Expression of the *psbA* Gene in *E. coli*

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Z. Naturforsch. **42c**, 755–757 (1987); received November 17, 1986

psbA Gene, Herbicide Binding Protein, Hybrid Protein, Antibody

A *psbA* gene fragment has been cloned into an expression vector in frame with the 3′end of the beta-galactosidase gene. Expression of this construct in *E. coli* results in an overproduction of a hybrid protein consisting of part of the herbicide binding protein and beta-galactosidase. An antiserum raised against this fusion protein specifically detects a 34 kDa polypeptide within the complex mixture of spinach thylakoid membrane proteins.

Introduction

The *psbA* gene coding for the herbicide binding protein (D1) is localized on the chloroplast genom and has been sequenced in several higher plants and algae. Isolation, purification and protein sequencing of the highly hydrophobic gene product have been problematic and not succeeded yet. The D1 polypeptide can be detected by photoaffinity-labeling [1–3] and pulse-labeling [4]. The employment of antibodies has been reported [5, 6], but production of the antibody has only been described for the alga *Bumilleriopsis filiformis* [7]. In this case thylakoid proteins were separated by PAGE, the D1 protein excised from the gel and used as antigen. However, a clear relationship between gene and gene product can most unambiguously be established by expression of the gene in an organism like *E. coli*. Possible problems in handling a hydrophobic polypeptide like D1 might be overcome by expressing only part of the gene and constructing a hybrid protein, the largest part of which is a naturally occurring protein in *E. coli* cells. In addition, induction of expression minimizes possible lethal effects of the foreign gene product. This technique has been used for a variety of proteins [8, 9]. In this communication a method is presented which allows the expression of part of the *psbA* gene in *E. coli* supplying high amounts of a chimeric beta-galactosidase :: D1 protein which is suitable for antibody production.

Abbreviations: bp, base pair(s); beta-gal, beta-galactosidase; IPTG, isopropyl-beta-D-thiogalactoside; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; ::, novel joint.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0600–0755 $ 01.30/0

Materials and Methods

Plasmid pUR278 and its host *E. coli* K12 strain F′11recA were kindly provided by B. Müller-Hill. The plasmid containing the spinach *psbA* gene was obtained from H. Bohnert.

Isolation of plasmid DNA, preparation of DNA fragments, ligation and transformation in *E. coli* were carried out as described in Maniatis *et al.* [11]. Plasmid-containing colonies were screened by the rapid boiling method of Holmes and Quigley [12].

The clone pFJ2 selected for further experiments contained the *psbA* gene as is shown by hybridizing a 5′end labeled synthetic DNA oligomer to nitrocellulose-bound *Hind* III fragments of pFJ2 (Fig. 2A). The oligomer sequence is complementary to the noncoding strand nucleotides 844–860 as described by Zurawski *et al.* [10]. Pre-hybridization and hybridization [11] was performed in 2 × Denhardt's solution, 5 × SSC, 1% SDS and 0.1 mg/ml yeast RNA at ambient temperature.

For isolation of the fusion protein a 200 ml culture containing the proper plasmid was grown to late log phase. Expression of the hybrid protein was induced by adding IPTG (0.5 mM) for 2 h. Cells were harvested, suspended in 4 ml 0.1 M dithiothreitol, 0.1 M Na_2CO_3, 2 mM PMSF. After sonication 2.7 ml 5% SDS, 30% sucrose was added, and the solution boiled for 5 min. Total cellular proteins were separated on preparative 7.5% SDS polyacrylamide gels and stained with Coomassie blue. The strong band running slower than beta-galactosidase was excised and electroeluted [15]. Rabbits were injected intradermally with the antigen emulsified with complete Freunds adjuvant and incomplete Freunds adjuvant for all subsequent injections [14]. Thylakoids were prepared according to Robinson and Yocum [17].

Western blot analysis and immunological detection of proteins were done according to Towbin *et al.* [13].

Results and Discussion

Part of the *psbA* gene was cloned in frame with the 3'end of the *lacZ* gene to allow expression of a hybrid protein consisting of beta-galactosidase and amino acid 167 to 353 of D1 (10; Fig. 1) following an idea of Ohad *et al.* [6]. The strategy was as follows: a *Hind* III fragment from a cloned spinach *psbA* gene including the natural translation termination signal was cloned into the *Hind* III site of expression vector pUR278 [8]. Clones with the correct orientation could be easily identified by cutting recombinant plasmids with *Xba I* generating a 550 bp or 300 bp fragment in correct and incorrect orientation, respectively. One of the clones carrying plasmid pFJ2 was chosen for further studies. By hybridization with a *psbA* specific DNA oligomer the presence of *psbA* sequences in pFJ2 could be demonstrated (Fig. 2A). Expression in *E. coli* cells carrying the desired plasmid was obtained upon induction by IPTG. High amounts of a polypeptide with a lower mobility than beta-galactosidase were produced (H in Fig. 2B,

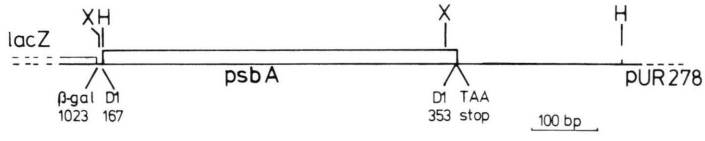

Fig. 1. Structure of the gene-fusion expression vector pFJ2. The *Hind* III (H)-fragment of the *psbA* gene contains the carboxyterminal part of D1 (amino acid 167 to 353) in frame with the beta-galactosidase carboxyterminus. X indicate sites for the restriction endonuclease *Xba* I.

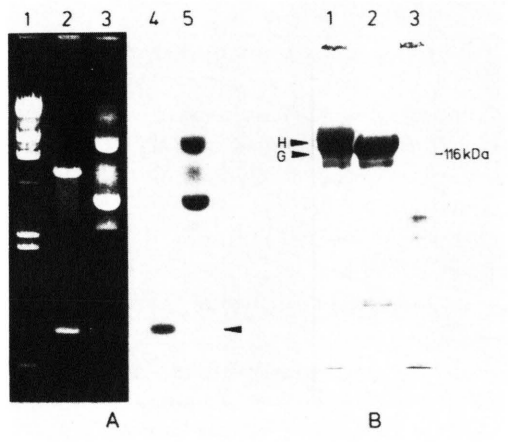

Fig. 2. A. Identification of the *psbA* gene in Vektor pFJ2. The ethidium bromide stained agarose gel was loaded with *Hind* III fragments of lambda DNA as marker, plasmid pFJ2 cut with *Hind* III (lane 2) and uncut pFJ2 (lane 2). Lanes 4 and 5 show the autoradiogram corresponding to lanes 2 and 3 obtained after blotting and hybridizing with a ^{32}P-labeled *psbA*-specific oligomer. Arrow points to the 800 bp *psbA* insert. B. High level expression of the beta-gal :: D1 hybrid polypeptide. Total cellular *E. coli* protein from cells growing in the presence (lanes 1 and 2) and absence (lane 3) of IPTG were separated on a 7.5% polyacrylamide gel and stained with Coomassie blue. Lane 1 shows the protein pattern of cells containing plasmid pFJ2, lanes 2 and 3 the protein pattern of cells containing plasmid pUR278 without insert. H indicates the hybrid protein, G the beta-galactosidase of MW 116 kDa.

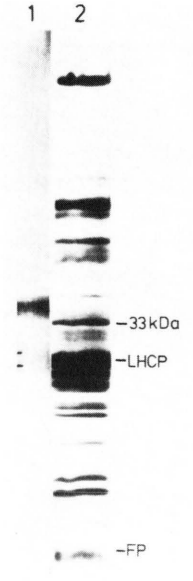

Fig. 3. Separation of spinach thylakoid membrane proteins on a 11–15% polyacrylamide gel after staining with Coomassie blue (lane 2) and detection of D1 by immunoblotting (lane 1) using the antibody raised against the hybrid protein (H in Fig. 2B). Antibody complexes were visualized by staining for peroxidase activity. The 33 kDa polypeptide involved in the water splitting complex and the light-harvesting chlorophyll *a/b* binding protein (LHCP) are indicated for reference. FP indicates the free pigment zone.

lane 1). An additional polypeptide slightly smaller than the fusion protein presumably is a breakdown product of the largest polypeptide. For antibody production total cellular proteins were separated by preparative PAGE and the Coomassie blue stained band containing the hybrid protein was excised and electroeluted. The antigen was used for production of antibodies in rabbits.

As shown in Fig. 3, the antibody clearly reacts with a 34 kDa polypeptide on Western blots. The protein is poorly stainable with Coomassie blue and runs as a diffuse band under these PAGE conditions [16]. Enriched photosystem II particles strongly react with the antiserum when blotted onto nitrocellulose (data not shown).

The technique described offers a convenient way to produce high amounts of D1 antigen covalently attached to a "carrier-protein". By using other restriction enzymes and/or *Bal*31 deletions it should be possible to express different epitopes of the herbicide binding protein. Antibodies raised against these polypeptides should allow the study of the topology of the D1 protein in the membrane.

Acknowledgements

The work was supported by Fonds der Chemischen Industrie. I thank Dr. R. J. Berzborn and F. Jasper for their help in this work.

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