FEDERAL RESEARCH CENTRE
«FUNDAMENTALS OF BIOTECHNOLOGY»
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Detection of the binding the stress HliA protein *Synechocystis* sp. with pigments

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Hli proteins are important for acclimatization, optimal photosynthetic activity, and survival of cyanobacteria cells under stress, but their exact role and mechanism of action are still unclear. Therefore, the preparation of purified HliA protein and the study of its binding to the pigments (chlorophyll a and beta-carotene) is important for understanding protection mechanism of the photosynthetic apparatus from light stress.

The aim is to study the binding of chlorophyll a and beta-carotene to the recombinant HliA protein.

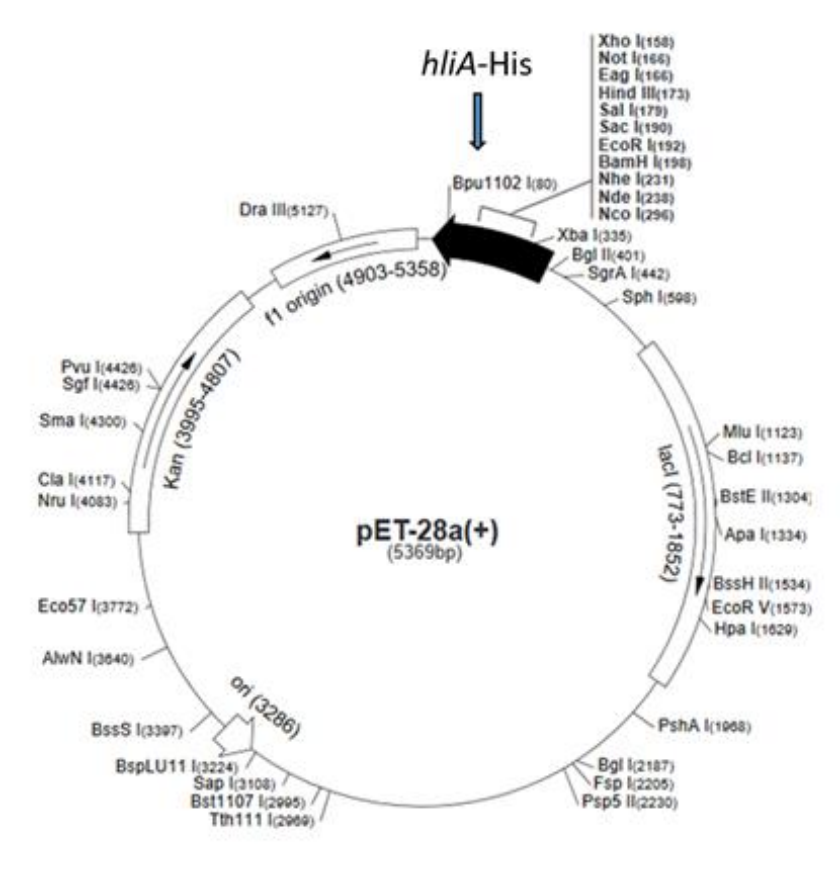


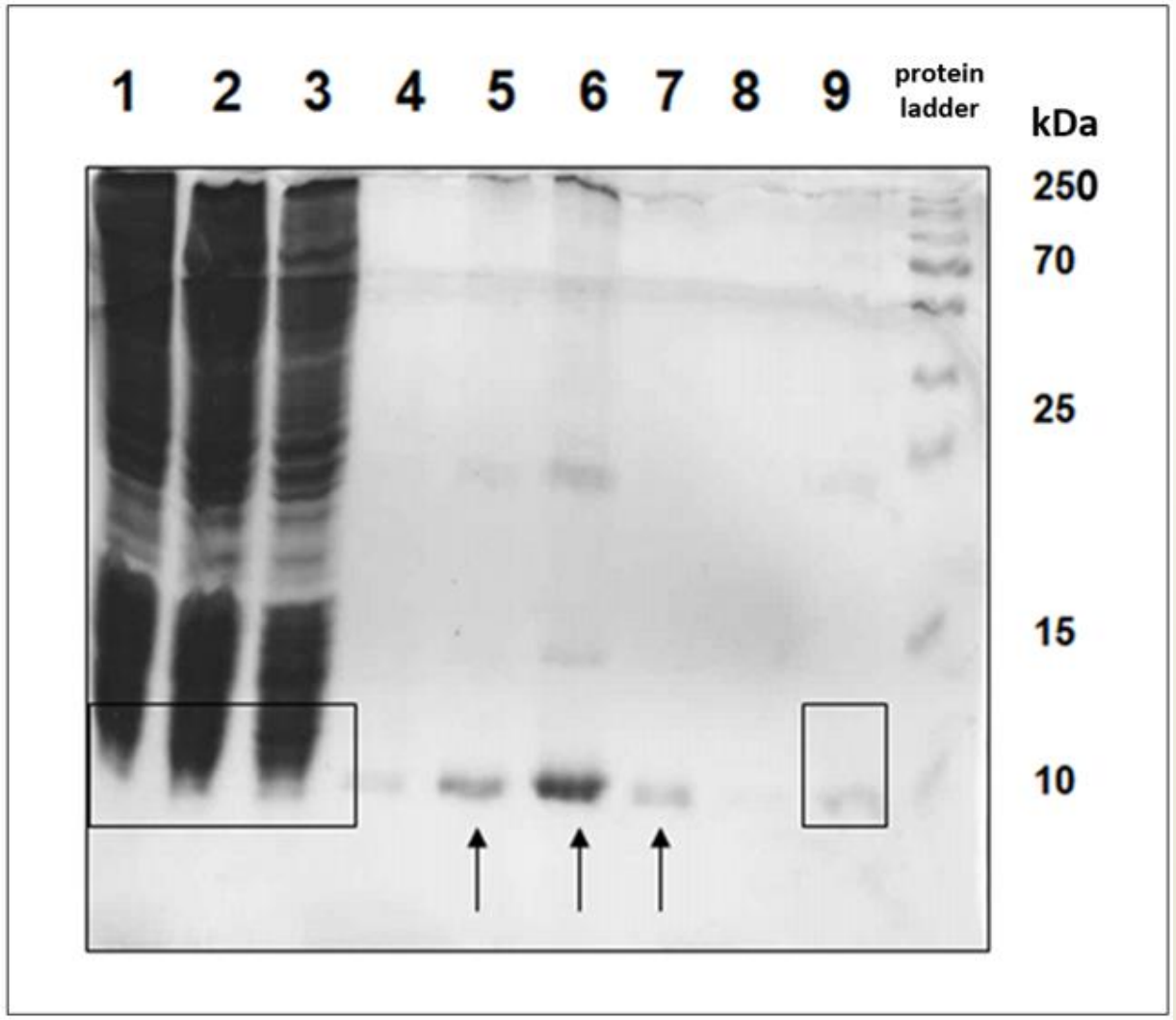
Fig. 1. pET28a vector, on the basis of which the plasmid is constructed.

Materials and methods:

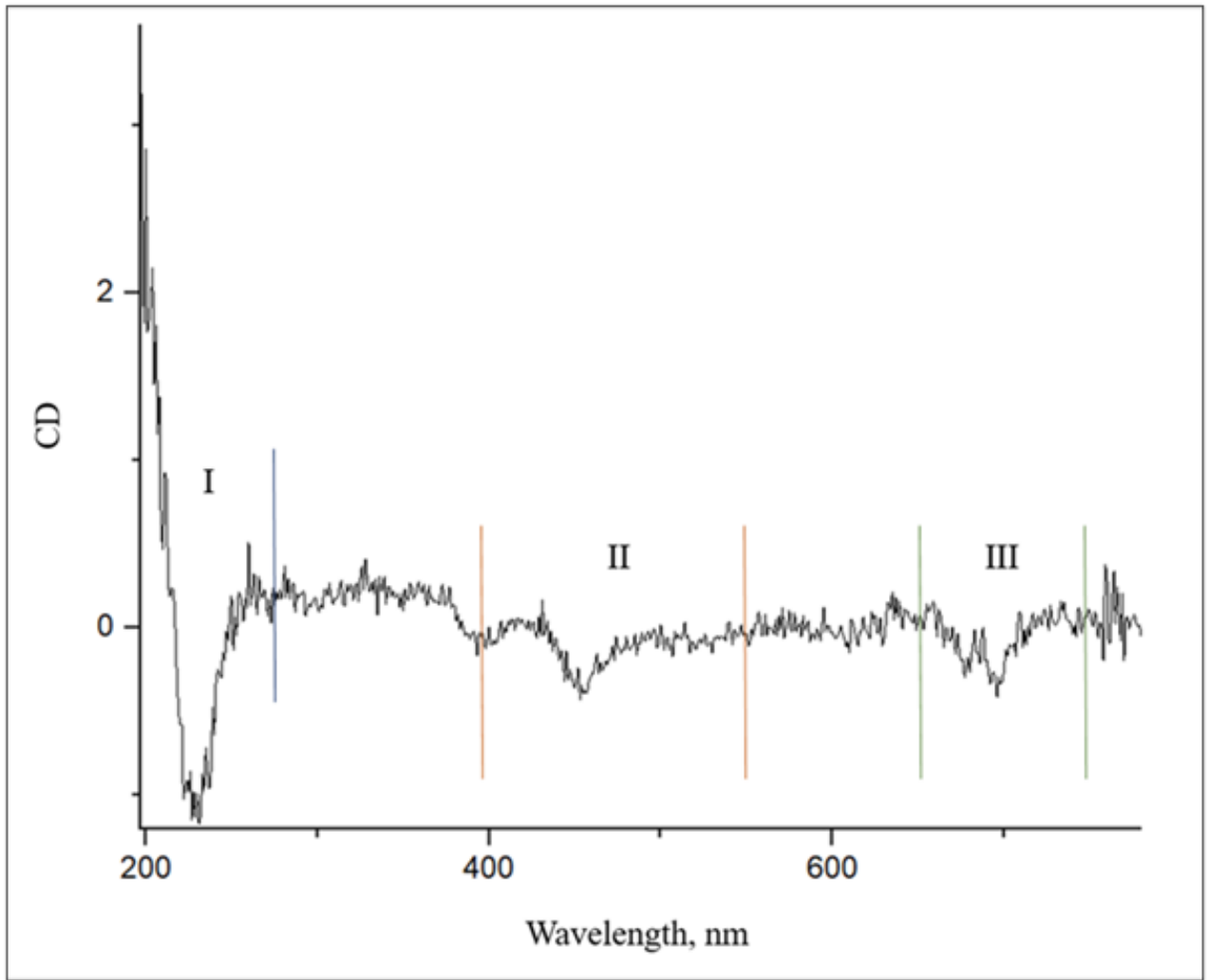
To isolate and purify the HliA protein, a plasmid carrying a synthetic HliA protein gene with the His6 label at the C-end of the protein was constructed on the basis of the pET-28a vector (Fig. 1). As a result of *E. coli* BL (DE3) transformation by this plasmid, the E. coli Yu-1 strain was obtained. The HliA protein was isolated by affinity chromatography. The pigments were extracted from *Synechocystis* sp cells. according to the method of Natali et al., 2014. Incubation of the recombinant HliA protein with pigments was performed according to the modified method of Grimm et al., 2020. The circular dichroism (CD) spectra were measured using a Chiroscan dichrograph (Applied Photophysics, UK).

Study of HliA protein binding to pigments, electrophoresis. When an alcohol extract of pigments was added to the fraction with purified recombinant HliA, a high yield of the target protein was observed, but the formation of a protein-pigment complex under denaturing conditions in the presence of 8 M urea was not observed (Fig. 2).

Fig.2. Content of HliA protein in fractions after affinity chromatography. Analysis of the formation of a pigment-protein complexes. Electrophoresis was performed in SDS-PAGE. The target protein HliA-His is found in the 1, 2, and 3 fractions of the eluate (Fig. numbered 5, 6, and 7, molecular weight 10 kDa, marked with arrows). The boxes indicate the areas where the fluorescence of chlorophyll that did not bind to HliA under denaturing conditions was observed.



Study of HliA protein binding to pigments. Reconstruction under milder conditions (beta-D-maltoside in low concentration (0.03%) and buffered medium). Analysis of the circular dichroism spectra of the protein in solution allows us to study its secondary structure. The formation of pigment-protein interactions was studied by CD spectroscopy. We conducted an experiment in which the CD spectra of the purified HliA protein preparation and the HliA preparation incubated with the pigment extract under mild conditions were measured (Fig. 3). The areas of the spectrum that primarily indicate the formation of pigment-protein bonds are marked with Roman numerals.



Changes in the CD spectrum of the protein+pigment preparation compared to the spectrum of the purified HliA protein suggested that the formation of a pigment-protein complex occurred during incubation. Further studies are needed to elucidate the structural changes of the protein molecule when binding to the pigment.

Fig.3. The CD spectrum of protein+pigment, λ 197-780 nm.

I-spectral region, λ 197-260 nm;

II-spectrum, λ 400-550 nm;

III-spectrum, λ 650-750 nm.

Results

- The expression of the hla gene in *E. coli* cells was obtained; the purified recombinant HliA protein was isolated by affinity chromatography;
- It was shown that for the formation of the protein-pigment complex in the solution, it should be carried out under mild conditions and reduce the content of denaturing agents used in protein isolation;
- The CD spectra of the protein+pigment preparation were obtained. Changes in the CD spectrum compared to the spectrum of the purified HliA protein suggested that the formation of a pigment-protein complex occurred during incubation. Further studies are needed to elucidate the structural changes of the protein molecule when binding to the pigment.

Thanks for your attention!