in *E. coli*, and the expressed protein was examined for its binding and functional properties.

Materials and methods

PCR amplification

Two sets of degenerate oligonucleotide primers, AGEPKM and GEPVYK, were synthesized based on the N-terminal sequences of the 20 kDa protein. Using these primers, cDNA fragments were amplified by RT-PCR, and their sequences were determined to confirm that it indeed corresponded the N-terminal part of the gene for the 20 kDa protein. Based on this information, the remaining fragments of the genes were amplified by RACE-PCR (3) performed using the Marathon cDNA Amplification Kit (Clontech, Palo Alto CA).

Cloning of PCR products and sequencing

The PCR fragments obtained were inserted into the plasmid pCR II (TA Cloning Kit, Invitrogen, San Diego, CA), and the DNA sequences were determined by the method of Dye Deoxy Terminator Cycle Sequencing method with a DNA Sequencer (Applied Biosystems, model 310).

Expression and purification of recombinant 20 kDa protein

The gene encoding the 20 kDa protein was cloned into the LIC site of plasmid pCAL-n-EK (Stratagene). The recombinant protein was expressed and purified according to the manufacture s instructions.

Reconstitution analysis

Rebinding and functional properties of the recombinant protein were examined by reconstitution experiments with $CaCl_2$-washed red algal PS II, according to Enami et al. (1998)

Results and discussion

Cloning and sequence analysis of the 20 kDa protein

The gene coding for the 20 kDa protein from *C. caldarium* was successfully cloned by the two-step PCR method described in the Materials and methods section. First, a 90-bp cDNA fragment corresponding to the N-terminal part of the protein was amplified from a *C. caldarium* cDNA library. The second PCR step was performed with the RACE procedure (3), by which DNA fragments including the 5 - and 3 -flanking regions of the 20 kDa protein were amplified using primers newly synthesized based on the 90-bp cDNA fragment. This second-step PCR resulted in 450-bp and 600-bp cDNA fragments from the 5 - and 3 -RACE, respectively. Sequencing of these cDNA fragments confirmed that they contain the cDNA for the 20 kDa protein. These sequences were combined with the partial sequence of the N-terminal part to yield the whole sequence of the gene. The resulted gene encodes a polypeptide of 218 amino acid residues with a total molecular mass of 24028 Da (the complete sequence of the gene will be published elsewhere and also in the DNA databases).

Fig. 1 shows hydropathy plot of the cloned gene for the 20 kDa protein. The N-terminal part of the mature 20 kDa protein starts at residue number 73; thus, residues 1-72 serve as leader sequences. Hydropathy analysis revealed that there are two characteristic domains in this leader sequence, one of which is residues number 1-51 that are enriched in basic, hydrophilic, as well as hydroxylated residues. These are consistent with the characteristic features of transit peptides for transport across the chloroplast envelope, suggesting that this domain functions to direct the 20 kDa protein transferring across the chloroplast envelope. The second domain is residues number 52-72 which has characteristic features of transit peptides for transfer through the thylakoid lumen, because its central part is enriched in hydrophobic residues and its C-terminus contains residues in position —3 and —1. Thus, we conclude that the 20 kDa protein is encoded by the nuclear DNA in the red alga.

Cleavage of the transit peptides resulted in a mature polypeptide of 146 amino acid residues with a calculated molecular mass of 16477 Da. A search in the Swiss-Prot database indicated that the deduced amino acid sequence of the mature form of the 20 kDa protein was 36.3% and 30.3% identical to the extrinsic 17 kDa protein from two green algae, *Volvox carteri* and *Chlamydomonas reinhardtii*, respectively (data not shown).

Expression and purification of recombinant proteins

The 20 kDa protein was expressed as a fusion protein with calmodulin binding-tag to permit affinity purification, using the pET expression system. The expressed protein was recovered in the supernatant of the lysate of the host cell (BL21, Novagen, WI) by centrifugation and subsequently purified by calmodulin affinity chromatography. The fusion protein was then treated with enterokinase and again applied to calmodulin affinity chromatography to remove calmodulin binding-tag. Using this recombinant 20 kDa protein, we have performed reconstitution experiments with the red algal PSII and our results have shown that the

recombinant 20 kDa protein can bind and function in the red algal PSII effectively (data to be published elsewhere).

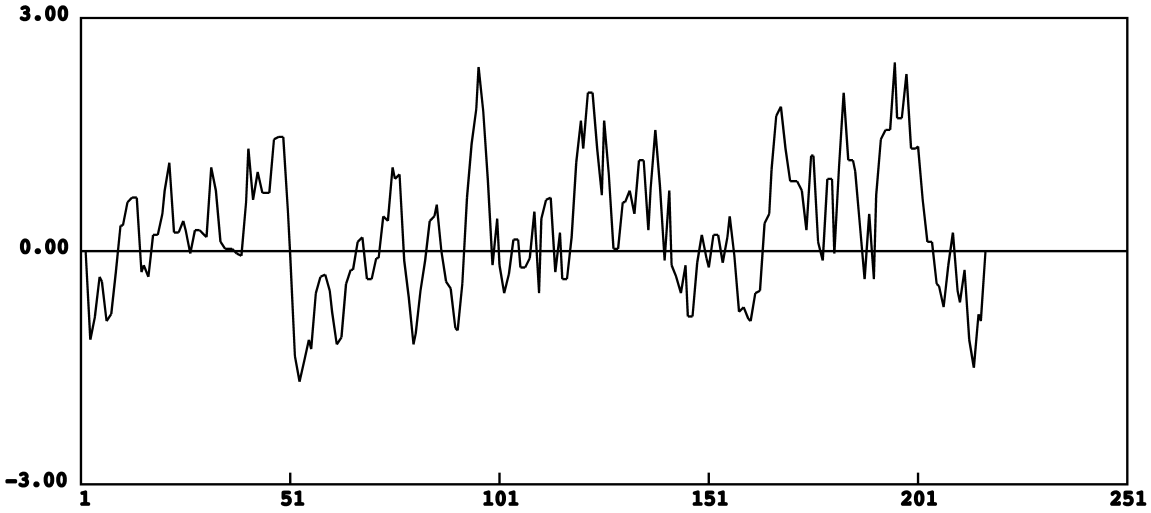

Fig. 1. Hydropathy plot analysis of the deduced amino acid sequence of the gene for the 20 kDa protein.

In conclusion, we have cloned for the first time the gene coding for the extrinsic 20 kDa protein involved in oxygen evolution from a red alga, *C. caldarium*, and determined the complete nucleotide sequence of the gene. Our results indicated that the gene of the 20 kDa protein was encoded in the nuclear genome. The protein was successfully expressed in *E. coli*, and the resulted recombinant protein retained its binding and reactivation abilities for oxygen evolution.

References

Enami I, Murayama H, Ohta H, Kamo M, Nakazato K and Shen J-R (1995) Isolation and characterization of a photosystem II complex from a red alga *Cyanidium caldarium:* Association of cytochrome *c*-550 and a 12 kDa protein with the complex. *Biochim. Biophys. Acta* 1232, 208-216.

Enami I, Kikuchi S, Fukuda T, Ohta H and Shen J-R (1998) Binding and Functional properties of four extrinsic proteins of photosystem II from a red alga, *Cyanidium caldarium*, As studied by release-reconstitution experiments. *Biochemistry* 37, 2787-2793

Frohman, M.A., Dush, M.K. and Martin, G.R. (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA* 85, 8998-9002.